Compendium-cum-Souvenir

8th Annual Convention

of

Society of Veterinary Biochemists and Biotechnologists of India (SVBBI)

&

National Symposium

on

Unlocking the Potential of Veterinary Biochemistry and Biotechnology for Food and Nutrition Security

Organized by

Department of Veterinary Biochemistry
College of Veterinary Science and Animal Husbandry
UP Pandit Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalya evam Go Anusandhan Sansthan,
Mathura-281 001 (U.P.) INDIA
and
Society of Veterinary Biochemists and Biotechnologists of India (SVBBI)

20-21
December, 2024

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Go Anusandhan Sansthan, Mathura-281 001 (U.P.) INDIA
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Dr. Ambika Sharma

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उ.प्र. पं. दीनदयाल उपाध्याय पशु चिकित्सा विज्ञान विश्वविद्यालय
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evam Go Anusandhan Sansthan (DUVASU), Mathura-281 001 (U.P.) INDIA

प्रो. (डॉ.) ए.के. श्रीवास्तव
कुलपति

Prof. (Dr.) A.K. Srivastava
Vice-Chancellor

Message

It gives me immense pleasure to know that the department of Veterinary Biochemistry, U.P. Pandit Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan (DUVASU), Mathura in association with Society of Veterinary Biochemists and Biotechnologists of India (SVBBI) is organizing 8th Annual Convention of SVBBI on the theme "Unlocking the Potential of Veterinary Biochemistry and Biotechnology for Food and Nutrition Security" on December 20-21, 2024.

Food Security exist when all people, at all time, have physical, social and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preference for an active and healthy life. Nutrition security is a broader concept that refer to access to individuals to nutrients and their utilization for optional health. Household food security, care of the vulnerable segments of the population and adequate health service,s and environmental hygiene are the underlying determinants of food and nutrition security that have a very close interrelationship. Further, the constant threat of newly emerging and re-emerging infectious disease ignited great research interest among scientist across the world in the field of molecular Nutrition and its impact on health, resistance and immunity, in addition to the newer areas of molecular diagnostics, therapeutics and vaccine production for achieving one health goal. Furthermore the importance of nutrition to combat these emerging infectious diseases cannot be ruled out. This Convention of Society of Veterinary Biochemists and Biotechnologists of India (SVBBI) will be a suitable platform to discuss the latest trends in biochemistry and biotechnology to takle the challenges to achieve the food and nutrition security.

This national level symposium will be immensely beneficial in enriching knowledge among budding biochemists, and biotechnologists as they interact with stalwarts of their field.

I hope that through wide ranging discussions and deliberations, the scientists, academicians and industrialists from different fields will be able to generate new ideas to meet the challenges in food and nutrition security in the country.

I wish the organizers a grand success in their efforts.

Srivastava
(A.K. Srivastava)

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केन्द्रीय होम्योपैथी अनुसंधान परिषद्
(स्वायत्त निकाय आयुष मंत्रालय, भारत सरकार)
CENTRAL COUNCIL FOR RESEARCH IN HOMOEOPATHY
(An Autonomous Body of Ministry of AYUSH, Govt. of India)

आज़ादी का अमृत महोत्सव

डॉ. सुभाष कौशिक
महानिदेशक
Dr. Subhash Kaushik
Director General

I am delighted to learn about this 8th National Symposium on Veterinary Science. The veterinary sector of India is gaining more and more importance with the advancements in understanding of the animal kingdom. DUVASU plays a substantial role in imparting high-quality education on this front in our country.

Homoeopathy offers immense potential in veterinary medicine as a holistic and sustainable approach to animal healthcare. Its non-toxic remedies are especially suited for livestock and companion animals, effectively minimizing side effects while addressing pressing issues such as antimicrobial resistance.

The Central Council for Research in Homoeopathy (CCRH), in collaboration with DUVASU, Mathura, is spearheading efforts to advance research in this field. This collaboration aims to develop evidence-based protocols for managing common animal diseases, boosting livestock productivity and immunity, and supporting eco-friendly agricultural practices. By leveraging CCRH's expertise in Homoeopathic research and DUVASU's strengths in veterinary science, this collaboration is paving the way for integrating homoeopathy into mainstream veterinary care. This initiative underscores CCRH's commitment to innovative, interdisciplinary approaches to ensure a bright future of veterinary healthcare.

I wish this symposium a great success and hope the deliberations in the scientific sessions will guide us the way forward in this direction.

With best wishes and compliments,

(Subhash Kaushik)

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डॉ त्रिवेणी दत्त
निदेशक एवं कुलपति
Dr Triveni Dutt
Director-cum-Vice Chancellor

MESSAGE

It gives me immense pleasure to know that U.P. Pandit Deen Dayal Upadhayaya Pashu Chikitsa Vigyan Vishwavidyalaya (DUVASU), Mathura, in collaboration with the Society of Veterinary Biochemists and Biotechnologists of India (SVBBI), is hosting the 8th Annual Convention on December 20–21, 2024, with the theme "Unlocking the Potential of Veterinary Biochemistry and Biotechnology for Food and Nutrition Security".

As the global population continues to rise, ensuring food and nutrition security has become a pressing challenge. One of the most promising avenues for addressing this challenge lies in the fields of veterinary biochemistry and biotechnology. These interdisciplinary sciences offer innovative solutions to improve animal health, enhance food production systems, and ensure the sustainability of our agricultural practices.

Veterinary biochemistry plays a crucial role in understanding the biochemical processes that influence animal health, growth, and productivity. By studying how animals metabolize nutrients, fight diseases, and adapt to changing environmental conditions, scientists can develop more efficient and sustainable farming practices. Improving the nutritional content of animal feed through biochemistry can lead to healthier livestock, higher-quality meat, and dairy products, which are vital sources of protein and other essential nutrients for human populations. Biotechnology, on the other hand, enables the development of advanced tools and techniques to address food security challenges. Genetic engineering, for instance, can help produce animals with enhanced disease resistance, faster growth rates, or improved reproductive performance. This can lead to more efficient production of livestock and fish, reducing the strain on natural resources and minimizing the environmental impact of food production. Additionally, biotechnology can assist in the creation of vaccines and diagnostic tools that help prevent and control animal diseases, ensuring a stable and safe food supply. Together, veterinary biochemistry and biotechnology hold immense potential for addressing the growing demand for food while improving nutritional outcomes and ensuring the sustainability of global food systems. Their applications extend beyond animal production; they also contribute to the development of functional foods, better food safety practices, and innovations in food processing that can directly benefit human nutrition.

I extend my heart-felt congratulations to organizing committee for arranging an unique conference with the aims to bring together the scientists, faculty members, entrepreneurs and post graduate students from different corners of the country to exchange knowledge and discussion on unique theme regarding food and nutritional security.

(Triveni Dutt)

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भाकृअनुप - राष्ट्रीय पशु आनुवंशिक संसाधन ब्यूरो
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डॉ बी. पी. मिश्रा
निदेशक
Dr. B. P. Mishra
Director

Message

It gives me immense pleasure that the Society of Veterinary Biochemists and Biotechnologists of India (SVBBI) is organizing its 8th Annual Convention and National Symposium (SVBBICON 2024) on 'Unlocking the Potential of Veterinary Biochemistry and Biotechnology for Food and Nutrition Security' at UP Pandit Deen Dayal Upadhyay Pashuchikitsa Vigyan Vishwavidyalya Evam Go Anushanthan Sansthan, Mathura (Uttar Pradesh) during 20-21 December 2024.

Animal based food occupy significant role in human diet and provides nutritional security to millions of people, globally. Animal produces and products are the most promising food to reduce the hunger and malnutrition, with enormous opportunity for their production and quality. In recent time, India has witnessed tremendous growth in the sector, with an annual growth of 5 to 8 percent for various animal produces including milk, meat and egg. New technology and innovation remained significant contributor to such a stellar growth of the sector in the country. Surely, biochemistry and biotechnology have laid a strong foundation for the innovations to enhance animal production and improving quality and value of the animal products.

Indigenous Animal Genetic Resources (AnGR), well known sources of functional food, may be vital for exploration of specific biomolecules and metabolites using biochemical and biotechnological research. Such research in the country has also gained momentum, in recent time and resulted in identification of some of the important biomolecules. However, the efforts to explore such attributes need to be hastened, and, the recent advances in biochemistry and biotechnology would be the most potent tools to explore the uniqueness of indigenous animal produces and products. Surely, new biomolecules would also be important for branding and value addition of indigenous animal products, besides providing nutraceutical benefits.

Hope this Symposium would provide a learning platform to the scientists, academicians, and young researchers from all parts of the country to discuss and deliberate this very theme on - Unlocking the Potential of Veterinary Biochemistry and Biotechnology for Food and Nutrition Security'. I hope the deliberations would be helpful in making effective recommendations for formulating future programmes on these lines.

I congratulate the organizers for arranging this Symposium and convey my best wishes for its great success.

[B.P.MISHRA]

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दिनांक/ Dated : **December 10, 2024**

Dr. T.K. Datta
Director, ICAR-CIRB

Message

I am delighted to know that UP Pandit Deen Dayal Upadhyaya Pashuchikitsa Vigyan Vishwavidyalya evam Go Anusandhan Sansthan, Mathura is organizing the National Symposium on "Unlocking the Potential of Veterinary Biochemistry and Biotechnology for Food and Nutrition Security" on December 20th and 21st, 2024.

Biomolecules—DNA, RNA, proteins, lipids, and metabolites—are the foundation upon which the modern world of "omics" has been built. The central role of these molecules in life processes has guided the development of genomics, proteomics, transcriptomics, metabolomics, and all other omics disciplines. The evolution of omics from its early beginnings to its current prominence has been transformative. Starting with Gregor Mendel's principles of inheritance in the 19th century and advancing through the discovery of DNA's structure by Watson and Crick in 1953, the field of genetics and biochemistry laid the groundwork for genomics.

The central dogma, with its elegant simplicity, presents a compelling framework for understanding biological processes. It posits that DNA encodes messenger RNA (mRNA), which in turn dictates protein synthesis, positioning genes as the fundamental blueprints of life. This concept has profoundly shaped research over the past half-century, guiding investigations into the molecular underpinnings of disease and phenotype. Furthermore, it has served as the foundation for developing laboratory tools to probe these biological mechanisms, solidifying its importance in molecular biology and genetics. With the advent of high-throughput DNA sequencing technologies, notably the completion of the Human Genome Project in 2003, genomics became a cornerstone of biological research. This technological leap enabled the identification, mapping, and analysis of entire genomes, providing a deeper understanding of genetic regulation and variability. It has expanded this paradigm, unveiling a previously uncharted domain of non-coding RNAs (ncRNAs). While not directly encoding proteins, many ncRNAs play vital roles in cellular processes. However, the functions of many ncRNAs remain elusive, with some displaying variable sequence conservation and inconclusive phenotypic effects when knocked out. This complexity presents challenges in fully understanding their roles but also opportunities for discovery. Emerging technologies like CRISPR/Cas9 offer precise tools to surgically remove genomic regions encoding ncRNAs, potentially shedding light on their biological significance and paving the way for innovative approaches to improve food security.

This symposium focussed on the biochemical and biotechnological approaches to enhance food security will shed light on innovative ways to battle the Global Hunger crisis. I believe the symposium will bring forth thorough understanding and a stronger collaboration between academia, industries, stakeholders, and policymakers. I applaud the organizers and extend my best wishes to all the participants of the symposium for a grand success.

(T.K. Datta)

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डॉ अशोक कुमार मोहंती/Dr. Ashok Kumar Mohanty
निदेशक/Director

MESSAGE

It is a matter of great pleasure for me that UP Pandit Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go-Anusandhan Sansthan, Mathura is organizing 8th Annual Convention of SVBBI on the theme 'Unlocking the Potential of Veterinary Biochemistry and Biotechnology for Food and Nutrition Security' on 20th – 21st December 2024.

Biochemistry and biotechnology has taken a big leap forward offlate where in Genetic engineering, Protein Science, Genomics, Proteomics, Metabolomics and lipidomics are discovering new frontiers in systems biology. These approaches have led to solution in all areas of biology in general including veterinary science with specific reference to meeting challenges on production, reproduction and diseases of animals.

The constant threat of emerging and re-emerging trans-boundary infectious diseases has sparked global research in molecular diagnostics, therapeutics, and vaccine development to achieve the One Health goal. Additionally, the crucial role of nutrition in boosting immunity to combat these diseases cannot be overlooked. The 8th Annual Convention of the Society of Veterinary Biochemists and Biotechnologists of India (SVBBI) will provide an excellent platform to explore the latest advancements in biochemistry and biotechnology to address these pressing challenges.

I wish you all a journey of brilliance and success as you shine in this event.

(A.K. Mohanty)

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डा. मनीष कुमार चेटली
निदेशक
Dr. Manish Kumar Chatli
DIRECTOR

MESSAGE

I am delighted to know that **Society of Veterinary Biochemists and Biotechnologists of India** is organizing 8th Annual Convention on the theme ***'Unlocking the Potential of Veterinary Biochemistry and Biotechnology for Food and Nutrition Security'*** on 20th – 21st December 2024 at DUVASU, Mathura. The selected theme resonates deeply with the pressing global challenges of food and nutrition security, highlighting the crucial role of veterinary biochemistry and biotechnology in fostering sustainable solutions. With the world's growing population and the increasing demand for safe, nutritious, and sustainable food, innovations in veterinary science offer transformative solutions. I hope that the scientific deliberations in the symposia will address global challenges in food and nutrition security, and will ensure healthier animals, safer food, and resilient ecosystems.

Your meticulous planning and commitment to excellence will ensure meaningful interactive sessions balanced with scientific rigor and practical relevance and structured feedback system to assess the event's success and gather insights for future improvements.

I wish you great success with the upcoming conference and am confident it will serve as a catalyst for innovation and collaboration in this vital field.

Manish Kumar Chatli

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SOCIETY OF VETERINARY BIOCHEMISTS AND BIOTECHNOLOGISTS OF INDIA (SVBBI)
Regd. No.22912/122

Message

I am delighted that the Society of Veterinary Biochemists and Biotechnologists of India (SVBBI) is holding its **8th Annual Convention & National Symposium on 'Unlocking the Potential of Veterinary Biochemistry and Biotechnology for Food and Nutrition Security' (**SVBBICON-2024) at the Veterinary University (DUVASU) Mathura on 20-21 December 2024. It is being organized jointly by the Department of Veterinary Biochemistry, UP Pandit Deen Dayal Upadhyaya Pashuchikitsa Vigyan Vishwavidyalya evam Go Anusandhan Sansthan, (DUVASU) Mathura-281 001 (U.P.) and the SVBBI, Bhubaneswar.

In the National Symposium, besides the Symposium on Nutrition and Food Security, there would be six Technical Sessions: Advancement in Animal and Fish Biochemistry (TS-I); Innovations in Biotechnology (Food, Animal, Fish, Environmental and Nano-technology) (TS-II); Contemporary Research in Molecular Diagnostics and Vaccine Production (TS-III); Advancement in Genomics, Proteomics and Metabolomics Research (TS-IV); Development in Alternative Medicine, Pharmaceutical and Nutraceutical Research for Animal Health and Production (TS-V); Innovations in Aquaculture, Wildlife and Veterinary Research Industry-Academia-Student Meet (TS-VI). This platform will facilitate great scientific discussions in Veterinary Biochemistry & Biotechnology for exchange of knowledge and ideas leading to better deliverables for the growth of animal husbandry and veterinary sector. Needless to say animal health is of paramount importance for global food, nutrition and livelihood security and also for achieving the Sustainable Development Goals (SDGs). To become a 'Viksit Bharat' (Developed Country), first step is food sufficiency and keeping the malnutrition and hunger away. In this context, the animal husbandry sector has the biggest role to play in fighting protein hunger and hidden hunger.

The SVBBICON-2024 aims to bring together scientists, research scholars, academicians from Biochemistry, Biotechnology and allied-disciplines as well as industry partners and entrepreneurs for scientific discussions, and knowledge sharing for better understanding of the challenges and opportunities of the sector. The scientific deliberations and interactions would comprise lead talks, invited lectures, research paper and abstract presentations. Several Awards like: the SVBBI-Dr. LN Singh Lifetime Achievement Award, SVBBI-Senior Scientist Award, SVBBI-Young Scientist Award, SVBBI-Best Teacher Award, Best Doctoral Thesis Award, Best Master's Thesis Award, and Fellowship of the Society have been introduced during the Mathura Convention (SVBBICON-2024) for the members aimed at stimulating the members for healthy scientific competition, peer recognition, and ultimately for doing excellent, basic and applied science.

A **Compendium** is being brought out to document the scientific deliberations. I am sure this compendium would be an amalgamation of current science, emerging science, new ideas and innovations for future scientific advancements.

I extend my Best Wishes to the participants and the organizers for the grand success of the Convention and National Symposium - SVBBICON-2024 and also to enjoy visiting the Holy Cities of Mathura and Vrindavan.

November 30, 2024

(Bimal Prasanna Mohanty)

Dr. Subhasis Batabyal
General Secretary SVBBI,
Prof. & Head,
College of Veterinary Science,
WBUAFS, Kolkata

Message of Secretary for SVBBICON 2024 Mathura

It is with immense pleasure and pride that I welcome you all to the 8th Annual Convention of the Society of Veterinary Biochemists and Biotechnologists of India (SVBBI) and the National Symposium on "Unlocking the Potential of Veterinary Biochemistry and Biotechnology for Food and Nutrition Security" (SVBBICON-2024).

This annual event has consistently served as a platform for researchers, academicians, industry professionals, and students to converge and share their latest research findings, innovative ideas, and practical experiences in the field of veterinary biochemistry and biotechnology. SVBBICON-2024 promises to be a landmark event, fostering interdisciplinary collaborations and stimulating insightful discussions that will shape the future of veterinary science.

As we navigate the complexities of the 21st century, the role of veterinary biochemistry and biotechnology becomes increasingly crucial. By harnessing the power of these disciplines, we can develop innovative solutions to address emerging challenges, such as antimicrobial resistance, zoonotic diseases, and food security. This book of research work showcases the collective efforts of researchers from across the nation who are dedicated to addressing critical challenges in animal health, food safety, and nutrition security. We are grateful to all the contributors for their valuable contributions and for sharing their knowledge with the scientific community.

I extend my heartfelt gratitude to the organizing committee, the scientific advisory board, and all the volunteers for their tireless efforts in bringing this event to fruition. I also thank our sponsors and collaborators for their generous support.

Let us come together at SVBBICON-2024 to celebrate the achievements of our field, explore new frontiers, and inspire future generations of scientists.

(Subhasis Batabyal)

UP Pandit Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalya evam Go Anusandhan Sansthan, Mathura-281 001 (U.P.) INDIA

Prof. Vikas Pathak
Dean,
College of Veterinary Science and A.H.
DUVASU, Mathura

Message

It is greatly satisfying to note that the Department of Veterinary Biochemistry, College of Veterinary Science, U.P. Pandit Deen Dayal Upadhayaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan, Mathura is organizing a National Conference and National Symposium on the critical topic "Potential of Veterinary Biochemistry and Biotechnology for Food and Nutrition Security". The Food and Nutrition Security involves physical, social and economic access to balanced and nutritious food, clean drinking water, nontoxic environment, and health care (preventive and curative) for an active and healthy life to all people, at all times. Nationwide assessments of diet show that Indian diets are qualitatively and quantitatively deficient not only in vitamins and minerals (hidden hunger) but also in proteins due to low intake of foods of animal origin. In this regard meat industry can play a notable and crucial role by using biotechnological tools as a weapon for augmenting the quality and quantity of animal food products through optimal animal health and production and thereby in attaining the food and nutrition security to all the individuals for national economic growth.

I hope that through wide ranging debates and discussions, stakeholders from diverse fields will be able to build connections, create collaborations, generate new ideas to meet the challenges and will join hands to create awareness among industry professionals, policymakers, and the general public about the significance of food and nutrition. It's my sincere urge to all the participants to utilize this prestigious forum to plan, discuss and derive the roadmap for producing safe, secure, healthy, nutritious and sufficient food for achieving the food and nutrition security for future generation.

Kudos to the organizers for putting this together and Best wishes for a successful event !!

(Vikas Pathak)
Chairman SVBBICON-2024
Dean, College of Veterinary Science and A.H.
DUVASU, Mathura

UP Pandit Deen Dayal Upadhyaya
Pashu Chikitsa Vigyan Vishwavidyalya
evam Go Anusandhan Sansthan,
Mathura-281 001 (U.P.) INDIA

Dr. Vijay Pandey
Organizing Secretary
Professor and Head
Veterinary Biochemistry

From the Desk of Organizing Secretary

On behalf of the Organizing Committee, with great enthusiasm and pleasure I profoundly greet the executive members of society, eminent invitees, professionals and budding and young scientists, 'the students' from all over the nation to the distinguished 8th Annual Convention of Society of Veterinary Biochemistry and Biotechnology and National Symposium at the most sacred city of India, Mathura from 20th to 21st December 2024.

The theme preferred for SVBBICON-2024 is “Unlocking the Potential of Veterinary Biochemistry and Biotechnology for Food and Nutrition Security" - A theme that reflects our commitment for recognizing and understanding significance of the real challenges of the Society and embracing the advancements to counter the challenge. Since food and nutrition plays pivotal role in enhancing immunity and resistance to combat the metabolic and infectious diseases. This novel concept demands strong and efficient health plans along with concrete research strategies in the field of food and nutrition for maintaining the health of the society. Besides the traditional approaches, the biotechnological tools may be taken up as weapon to defy the obstacle in achieving the food and nutrition security and thereby alleviating the socio-economic status of rural masses.

We extend a warm and heartfelt welcome to all delegates and faculty for joining us in this special and unique scientific extravaganza to share their research findings, ideas, and rationally explore the challenges for attaining the food and nutrition security in the country. With these insights in mind the SVBBICON-2024 is organized to congregate group intellectuals on common platform to share their experiences, knowledge and innovative solutions to ease out the forbidding situation.

I express heartfelt gratitude and thanks to Prof. A.K. Srivastava, Hon'ble Vice Chancellor of University for his persistent motivation, encouragement and genuine guidance for successfully organizing this SVBBICON-2024. I would like to express my deep gratitude to our Dean College of Veterinary Science and Chairman of the Committee Prof Vikas Pathak who has been the driving force behind this whole process. I am extremely thankful to executive members of SVBBI for showing confidence on us and providing us the opportunity to host this event. I genuinely appreciate the ever-willing help and support provided by faculty colleagues and friends Dr(s) Pawanjit Singh, Ambika Sharma, Amit Singh and Anand Singh who worked day and night to make the program a great success. We look further for valuable deliberations during different scientific sessions with the intent of evolving fruitful commendation for drawing the future roadmap for current and prevailing challenges to food and nutrition security in India.

With adoring regards to one and all,

(Vijay Pandey)


प्रेम मन्दिर

About University

Uttar Pradesh Pandit Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go-Anusandhan Sansthan, Mathura, find first of its kind in the State and fourth in the Country, was established by Government of Uttar Pradesh vide U. P. Act. No. 27 of 2001 on 25.10.2001 with the College of Veterinary Science & AH, the erstwhile U.P. College of Veterinary Science & Animal Husbandry, Mathura as its main constituent College.

Brij area is known since time immemorial for lord Krishna and its animal wealth, particularly cows. There are large numbers of Gau-shalas in and around Mathura having hundreds and thousands of cows. The whole of Brij Kshetra is famous for its milk and products (doodh, dahi, makhan, pera etc.) Recognizing the important of live-stock of this area, the erstwhile U.P. College of Veterinary Science and Animal Husbandry was established in Mathura by Govt. of U.P. in 1947. It was the first Veterinary College in Asia to confer the degree in Veterinary Science. Ever since the establishment of this College, it has contributed significantly not only in terms of number of graduated and postgraduated of high scholastic order, but also quality research of national and international standards. This College has a glorious past and the distinction of having its alumni holding high positions in India and abroad as teachers, research workers, policy marker, consultants and administrators. In the year 1975, this College was made a constituent College of the newly established C.S. Azad University of Agriculture and Technology, Kanpur However, keeping in view the requirement of trained and competent manpower in the field of Veterinary Science, Animal Husbandry, Fisheries and other allied disciplines and also to give a fillip to research on different aspect of cattle production, Govt. of Uttar Pradesh established U.P. Pandit Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Exam Go-Anusandhan Sansthan, Mathura, the fourth Veterinary University in the country and the Veterinary College Mathura became the first constituent College of the University. The University is located on the Mathura-Agra road and is about 5 km from Mathura Junction railway station and 4 km from new bus stand. The main campus of the University is spread over a vast land area of 782.32 acres in Mathura Cantt and about 1400 acres at Madhurikund, about 20 km from the main campus.

Objectives of the University:

- Produce competent and skilled human resources who are socially sensitive and responsible professional in the field of animal health, production and allied sectors.
- Undertake region-based, need-based, and basic research for improving animal health and productivity by adapting modern technology including value addition.
- Validate Indigenous Traditional Knowledge (ITK) on scientific basis.
- Provide efficient extension services at the door step of poor and marginal farmers and livestock owners and motivating them to adopt animal husbandry, poultry, fishery and related vocations as an engine of economic growth and social empowerment.
- Empower women to become," Knowledgeable Livestock Stakeholder" giving them economic identity.
- Interface industry and stakeholder in the newer perspective of open global market.
- Ensure enhanced production from rural and urban livestock through effective diseases surveillance and diagnosis, health care, vaccination and production programs.

Targets of the University:

A) Teaching

- To revamp teaching programmers and "Teaching Methodologies", set up e-learning class-rooms, introduce net-based "virtual class-rooms" and promote e-teaching and learning;
- To set up "State of the Art" Instructional Livestock Farms, Demonstration Units, Veterinary Clinical Complex, Disease Investigation and Research Laboratories;

- To achieve at least 15 per cent increase per annum in the number of University graduate and postgraduate students qualifying for national competitive examinations;
- To produce competent and skilled clinicians, entrepreneurs and livestock business managers and team leaders;
- Faculty up-gradation, filling vacant teaching posts and creating faculty positions in newer proposed faculties in the University;
- Encourage faculty members to garner more financial assistance from outside agencies through externally funded research projects and support at least one University funded research project in each department to give impetus to research;
- As per University Act, to obtain state support for generating trained and competent human resource in fisheries, biotechnology, livestock products technologies and industry and business management through designated colleges/faculties; and
- To augment University receipts.

B) Research:

- To coordinate, assess & review the ongoing research work in the University.
- To visualize and implement the future research strategies of DUVASU.
- To conduct research work in the emerging areas of Veterinary and Animal Sciences
- To provide research and development support for generation of technologies and knowledge for the growth of livestock

C) Extension:

- To develop knowledge, skill and managerial aspect of extension functionaries.
- To develop capacity of the rural mass in certain economic activities in Animal Husbandry sustainable manner for boosting rural economy.
- To transfer the lab to land technology to the farming community and solve their problems.
- To develop liaison and co-ordinate with the other appropriate agencies for successful conduction of Extension work to the beneficiaries.

About Department of Veterinary Biochemistry

- Department of Veterinary Biochemistry came into existence after bifurcation of Department of Veterinary Physiology and Biochemistry in the year 1984. Apart from teaching of undergraduate students, department has good facilities for post graduate teaching and research.
- The mandate of the Department is to undertake region-based, need-based, applied and basic research in strategic and emerging areas for improving animal health and productivity by adapting modern technology as well as to impart undergraduate and postgraduate teaching and research in Veterinary Biochemistry. Since inception, 49 and 9 students have completed their MVSc and PhD degrees, respectively from this department and presently there are 4 students on roll. The main focus of the research is clinical biochemistry, reproductive biochemistry, molecular genetics, antimicrobial peptides, disease diagnosis and alternative medicines. Department has the distinction of having handled research projects awarded from International Foundation for Science, Sweden; Indian Council of Agriculture Research (ICAR), New Delhi, Central Council for Research on Homeopathy (CCRH), New Delhi and in organizing Summer institute in 1983, international symposium in 1984, Conference of Society of Veterinary Science and Biotechnology (SVSBT) in 2014 and e-Conference of Society of Veterinary Biochemists and Biotechnologists of India (SVBBI) in 2021.

- **Extra Mural Research Project:**

S. No.	Project Title	Investigator	Funding Agency	Budget
1	Management of otitis externa in dogs with homeopathic intervention vs the standard treatment–single blind, double armed, randomised clinical trial" has been sanctioned from Central Council for Research on Homeopathy, New Delhi	Dr. Vijay Pandey	Central Council for Research in Homeopathy, New Delhi	23.79 Lakh

Faculty Position

S. No.	Name of the Faculty	Designation
1	Prof. Vijay Pandey	Professor & Head
2	Dr. Pawanjit Singh	Associate Professor
3	Dr. Ambika Sharma	Associate Professor

SOCIETY OF VETERINARY BIOCHEMISTS AND BIOTECHNOLOGISTS OF INDIA (SVBBI)

Regd. No.22912/122

SVBBI HQs,
C/o: Department of Veterinary Biochemistry,
Faculty of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology (OUAT), Bhubaneswar 751003, ODISHA.
web: www.svbbi.in

About SVBBI, The Society

Inception: Visionary teachers and scientists in the field of Veterinary and Animal Biochemistry of the nation, including Late Prof. LN Singh, Late Prof. R.K. Srivastava, Prof. R.L. Prasad, Prof. Ashok Kumar, Prof. P.C. Bisoi and many more dreamt of a platform for overall development of the subject, Veterinary Biochemistry and Biotechnology. They convened the 1st formal meeting at Indian Veterinary Research Institute (IVRI) in the year 1995 and decided to form a Society named as "*Society of Veterinary Biochemists and Biotechnologists of India*" (SVBBI). The official registration process of the society was assigned to Prof. P.C. Behera, Department of Veterinary Biochemistry, Faculty of Veterinary Science and Animal Husbandry, OUAT, Bhubaneswar, Odisha who with the help of other active and enthusiastic members, registered the Society vide Regd. No.22912/22 of 2008 under Society Registration Act, 1960. Prof. R.L. Prasad was nominated as President, Prof. S. Batabyal, Vice President, Prof. P.C. Bisoi, Secretary, Dr. J.K. Ray, Joint Secretary and Prof. P.C. Behera, Treasurer as the Office Bearers of the Society.

Activities: As per the mandate of SVBBI, the academic and research activities in the subject of Veterinary Biochemistry and Biotechnology have been updated and expanded through organization of National Conferences and Symposia for mutual sharing of advanced knowledge and Annual Conventions for formulating the future plan of actions of the Society. In this context, Prof. P.C. Behera, as Organizing Secretary, hosted the 1st Annual Convention and National Symposium at Bhubaneswar during 11-12 March, 2016. Thereafter, the Annual Conventions and National Symposia were organized in different states across the nation.

1. 1st Convention - Bhubaneswar, 2016
2. 2nd Convention - Bengaluru, 2017
3. 3rd Convention - Hissar, 2018
4. 4th Convention - Tirupati, 2019
5. 5th Convention - Mathura (E-conference), 2021
6. 6th Convention - Jabalpur, 2023
7. 7thConvention - IVRI, Izatnagar, 2023

The 8th Annual Convention and National Symposium on 'Unlocking the Potential of Veterinary Biochemistry and Biotechnology for Food and Nutrition Security' (SVBBICON-2024) is being organized jointly by the Department of Veterinary Biochemistry, UP Pandit Deen Dayal Upadhyaya Pashuchikitsa Vigyan Vishwavidyalya evam Go Anusandhan Sansthan, (DUVASU) Mathura (U.P.) and the SVBBI, Bhubaneswar.

In the Annual Conventions, the SVBBI has felicitated the retired Professors and Scientists in recognition of their significant contributions in the field of teaching and research. Further, the young members are being encouraged for teaching and research excellence by conferring the “Teacher of the Year” and the “Scientist of the Year” Award, since 2021.

The journey ahead for the SVBBI is filled with possibilities, and its success lies in achieving the society's mission and in safeguarding the professional well-being of its members. Although hosting conventions, symposia, conferences, and felicitations for retiring members and such other activities have been conducted in regular manner, many other features planned last year have been achieved.

The Society has its own web-site (www.svbbi.in) now, which was launched on Saturday 27 April 2024, on the occasion of the World Veterinary Day - 2024.

From this 8th Annual Convention at DUVASU, Mathura (SVBBICON-2024) several Society Awards such as the SVBBI-Dr. L.N. Singh Lifetime Achievement Award, SVBBI-Senior Scientist Award, SVBBI-Young Scientist Award, SVBBI-Best Teacher Award, Best Doctoral Thesis Award, and Best Master’s Thesis Award Society are being introduced for the Life Members of the Society. These awards are for all age groups of members. This is aimed at stimulating the members for healthy scientific competition, peer recognition, and ultimately for doing excellent, basic and applied science for One Health under the triad “Animal Health - Human Health - Ecosystem health).

The Fellowship of the Society (FSVBBI) will be awarded to the members of the society from 2024 (SVBBICON-2024) onwards. There is a laid-down procedure and format for selecting the awardees for the awards and fellowship from amongst the nominations.

Publishing the Society’s journal “Indian Journal of Veterinary Biochemistry and Biotechnology” remains the next objective that is a cherished desire of the members of SVBBI.

Office Bearers of the SVBBI

Executive Committee:

 **Dr. B. P. Mohanty** (President)	 **Dr. P. E. Prasad** (Vice president)	 **Dr. Subhasis Batabyal** (Secretary)	 **Dr. P. C. Behera** (Treasurer)
 **Dr. Vijay Pandey** (Joint Secretary)	 **Dr. Chanchal Singh** (Joint Secretary)	 **Dr. K. Padmanath** (Joint Secretary)	 **Dr. Anil Gattani** (Joint Treasurer)

Advisory Committee:	**Selection Committee:**	**Editorial Committee:**
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Dr. P.C. Bisoi (Co-Chairman)	Dr. T. P. Rao (Member)	Dr. P.S.L. Sesh (Co-Chairman)
Dr. R. Nigam (Co-Chairman)	Dr. S.V. Perumal (Member)	Dr. Barkha Gupta (Member)
	Dr. Ramesh D. (Member)	Dr. Pratiksha Raghuwanshi (Member)

Zonal Representatives:

Sl. No.	Zone name	Zonal representatives	States
1.	North Zone	Dr. Pawanjit Singh (Mathura) Dr. Ajay Kumar (IVRI)	Jammu and Kashmir, Ladakh, Himachal Pradesh, Punjab, Uttar Pradesh, Uttrakhand
2.	South Zone	Dr. K. Padmanath (TN) Dr. Ramesh D. (Karnataka)	Karnataka, Kerala, Andhra Pradesh, Tamil Nadu, Pondicherry
3.	East Zone	Dr. Ajeet Kumar (Bihar) Dr. Apratim Maity (WB)	Bihar, Jharkhand, Orissa, West Bengal
4.	West Zone	Dr. Barkha Gupta (Rajasthan) Dr. Surbhi (Haryana)	Rajasthan, Gujarat, Haryana, Goa
5.	Central Zone	Dr. T. Prasad Rao (A.P.) Dr. Sonali Borker (Maharashtra)	Maharashtra, Chhattisgarh, Madhya Pradesh, Telengana
6.	North-East Zone	Dr. ParthaSarthi Behera (Aizwal) Dr. Shantanu Tamauli (Assam)	All North Eastern States

SVBBI Executive Committee

Organizing Committee of the SVBBICON-2024

8th Annual Convention

of

Society of Veterinary Biochemists and Biotechnologists of India (SVBBI)

&

National Symposium

on

Unlocking the Potential of Veterinary Biochemistry and Biotechnology for Food and Nutrition Security

from

20-21 December, 2024

Department of Veterinary Biochemistry
College of Veterinary Science and Animal Husbandry
UP Pandit Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya evam Go Anusandhan Sansthan,
Mathura-281 001 (U.P.) INDIA

Prof. A.K. Srivastava
Patron
Vice-Chancellor
DUVASU, Mathura

Prof. Vikas Pathak
Chairman
Dean
College of Veterinary Science and Animal Husbandry

Prof. Vijay Pandey
Organizing Secretary
Professor and Head
Veterinary Biochemistry

Dr. Pawanjit Singh
Joint-Organizing Secretary (Treasurer)
Associate Professor
Veterinary Biochemistry

Dr. Amit Singh
Technical Secretary
Professor & Head,
Veterinary & Animal Husbandry Extension

Dr. Ambika Sharma
Technical Secretary
Associate Professor
Veterinary Biochemistry

Dr. Ajay Pratap Singh
Technical Secretary
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Dr. Shanker Singh
Technical Secretary
Associate Professor
Veterinary Medicine

Dr. Soumen Choudhury
Technical Secretary
Associate Professor
Veterinary Pharmacology

Dr. Sanjay Purohit **Member** Professor & Head, Veterinary Surgery	**Dr. Rajneesh Sirohi** **Member** Associate Professor, Livestock Production Management	**Dr. Neeraj Gangwar** **Member** Associate Professor, Veterinary Pathology	**Dr. Udit Jain** **Member** Associate Professor, Veterinary Public Health

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Innovations in Biotechnology (Food, Animal, Fish, Environmental and Nano-technology) (IBT)

Innovations in Aquaculture, Wildlife and Veterinary Research (AWVR)

National Symposium:

Unlocking the Potential of Veterinary Biochemistry and Biotechnology for Food and Nutrition Security

01-NS

Food and Nutrition Security: Livestock sector is most vital

Talk by Prof. A.K. Srivastava, Hon'ble Vice Chancellor, DUVASU, Mathura

02-NS

Next-Gen Technologies and Software/Databases for Proteomics: Transforming Aquaculture Research

Prof. Sanjeeva Srivastava
Department of Biosciences and Bioengineering
Indian Institute of Technology Bombay, Powai, Mumbai 400076, India

Recent advancements in proteomics, including high-resolution mass spectrometry, targeted approaches, and sophisticated software tools, have revolutionized large-scale analysis of protein expression, modifications, and interactions. These innovations, complemented by user-friendly databases and interactive platforms, have significantly expanded proteomics applications across diverse fields. However, their potential in veterinary and fisheries science remains underutilized. The second part of this presentation will showcase the application of next-gen proteomics technologies in aquaculture through case studies on Labeorohita, a key species in global fisheries. Comprehensive proteomic profiling of 17 tissues, blood plasma, and embryos identified 8,498 proteins, representing 26% of the annotated protein-coding sequences in Labeorohita. This study revealed tissue-specific expression patterns, validated biologically significant proteins, and performed an extensive analysis of global post-translational modifications (PTMs) such as acetylation, methylation, and phosphorylation. To enhance accessibility, a dedicated web-based portal (www.fishprot.org) was developed, offering researchers an interactive proteomic resource for this species. This work underscores the transformative potential of next-gen proteomics technologies, integrated with advanced software and databases, to drive innovation in aquaculture research and address critical global challenges such as food and nutritional security.

03-NS

Newer insights into pro-and prebiotics functional efficacy

Ankur Rastogi[1], Pratiksha Raghuwanshi[2], R K Sharma[1], Imran Ahmed Ganai[1], Kanwaljeet Kour[1], Yadvendra Singh Parihar[1], Mahima Khajuria[1] and Chaitanya K Sonule[1]
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[2]Division of Veterinary Physiology & Biochemistry, FVSc& AH, SKUAST-Jammu

Prebiotics and probiotics research and applications have been fueled by their role in optimizing gut health, preventing dysbiosis and a resultant increase in available nutrients, reducing infection stress and improving growth rate and efficiency. They are extensively used to replace antimicrobial growth promoters in feed in the pigs and poultry industry and reduce infant mortality in the dairy and human healthcare industries.

In the mutualistic relationship between microbial communities and the human host, the host provides the resources for bacterial growth while the microbial communities defend against infections, aid in the digestion of food and fiber, generate vitamins, and metabolize xenobiotics. On the other hand, prebiotics, such as fructooligo saccharides (FOS) and inulin, are non-digestible carbohydrates that stimulate the growth of probiotic microorganisms. Furthermore, synbiotics, a combination of probiotics and prebiotics, have been shown to have greater effectiveness than either alone.

Probiotics and prebiotics, or their combined supplemented synbiotics (all three referred to collectively as biotics), are known to benefit the immune system. They have been shown to reduce harmful bacterial colonization, prevent infections, and improve the gastrointestinal tract. Recently, probiotics have gained attention for their potential to improve health outcomes in skin conditions, genital tract disorders, and infertility. These beneficial effects are attributed to several key mechanisms by which probiotics eradicate pathogens and maintain a healthy balance of gut flora. These mechanisms include competing with pathogens for nutrients and adhesion sites in the gut, enhancing intestinal barrier functions, improving the immune system, and producing neurotransmitters, which makes it difficult for harmful pathogens to thrive. Probiotics also function as antimicrobial agents by producing substances, such as organic acids and hydrogen peroxide, which combat the pathogenic bacteria in the gut. In addition, probiotics increase the production of mucin proteins, which strengthen the function of the intestinal barrier.

Research on biotics has focused predominantly on these areas; however, the mode of action for most of the effects credited to these molecules is still unclear. Evidence is emerging that pre-and probiotics could exert a vast array of effects involving most physiological systems rather than being limited to the gut and related organs. These far-reaching effects have answered some underlying questions regarding their mode of action but have raised many more questions.

Recent studies have affirmed that these molecules have manifold effects that could affect the health and productivity of animals and humans more holistically than we have imagined.

Immune system interactions

The gastrointestinal system is home to 70-80% of the body's immunological population. Dendritic cells, innate lymphocyte cells, intra-epithelial lymphocytes, macrophages, T and B cells, and other immune cell types are clustered inside the mucosa and lamina propria. Together, intestinal immune cells control the permeability and integrity of the intestinal barrier to create a highly effective and functional immunological barrier against bacterial invasion. It is closely related to the gut microbiota, and the two have evolved together to shield the host from outside threats. Cytokines enhance the mucosal barrier by inducing innate lymphoid cells to generate IL-22 in response to commensal microorganisms. As a result, epithelial cells absorb more lipids, enhancing their barrier function. Due to its high degree of flexibility and capacity to be altered by dietary interventions, the

gut microbiota presents a promising target for therapeutic modification. Probiotics improve the function of the gut barrier, reduce inflammation by activating Tregs, and increase the expression of tight junction proteins in intestinal epithelial cells. Dysregulations in the immune system, metabolism, and gut hormones have all been connected to disruptions to these microbial communities, also called gut microbial dysbiosis. As a result of these dysregulations, autoimmune and inflammatory illnesses like colitis may eventually arise.

The gut microbiota's composition influences immune function and controls the local generation of antibodies. The glycocalyx and inner mucous layer keep apart enterocytes and gut bacteria. Still, intestinal dendritic cells can extend their dendrites into the intestinal lumen to sample the microbiota. Macrophages eliminate most of these invasive germs, and some are also exposed to B cells. IgA, produced in the lumen by the B cells, binds to bacteria and initiates specific bacterial death.

Depending on the cues from the microbiota, intestinal helper T (Th) cell precursors can differentiate into Treg or Th17 cells. Th17 cell production is inhibited at homeostasis, Treg cell production is promoted, and gut wall inflammation is at its lowest. Unchecked effector T cells will react to microbial antigens and cause inflammation in the absence of Treg cells. Certain bacterial groups can affect this process. For instance, it has been demonstrated that segmented filamentous bacteria can induce Th17, generating pro-inflammatory signals and that members of Clostridium groups IV and XIVa can stimulate the induction of Treg, inducing an anti-inflammatory response.

Further, immune system-microbiota effects could also be mediated by microbial fermentation by-products. The role of short-chain fatty acids (SCFAs) is well documented. Still, it has been demonstrated that the amino acid tryptophan's breakdown by the microbes produces indole, which enhances immune homeostasis in the host microbiome, reduces inflammatory markers, and increases epithelial-cell tight-junction resistance. Additionally, the metabolite indole-3-propionate, which is related to indole, enhances gut barrier integrity.

The primary components of the bacterial cell wall, peptidoglycan and teichoic acid (TEIA), have been demonstrated to have immunomodulatory properties. Lipoteichoic acid (LTEIA) and wall-teichoic acid (WTEIA) are the two groups that make up TEIA. WTEIA creates covalent interactions with peptidoglycans, while LTEIA binds to the bacterial membrane via glycolipids. Peptidoglycans from *Lactobacillus* spp. prevent the release of inflammatory cytokines. Furthermore, by generating defensins and cathelicidin, LTEIA can effectively treat skin infections and prevent bacterial and viral infections.

Endocrine system interactions

The gut microbiome interacts directly with the gut's endocrine system and other endocrine glands via several complex mechanisms. Several mechanisms have been proposed for the beneficial effects of probiotics in restoring and maintaining hormonal homeostasis *in vivo*.

Probiotic metabolites trigger the release of signalling molecules that control hormone production in intestinal epithelial cells and other organs. The synthesis of GI hormones such as leptin, ghrelin, and GLP-1 is influenced by specific microbiota strains, indicating a role for the microbiota in appetite regulation.

They can also influence the release of key metabolically active hormones such as serotonin, glucagon-like peptide-1 (GLP-1), peptide YY (PYY), and cholecystokinin (CKK) from enteroendocrine cells in the gut. These hormones regulate essential metabolic processes, including glucose metabolism, insulin sensitivity, adiposity, and appetite.

SCFAs, in particular, are known to modulate the secretion of these gut peptides. GLP-1 and PYY, secreted by L-cells located primarily in the ileum and colon, play essential roles in regulating food intake and satiety.

The gut microbiota plays a pivotal role in thyroid disorders, including Grave's disease (GD). Microbes influence thyroid hormone levels by regulating iodine uptake, degradation, and enterohepatic cycling. Biotics may modestly reduce thyroid-stimulating hormone receptor antibody levels in patients with hyperthyroidism. The gut microbiota can produce neurotransmitters, such as

dopamine, which can regulate the hypothalamus-pituitary axis (HPA) and inhibit TSH. Studies have shown that *Lactobacillaceae* and *Bifidobacteriaceae*are often reduced in hypothyroidism and hyperthyroidism. In GD patients, the composition of the gut microbiota differs from that of healthy individuals, with a significant decrease in the relative abundance of *Faecalibacteriumprausnitzii*, *Butyricimonas faecalis*, *Bifidobacterium adolescentis* and *Akkermansiamuciniphila* compared to the control. Furthermore, a diagnostic model was developed using metagenome-assembled genomes of the gut microbiome, which may serve as a predictor for GD.

Probiotics *bifidobacterium longum* supplementation in GD patients significantly reduced clinical thyroid indexes, including fT3, fT4, and thyrotropin receptor antibody, while TSH levels increased compared to baseline. Synbiotics have shown potential in reducing TSH and increasing fT3 in patients with hypothyroidism. Stress can disrupt the HPA axis, leading to changes in cortisol levels, the primary stress hormone. Probiotics can regulate the HPA axis and reduce cortisol levels, thereby mitigating the adverse effects of stress. Biotics supplements decrease waking salivary cortisol reactivity.

Recent evidence highlights the role of the gut microbiota in regulating sex steroid levels. It affects estrogen metabolism through the estrobolome, a collection of bacterial genes encoding enzymes such as β-glucuronidase. These enzymes deconjugate estrogens, impacting their bioavailability and circulating levels. Additionally, the gut microbiota has been identified as a major regulator of androgen metabolism in the intestines, leading to relatively high levels of free dihydrotestosterone, the most potent androgen, in the colonic contents of young and healthy mice and men.

A disrupted gut microbiome, in turn, can have detrimental effects on reproductive and metabolic health through hormonal fluctuations and inflammation. Dysbiosis can lead to fluctuations in circulating estrogens by altering β-glucuronidase activity, which may contribute to metabolic complications and female infertility. Additionally, dysbiosis may promote PCOS development by increasing gut permeability, leading to systemic inflammation and insulin resistance. Dysbiosis-induced hypoestrogenemia could also influence the progression of endometriosis and androgen synthesis dysfunction. Intestinal dysbiosis may affect the secretion of multiple hormones and vitamins, including vitamin D, thyroid hormones, and insulin. Primary hypothyroidism is associated with altered bacterial diversity and reduced SCFA production, which may contribute to thyroid dysfunction by lowering thyroxine levels.

Mineral metabolism interactions

Probiotics have a role in modulating the absorption of most of the nutrients, but this effect is more pronounced with respect to minerals. Some strains of probiotics have been found to enhance the absorption of certain minerals, while others may inhibit or have no effect on mineral absorption.

The gut microbiome can interact with minerals in several ways. They may produce enzymes that can break down complex minerals, making them more accessible to the host for absorption. For example, bacteria in the gut produce phytases that humans or monogastric animals cannot endogenously produce. Phytases can break down phytic acid, a compound in many plant-based foods that can bind to minerals and prevent their absorption. By breaking down phytic acid, these bacteria can release minerals like Ca, Mg, and Zn, making them available for absorption in the small intestine. Phytase hydrolyses phosphomonoester linkages in phytate during enzymatic conversion, resulting in an inorganic phosphate and a derivative of myo-inositol phosphates. Gut dysbiosis can lead to decreased phytase production, resulting in reduced mineral absorption. It was observed that the phytases resulting from microorganisms are more effective in mineral absorption in animals than in plants or animal tissues.

Another way in which microbes can influence mineral absorption is by altering the gut environment. Fermentation of dietary fibers by microbes increases the surface area accessible for mineral absorption through epithelial cell hypertrophy. Further, by-products SCFAs can lower the pH of the gut, which can increase the solubility of certain minerals (Mg, Ca, Se, Zn, etc.), making them more accessible for the host to absorb. For example, reducing pH in the intestine leads to the

dissolution of Ca ions, facilitating their delivery to cells through the paracellular pathway and regulating the mineral density of bones. The production of SCFAs can influence Ca absorption due to their ability to increase the surface area of the cecal villi and, thus, the absorption. SCFAs can enhance Ca metabolism by increasing the Ca-binding protein expression and paracellular transport. Likewise, a high level of SCFAs can lower the pH of the colon and cecum, improving Ca solubility. The reduction in pH leads to the dissolution of minerals and a facile delivery of Ca ions to cells through the paracellular pathway, regulating the mineral density of bones. Also, SCFAs generated the hormone-insulin-like growth factor 1 (IGF-1, or somatomedin C), which benefits bone development, highlighting a link between the microbiota and bone metabolism.

Moreover, gut microbes can affect the expression of genes involved in mineral absorption in the host cells. For instance, certain bacteria can promote the expression of genes that increase the host cell's ability to absorb iron (Fe). Biotics supplementation leads to an upregulation of the expression of genes for Fe transporters, ferritin, and enzymes in the gut enterocytes.

It was shown that a healthy gut microbiome could increase Ca absorption and modulate gut production of serotonin. The monoamine neurotransmitter serotonin is believed to regulate bone metabolism through its interaction with bone cells, possibly mediating the microbiota and bones. Probiotics improve bone health by modulating osteoblast bone formation by osteoblasts and osteoclast bone resorption. *L. plantarum* has been shown tomodulate the transcellular pathway and enhance the expression of vitamin D receptors and Ca transporters such as claudin-2. When supplemented with *L. rhamnosus*strain, broilers predisposed to tibial dyschondroplasia demonstrated development of the tibial growth plate alongside a restored Ca balance.

GM has been shown to influence selenium status and SeP expression in mice. It was found that GM can isolate Se and limit its accessibility in the host. When Se amounts in the host are limited, microorganisms can compete with the host for Se. Se-enriched probiotic strains have been shown to significantly increase Se concentration *in vivo*, with SeCys as the predominant form of Se in the liver and kidneys of rats. An increased absorption and distribution of Se into the pancreas was observed after administering Se-*B. longum* to rats, in comparison to the Na2SeO3 formulation. Although Na_2SeO_3 exhibited the fastest time to reach the maximum absorbable concentration, supplementation with the organic Se formulations (selenized yeast and Se-*B. longum*) showed more efficient absorption, as these formulations had a higher accumulation and longer retention time of Se in the blood.

Recent studies suggest that certain probiotics may enhance Zn absorption through the expression of specific proteins involved in Zn transport across the intestinal wall or through the production of SCFA by lowering the gut's pH, which can increase solubility and promote its absorption.

Several studies have also investigated the relationship between probiotics and K absorption. The administration of probiotics to pigs positively impacted Sodium-Potassium ATPase enzyme activity within the intestinal tract. This enzyme plays a crucial role in maintaining electrochemical balance in cells, pumping sodium ions out of the cell and K ions into the cell.

The dosage of probiotics can play a role in their efficacy. Higher doses of probiotics may lead to more significant effects on mineral absorption.

In a study on the effect of a rumen liquor-derived prebiotic (RL) in the authors' laboratory, significant improvement in calcium balance was seen in broilers on RL supplementation. Further, significantly higher total ash (%) and calcium (%) were recorded in the tibia of broiler chickens of the supplemented group.

Cardiovascular interactions

Cardiovascular disease (CVD) encompasses a range of pathological disorders affecting the heart and blood vessels. Biotic supplements have demonstrated protective effects against CVD through cholesterol reduction, mitigation of oxidative stress, modulation of functional and structural alterations in the gut microbiota, and enhancement of immunological responses.

The gut microbiota metabolites, including trimethylamine N-oxide (TMAO) and SCFAs, have significant implications for predicting, improving, and mitigating cardiovascular disease. TMAO is a

potential metabolite that is a non-traditional CVD biomarker. TMAO increases atherosclerotic plaque size, triggers prothrombotic platelet function, and promotes arterial thrombus growth. Changes in the ratio of *Firmicutes* to *Bacteroidetes*, indicative of intestinal dysbiosis, change the production of SCFA and TMAO and significantly affect host physiology. A decrease in butyrate-producing bacteria, such as *Bacteroides, Butyrivibrio* and *Eubacterium*, results in various inflammatory diseases, such as hypertension, obesity, and diabetes, all of which are risk factors for cardiovascular disease.

The ratio of *Firmicutes* to *Bacteroidetes* (F/B ratio) and the diversity of gut microbiota decreases in cardiovascular disorders. The population of pathogens like *Shigella*, *Campylobacter*, and *Streptococcus spp.* were increased in the stool samples of patients with CVD.

In addition, the microbial cell wall component of Gram-negative bacteria, lipopolysaccharide (LPS), may compromise cardiovascular function and increase CVD risk.

Dyslipidemias, or abnormal levels of cholesterol and triglycerides, are frequently associated with type 2 diabetes. The administration of probiotics is associated with improvement in the lipid profile of patients with dyslipidemias.

Biotics exhibit beneficial effects on CVD through several mechanisms involved in the alteration of the gut environment, histone deacetylation, improvement of gut epithelial permeability contributing to reduced total and LDL cholesterol and hs-CRP, and lowering the incidence of CVD risk factors such as hypertension, obesity, and diabetes.

Supplementation of biotics with vitamin D or selenium for diabetic patients with CVD could significantly improve the metabolic profiles, such as hs-CRP, nitric oxide, LDL or total cholesterol, and the parameters involved in inflammation and oxidative stress. *L. plantarum* 299v supplementation ameliorated vascular endothelial function and attenuated system inflammation in Coronary artery disease (CAD). The intake of *Lactobacillus rhamnosus* GG also exhibited beneficial effects in reducing metabolic endotoxemia and mega inflammation in participants with CAD.

Further, SCFAs bind to deorphanized G protein-coupled receptors, such as acetate and propionate bind to free fatty acid receptors (FFAR) 2 and 3 and olfactory receptor 78 (Olfr78) to regulate blood pressure. Butyrate attenuated blood pressure by reducing the expression of renal protein receptors and renin.

Hyperglycemia in obesity and insulin resistance triggers increased gut permeability, contributing to an inflammatory cascade. SCFAs are crucial for maintaining a healthy gut, particularly in modulating epithelial integrity via tight junction proteins. Butyrate regulates proteins of the tight junction complex via acting on nucleotide-binding oligomerization domain-like receptors, which subsequently modulate inflammation.

Gut-brain interactions

It is recognized that the communication between the gut and the brain is mediated by multiple signals from neural, immune, and endocrine pathways. The neurological pathway includes the enteric nervous system (ENS), vagus nerves (VN), and gastrointestinal neurotransmitters. This is a bidirectional communication network called the gut-brain axis (GBA). Gut dysbiosis may alter brain structure, brain vascular physiology, and blood-brain barrier (BBB) permeability, which may cause neurological disorders and cognitive impairment. Gut microbes may impact the availability of nutrients, resulting in the alteration of peptide release from enteroendocrine cells and affecting the GBA. The dysbiosis of the gut microbiome may cause inflammation and release of cytokines, which also influences the GBA. The gut microbiome influences various brain growth and operations elements, such as microglia and astrocyte polarisation, maturation, and control of neurotransmission, neurogenesis, and myelination. A group of probiotics are known to improve psychological and mental health, influence behaviour, relieve stress and anxiety, and improve cognitive functions and are aptly titled "psychobiotics".

There are several interlinked systems by which biotics can potentially alter brain function through direct and indirect multi-modal action. In particular, these include the endocrine, the parasympathetic autonomic (e.g. vagus nerve), and the immune system. As ENS is separated from the

gut microbiota by the mucous cell layer; intestinal microbes do not have direct access to this local nervous system. Microbiota may communicate indirectly with ENS by transmitting them from the intestinal lumen to the lamina propria via the microfold cells or dendritic cells, given the direct entry of resident microbes invasively causing ulceration and perforation in the intestine.

The vagus nerve is an important pathway for neural communication between gut microbes. The discovery of the morphology of enteroendocrine cells, which act as sensors of the luminal content, suggests a complex interaction between these cells and vagal afferent neurons, acting via axon-like basal processes known as neuropods. These neuropods help the enteroendocrine cells communicate directly with enteric nerves, including the vagus nerve, to produce fast neurotransmission. However, there is also evidence that vagotomy does not disrupt the anti-anxiolytic effects of a relative increase in abundance of Lactobacilli in the innately anxious male BALB/c mice. This demonstrates that the vagus nerve is likely only one of the mechanisms through which gut bacteria can impact the brain.

Another possible pathway is intestinal bacterial secretions and metabolites such as SCFAs, exopolysaccharides (EPS), LPS, and glutamate that can cross the intestinal cell wall and directly affect the ENS, and can interact with some receptors, e.g., G-protein coupled receptors (GPCRs), and Toll-like receptors (TLRs). GPCRs are the receptors in the CNS striatum, that are crucial for regulating and controlling metabolism and the inflammatory process in mental disorders. SCFAs produced in the gut, stimulate and activate GPCRs at the ENS and CNS. TLRs are stimulated and activated by EPS and LPS. The activity of microbiota via TLR2, strengthen and regulate ENS integrity, stimulate glial cell line-derived neurotrophic factor, enhance the number of glial cells and enteric neurons, and ultimately strengthen several neurons. SCFAs can also induce hormone and neuropeptide production, such as glucagon-like peptide 1 (GLP-1) and peptide YY (PYY) from intestinal enteroendocrine cells. In addition, SCFAs are also epigenetic regulators, acting as histone deacetylase inhibitors, a mechanism implicated in learning and memory processes. Evidence shows that SCFAs may enter brain tissue, thus raising the possibility of direct CNS action.

Optimal balance of intestinal microbiome and strengthening of ENS and vagus nerve by biotics increases metabolites such as tryptophan and SCFAs that directly affect brain, and the level of some factors such as gamma-aminobutyric (GABA), serotonin/5 hydroxy tryptamine, brain-derived neurotrophic factor, and dopamine. GABA is the mammalian nervous system's primary inhibitory neurotransmitter, dampening overall mature brain activity and function. Although these neurotransmitters do not cross the blood-brain barrier under normal circumstances, alterations in the GABAergic system are associated with psychiatric manifestations, as observed in schizophrenia and the pathophysiology of depression.

Serotonin levels are closely correlated with mood, appetite and sleep, and dysregulation can lead to anxiety and depression. Gut bacteria such as Candida, Streptococcus, Escherichia, and Enterococcus can produce serotonin. Prolonged supplementation of probiotics increases the concentration of tryptophan, the precursor of serotonin, in the peripheral system and improves mental health.

The neurotransmitter dopamine is produced by spore-forming Bacillus. Dopamine is another key modulatory neurotransmitter and a precursor for other catecholamines (norepinephrine and epinephrine) and is closely associated with motivated behaviours. Together with norepinephrine, it is also known for its role in alertness, as well as cognitive functions such as memory, learning, and attention.

Another neurotransmitter that gut bacteria, such as Lactobacillus, can produce is acetylcholine, which plays a key role in learning, memory, and motor function. Acetylcholine is also a molecular target for conditions where these cognitive elements have been impaired, such as Alzheimer's disease.

The grade of psychological stress may be increased by dysbiosis in the gut. Conversely, chronic psychological stress may increase dysbiosis. The hypothalamic–pituitary–adrenal tension feedback, which regulates behaviour, is weakened by some probiotics, dropping corticosteroid amounts.

The association of gut microbiota with the cognitive function of infants in humans has been reported. Three different microbial clusters have been identified based on the variations in the

prevalence of three important bacterial species: Faecalibacterium, Bacterioides, and Ruminococcaceae. The study showed the differences between the three groups in the Early Learning Composite Score. The newborns with a comparatively high abundance of Bacteroides had the most significant scores. In contrast, newborns with a reasonably high abundance of Faecalibacterium had the lowest cognitive and motor development scores. Gut microbial diversity at the first year of life also reflected cognitive development in the second year of age.

In a landmark study, Sudo demonstrated that mice raised in sterile environments showed inflated physiological reactions to stress compared to control mice (Sudo, 2014). The exaggerated responses to stress were reversed by bacterial recolonization following probiotic administration.

Interestingly, the production and release of neurotransmitters have been shown to have a gender bias. Lactobacillus and Bifidobacterium, known to produce GABA and serotonin, are more prevalent in females than males. The term used for this sexually dimorphic microbiome is "microgenderome."

Conclusion

Gut microbiome appears to affect almost every aspect of mammalian physiology, and therefore, biotics offers immense opportunity to intervene and restore balance in metabolic errors, with minimum side effects. This microbial universe's interaction with the environmental factors and food and its interaction with host metabolic pathways have also increased our understanding regarding environmental effects over pathophysiological reactions in the body. Arguably, it may not be too early to predict that biotics supplementation will be the treatment of choice and possibly a prevention tool for a multitude of diseases in the near future.

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Metabolomics in Fisheries Research: Applications and Challenges

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Fisheries and aquaculture are sector are set to be the game changer in the fight against food insecurity as the word population continues to rise. Advances in fisheries research are need of the hour to ensure sustainable fish production by generating domain-specific knowledge for making policies and developmental interventions. Climate change is pushing fisheries and aquaculture industries to implement strategies that will help them cope with the environmental changes and continue producing sustainably. Understanding the physiological adaptations of organisms to different environmental stressors of varying nature and intensity as well as diseases in aquaculture systems. Research-based evidence is essential in planning for and enhancing the further development of the sector. Metabolomics is an extensive data-dependent method that will give robust valuable insights for understanding complex biological processes. It is increasingly being used with other omics techniques, such as transcriptomics, genomics, and proteomics, to understand life processes better. Unlike the conventional reductionist approaches, systems biology and omics approaches give a larger picture of the biological process. Fisheries and Aquaculture research greatly benefitted from this technique, from understanding the basic physiology underpinning key biological processes to identifying biomarkers under different stress conditions.

1. Systems Biology and Omics

Systems biology adopts mathematical and computational models to dissect and comprehend biological systems. This interdisciplinary field merges engineering, physics, computer science, and mathematics with experimental data. Omics techniques enable scientists to dissect and untangletheimmense intricacy of biological processes. Omics approaches in biology are extensively used to study complex biological processes. The term "Omics" is a suffix that refers to the study of the totality of a particular type of biological molecule or process. It is believed to come from the Greek word "ome," meaning "all" or comprehensive. Basically, the suffix omics is applied to certain types of biomolecules being examined, such as DNA to represent genomics, RNA as transcriptomics, protein as proteomics, and metabolites as metabolomics as a comprehensive and integrated approach to studying biological systems. Metabolomics is the study of small molecules called metabolites with molecular weight < 1500 da in an organelle, cell, fluids, and tissues in an organism in a given time using advanced spectroscopy techniques such as Mass spectrometry and NMR (Nuclear Magnetic Resonance) spectrometry (Markley *et al.,* 2017; Wishart, 2019). Metabolites are the substrates, intermediaries, or products of the cellular pathways, such as organic acids, carbohydrates (monosaccharides), amino acids, fatty acids, nucleosides, nucleotides, steroids and steroid derivatives, terpenoids, carotenoids, or flavonoids (Roques *et al.,* 2018) among several others i.e. glucose, lactate, di-tri peptides, phospholipidsetc. Among omics techniques, metabolomicsis particularly well suited to providing a closer view of an organism's phenotype. Because metabolites are highly responsive to environmental changes, they reveal real-time insights into metabolic and physiological activities (Patti *et al.,* 2012).

There are two approaches in metabolomics: Targeted and Untargeted metabolomics. Targeted metabolomics is a quantitative approach wherein selective metabolites of interest are quantified in the sample matrix using a standard of the metabolite of interest. Untargeted metabolomics, on the other hand, gives an entire snapshot of all metabolites that are present in the sample matrix and gives a relative abundance of metabolites across treatment groups against control. A targeted metabolomics experiment measures the levels of specific metabolites (e.g., metabolites in the TCA cycle). In contrast, an untargeted experiment will measure any molecule that ionises within a specific range of mass values. Targeted experiments provide better quantitation, typically through the use of internal

standards and specific mass spectrometer conditions, while untargeted experiments provide broader coverage.Untargeted metabolomics is used initially to understand the different metabolic pathways that are upregulated or downregulated and helps find the biomarker metabolites that are significantly altered in the sample due to the experiment or disease condition. Then, these validated metabolite biomarkers can be quantified using reference standards for accurate quantification.

2. Procedure or workflow of metabolomics study

A typical metabolomics study involves the following steps: (i) Clear experimental design, (ii) sample collection and preparation, (iii) Analytical measurement and data acquisition, (iv) bioinformatics (data integrity checking and metabolite identifications), (v) statistical analyses and (vi) biological interpretation and biomarker validation (Young and Alfaro, 2018; Kovacevic and Simpson, 2020).

2.1. Experimental design:

A well-crafted experimental design is crucial for a successful metabolomics study. Ensuring sufficient controls and replicates in each treatment group is vital for robust statistical analysis. Control and treatment groups must share the same genetic background and be matched for factors such as gender, age, size, and developmental stage (Alfaro and Young, 2018). Minimising biological, technical, and experimental variability is critical, as the metabolome can swiftly change in response to even minor environmental shifts.

2.2. Sample Preparation:

Metabolite extraction should be swift, robust and reliable to prevent sample degradation and metabolite modification (Allwood *et al.,* 2013). Caution must be taken to collect samples during the sample time of the day for repeated or time interval sampling to avoid errors. The sample preparation method depends on the biological sample type and the analysis instrument. Sample preparation can vary from using solvent to extractthemetabolitestomaking them more stable through robust derivatisation. Major solvent extraction methods include:

- Extraction of polar and nonpolar metabolites with a methanol, water, and chloroform mixture.
- Extraction of polar metabolites using methanol alone or with water.
- Extraction of polar metabolites using perchloric acid (Alfaro and Young, 2018).

The extracted metabolite samples are processed according to the analytical platform used for analysis, such as the derivatisation of samples for GC-MS or the reconstitution of metabolites in the mobile phase of interest in LC-MS. An internal standard of known concentration is usually spiked inside the samples for untargeted metabolomics analysis to achieve relative quantification of the analysts and method validation. A pooled quality control sample containing equal aliquots from all samples for analysis is used for method validation and accuracy for reproducible results between each sample run.

2.3. Analytical measurements:

Nuclear magnetic resonance (NMR) and Mass spectrometry (MS) are the most commonly used analytical techniques for high-throughput and high-resolution metabolomic studies. There is not a single platform that can analyse all the metabolites that are present in the samples, and some instruments offer coverage for a specific class of metabolites. The choice of instrumentation should be pre-determined based on the study objectives. Untargeted metabolomics is usually carried out using High-resolution Mass spectrometers such as Orbitrap or TOF (Time of Flight) coupled with Gas Chromatography (GC-MS) or Liquid Chromatography (LC-MS) for analyte separation before mass detection using MS or Tandem MS (LC-MS/MS and GC-MS/MS). Targeted metabolomics is usually carried out based on the analytes of interest using MS of any choice. The most commonly used ionisation is ESI (Electron spray ionisation) for LC-MS-based metabolomics analysis and electron impact or chemical ionisation for GC-MS-based metabolomics (Li *et al.,* 2015).

2.4. Bioinformatics and statistical analysis:

The raw data obtained from the metabolomics analytical platform in mzXML, mzData, mzML and netCDF formats is processed using vendor-specific software or open-source software such as MS-dial to generate a list of metabolite features table. Data conversion, spectral processing (e.g. deconvolution, alignment, noise reduction), feature selection, metabolite identification via database matching, metabolite quantification and quality control procedures are carried out using open-source software such as the R package for XCMS, MS-DIAL, Metaboanalyst, Galaxy or vendor-specific software such Progeneis QI (Non-linear dynamics, Waters^{TM}, USA) or Analyst® Software (Sciex). Subsequent statistical analyses are carried out using statistical software such as R, SIMCA, SPSS, etc. Generally, Univariate and Multivariate statistical analysis are used for metabolomics data analysis, wherein data dimension reduction techniques such as PCA (Principal components analysis), OPLS-DA (Orthogonal Polynomial Least square-discriminant analysis), PLS-DA (Projection to latent structures discriminant analysis) Hierarchical Cluster Analysis (HCA) and k-means clustering are used to identify the biomarker metabolites in the sample. The open-source web-based Metaboanlyst platform provides comprehensive tools for metabolomics analysis, from data processing to statistical analysis to pathway analysis to multi-omics integration.

2.5. Biological interpretation of results:

Various bioinformatics tools were available for the proper interpretation of metabolomics results, and Metaboanalyst is often widely used. Several algorithm-based analyses were developed for this purpose, such as Correlation network analyses for biological interpretation of results, which maps the relationship between every metabolite within the metabolite network. Metabolite Set Enrichment Analysis (MSEA) detects small but consistent changes among metabolite groups within biological pathways (Persicke *et al.,* 2012). Pathway enrichment techniques aim to identify altered metabolic pathways or networks. PAPi (Pathway Activity Profiling), an R package, quantitatively compares metabolic pathway activities between different sample groups, showing which pathways are upregulated or downregulated (Alfaro and Young, 2018).

3. Application of metabolomics in Fisheries and Aquaculture

Metabolomics, being a highly comprehensive and sensitive method, will be helpful in various disciplines in fisheries science: fish physiology, fish nutrition, fishery products and their processing, aquaculture environment, fish biotechnology, fish behaviour, and fish welfare.Metabolomics has been applied in fish research to study physiology and development (Kullgren *et al.,* 2013; Zhou *et al.,* 2017), infections by pathogenic microorganisms (Jiang *et al.,* 2019), and nutrition (Gil-Solsona *et al.,* 2019; Roques *et al.,* 2020).

4.1 Metabolomics in biochemical adaptions and stress physiology

Young *et al.,*. (2015) used a metabolomics approach to assess the physiological condition and quality of mussel (*Perna canaliculus*) larvae during hatchery production using Gas Chromatography-Mass Spectrometry (GC-MS). Robles *et al.,*. (2013) employed High-Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS) to measure over 80 metabolites in seabream intestines before and after a butyrate-supplemented diet. NMR-based metabolomics was used to monitor shrimp (*Litopenaeusvannamei*) health from nursery to harvest (Schock *et al.,* 2013) and to investigate spermatozoa energy metabolism during the swimming phase. Dreanno *et al.,*. (2020) used 31P and 1H NMR to study turbot (*Psetta maxima*) sperm before and after motility onset, aiming to identify molecular pathways providing motility energy. Metabolomics also helped differentiate between wild and farmed sea bream from various locations, marketed as the same high-quality product (Melis *et al.,* 2014), and investigate the effects of ocean acidification on mussels (Ellis *et al.,* 2014) and oysters (Wei *et al.,* 2015), thermal stress on mussels (Ellis *et al.,* 2014; Dunphy *et al.,* 2015), and sea cucumbers (Shao *et al.,* 2015).

Esmaeili *et al.,* 2023 investigated the feeding efficiency of chinook salmon in seawater using an integrated multi-omics approach using proteomics and metabolomics. They found that several metabolic pathways were activated over time, and the pathways involved in protein turnover were vital components. GC-MS-based metabolomics showed that killifish (*AustrofundulusLinnaeus*)

embryo metabolism is dominated by glycolytic production of lactate, with minor contributions from succinate and alanine during hypoxia (Podrabsky *et al.,*2007). Raposo deMagalhães *et al.,* 2022 reported metabolic reprogramming in the liver tissue of *Sparus aurata* under chronic stress using integrated proteomics and untargeted metabolomics. Integrated transcriptomics and metabolomics approach was used by Cao *et al.,* 2022 to assess the metabolic altercations when replacing fish protein and Fish oil with terrestrial proteins in the diets of Rainbow trout and found adenylosuccinate and adenosine monophosphate as potential biomarkers. Yang *et al.,* 2022 investigated the effects of hypoxia in the intestinal tissue of cobia using integrated transcriptomics and untargeted metabolomics approach, wherein abnormalities in antioxidant activities, protein and lipid metabolism. Wang *et al.,* 2024 elucidated the liver metabolism in response to hypoxia in yellow catfish (*Pelteobagrusfulvidraco*) using transcriptomics and LC-MS-based metabolomics.

Similarly, Ma *et al.,.* (2021) used a multi-omics approach to reveal the glycolipid metabolism response mechanism under hypoxia stress in the liver of GIFT tilapia. The effects of thermal stress in the kidney of *Scophthalmus maximus*were investigated by Yang *et al.,.* (2020), who found decreasing levels of essential amino acids. LC-MS/MS-based metabolomics was used to ascertain the metabolic adaptation of Atlantic salmon when transferred from freshwater to seawater through stages of pre-smolt, smolt and post-smolt stages (Su *et al.,* 2024). Van Nguyen *et al.,.* 2020 elucidated the effects of thermal stress (summer mortality) in *Perna canaliculus* using GC-MS based metabolomics approach. They found metabolic perturbations in stressed groups compared to control. Azizan *et al.,* 2023 investigated the metabolic perturbation of *Perna canaliculus* to marine heat wave scenarios and found altercations in major energy production pathways using LC-MS-based metabolomics.

4.2 Metabolomics in fish nutrition

The serum NMR study by Schock *et al.,.* (2012) shows an early change in metabolites that distinguishes fish and plant-based diets from those fed marine resources over a 98-day trial in cultured cobia. The serum LCMS study by Gil-Solsona *et al.,.* (2019) uncovers unexpected changes in vitamin metabolites in fish on low fishmealandfish oildiets, highlighting the value of a metabolomic approach to detect nutritional deficiencies.Van Nguyen *et al.,.* 2023 demonstrated size-dependent variation in metabolite profiles using GC-MS-based metabolomics in Blackfoot abalone. Lin *et al.,* 2024 used LC-MS-based metabolomics to study the antioxidant properties of dietary *Caulerpa lentillifera* on diquat-induced oxidative damage in zebrafish liver. Alfaro *et al.,.* (2022) studied the immunostimulatory effects of probiotic mixtures in *P. vannamei* using GC-MS-based metabolomics profiling of the haemolymph. They found significant changes in metabolic pathways between treatment groups.

4.3 Metabolomics in Fish Health Management

Metabolomics can be used for disease diagnosis and to elucidate the underlying metabolic mechanisms of diseases. Also, lipidomics, a branch of metabolomics which includes the concept of a global study of lipids, has received much attention in the manifestation of many diseases. There is a link between lipids and many diseases, and in such cases, the information provided by transcriptomics and proteomic studies may not be able to explain the clear picture. In such cases, metabolomics, especially lipidomics, is beneficial in establishing lipid biomarkers for diseases and their underlying mechanisms. Tao *et al.,.* (2020) elucidated the metabolic impairments in hepatic steatosis induced by a high-fat diet in GIFT tilapia. GC-MS-based metabolomics revealed that L-proline is a critical supplement enhancing the survival of tilapia against *Streptococcus agalactiae*infection under high water temperature.

4.4 Metabolomics in Fishery processing technology and products

Metabolomics can be used to evaluate the quality and authenticity of fishery products. Untargeted lipidomic and chemometric analysis has been used to identify three species of loliginid squids based on lipid biomarkers (Haridas *et al.,* 2024). Thus, metabolomics-based species authenticity will pave the way for rapid detection of seafood mislabelling and adulteration that hampers the export markets. The metabolites formed after as well as to optimise processing methods.

4.5 Metabolomics in Environmental Toxicology

Metabolomics can be used to assess the biological effects induced by chemical pollutants on aquatic ecosystems. Nazar *et al.,*. (2024) investigated the harmful effects of 2-Phenylphenol, an endocrine-disrupting chemical often found in aquatic environments, using an Untargeted metabolomics approach. Li *et al.,*. (2021) studied the specific key events (KEs) in an AOP (Adverse outcome pathways) associated with brain toxicity induced by selenium in zebrafish (*Danio rerio*) using metabolomics. Dietary seleno-L-methionine influenced the activity of neurotransmitters, metabolites, and gene transcripts associated with dopamine, serotonin, gamma-aminobutyric acid, acetylcholine, and histamine signalling pathways. An additional illustration of the value of metabolomics in elucidating the mechanisms of chemical toxicity and the development of adverse outcome pathways is provided by the work of Davis *et al.,*. (2017), who investigated the biochemical responses of the pharmaceutical spironolactone in fathead minnows (*Pimephalespromelas*). The effects of 21-day exposure to spironolactone on polar metabolite profiles in fish livers and links to those changes to declines in fecundity and other reproductive parameters have been studied. The study suggested additional potential impacts and AOPs induced by spironolactone. For example, spironolactone was observed to reduce the biosynthesis of L-carnitine, which in turn altered lipid and amino acid metabolism, membrane transport, and other biological functions. The application of an untargeted metabolomics approach in this study thus enabled the elucidation of other pathway interactions and associated effects occurring in parallel with androgen receptor activation

Strengths and Limitations of Metabolomics

All analytical methods come with their advantage and disadvantages. Metabolomics, being an emerging field in biology, has a significant impact on the capacity to generate and develop knowledge. Both Targeted and Untargeted metabolomics have their strength based on their applications and research problems. Some of the advantages of metabolomics are

- Comprehensive and High-throughput Analysis: Untargeted metabolomics provides a holistic view of the metabolome, capturing a wide range of metabolites and their interconnected metabolic pathways
- Biomarker Discovery: Helps identify potential biomarkers for diseases and stress conditions, leading to early diagnosis and stress mitigation
- Systems Biology: Metabolomics data can be integrated with genomics and proteomics to get a holistic view of biological systems and pathways
- Sensitivity: Advanced instrumentation and software detect subtle changes in metabolite levels, making it helpful in studying dynamic biological processes.
- Non-Invasive: Unlike conventional biochemistry approaches, metabolomics requires minimal sample inputs and paves the way for the analysis of non-invasive samples such as blood, serum, plasma, and faeces.

Metabolomics studies have a few disadvantages, such as

- Cost: High-throughput instrumentation facilities for analysis, limited skilled manpower, and software increase the cost of analysis per sample
- Complexity: Data analysis can be complex, requiring advanced bioinformatics tools and expertise in statistical functions and programming languages, often engaging experts from different backgrounds for results interpretation
- Variability: Biological variability and environmental factors with regard to animal/fish can affect results, requiring careful experimental design and standardised working protocol
- Standardisation: Lack of standardised protocols can lead to inconsistencies across studies
- Data Overload: The vast amount of data generated can be overwhelming and challenging to interpret with open-source options available.

4. Conclusion and prospects

Metabolomics is a new field in fisheries and aquaculture research with growing application areas such as fish nutrition, physiology, stress mitigation, disease management and environmental monitoring. Integrating metabolomics with other omics technologies such as phenomics, transcriptomics, and proteomics will facilitate the generation of a holistic, large-scale picture of the actual biological process under investigation. Rapid growth in computing capabilities like cloud computing and open-source software and programs will pave the way for easy adoption of this technique in many research domains. It is imperative to develop dedicated protocols for the preparation of samples from fish tissues for metabolomic analysis. Finally, the creation of species and organ-specific metabolome databases could also enhance several aspects related to fish biological research mainly due to influence from their inherently distinct metabolic signatures of fish. Additionally, the targeted metabolomics provides a focused approach and can contribute in discovery of novel non-invasive biomarkers for different stress and disease conditions that will have implications on fish welfare and fisheries resource management.

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Current advances in metabolomic fingerprinting for the assessment of poultry meat quality

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There has been a notably increased poultry consumption and production worldwide (Del Bosque *et al.,* 2021). Consumers play a pivotal role in shaping the poultry industry as their demand serves as the preliminary driving force behind its growth. Poultry meat has become a popular choice among consumers due to its high content of proteins, minerals, low fat content and as well as its affordable price point (Adamski *et al.,* 2017). The FAO also highlights the significance of pale, soft, and exudative meat and dark, firm, and dry meat in evaluating meat quality, underscoring the importance of proper meat care post-slaughter, during transportation and storage. The escalating demand of poultry meat has stimulated the emergence of poultry industry, each striving to meet the poultry product purchase specifications. Furthermore, the increasing health consciousness among consumers and concerns about the welfare of poultry animals should also be considered when the poultry industry is progressing. Acknowledging these aspects is crucial for the advancement of the poultry industry.

Meat quality is the key factor considered by consumers when making purchasing decisions, thus prompting the poultry industry to focus on producing high-quality meat (Choi *et al.,* 2023).Consequently, there is a surge in research and innovation aimed at ensuring and enhancing poultry product quality.

Poultry Product Quality and Parameters

Factors Affecting Poultry Product Quality

Feed

Feed consumption have significant contribution in determining poultry product quality and has been proven to enhance poultry growth and meat quality (Cramer *et al.,* 2018). Feed intake with vitamin E supplementation has been found to be beneficial for preventing lipid oxidation which affects meat quality. Additionally, a diet with high lipid content was found to enhance overall performance and nutrient utilization in broilers (Saleh *et al.,* 2020).

Environment

It has been reported that heat stress is one of the primary concerns of climate change that signifcantly impacts poultry growth, productivity and meat quality (Abdel-Moneim *et al.,* 2021). Gu *et al.,* (2008) reported that high temperatures can substantially reduce meat protein content. Conversely, high temperatures can increase the carcass content of fat, abdominal fat in particular, while low temperatures have the opposite effect.

Age

Li *et al.,*. (2020) revealed that older chicken meat had a higher pH and appear darker and redder. A higher pH value increases the water-holding capacity of the meat, resulting in increased juiciness and taste. Juiciness, along with appearance and texture, serves as a key indicator of meat quality.

Breed

Genotype is another factor affecting body protein and fat percentage in broilers. Crossbreeding enables the production of specific meat with favourable metabolite content to improve taste to meet consumer demands. Morri *et al.,* (2020) reported that genetic diversity plays a vital role, specific genes also make significant contributions in producing better-quality poultry products.Meluzzi*et al.,*

(2009) found the highest protein content in the slow-growing hybrid, than in medium- and fast-growing ones, and a 30% higher fat content in the fast-growing hybrid

Slaughtering method

Slaughtering holds a prominent position and affects meat quality. According to Ahmed *et al.,*. (2018), the amount of blood removed during slaughter determines the method's effectiveness and enhances the quality of the meat as an end-product. The quality of meat produced through halal slaughtering is better as the meat has a better shelf-life (Hafz*et al.,* 2015). It has also been reported that halal-slaughtered chicken contains lower amounts of iron, reduced lipid oxidation, and lower total bacterial count (Sohaib *et al.,* 2020).

Multi-omics in in meat Quality Assessment

Meat and meat products are significant sources of nutrition for humans, including proteins, lipids, minerals, and vitamins. The knowledge gaps in understanding the components that affect meat quality could limit progress in the development of rapid and accurate quality assessment methods.

Recent advances in high-throughput sequencing technology and high-resolution mass spectrometry are enabling a systematic understanding of meat quality and taste characteristics at the molecular level through intensive multi-omics studies. Commonly used omics techniques related to the quality and taste of meat are mainly genomics, including transcriptomics, proteomics, and metabolomics. Multi-omics techniques integrating these individual omics are very useful for exploring related genes, proteins, and metabolites that can improve meat quality and taste (Ramanathan *et al.,* 2020). Differentially expressed biomolecules identified using omics can not only be used as biomarkers to predict meat quality, but also can be applied to find interactions at the molecular level. For these reasons, a number of recent studies using multi-omics technologies have successfully explored impacts of various physiological processes on meat quality during meat production (Kim and Kim, 2021; Purslow *et al.,* 2021).

Moreover, multi-omics technologies have been used to investigate biomarkers and biological processes related to meat quality. However, there is still a lack of reviews on multi-omics technologies in relation to meat quality and taste characteristics.

Metabolomics is the systematic study of compounds with molecular weight of less than 1500 Da that are present in biological systems (Wen *et al.,* 2020), has gained attention in the areas of food quality and safety. Metabolic profile of food influences its taste, aroma, nutrition content, quality, and authenticity. Metabolites in cells, tissues and biological fluids are the result of gene expression derives from the interaction between the genomic system and the environment (Crestani *et al.,* 2020). The fingerprint characteristics of metabolites such as amino acids, sugars, organic acids, nucleic acids, and their derivatives in meat, could be provided through metabolomics.

All the biological traits are associated with complex regulation of genes and proteins. Meat quality traits are closely related to the biological sciences, including genetics, physiology, cell biology and biochemistry has been widely employed for decades to characterize the biological mechanisms behind major variability of meat quality (Wu *et al.,* 2020.

Increase levels of L-carnitine and acetyl-carnitine, while lowering contents of inosine monophosphate and taurine can decrease meat color stability. In addition, oxidation of PUFA produces volatile carbonyl and accumulation of PUFA promotes mitochondrial uncoupling, continuously increasing the content of acylcarnitine, which undergoes β-oxidation and causes cellular heat production, thus reducing the meat quality (Erban *et al.,* 2019).

Metabolomics Applications in Quality Assessment

The metabolomics approach can be used for quality measurement of meat from its raw form to processed form. The metabolites can vary and change due to many factors, such as stress, storage, pre- and post-slaughtering processes, and geographical origin. The metabolomics approach can also be used to determine meat quality prior to processing, the effects of stress conditions, and the welfare status of animals (Muroya*et al.,* 2020).

The molecular mechanisms or biological changes in meat is important to understand the technical principle of approaches used to analyze the quality and taste of meat, to improve the accuracy to better reflect changes in quality and taste, and to identify the molecular mechanisms responsible for high-quality and delicious meat (Gagaoua*et al.,* 2021; Kim *et al.,* 2023b).

Chromatography in mass spectrometry is usually being used to identify and separate metabolites. The time of flight-mass spectrometry apparatus coupled to the chromatography device, making it easier to get the research data. The data could be compared to the Compound Discovered database, and multivariate statistics could be used to interpret the results.

Lipidomics in meat product evaluation

Lipidomics has been used to evaluate functional ingredients in food, and play a significant function in promoting health. Bioactive lipids, including omega-3 fatty acids, conjugated linoleic acid, carotenoids, and phytosterols, exert significant effects on enhancing overall health. The human body lacks the ability to produce these chemicals, necessitating their acquirement through dietary sources, such as meat. Bioactive lipids play crucial roles in maintaining health, including their functions as antioxidants and anti-inflammatory properties (Costa *et al.,* 2023).

Proteomic and poultry meat quality

Proteomics is an important cornerstone in post-genome sciences, and the aim of this review is to introduce the developing field of proteomics, and to discuss its use in meat science projects.

The LC-MS method that integrates metabolomic and lipidomic analysis in pork and beef is a new technology with excellent sensitivity and provides the fingerprint of metabolites and lipids in biological samples.

The proteomic technique is another method for identifying the validity of meat products.The goal is to validatemeat products by examining for proteins, biological activity, post-translational modifications, and interactions in cells. Liquid chromatography quadrupole time of flight mass spectrometry (LC-QTOF-MS) is a method for determining the type of meat using a powerful tool for identifying protein peptides (Sarah *et al.,* 2016). Protein extraction precedes mass spectrometry (MS) or LC-QTOF-MS analysis in the proteomic analysis method. In proteomics, mass spectrometry has a wide range of applications, including meat science research, but it is hampered by the large biochemical heterogeneity of proteins and the inability to detect low protein levels.

Unfortunately, very few studies reporting results concerning poultry muscle development and/or meat quality. A paper published by Doherty *et al.,*. (2004) reported the characterization of the proteome of chicken pectoralis muscle in relative expression levels of several proteins during growth. Nevertheless, it was a first interesting step towards a better understanding of muscle modifications, in poultry, during growth.

Meat proteins represent the main muscle constituents, and their substantial changes upon different heat processing will therefore determine the structure and quality of the cooked meat (Yu *et al.,* 2017). Molecular understandings of protein and protein-induced biochemical/physicochemical changes involved in heat treatments will help to decipher the underlying mechanisms responsible for meat quality attributes (Theron *et al.,* 2019). Proteins and lipids of muscle tissue are important meat quality parameters. They contribute substantially to the nutritional characteristics of meat.

During the transportation of chicken, stress factors such as fasting, transportation time and high temperature, might affect the meat quality. In the pre-slaughter transportation process, the biological processes associated with protein regulation; mainly related to metabolic cell division and apoptosis control. Pre-slaughter transportation time affects the expression of THOP1, HSPB1, KPYK, and G3P protein of Ross chicken (Carvalho *et al.,* 2014)

Doherty *et al.,* (2004) isolated and identified the expression of soluble proteins in the skeletal muscle of chickens of different ages and found a total of 53 differentially abundant proteins mainly β-enolase, G3P DHG, LD, TPI, CK, and other metabolism-related proteins. These results explored the possibility for the application of proteomics in the growth of poultry. The expressions of metabolic

proteins (PGAM1, TPI1, APOA1, and FABP3) and stress proteins (HSP25)(Desai *et al.,* 2014)] PGAM1 and TPI1 were important in glycolysis and were closely related to meat tenderness and toughness (Solem *et al.,* 2008] The results showed that energy metabolism and stress proteins were important for chicken development. FASN and ME1 proteins were closely related to the lipid metabolism at different stages of growth (Jung *et al.,* 2008).

Protein markers in meat quality assessment

Content	Protein biomarker	Reference
Water Holding capacity	HSP70, PKM, TPI	Shao *et al.,* 2016
Tenderness	PKM2, PGAM1, TPI1	Supamit *et al.,* 2010
Flavour	PGM1, MyHC. DNA MTase3, HSP27, ENSGA, Glyoxylase1,UQCRC1	Liu *et al.,* 2012
Effect of growth rate on chicken quality	Actin, Chain A1, Chain A2, Chain A3, M-CK	Phonga-Ngan *et al.,* 2011
Intramuscular fat (IMF) accumulation	ACADL, ACAD9, HADHA, HADHB	Liu *et al.,* 2017
Pre-slaughter transport	THOP1, G3P, HSPB1, KPYK	Xing *et al.,* 2017

Conclusion

To address the challenges of multi-omics, meat quality regulatory mechanism must be better explained by focusing on dynamic alterations of intracellular substances. Meat quality-related mechanisms acan be explored with the validation of potential molecular biomarkers in a reasonable manner and state-of-art omics technologies such as third-generation transcriptome sequencing, “4D” proteomics, and spatial omics should be applied to future meat quality studies. In addition, a multi-omics database with integrated algorithm and software for multiomics data should also be develop. Additionally, improving food quality through the metabolomics approach will help to improve consumer health and reduce health risks in the population. In conclusion, metabolomics pave ways towards improving the quality and authenticity of meat and products systematically.

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AAFB-LP-3

Impact of Heavy Metal Pollution on Animal Health: Insights into Metabolic Disruptions and Strategies for Resilience in Contaminated Environments

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Heavy metal pollution poses a significant threat to livestock health, particularly in industrially impacted regions. Recent studies highlight the effects of heavy metal exposure in buffaloes, focusing on health, metabolic profiles, milk quality and oxidative stress responses. Seasonal analysis reveals that buffaloes exposed to high levels of metals such as lead, cadmium, and chromium exhibit altered biochemical and antioxidant profiles, with increased oxidative stress markers during high-stress summer months. The contamination of drinking water, blood, and milk not only threatens animal health but also raises concerns for human consumers.Further, molecular studies indicate metallothionein expression as a natural defense mechanism against metal-induced cellular damage in buffaloes. Research suggests that dietary supplements, such as Vitamin E, selenium, and probiotics, can mitigate some adverse effects of metal exposure, offering potential strategies for enhancing animal resilience. This summary of recent findings underscores the importance of implementing targeted nutritional and environmental management practices to reduce contamination risks and enhance livestock welfare in heavy metal-polluted areas.

AAFB-LP-4

Cutting-Edge Bimolecular Techniques in Veterinary Diagnostics

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Introduction

Veterinary medicine relies on various diagnostic tests to inform clinical decisions and guide treatment strategies. Biochemical diagnostics, which analyse biochemical parameters in bodily fluids (such as blood, urine, and saliva), are integral in diagnosing metabolic disorders, infectious diseases, and monitoring therapeutic responses. These diagnostic tools help veterinarians to identify health issues at an early stage, enabling prompt and appropriate interventions that can improve animal welfare.

In veterinary practice, the ability to rapidly diagnose conditions using biochemical parameters directly impacts patient outcomes. For instance, timely identification of renal failure through serum biochemistry allows for immediate management strategies that can significantly affect prognosis. Thus, advancements in biochemical diagnostics not only enhance diagnostic accuracy but also contribute to better overall care for animals.

The past decade has witnessed substantial progress in veterinary diagnostics for biochemical parameters. These advancements encompass a variety of innovations, from the development of sophisticated analytical techniques to the integration of artificial intelligence in interpreting complex data. This paper will explore these advancements in detail, highlighting their implications for veterinary practices.

Technological Innovations in Veterinary Biochemical Diagnostics

Point-of-Care Testing (POCT)

Point-of-Care Testing (POCT), often known as near-patient testing, refers to diagnostic testing conducted at or near the site of patient care. Unlike traditional laboratory tests, which are usually performed in centralized laboratories and may require significant time for transport and analysis, POCT enables immediate analysis, facilitating rapid decision-making. This can be particularly valuable in veterinary care, where timely diagnosis and treatment are critical. POCT involves performing diagnostic tests at the point of care (e.g., bedside, clinic, or field site) rather than a central laboratory. The results are available within minutes, allowing clinicians to make informed decisions promptly. For animals, especially in emergency or critical care situations, rapid diagnostics are crucial in delivering effective care. In livestock, POCT can help in monitoring herd health and in the rapid identification of diseases that might otherwise spread quickly.The future of POCT in veterinary medicine is promising, with emerging technologies that enable multiplexed testing, molecular diagnostics, and even real-time monitoring through wearable devices. Innovations in biomarker discovery, miniaturized lab-on-chip platforms, and data integration with electronic health records (EHRs) will enhance the diagnostic capacity of POCT.

Types of Tests Available

POCT devices are available for a range of diagnostic tests, including:

a) Blood Glucose Monitoring: Common in diabetic animals and critical care cases.

b) Electrolyte and Blood Gas Analysis: Essential in emergency care, especially for acid-base balance.

c) Urinalysis and Blood Chemistry: Used in routine health screenings and to monitor liver and kidney function.

d) Coagulation Testing: Useful in animals suspected of coagulopathies or those undergoing surgical procedures.

e) Infectious Disease Detection: Tests for common pathogens, such as parvovirus in dogs or FeLV/FIV in cats, can yield results within minutes.

Technological Advances in POCT

Advances in biotechnology have significantly improved POCT. Current devices use technologies such as biosensors, microfluidics, and miniaturized analytical platforms. Newer POCT devices also connect to smartphones or cloud-based platforms, making it easier to store, share, and analyse results. In veterinary care, portability and ease of use are critical, as veterinarians may need to transport devices to farms or remote areas.

Applications in Veterinary Care

a) Companion Animals: POCT is commonly used for monitoring chronic conditions (e.g., diabetes in dogs and cats) and for emergency diagnostics.

b) Livestock and Equine Health: POCT can aid in the rapid diagnosis of infectious diseases that could otherwise decimate herds. In equine medicine, POCT helps assess performance parameters and detect conditions like dehydration or electrolyte imbalances.

c) Wildlife and Exotic Animals: POCT devices are used in zoos, wildlife rehabilitation centres, and during fieldwork. They enable quick health assessments without the need to transport animals to a laboratory, which could cause additional stress.

Advantages and Challenges

Advantage

POCT provides rapid results, convenience, and accessibility, particularly in remote areas. It also enables immediate patient management and reduces the reliance on centralized labs.

Challenges

POCT devices may face issues with accuracy compared to laboratory-based testing, especially under suboptimal conditions. Quality control, maintenance, and calibration are essential to ensure reliable results.

High-Throughput Screening (HTS)

High-Throughput Screening (HTS) is a powerful and automated technique used extensively in biomedical research, pharmacology, and veterinary biochemistry for rapidly testing thousands to millions of compounds to identify potential biological activity or interactions. HTS is integral in drug discovery and diagnostics, allowing researchers to screen large libraries of chemical compounds against biological targets. This process can uncover potential therapeutic agents, novel biochemical pathways, or diagnostic markers.Precision medicine in veterinary science focuses on tailoring treatments to individual animals or species. HTS can contribute to this by identifying biomarkers or genetic markers specific to animal breeds, enabling the development of more targeted therapies. This approach is especially promising in veterinary oncology, where tailored treatments can improve outcomes for companion animals.

Purpose and Importance

HTS allows researchers to accelerate the drug discovery and development process by screening large volumes of compounds in a relatively short period. In veterinary medicine, HTS plays a role in discovering new treatments for animal diseases, identifying pathogens, and exploring how compounds interact with various animal-specific biological targets. By streamlining this process, HTS makes it easier to identify promising leads and develop therapeutic agents that can advance both human and animal health.

HTS Workflow

The HTS process generally involves the following stages:

a) Compound Library Preparation: Chemical compounds or biological samples are organized into libraries, sometimes containing millions of unique substances.

b) Assay Development: Researchers design a test, or assay, to measure the desired biological activity, such as enzyme inhibition, cell viability, or receptor binding. Assays are optimized for automation and reproducibility.

c) Automation and Miniaturization: HTS requires automated robotic systems to handle thousands of samples simultaneously. These systems often work with 96-, 384-, or even 1,536-well plates, where each well contains a unique compound.

d) Detection and Data Analysis: HTS relies on sensitive detection systems to measure biological activity. Techniques include fluorescence, luminescence, or absorbance. Advanced data analysis software is then used to analyze results and identify hits, or compounds that show promising activity.

HTS Assay Types

HTS assays are designed based on the biological target or activity in question. The main types include:

a) Biochemical Assays: Measure the direct interaction of compounds with a target molecule, like enzyme activity or receptor binding.

b) Cell-based Assays: Examine how compounds affect cellular functions, such as cell viability, apoptosis, or intracellular signaling pathways. Cell-based assays are particularly relevant for studying complex biological interactions.

c) Genomic and Proteomic Assays: Focus on identifying compounds that affect gene or protein expression. These are useful in target discovery and understanding the molecular mechanisms of action.

Advantages of HTS

a) Speed and Efficiency: HTS can screen thousands of compounds in a matter of days, which is much faster than traditional screening methods.

b) Scalability: The automated, miniaturized nature of HTS enables researchers to scale up their experiments significantly, allowing for extensive exploration of chemical space.

c) Data-rich Output: HTS generates large datasets, which can reveal potential trends, mechanisms of action, and dose-response relationships.

Challenges and Limitations

a) Complexity of Biological Systems: Not all biological interactions can be accurately replicated in HTS assays, particularly when using isolated molecules or simplified cell models. This can limit the predictability of HTS results for in vivo scenarios.

b) False Positives and False Negatives: The high volume of testing increases the likelihood of false results. Hits identified in HTS may not always be viable in further testing, requiring rigorous validation and follow-up experiments.

c) Cost and Infrastructure: HTS requires substantial investment in robotic systems, assay reagents, and data handling infrastructure, which can be cost-prohibitive for smaller laboratories.

Applications of HTS in Veterinary Science

HTS has diverse applications in veterinary research, including:

a) Drug Discovery for Animal Diseases: HTS helps identify compounds that can treat common diseases in animals, such as infectious diseases or cancer.

b) Antibiotic Resistance Screening: HTS is used to discover new antibiotics and study their effectiveness against resistant pathogens in livestock, an area critical to both animal and human health.

c) Vaccine Development: HTS can assist in the discovery of adjuvants and antigens that could enhance immune responses in veterinary vaccines.

d) Environmental Toxicology: HTS assays are used to evaluate the impact of environmental contaminants on animals, particularly in agricultural and aquatic settings, helping assess safety levels.

Technological Advances in HTS

The future of HTS is marked by continuous innovation:

a) High-Content Screening (HCS): HCS combines HTS with advanced imaging techniques to provide a more comprehensive analysis of cellular events and responses.

b) AI and Machine Learning: Machine learning algorithms analyze HTS data to identify patterns and improve hit-prediction accuracy, aiding in the selection of potential lead compounds.

c) Miniaturized Assays and Microfluidics: These technologies allow even greater efficiency by reducing reagent volumes and enabling the study of small cell populations or single-cell responses.

Mass Spectrometry (MS)

Mass Spectrometry (MS) is a powerful analytical technique used in biochemistry, pharmacology, environmental science, and various fields of veterinary medicine. MS allows scientists to measure the masses of particles, identify the chemical structure of molecules, and quantify compounds within a sample. In veterinary biochemistry, MS is a critical tool for analysing complex biological samples, diagnosing diseases, identifying contaminants, and monitoring drug levels.Mass spectrometry operates by ionizing chemical compounds to generate charged particles (ions) and then measuring their mass-to-charge ratios (m/z). MS can analyse everything from small organic compounds to complex proteins, making it versatile for biological applications. MS instruments detect these ions, producing a mass spectrum that represents the molecular profile of the sample. Toxicology is a key area where MS plays a crucial role. Detecting toxic substances, such as pesticides, heavy metals, and mycotoxins, is essential to protect animal health and prevent harmful residues in animal products. MS allows for rapid, sensitive, and specific analysis of these contaminants.

Mass Spectrometry Workflow

The MS process generally involves three key steps:

a) Ionization: The sample is ionized, typically through techniques like electron ionization (EI), electrospray ionization (ESI), or matrix-assisted laser desorption/ionization (MALDI). The type of ionization method depends on the sample and desired analysis.

b) Mass Analyser: Ions are separated based on their mass-to-charge ratios using a mass analyser. Types of mass analysers include quadrupole, time-of-flight (TOF), ion trap, and Fourier transform ion cyclotron resonance (FT-ICR) analysers.

c) Detection: Ions are detected and measured, producing a mass spectrum that shows the m/z of detected ions, which researchers used to identify and quantify compounds.

Types of Mass Spectrometers

There are several types of MS systems, each suited for different analytical needs:

d) Quadrupole MS: Commonly used for quantitative analysis; it filters ions based on their m/z, suitable for single-compound analysis.

e) Time-of-Flight (TOF) MS: Offers high-resolution and accuracy, ideal for analysing complex mixtures.

f) Ion Trap MS: Captures ions within a confined space, allowing for fragmentation and further analysis, useful in structural elucidation.

g) Fourier Transform MS (FT-MS): Provides the highest resolution and sensitivity, suitable for detailed molecular analysis, including proteomics.

h) MALDI-TOF: Combines MALDI ionization with TOF analysis and is widely used in microbiology for bacterial identification.

Applications of MS in Veterinary Medicine

Mass spectrometry has diverse applications in veterinary diagnostics and research:

a) Pharmacokinetics and Drug Monitoring: MS quantifies drug concentrations in blood or tissue samples, allowing veterinarians to monitor therapeutic drug levels and ensure proper dosing. This is particularly important in cases where animals metabolize drugs differently from humans.

b) Proteomics and Metabolomics: MS is essential for studying proteins (proteomics) and metabolites (metabolomics) in veterinary species, helping identify biomarkers for disease diagnosis and understanding molecular mechanisms of disease.

c) Disease Diagnosis: MS can detect biomarkers of specific diseases or pathogenic agents, aiding in the diagnosis of infections, metabolic disorders, and other health conditions in animals.

d) Environmental Contaminants: MS detects toxins, heavy metals, and pesticides in animal feed and environmental samples, ensuring safety in the food supply chain.

e) Nutritional Analysis: MS is used to assess nutrient composition in animal feed and to study metabolic responses to different diets in livestock.

Advantages of Mass Spectrometry

a) Sensitivity and Specificity: MS is highly sensitive, capable of detecting trace amounts of compounds, and highly specific, able to differentiate between molecules with slight structural differences.

b) Versatility: Applicable to a wide range of compounds, from small molecules to proteins and nucleic acids.

c) Quantitative and Qualitative Analysis: MS not only identifies compounds but can also accurately quantify them, allowing for precise concentration measurements.

Challenges in Mass Spectrometry

a) Complex Sample Preparation: Biological samples often require extensive preparation to reduce complexity and enhance ionization efficiency.

b) Instrumentation Cost and Maintenance: High-quality MS instruments are expensive and require significant maintenance, which may limit access in smaller veterinary labs.

c) Data Analysis and Interpretation: MS generates large datasets that require expertise and advanced software for analysis, particularly in proteomics and metabolomics.

Advances in Mass Spectrometry

a) High-Resolution MS (HRMS): Developments in HRMS have improved sensitivity and accuracy, enabling more detailed molecular profiling.

b) Coupling with Chromatography: Liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS) are common techniques that combine separation with mass analysis, enhancing the ability to analyze complex samples.

c) Imaging Mass Spectrometry (IMS): Allows spatial mapping of compounds within tissue samples, useful in pathology for studying disease distribution at the molecular level.

d) Biomarker Discovery: MS aids in identifying biomarkers for specific animal diseases, advancing precision medicine and personalized veterinary care.

Nucleic Acid Testing (NAT)

Nucleic Acid Testing (NAT) is a molecular diagnostic technique used to detect specific nucleic acids—either DNA or RNA—in biological samples. NAT is fundamental for diagnosing infectious diseases, genetic disorders, and some cancers. By detecting the genetic material of pathogens or mutations, NAT provides high sensitivity and specificity, making it a valuable tool in veterinary diagnostics, medical diagnostics, and environmental testing.

Principles of NAT

NAT works by detecting and amplifying specific sequences of DNA or RNA. The technique is highly sensitive, capable of identifying even minute quantities of genetic material. This is particularly useful in early-stage infections, when pathogen levels are low, and in the detection of asymptomatic carriers. Unlike serological tests, which detect antibodies or antigens, NAT identifies the genetic material of pathogens, providing a direct and accurate diagnosis.

Common NAT Techniques The most widely used NAT techniques include:

a) Polymerase Chain Reaction (PCR): Amplifies specific DNA sequences, making even small amounts detectable. Variants like quantitative PCR (qPCR) provide quantitative data, while reverse transcription PCR (RT-PCR) is used to detect RNA by first converting it to complementary DNA (cDNA).

b) Loop-mediated Isothermal Amplification (LAMP): Amplifies DNA at a constant temperature, making it faster and more cost-effective than PCR. It's especially useful in low-resource settings.

c) Next-Generation Sequencing (NGS): Allows comprehensive sequencing of DNA or RNA in a sample, enabling detailed analysis of genetic mutations and pathogen diversity. Though more expensive, NGS is valuable in research and for pathogen discovery.

d) Transcription-Mediated Amplification (TMA): Similar to PCR but focuses on RNA, making it ideal for detecting RNA viruses. TMA is faster than traditional PCR and can provide high sensitivity.

Applications in Veterinary Medicine

NAT is crucial in veterinary diagnostics due to its precision and adaptability. Key applications include:

a) Infectious Disease Diagnosis: NAT can rapidly detect viral, bacterial, and parasitic pathogens in animals, such as foot-and-mouth disease virus (FMDV) in livestock or feline immunodeficiency virus (FIV) in cats. It enables early detection and control, helping prevent disease outbreaks.

b) Zoonotic Disease Surveillance: Many zoonotic pathogens (e.g., rabies, avian influenza) can jump from animals to humans. NAT plays an essential role in monitoring these pathogens, aiding in public health efforts.

c) Genetic Testing and Breed Identification: NAT is used to identify genetic mutations linked to diseases or specific traits in animals, enabling breeders and veterinarians to manage breed-specific health conditions and aid in breed identification.

d) Antibiotic Resistance Testing: NAT identifies genetic markers of antibiotic resistance, helping veterinarians choose the most effective treatments and manage the spread of resistant strains in animals and the environment.

Advantages of NAT

a) Sensitivity and Specificity: NAT can detect very low levels of DNA or RNA, providing highly sensitive results even in the early stages of infection.

b) Speed: Many NAT tests, particularly PCR-based methods, deliver results in hours. This rapid turnaround is critical in clinical settings and during outbreaks.

c) Quantitative Capabilities: Techniques like qPCR allow for the quantification of pathogens, providing insight into infection severity and monitoring treatment progress.

d) Versatility: NAT can be used to detect a wide range of pathogens and genetic markers, from viruses and bacteria to complex genetic mutations.

Challenges in NAT Implementation

a) Technical Expertise: NAT requires trained personnel and specialized equipment, especially in PCR and NGS. This can be challenging in low-resource settings and smaller veterinary clinics.

b) Cost and Infrastructure: While costs have decreased over time, NAT can still be relatively expensive, particularly with advanced techniques like NGS, which require significant infrastructure.

c) Contamination Risks: NAT is highly sensitive, so contamination of samples can lead to false positives. Stringent laboratory protocols and controls are required to minimize this risk.

d) Interpretation of Results: Quantitative data from qPCR and NGS can be complex, requiring careful analysis to understand the clinical relevance of findings.

Technological Advances in NAT

a) Point-of-Care NAT: Portable devices and isothermal amplification methods are advancing NAT accessibility in the field, enabling faster diagnosis in clinical and remote settings.

b) Digital PCR: Offers increased sensitivity by partitioning the PCR reaction into thousands of droplets, making it possible to detect low-abundance targets with high precision. It's particularly useful for applications requiring extreme sensitivity.

c) CRISPR-Based Diagnostics: CRISPR technology is being developed for NAT applications, offering a potential tool for rapid, on-site testing with high specificity. It is gaining attention for applications in infectious disease diagnostics and genetic testing.

d) Nanopore Sequencing: A form of NGS, nanopore sequencing provides real-time sequencing and does not require PCR amplification, making it a promising approach for rapid, low-cost NAT.

NAT in Veterinary Disease Surveillance and Control

NAT's precision in detecting specific genetic material makes it invaluable for monitoring the spread of diseases within and between animal populations. This is particularly critical for livestock health, where outbreaks can have significant economic and food security impacts. NAT also plays a role in identifying emerging pathogens and monitoring genetic shifts in pathogens, which can inform vaccination and treatment strategies.

NAT in Animal Research and Genomics

Beyond diagnostics, NAT aids in veterinary research, particularly in genomics. For instance, it helps identify disease resistance genes in livestock and companion animals, enabling selective breeding for improved health. It also facilitates studies on the genetic basis of behavior and physiological traits, advancing the understanding of animal biology and improving animal management and welfare.

Emerging Biomarkers in Veterinary Medicine

Emerging biomarkers in veterinary medicine are becoming vital for early diagnosis, treatment monitoring, and prognosis of various diseases in animals. These biomarkers, often measurable molecules or biological indicators, offer insights into physiological and pathological processes, helping veterinarians tailor treatment strategies and improve animal health management. Biomarkers can be proteins, metabolites, genes, or even imaging findings that reveal specific aspects of disease states in animals.

Types of Emerging Biomarkers

Proteomic Biomarkers

Proteins are a primary focus for biomarkers due to their active role in biological processes. Proteomic biomarkers are proteins associated with specific disease states and can be identified through technologies like mass spectrometry. For example:

a) Acute Phase Proteins (APPs): Proteins like serum amyloid A (SAA) and C-reactive protein (CRP) are involved in the immune response and inflammation. Elevated levels of APPs are indicative of infections or inflammatory diseases in animals, such as equine colic or canine arthritis.

b) Enzymes and Hormones: Enzymes like ALT and AST serve as liver function biomarkers, while hormone levels, such as cortisol, can reflect stress or adrenal issues.

Genomic and Epigenomic Biomarkers

DNA-based biomarkers include mutations, gene expression levels, or specific gene sequences that correlate with disease susceptibility or prognosis.

a) Genetic Mutations and Variants: Mutations in genes, such as those associated with hereditary diseases, provide insight into breed-specific conditions. For example, mutations in the ABCB1 gene are linked to drug sensitivity in certain dog breeds, which helps veterinarians personalize medication choices.

b) Epigenetic Modifications: DNA methylation patterns, for instance, are emerging as biomarkers for cancer in animals. Changes in gene expression regulation, without altering the DNA sequence, can indicate cancer progression and environmental influences on animal health.

Metabolomics Biomarkers

Metabolomics studies small-molecule metabolites in biological samples, providing a comprehensive view of metabolic processes in health and disease.

a) Lipid Metabolites: Elevated lipid metabolites may indicate metabolic disorders like obesity and diabetes in companion animals. For example, increased ketone bodies can suggest insulin dysregulation or energy balance issues.

b) Amino Acids and Organic Acids: Certain amino acid levels, such as tryptophan, can reflect liver function, while elevated organic acids may indicate metabolic acidosis or specific enzyme deficiencies.

Microbiome Biomarkers

The micro biome or community of microorganisms in the body, has a significant impact on animal health. Microbiome imbalances are associated with gastrointestinal (GI) disorders, metabolic issues, and immune dysfunctions.

a) Gut Microbiota Composition: Shifts in the diversity and composition of gut bacteria can be biomarkers for GI diseases, such as irritable bowel syndrome (IBS) in dogs and inflammatory bowel disease (IBD) in cats. By analysing microbial DNA, veterinarians can detect these imbalances and make dietary or treatment recommendations.

b) Fecal Biomarkers: Microbial metabolites in feces, like short-chain fatty acids, offer insights into GI health and diet-related metabolic changes.

Immunologic Biomarkers

Immunologic biomarkers include cytokines, chemokines, and antibodies that indicate immune responses in infections, allergies, or inflammatory conditions.

a) Cytokine Levels: Pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) are potential biomarkers for inflammatory diseases in animals, especially in conditions like arthritis or autoimmune diseases.

b) Autoantibodies: In cases of autoimmune disease, specific autoantibodies can serve as biomarkers for conditions such as lupus in dogs. Elevated levels of antibodies against self-antigens can indicate underlying immune dysregulation.

Cancer Biomarkers

Cancer biomarkers are molecules that indicate the presence or progression of cancerous cells.

a) Tumor Markers: Tumor-specific antigens, such as prostate-specific antigen (PSA) in prostate cancer, are detectable in various cancers. In veterinary oncology, new tumor markers are being studied for canine lymphomas and feline mammary tumors.

b) Circulating Tumor DNA (ctDNA): ctDNA, found in the blood, offers a non-invasive means of detecting genetic mutations associated with cancer, providing insights into prognosis and treatment monitoring.

Cardiac Biomarkers

Biomarkers of cardiac function and stress are essential for detecting cardiovascular diseases in animals.

a) Cardiac Troponins (cTnI and cTnT): Troponins are proteins released into the bloodstream when the heart muscle is damaged. Elevated troponin levels in cats and dogs can indicate heart diseases such as myocarditis or heart failure.

b) N-terminal Pro-B-type Natriuretic Peptide (NT-proBNP): This peptide is released in response to heart strain, making it a valuable biomarker for congestive heart failure and other heart conditions in dogs and cats.

Biomarkers for Infectious Diseases

In veterinary medicine, infectious disease biomarkers are critical for detecting pathogens early and accurately.

a) Pathogen-Specific Nucleic Acids: PCR and other nucleic acid-based assays detect DNA or RNA from pathogens like parvovirus in dogs or feline herpesvirus. These biomarkers enable rapid and precise diagnosis.

b) Viral Antigen Levels: Specific viral antigens, like those for avian influenza or bovine respiratory disease, are measurable in blood or tissue samples, allowing timely intervention in outbreaks.

Applications of Emerging Biomarkers in Veterinary Medicine

1. Early Disease Detection and Diagnosis

Biomarkers enable early diagnosis of conditions like cancer, kidney disease, and metabolic disorders, often before clinical symptoms appear. This can lead to timely treatment, reducing the impact of the disease on animal health and quality of life.

2. Personalized Veterinary Care

Biomarkers allow veterinarians to tailor treatment strategies based on an individual animal's specific biomarkers. For instance, certain genetic biomarkers can inform drug selection and dosage, optimizing treatment efficacy and minimizing side effects.

3. Disease Monitoring and Prognosis

Biomarkers help monitor disease progression and treatment response, especially in chronic conditions such as cancer or cardiovascular diseases. For example, tracking cardiac biomarkers like NT-proBNP can inform prognosis and treatment adjustments in heart failure patients.

4. Improving Animal Welfare and Preventive Care

Biomarkers can also be used to monitor stress, inflammation, and general health in livestock, helping to improve welfare, manage diseases, and enhance productivity in animal husbandry. They are also increasingly used in preventive care programs for companion animals.

5. One Health and Zoonotic Disease Surveillance

Many biomarkers are instrumental in zoonotic disease surveillance, detecting pathogens that can spread between animals and humans. By monitoring these biomarkers, veterinarians contribute to public health efforts and the prevention of zoonotic disease outbreaks.

Challenges in Emerging Biomarkers

While biomarker research in veterinary medicine is promising, challenges remain:

1. Validation and Standardization: Biomarkers need to be validated across different species and conditions to ensure accuracy and reproducibility.
2. Cost and Accessibility: Biomarker tests can be costly and may not be widely available, limiting their use in routine veterinary practice, especially in resource-limited settings.
3. Data Interpretation: Biomarker data can be complex, requiring skilled interpretation to understand its clinical implications fully.

The future of biomarkers in veterinary medicine is moving toward multi-biomarker panels, where several biomarkers are assessed together to provide a comprehensive view of an animal's health status. This approach, along with advances in diagnostic technology, holds promise for more precise, timely, and personalized veterinary care.

Artificial Intelligence and Data Analytics in Veterinary Diagnostics

Artificial Intelligence (AI) and Data Analytics are revolutionizing veterinary diagnostics by enhancing diagnostic accuracy, improving patient care, and enabling personalized treatment plans for animals. By leveraging large datasets and advanced machine learning models, AI and data analytics offer new insights into animal health, disease progression, and treatment responses. In veterinary diagnostics, AI assists in automating processes, predicting outcomes, and identifying complex patterns that are often beyond human recognition.

Key Applications of AI and Data Analytics in Veterinary Diagnostics

1. Image Analysis for Diagnostics

AI-powered image analysis is transforming veterinary radiology, pathology, and dermatology by automating the interpretation of imaging studies.

i. Radiology and Imaging: AI algorithms analyze X-rays, CT scans, MRI images, and ultrasound data, detecting abnormalities like fractures, tumors, and organ lesions. For example, deep learning models can assess radiographic images to identify signs of respiratory diseases in horses or detect joint abnormalities in dogs with arthritis.

ii. Pathology: Digital pathology supported by AI helps in diagnosing conditions based on tissue samples. Algorithms analyzehistopathological images to detect cancerous cells, inflammation, and other abnormalities in animal tissues. This speeds up the diagnosis and aids in accurately staging cancers.

iii. Dermatology: AI systems can analyze skin images to identify dermatological conditions in animals, such as fungal infections, mites, and dermatitis, enabling faster, more accurate diagnosis.

2. Predictive Analytics in Disease Surveillance and Management

Data analytics combined with AI enables predictive modeling for disease surveillance, outbreak prediction, and overall management of animal health.

i. Epidemiology and Disease Prediction: AI algorithms analyze data from various sources (e.g., climate data, animal movement, and historical disease patterns) to predict potential disease outbreaks, such as avian influenza or African swine fever. This helps veterinarians and animal health authorities prepare and implement control measures to prevent outbreaks.

ii. Health Monitoring and Early Detection: Predictive models identify early warning signs of diseases based on patterns in physiological data, clinical symptoms, and diagnostic results. This is especially valuable in livestock management, where early detection can reduce disease spread and economic losses.

3. Electronic Health Records (EHR) Analysis

AI and data analytics improve the utilization of Electronic Health Records (EHRs) by extracting valuable insights and enabling personalized treatment.

i. Data-Driven Insights for Treatment: Machine learning algorithms analyze EHR data to identify treatment responses and adverse effects, helping veterinarians choose optimal treatment strategies. For instance, analyzing EHR data can help predict which antibiotics will be most effective for bacterial infections in livestock.

ii. Personalized Treatment Plans: By leveraging EHR data, AI models predict individual animals' responses to treatments based on breed, age, genetics, and medical history, supporting personalized care approaches.

iii. Diagnostic Decision Support: AI-powered decision support tools suggest probable diagnoses and treatment options based on an animal's symptoms, lab results, and history, helping veterinarians make informed decisions and reducing diagnostic errors.

4. Genomic Data Analysis

The analysis of genomic data with AI is critical in understanding genetic disorders, enhancing disease resistance, and improving breeding practices.

i. Genetic Disorder Screening: AI algorithms analyze genetic data to screen for breed-specific genetic disorders, such as hip dysplasia in dogs or hypertrophic cardiomyopathy in cats. Early identification of genetic mutations helps manage hereditary diseases and guide breeding decisions.

ii. Marker-Assisted Selection in Breeding: Data analytics in genomics enables the identification of genetic markers associated with desirable traits, such as disease resistance or productivity in livestock. This information supports selective breeding programs that enhance animal health and reduce the prevalence of genetic disorders.

5. Natural Language Processing (NLP) for Veterinary Literature and Case Analysis NLP, a branch of AI that processes human language, helps veterinarians stay up-to-date with the latest research and clinical guidelines.

i. Research Analysis: NLP algorithms analyze vast amounts of veterinary literature, extracting relevant information for specific diseases, treatments, and animal species. This helps veterinarians quickly access the latest findings and apply evidence-based treatments.

ii. Automated Case Analysis: NLP can analyze veterinary records, case reports, and notes, identifying trends or patterns in clinical cases. This is particularly useful for rare or complex cases where collective insights from past cases can guide diagnosis and treatment.

6. Wearable and IoT Data Integration for Real-Time Monitoring with the rise of IoT and wearable devices in veterinary care, AI analyzes continuous data from sensors, wearables, and monitoring devices to provide real-time insights into animal health.

i. Livestock Monitoring: IoT devices on farms collect data on animal behavior, movement, and physiological parameters like temperature and heart rate. AI algorithms process this data to detect signs of illness, reproductive cycles, or stress, enabling timely interventions and enhancing productivity.

ii. Pet Health Monitoring: Wearable devices for pets, like smart collars, track parameters such as activity levels, heart rate, and sleep patterns. AI models analyze this data to alert pet owners and veterinarians of potential health issues before clinical symptoms become apparent.

7. Drug Discovery and Precision Medicine

AI and data analytics are integral in drug development, reducing costs and accelerating the discovery of new treatments for veterinary use.

i. Target Identification and Drug Screening: AI models analyse biological data to identify molecular targets for drug development. By predicting how different compounds interact with these targets, AI accelerates drug discovery for animal diseases.

ii. Precision Medicine: AI and data analytics enable the development of precision medicine in veterinary care, tailoring treatment regimens to individual animals based on genetic, environmental, and clinical data. For instance, specific cancer treatments for companion animals can be personalized based on tumor genetics, improving outcomes.

8. AI-Powered Biomarker Discovery

AI supports the discovery of novel biomarkers for various diseases by analysingomics data (genomics, proteomics, metabolomics) and identifying patterns indicative of disease states.

i. Biomarker Identification for Diagnostics: AI algorithms analyse large datasets to discover biomarkers associated with specific conditions, such as renal failure in cats or respiratory diseases in cattle. Biomarkers can be used in diagnostic tests to detect diseases early or monitor treatment progress.

ii. Prognostic Biomarkers: AI analyses clinical and molecular data to identify biomarkers that indicate disease progression, enabling veterinarians to predict disease outcomes and adjust treatment accordingly.

Benefits of AI and Data Analytics in Veterinary Diagnostics

i. Enhanced Diagnostic Accuracy: AI algorithms reduce the chance of human error by detecting patterns in data that may not be evident to the human eye.

ii. Early Disease Detection: Data analytics enable earlier detection of diseases, improving the chances of successful treatment.

iii. Personalized Veterinary Care: AI-driven insights allow for personalized treatment plans, considering each animal's unique health profile.

iv. Improved Workflow Efficiency: Automating routine diagnostic tasks allows veterinarians to focus on complex cases, enhancing workflow efficiency in veterinary clinics and laboratories.

Challenges in Implementing AI and Data Analytics in Veterinary Medicine

i. Data Privacy and Security: Veterinary data, like human medical data, requires robust security protocols to protect animal owners' privacy and prevent unauthorized access.

ii. Data Standardization and Integration: Integrating data from various sources (EHRs, imaging, wearables, and genomics) requires standardization to ensure compatibility and effective analysis.

iii. Limited Data Availability: AI models rely on large datasets to perform accurately, but data availability can be limited in veterinary medicine, particularly for rare species or diseases.

iv. Ethics and Bias in AI: AI models must be developed carefully to avoid biases that could impact animal welfare. For instance, diagnostic tools should be validated across breeds and species to ensure fair treatment.

Regulatory Considerations:

FDA Guidelines: In the United States, the FDA regulates veterinary diagnostic tests under the Federal Food, Drug, and Cosmetic Act. Manufacturers must navigate the regulatory process to ensure that their products meet safety and efficacy standards.

Quality Assurance: Manufacturers must implement quality assurance programs to monitor the performance of diagnostic tests post-market. This includes ongoing surveillance for adverse events and product recalls when necessary.

Future Directions in Veterinary Biochemical Diagnostics

Integration of Multi-Omics Approaches

The future of veterinary diagnostics will likely involve the integration of multi-omics approaches, combining genomics, proteomics, metabolomics, and microbiomics.

Benefits:

Holistic Insights: Analyzing data from multiple biological layers will provide a more comprehensive understanding of animal health and disease mechanisms.

Precision Medicine: Tailored treatment plans based on an individual animal's biological profile will become increasingly common, improving treatment outcomes and animal welfare.

Telemedicine and Remote Diagnostics

The rise of telemedicine presents new opportunities for veterinary diagnostics, particularly in remote and underserved areas.

Advantages:

Access to Care: Telemedicine enables veterinarians to provide remote consultations and diagnostic services, improving access to veterinary care for clients in rural regions.

Monitoring and Follow-Up: Remote monitoring tools allow for ongoing assessment of chronic conditions, enabling timely adjustments to treatment plans based on real-time data.

Sustainability in Diagnostics

As the veterinary field increasingly emphasizes sustainability, there is a growing need for environmentally friendly diagnostic practices.

Key Strategies:

Waste Reduction: Innovations that minimize waste generation and promote recycling of materials will be critical in advancing sustainability.

Resource Efficiency: Developing diagnostic assays that require fewer reagents and reduce energy consumption will contribute to more sustainable veterinary practices.

Conclusion

Technological innovations, such as point-of-care testing, high-throughput screening, and mass spectrometry in veterinary diagnostics for biochemical parameters have significantly improved the ability to diagnose and manage diseases in animals. They have enhanced diagnostic accuracy and efficiency. The identification of novel biomarkers, coupled with the integration of artificial intelligence and data analytics, is transforming clinical practice. POCT represents a transformative tool in veterinary care. It enhances diagnostic efficiency, allowing veterinarians to offer high-quality, timely care across a wide range of animal species. HTS accelerates research in drug discovery, vaccine development, and toxicology, ultimately contributing to advancements in animal health and welfare and provide a deeper understanding of veterinary pharmacology, helping to bridge the gap between human and animal health research. MS provides valuable insights into the molecular and biochemical makeup of animals, helping to ensure their health and animal welfare; also offer precise analytical capabilities that support diagnostics, drug monitoring, environmental safety, and biomarker discovery. Advances in NAT, such as point-of-care testing and CRISPR-based methods, will continue to broaden its accessibility and utility, making it an increasingly integral part of modern veterinary medicine. AI and data analytics are rapidly advancing veterinary diagnostics, offering new tools for precise, efficient, and personalized care. AI is poised to expand, bringing improved diagnostic and treatment capabilities to veterinarians across the globe. Therefore, these advancements extend beyond diagnostic accuracy and facilitate earlier disease detection, improve treatment outcomes, and ultimately enhance animal welfare.

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TECHNICAL SESSION

Advancement in Animal and Fish Biochemistry (AAFB)

ORAL PRESENTATIONS

1-AAFB-OF-1

Supplementation of wheat flour with processed rohu fish powder; influence on dough characteristics and chapati quality

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The study aims to assess the utilization of processed Rohu fish powder (PRP) to supplement wheat flour (WF) and its influence on dough characteristics and chapati quality. The PRP was obtained after its initial processing to minimise fishy odour and flavour. This was used to supplement WT at 0, 2.5, 5, 10 and 20% of the wheat flour to make them into chapati. The chapatis were incubated at room temperature before evaluating their proximate composition, color profile, water activity, texture, and sensory attributes. The chapatis added with PRP had significantly higher protein content ($p \leq 0.05$) (Increased from9.62±0.03 to 21.88±0.08%) indicating enhancement of its nutritional value. The colour values of chapatis from different lots showed no significant difference. The hardness value was highest (2644.92±406.94Kg Force) for chapattis with 5% PRP, which further decreased with increasing PRP content, which might be due to the well-known combination effect that indicates that the interaction of proteins with other food components at a particular concentration could be more than the interaction at higher protein concentrations. The sensory scores of the PRP-supplemented chapatis did not show any significant difference ($p>005$) in any of the attributes. The chapatis could be successfully supplemented with PRP upto 20% level without significant changes in physical properties and sensory acceptability. The study indicates the possibilities of using processed fish powders in food systems to enhance the nutritional profile of low-protein diets.

2-AAFB-OF-2

In vivo Antioxidant Status of Cancer Patients

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The *in vivo* antioxidant status of different types of cancer patients in different age groups were studied by analyzing the blood samples collected from the cancer patients who are currently undertaking treatment. The antioxidant levels of the patients were altered compared to the levels reported for normal subjects. The analysis of the level of individual antioxidants in different cancer cases revealed that the levels of reduced glutathione, total antioxidant capacity, activities of catalase and SOD decreases in all the cancer cases while the level of malondialdehyde which is the product of lipid peroxidation is increased though the extent of decrease or increase in these parameters is different for each type cancer patients. The cancer cases for which the in vivo antioxidant status was evaluated includes oral cancer, breast cancer, esophageal cancer, lung cancer, gastric cancer, endometrial cancer, colorectal cancer, prostate cancer and AML cancer. The lowest level of reduced glutathione was observed among oral cancer patients while the lowest level of total antioxidant capacity was observed among AML cancer patients. The lowest levels of catalase and SOD activity were observed among the gastric cancer patients and AML cancer patients. The highest lipid peroxidation was observed among the lung cancer patients.

3-AAFB-OF-3

Morin Mitigates LPS-Induced Neuroinflammation in HMC3 and SH-SY5Y Cell Lines via Modulation of NF-κBSignaling Pathways

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Neuroinflammation, a crucial process involving immune responses in the central nervous system, is integral to various neurological disorders. Microglia and astrocytes, key players in neuroinflammation, release pro-inflammatory molecules upon activation, contributing to the inflammatory milieu. The Nuclear Factor-kappa B (NF-κB) pathway emerges as a central player in regulating immune and inflammatory gene expression in neuroinflammation. Understanding this pathway's activation is pivotal in comprehending the inflammatory cascade within the CNS, offering potential therapeutic targets for neurological pathologies. Our study investigates the potential ofMorin- potent flavonoids, in mitigating lipopolysaccharide (LPS)-induced neuroinflammation and NF-κB-mediated inflammatory damage in HMC3 and SH-SY5Y cell lines. The results reveal Morin do not significantly affect cell viability and the compounds demonstrate a reduction in LPS-induced reactive oxygen species (ROS) production in a dose-dependent manner. Additionally, Morin exhibit anti-inflammatory effects by reducing the activity of proinflammatory cytokines (TNF-α, IL-6, IL-1β) and NF-κB in HMC3 cells. Furthermore, the study explores the impact of these flavonoids on the nuclear fraction of NF-κB activity, indicating a significant decrease in NF-κB levels. Overall, the findings suggest the potential therapeutic role of Morin using in vitro models in mitigating neuroinflammation through modulation of NF-κBsignaling pathway.

4-AAFB-OF-4

Heat adaptability dynamics of kosali cattle in chhattisgarh

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The heat stress experienced by dairy animals has intensified as temperatures have risen in connection with climate change. The animals' capacity to adapt to climate stress mainly relies on different factors such as respiration rate, body temperature, neutrophil lymphocyte ratio, and serum cortisol level. Genetic adaptation has led *Bos indicus* cattle to acquire thermotolerant genes as compare to *BosTaurus.* The present objective was to analyze the physiological and hormonal responses of Kosali cattle in comparison to Sahiwal and crossbred cows.A total of 36 adult cows from each breed of Kosali, Sahiwal, and Crossbred were selected randomly from the surrounding areas of the college and the dairy farm of College of Veterinary Science and Animal Husbandry, Anjora, Durg, Chhattisgarh, all of which appeared to be healthy. The study was conducted across three different seasons - summer (THI = 80.310.12), rainy (THI = 77.611.0), and winter (THI = 69.280.89) - to observe changes in the respiration rate (RR), rectal temperature (RT), neutrophil lymphocyte ratio (NLR), and serum cortisol levels in three cattle breeds due to seasonal variations.Thespecific pattern in the respiration rate, rectal temperature, serum neutrophil lymphocyte ratio, and cortisol concentration were observed during the rainy and summer seasons among all the three breeds of cattle. The cross-breed cattle showed significantly ($P>0.01$) higher respiration rate, rectal temperature, serum neutrophil lymphocyte ratio, and cortisol concentration followed by Sahiwal and Kosali cattle.Based on current findings, it was concluded that the Kosali cattle are highly suited to the challenging dietary and weather conditions in the rural areas of Chhattisgarh.

5-AAFB-OF-5

Serum thyroid hormones, macro minerals and electrolyte levels and their correlation at different lactation stages of Murrah buffalo

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The study was undertaken to estimate the serum levels thyroid hormones, electrolytes and macrominerals profile and their correlation with stages of lactation in Murrah buffalo. Blood samples were collected twice at month interval from 24 healthy lactating Murrah buffaloes during early, mid and late stages of lactation. Estimation of thyroid hormones done by Radioimmunoassay, Electrolytes by ISE and macrominerals by standard biochemical methods.There was significant increase in the levels of total triiodothyronine (TT3) during late stage of lactation. Total thyroxine (TT4) levels remained same during early and late stage of lactation, but the levels decreased significantly during mid-lactation stage. The mean±SE levels of free thyroxine (ng/dl) during early, mid and late stages of lactation were 0.86±0.04, 0.93±0.02 and 0.93±0.02 respectively.There was no significant variation in the levels of fT_4 during early and late lactation but the levels increased significantly ($P < 0.005$) in mid lactation stage.Themean±SE levels of Calcium (mg/dl) during early, mid and late stages of lactation were 9.98±0.23, 10.70±0.25 and 10.47±0.23 respectively with no significant variation during different stages of lactation. The values of phosphorus (mg/dl) ranged between 4.21-7.9, 4.72-7.23 and 4.56-6.99 during early, mid and late stage of lactation respectively. The levels of phosphorus show nonsignificant increased from early to mid-lactation. The mean ±SE serum levels of magnesium (mg/dl) during early, mid and late stages of lactation were 2.91±0.07, 2.95±0.06, 2.96±0.05 respectively. No significant difference was observed in magnesium level during different lactating stages. The mean±SE levels of ionized calcium (mg/dl), Sodium(mmol/L), Chloride (mmol/L) and Potassium(mmol/L) during early, mid and late stages of lactation were 4.41±0.07, 4.58±0.05 and 4.85±0.09.,134.40±0.57, 138.86±0.41, 141.47±0.43, 95.63±0.33, 101.88±0.83, 101.89±0.54 and 4.71±0.15., 5.01±0.06, 5.07±0.06, respectively. Serum triiodothyronine hormone was positively correlated with sodium (r=0.22), chloride (r=0.11), total calcium (r= 0.11), phosphorus (r= 0.15), magnesium (r= 0.1), free thyroxine (r= 0.25) and thyroxine (r= 0.11). However,it was negatively correlated with potassium (r= -0.064), ionized calcium (r= -0.15).

6-AIFB-OF-6

Some histological and histochemical observations of bursa of Fabrcius inTurkey during summer seasons

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The study was aimed to provide the basic findings about effect of summer season on histological changes of bursa of Fabriciusin the Turkey. For this study the Turkey were divided into two groups according to seasons. Six Turkey of 8 weeks of agewere selected in each group in both seasons i.e. autumn and summer seasons. Birds from bothgroups were sacrificed and bursa was exteriorized. For routine histological investigations tissues from different respective area were processed by paraffin techniques. Histologically the bursa wall was made up of tunica mucosa, tunica muscularis and tunica serosa. The epithelium was lined with psedostratified columnar epithelium and divided in to follicle associated epithelium and inter follicular epithelium. Tunica mucosa projected in to lumen called plicae filled with follicles of different shape and size. The epithelial height, length and width of bursal follicles, length of primary plicae were decrease significantly in the summer season. Areas of

epithelial attenuation, an increased convolutions of the IFE vacuolation and folding and detachment were noted over the plical surface were observed in the summer season. Histochemically, the epithelial lining, showed mild to moderate PAS positive reaction, mild PAS positive reaction were observed in the cortex, medulla, corticomedullary junction which decreased in summer seasons. The epithelial lining showed moderate to intense activity of acid mucopolysaccharides, alkaline phosphatase and Acid Phosphatase activity decreased in summer seasons. From above observation it can be concluded the in summer season adversely affect on the normal histology and histochemistry of bursa of fabricus in summer seasons.

7-AAFB-OF-7

Effect of Season and Breed on Antioxidant Activity of Raw Camel Milk (*Camelus dromedarius)*

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This study evaluated the impact of season and breed on the antioxidant activity of raw camel milk (*Camelus dromedarius)* in Rajasthan. Antioxidant activity in raw milk of 60 Bikaneri and Jaisalmeri camel (30 each) was observed as DPPH (PSA) and FRAP (TAC) assay during various seasons i.e. summer, rainy and winter. The ability to scavenge 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical or Percent Scavenging Activity (PSA) of milk was determined by DPPH assay according to Brand-Williams *et al.,.* (1995) with minor modifications. The Total Antioxidant Capacity (TAC) of milk was determined by Ferric Reducing Antioxidant Power (FRAP) assay according to Benzie and Strain (1996) with some modifications. The antioxidant activity showed a non-significant ($P>0.05$) effect of the breed i.e. Bikaneri and Jaisalmeri camel while these assays were significantly influenced by the seasons. Percent Scavenging Activity /DPPH ($P<0.01$) and Total Antioxidant Capacity/FRAP ($P<0.05$) were significantly higher in the winter season than in other seasons in both Bikaneri and Jaisalmeri camel. This might be due to favorable nutritional, physiological and environmental conditions. Our findings suggest that the season has an impact on the physiochemical and antioxidant properties of camel milk.

8-AAFB-OF-8

Nutritional Status and Biochemical Profile of Santal Lactating-Mother of Kharagpur Subdivision

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The nutritional and health status of the lactating mothers has a significant public health importance particularly in the developing countries. The situation is most vulnerable among the tribal mothers. India has the world's largest tribal population, with tribal people accounting for 8.6% of the country's total population. They are considered as vulnerable group due to the nutrient demands are increased during this time though most of the mothers residing in the developing countries cannot meet this high demand of high-quality nutrients. The Santals are one of the Munda peoples who live mainly in the state of Jharkhand, Bihar, west Bengal, Odisha and Assam. Several recent studies have highlighted the nutritional status of the various tribes of India. But pragmatic information on the nutritional status of lactating women of the Santal- tribe is lacking.The present study was conducted among thirty Santal lactating mothers residing in Kharagpur Sub-division of Paschim Medinipur

District to find out the association between socioeconomic status, nutritional status and biochemical profile of the Santal lactating mothers. The anthropometric measurement and biochemical assessments were carried out following standard protocol to assess the nutritional and health status. The present study showed that the prevalence of underweight, anaemia and hypercholesterolemia among the Santal lactating mothers were 20.00%, 80.00% and 10.00% respectively. There is no case of diabetes was found. The study found that the body adiposity index (BAI) is significantly associated with the triglyceride (GL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL). Therefore, from the results of the present study is helpful in assessing very high prevalence of anaemia when compared to other parameters of health which needs to be addressed to improve the health status of the lactating mothers of Santhal tribe.

9-AAFB-OF-9

Analysis of Serum metabolic profile of healthy and subclinical mastitis Barbari goats during summer season

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This study was carried out on lactating Barbari goats reared at goat farm of College of Veterinary Science, DUVASU Mathura. Total 50 samples of milk were collected and were stored at -20°C prior to analysis. The animals were divided in to two subgroups, namely control/healthy group, subclinical mastitis group on the basis of screening by using somatic cell count (SCC), Electrical Conduction and pH on the milk samples. The Serum samples were collected (Healthy-30 & Subclinical mastitic-20) and analysed for Total Protein, Albumin, Globulin, Albumin/Globulin Ratio, Sodium, Potassium, Aspartate transaminase (AST) Lactate dehydrogenase (LDH) Alkaline phosphatase (ALP). Serum metabolic profile of healthy and subclinical mastitis Barbari goats found significant difference in Total protein (g/dl) Healthy (6.85±0.04) Subclinical mastitis (8.53±0.03) Alb (g/dl) Healthy (3.47±0.03) Subclinical mastitis (3.74±0.09), A/G ratio Healthy (1.01+ 0.01) Subclinical mastitis (0.84+0.02) AST(IU/L) Healthy (93.48±1.07) Subclinical mastitis (77.22+0.55) LDH(IU/L) Healthy (158.12±9.20) Subclinical mastitis (159.33±5.58) and ALP(IU/L) Healthy (163.28 0.60) Subclinical mastitis (190.27 3.68) values during summer season.

10-AAFB-OS-10

A study on diagnostic serum markers in dogs affected with canine mammary gland tumour

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The early diagnosis of cancer is promising in prolonging the cancer survival period. Every cancer cell expresses specific proteins known as tumour-associated antigens. In patients with tumours, these antigens are called biomarkers as they can be detected in other tissues, serum or urine, in concentrations that are different from normal. In this study, a total of Thirty-six cases of Canine mammary tumours were presented to the Veterinary Clinical Complex, DUVASU, Mathura from January 2024 to October 2024. Blood samples of affected dogs were collected and serum biomarker level was estimated. The FNAC of samples was done which shows a high nucleus-to-cytoplasmic ratio, cluster of cells seen together, and highly pleomorphic cells to confirm neoplastic condition. Histopathological examination revealed various tumor grades that correlated with serum biomarker

levels, specifically Cancer Embryonic Antigen (CEA) and Cancer Antigen 15-3 (CA 15-3). In our findings, we were found that CA15-3 can be considered a better serum tumour marker than CEA for the diagnosis of mammary gland cancer, which has a high value in tumour diagnosis and a positive correlation with the clinical stage. The expression of serum markers in the malignant mammary gland tumour group was significantly higher than that of the benign group and healthy control group. The sensitivity and specificity of CEA and CA 15-3 improve when these serum markers are used together. This helps in detecting cancers earlier, allowing more effective treatment.

11-AAFB-OS-2

Evaluation of impacts of cholesterols on P2RX7 global dynamics in P2RX7-POPC lipid bilayer

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Membrane cholesterol is highly plastic, consistently alters in outer and inner leaflets of plasma membrane. It forms the 70-80% of total cellular cholesterol, regulates the membrane fluidity and receptors signaling. P2RX7 is a cationic, double spanning transmembrane purinergic receptor, upon activation by extracellular ATP (eATP), allowing the intracellular entry of multivalent cations non-selectively. Cholesterol negatively affects the P2RX7 activations and receptor desensitization. In our present study, we generated the transmembrane model of human P2RX7 with different concentration of cholesterol ranging from 0 to 50% in POPC lipid bilayer and analyzed the effects of different concentrations of cholesterol on stability, per-residue flexibilities and compactness of P2RX7 during microseconds in all atom molecular dynamic simulations using three metrics RMSD, RMSF and Radius of Gyration. These metrics which we used in our study, can also be employed to sanity check of molecular systems before going into in depth characterization of certain molecular features. In our study, the stability of P2RX7 was measured by backbone RMSD which shows, both open and closed forms of the P2RX7 system evolved well and seemed quite stable during simulations, however, open forms of P2RX7 showed more heterogeneity with different concentrations of cholesterol. The per-residue flexibility quantification by RMSF shows certain pockets in P2RX7 are more flexible in both open and closed forms, however the closed forms show little higher RMSF. Most of these regions are disordered and belong to the C-terminal domain. The compactness of the molecular systems examined by radius of gyrations (Rg), reproduce the RMSD results as well as added the information that open forms of P2RX7 show more extended forms after addition of the cholesterol which has not been observed in closed forms of P2RX7. Our study demonstrates that varying cholesterol concentrations in the P2RX7-POPC lipid bilayer system influence the global dynamics of the receptor differently for its open and closed forms. While the closed forms maintain global stability despite high per-residue flexibility, the open forms exhibit increased conformational activity and heterogeneity with rising cholesterol levels.

12-AAFB-OS-3

Characterization of serum biochemical marker for canine mammary tumor

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This study was intended to explore and identify the serum biochemical marker for canine mammary tumor. For that blood samples were collected from five healthy and sixteen dogs (irrespective age and breed) suspected for having mammary tumor presented at Veterinary Clinical Complex of DUVASU, Mathura. Blood samples were subjected for harvesting the serum and stored in −80°C for further proteome analysis. The serum samples were analyzed for Total serum protein (TSP), albumin (alb), globulin (glob), AG ratio (AG), alanine aminotransferase (ALT), aspartate aminotransferase (AST), Alkaline phosphatise (AKP), creatitine (Cre), urea (Ure), and cholesterol. In addition serum proteins were estimated by Bradford method and subjected to 10% SDS-PAGE for separation of proteins on the basis of molecular weight using vertical slab gel assembly. The serum biochemical analysis revealed significant changes in serum Golb, Ag, Cre, Ure, AKP and cholesterol. The SDS-PAGE analysis of serum proteins (following TCA-Acetone precipitation) from healthy dogs revealed 24 bands while serum of dogs bearing mammary tumor revealed 20 bands in 10% polyacrylamide gel. It can be concluded from the study that significant changes in serum Glob, AKP and Chol along with changes in serum SDS-PAGE profile can be used for marker for tumor marker. However further characterization of accurate serum metabolite and differentially expressed serum proteins exhibited in SDS-PAGE profile in healthy and tumor affected dogs need further thorough analysis by some sophisticated and high throughput tools for characterization of biochemical marker for diagnosis of canine mammary tumor.

TECHNICAL SESSION

Advancement in Animal and Fish Biochemistry (AAFB)

POSTER PRESENTATIONS

1-AAFB-PF-1

Symmetric dimethylarginine (SDMA): Aforefront biomarker revolutionising renal function in veterinary science

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Symmetric Dimethylarginine (SDMA) is a vital biomarker for renal function assessment. SDMA is considered a remarkable indicator of glomerular filtration rate and plays a vital role in the early diagnosis of diseases such asacute kidney injury (AKI), chronic kidney disease (CKD) and more. The rise of SDMA levels during mildly impaired renal function makes it a sensitive and specific biomarker for such premature kidney diseases, unlike creatinine. It is also nominally influenced by additional factors like age, breed, and muscle mass of animals making it more consistent than other traditional biomarkers across diverse species primarily in dogs and cats. SDMA competes with L-arginine for cellular uptake which leads to the decreased synthesis of nitric oxide causing endothelial dysfunction therefore indirectly contributing to elevated cardiovascular health risks.SDMA also has other roles as a biomarker such as hypertension, cardiovascular diseases and other systemic abnormalities. Manifold diagnostic strategies such as Enzyme-Linked Immunosorbent Assay (ELISA), Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS), immunoturbidimetric assays, microfluidic and Lab-on-Chip technologies are used for the estimation of SDMA. Therefore, SDMA could provide a better platform for early diagnosis of renal and cardiovascular functions in the near future.

2-AAFB-PF-2

Lipid Dysregulation and demographic trends in chronic kidney disease among canines: A correlation study on serum creatinine and lipid Profiles

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Chronic Kidney Disease (CKD) is a common and progressively debilitating condition impacting the canine population of various age groups, with serious consequences for health outcomes and quality of life. This study aimed at elucidating the relationship between CKD progression and lipid metabolism, as well as to identify various demographic and seasonal patterns associated with CKD incidence in dogs. This observational study included 100 dogs, comprising 50 with CKD and 50 healthy controls. Dogs with CKD were divided into three groups based on serum creatinine levels (Group 1: 1.7–10 mg/dL, Group 2: 10–20 mg/dL, and Group 3: >20 mg/dL) representing severe and progressive renal impairment. Retrospective blood samples data were collected from all subjects for biochemical and haematological tests. Later, lipid profiles were evaluated using Fujifilm Dri-Chem analyser with dry slide technology, with cholesterol and triglyceride levels measured to examine potential lipid dysregulation in relation to CKD severity. Data were analysed for demographic trends, and seasonal patterns in CKD incidence. Lipid profile markers, specifically cholesterol and triglycerides, were measured across all groups, with a correlation analysis conducted to assess the association between CKD severity and lipid levels. Results indicated a significant ($p<0.05$) positive correlation between increased CKD severity and elevated cholesterol and triglyceride levels. Group 3 exhibited the highest lipid values, suggesting lipid dysregulation as renal function deteriorates. Demographic analysis revealed a higher incidence of CKD among older dogs and female dogs, with most cases being diagnosed at advanced stages of the disease. Seasonal trends revealed a post-

monsoon increase in CKD diagnoses, highlighting potential environmental or dietary influences on disease incidence, which warrant further investigation. This study underscores the value of regular biochemical and lipid profile monitoring in managing CKD in canines. The correlation of dyslipidemia with advanced CKD stages emphasizes the need for integrative therapeutic approaches that address both renal function and lipid regulation, particularly in high-risk seasons and susceptible demographic groups. Findings also advocate for a closer examination of environmental and dietary factors as potential contributors to CKD progression. This research highlights the significance of a holistic approach to CKD management, promoting improved quality of life for affected dogs through proactive monitoring and intervention strategies.

3-AAFB-PF-3

Influence of grape seed extract in extending the shelf life of fish sausage stored at refrigerated temperature

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In this study, the potentials of grape seed extract (GSE) as a natural antioxidant and antimicrobial agent were evaluated for enhancing the shelf life of fish sausage in refrigerated conditions(4±2°C). GSE was extracted and analyzed for its total phenolic and flavonoid content, and its antioxidant activity was assessed through DPPH, ABTS, ferrous ion chelation (FIC), ferric reducing power (FRP), and the β-carotene bleaching test. Antimicrobial activity was also determined by using the disc diffusion method. Fish sausage prepared in cellulose casing was treated with GSE at a concentration of 3000 mg/L, and its storage stability was evaluated. Antioxidant activity of GSE was comparable to that of synthetic antioxidant (BHA), with strong inhibition of DPPH, ABTS, and FRP. The antimicrobial activity of GSE effectively inhibited Gram-positive bacteria than Gram-negative bacteria. Biochemical analyses revealed a significant reduction in peroxide value (PV), free fatty acids (FFA), thiobarbituric acid reactive substances (TBARS), total volatile basic nitrogen (TVB-N), and trimethylamine nitrogen (TMA-N) in the GSE-treated samples. The shelf life of the fish sausage was extended to 24 days with GSE treatment, a 7-day improvement over the control. This study demonstrates that GSE, a by-product of the fruit processing industry, can serve as an effective natural alternative to synthetic antioxidants, enhancing the shelf life and quality of fish sausage during refrigerated storage.

4-AAFB-PF-3

Assessment of oxidative stress biomarkers in moneiziosis in goats

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Moniezia is a serious parasitic challenge to the small ruminant population worldwide and affects more than 12% of the population in the eastern part of India. The parasite inhabits the small intestine of ruminants causing diarrhoea and weight declines leading to the decrease in productive

efficiency. The host reacts against parasites through the production of reactive oxygen species (ROS) during normal aerobic metabolism and by activated phagocytic cells with increased oxygen consumption, known as oxidative burst. The onset and progression of parasitic infections in the host organism are significantly influenced by oxidative stress. Oxidative stress develops due to an imbalance of free radicals and antioxidants within the organisms that leads to cell damage by damaging the various biomolecules i.e by the oxidation of lipids, proteins and DNA. The present study was conducted on 10 Black Bengal goats naturally infected with *Moneiziaexpansa* and 10 heathy Black Bengal goats with aim to assess the antioxidant status. The confirmation of the infection by parasite *Moneiziaexpansa* was done by PCR. Activity of lactate dehydrogenase (LDH), catalase (CAT), superoxide dismutase (SOD), Thiobarbituric acid reactive substances (TBARS) and reduced glutathione (GSH) concentration were estimated in serum of both healthy and infected goats by following the standard method. Activity of LDH, CAT, SOD and TBARS concentration were significantly ($P \leq 0.05$) higher in infected goats than healthy while GSH concentration was significantly ($P \leq 0.05$) higher in healthy goats than infected. Thus, we have recorded the negative impact of monieziosis in the development of oxidative stress andit should be taken into consideration when treating and planning a deworming approach.

5-AAFB-PS-1

Influence of season on oxidative status and ROMO1 gene expression associated with semen quality in Bucks

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Seasonal oxidative stress influences the semen quality and fertility. In this study we determined influence of season on oxidative stress biomarkers and ROMO1 gene expression in Barbari and Sirohi bucks. 12 adult breeding bucks of Barbari and Sirohi breed (six each) maintained at Amanala Goat Farm, Jabalpur. The average meteorological variables were recorded during summer and winter season. Fresh semen samples were assessed during summer and winter season to ascertain the oxidative stress in different season by evaluating the level of SOD, CAT, GSH, MDA and TAC. Spermatozoa were separated out from fresh semen by centrifugation and used to evaluate the ROMO1 gene expression during both seasons in comparison to GAPDH as housekeeping gene. No significant variation was observed for SOD, CAT, GSH, MDA and TAC in between two breeds. A significant ($p<0.05$) higher value of SOD and MDA were observed in summer than winter in both the breeds. The catalase activity, reduced glutathione and mean total antioxidant capacity (TAC) concentration were significantly ($p<0.05$) higher in winter as compared to summer in both the breeds. ROMO1 is one of the most important proteins which control the generation of reactive oxygen species (ROS) in the mitochondria, particularly in cells with high metabolic rates, like sperm cells. ROMO1 gene expression level was significantly ($p<0.05$) lower during the winter season in comparison to summer season. So, we could suggest that elevation of ROMO1 gene expression during summer season influence the antioxidant defense mechanism in the seminal plasma.

6-AAFB-PS-2

Studies on the variations in blood stress parameters of the Sonali breed of poultry in humid and dry winter seasons

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The aim was to examine variations of the different stress parameters in Sonali breed poultry during both humid and dry winter periods. Blood was drawn from 48 healthy birds, aged 55 days, with an equal number of males and females per season. The blood samples were collected in sterile tubes containing heparin, EDTA, and clot activator. The clot activator tubes were used to separate the serum, after which various stress indicators blood sugar level, hemoglobin, packed cell volume (PCV), and the heterophil-lymphocyte ratio (HLR) through were measured. Additionally, the levels of interleukin-6, C-reactive protein, and cortisol were analyzed through external services were measured. The findings revealed significant differences ($P<0.05$) in packed cell volume (PCV) between the humid and dry winter seasons. However, there were no significant variations ($P>0.05$) in hemoglobin, glucose, HLR, C-reactive protein, cortisol, and interleukin-6 levels between male and female Sonali birds. Male birds showed a significantly higher PCV compared to females. In the dry winter season, there were no significant differences ($P>0.05$) in stress indicators between males and females. Significant seasonal variations ($P<0.05$) in HLR, C-reactive protein, and interleukin-6 levels were observed, whereas hemoglobin, PCV, glucose, and cortisol levels did not show significant differences between males. Females had higher levels of PCV, glucose, HLR, C-reactive protein, and interleukin-6 during the humid winter season compared to the dry season. Based on the observations, it can be concluded the Sonali breed of poultry is well adapted in the rural area of Chhattisgarh and can survive in normal nutritional and housing management under harsh climatic condition.

7-AAFB-PS-3

Unraveling the complexities of hyperbilirubinemia in canines: A clinical report of three distinct cases

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Hyperbilirubinemia, characterized by elevated bilirubin levels in the bloodstream, can arise from prehepatic, hepatic, or posthepatic causes, each with distinct clinical implications. In this study, we evaluated three canine cases—two Pomeranians and one German Shepherd—presented with a history of yellowish pigmentation of the skin, gums, and sclera, raising suspicion of hyperbilirubinemia and jaundice. Comprehensive diagnostic tests, including liver function tests (LFT), kidney function tests (KFT), using Fujifilm dry slide chemistry analyzer and complete blood count (CBC), were performed to identify the underlying causes and further confirmed by ultrasonography.The first Pomeranian exhibited markedly elevated direct bilirubin levels, consistent with post-hepatic obstructive jaundice, potentially due to biliary tract obstruction. The second Pomeranian displayed elevated indirect bilirubin levels and was diagnosed with prehepatic jaundice associated with *Babesia spp.* infection, a hemoprotozoan parasite. The German Shepherd showed increased levels of both direct and indirect bilirubin, indicating hepatic jaundice, suggestive of intrinsic liver dysfunction.This case study highlights the importance of differentiating the types of hyperbilirubinemia in dogs through targeted biochemical profiling and etiological analysis. Each case's diagnostic profile underscores the need for precise laboratory investigations to guide appropriate therapeutic interventions.

8-AAFB-PS-4

Haemato-biochemical modifications in dogs affected with gastroenteritis

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Around the world, pets like dogs and cats play significant roles in communities. The most prevalent gastrointestinal illness in dogs, gastroenteritis, can affect dogs of all ages and breeds. The present study was conducted in the Department of Veterinary Biochemistry, SVPUA&T Meerut and a total of 12 dogs were included in the study out of which 6 were suffering from gastro-enteritis whereas 6 healthy dogs were chosen to serve as healthy control.Blood biochemical markers were examined in this study, which could aid in determining how well a patient is responding to treatment and modifying treatment plans accordingly.These parameters showed a significant decrease (Minimum 38.2 mg/dl) and increase (Maximum 313 mg/dl) in values of plasma glucose. A significant increase in plasma Uric Acid 0.2-0.8 mg/dl and non-significant increase in Cholesterol86.5-320 mg/dl was observed in enteritis which might be due to marked decline in diet intake, malabsorption and ongoing protein losing enteropathy. Alanine aminotransferase (ALT) 8.6-38.8 U/L and aspartate amino-transferase (AST) 4.2-67.8 U/L were found to be elevated non-significantly. This increase may be due to reactive hepatopathy. These biochemical changes are critical for veterinarians to monitor, as they guide diagnosis, prognosis, and treatment strategies.In conclusion, gastroenteritis in dogs leads to a cascade of biochemical changes that require careful monitoring and management. Recognizing these changes and understanding their implications allows for timely and effective intervention, improving outcomes for affected canines.

9-AAFB-PS-5

Identification of upper critical temperature (UCT) on the basis of plasma biochemical parameters in buffalo calf

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The present study aimed to identify upper critical temperature (UCT) on the basis of plasma biochemical parameters in buffalo calf. Six Murrah buffalo calves, aged between 8 and 12 months and weighing 100 to 150 kg, were used for the study. The experiment was conducted from May to July in a psychrometric chamber. The calves were subjected to six progressively increasing temperatures of 25°C, 28°C, 31°C, 34°C, 37°C, and 40°C, with corresponding Temperature-Humidity Index (THI) values ranging from 73 to 90. At each temperature, the calves were exposed for 10 days, and blood samples were collected on the 10th day at 1500 hours. Plasma was harvested from the blood samples for biochemical analysis. The upper critical temperatures (UCT) for various parameters were determined using segmented regression analysis (SegReg software). The results revealed that alkaline phosphatase (ALP), glucose, and lactate dehydrogenase (LDH) levels did not change significantly ($p>0.05$) with increasing temperatures. However, albumin and creatinine showed a UCT at 31.75°C, while urea and alanine aminotransferase (ALT) exhibited UCT at 31.15°C and 37.0°C, respectively. The UCT for aspartate aminotransferase (AST) was identified at 28.45°C, and for total protein, it was recorded at 34.15°C. ANOVA revealed a significant ($p < 0.05$) decrease in total protein and albumin levelsat 31°C, which remained constant at 34°C, 37°C, and 40°C. ALT levels progressively decreased with increasing temperature, showing a significant decline at 28°C, remained

same at 31°C and 34°C, and reached its lowest at 37°C, and remained same thereafter at 40°C. In contrast, glucose, AST, ALP, and LDH levels remained unchanged across all temperatures. Creatinine levels decreased significantly ($p<0.05$) at 34°C as compared to previous temperatures and remained constant at 37°C and 40°C. The results show that UCT for different plasma biochemical parameters were different and results also exhibited a differential dynamics of plasma biochemical responses with increasing temperature.

10-AAFB-PS-6

Comparative assessment of pre-weaning learning effects on biochemical parameters in dairy calves

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Effective dairy animal management faces the critical challenge of optimizing species-specific behaviours within the constraints of commercial farming systems, which differ significantly from animals' natural habitats. Understanding the cognitive needs and capabilities of these animals is essential for enhancing daily management practices. So, on the basis of this the, experiment was conducted for comparative assessment of pre-weaning learning effects on biochemical (cortisol) on Hariana and Sahiwal calves at ILFC of DUVASU, Mathura from March to August. For this study, fourteen new born calves after competition of the colostrum feeding phase were selected and randomly divided into two groups (7 hariana& 7 Sahiwal). They were provided with general feeding and other management routine practices followed at the farm.It was observed that when calves were observed at every fortnight interval for their apprehensive behavior in the presence of a novel setting object. The overall tendency to escape and number of vocalizations was significantly higher ($P<0.01$) in Hariana calves compared to Sahiwal calves. During the analysis of blood biochemical the, overallresults for plasma cortisol levels at every fortnight interval were significantly higher ($P<0.01$) in Hariana calves (12.75±0.24 ng/ml) compared to Sahiwal calves (11.83±0.24 ng/ml). The study concluded that repeated exposure to novel settings significantly reduced stress levels in calves, positively influencing their learning abilities. This adaptation to new environments plays a crucial role in enhancing daily farm management practices, promoting better behavioural responses, and improving the overall welfare and efficiency of dairy operations.

11-AAFB-PS-7

Analysis of Milk metabolic profile of healthy and subclinical mastitis Barbari goats during summer & winter season

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This study was carried out on lactating Barbari goats reared at goat farm of College of Veterinary Science, DUVASU Mathura. Total 50 samples of milk were collected and were stored at -20°C prior to analysis. The animals were divided in to two subgroups, namely control/healthy group, subclinical mastitis group on the basis of screening by using somatic cell count (SCC), Electrical Conduction and pH on the milk samples. The Milk samples were collected (Healthy-50 & Subclinical mastitic-50) and analysed for Total Protein, Albumin, Globulin, Albumin/Globulin Ratio, Sodium, Potassium, Aspartate transaminase (AST) Lactate dehydrogenase (LDH) Alkaline phosphatase (ALP). Serum metabolic profile of healthy and subclinical mastitis Milk metabolic profile of healthy Barbari

goats found significant difference in A/G ratio values during summer (1.82*+ 0.10) and winter season but the other parameters show no significant difference. Milk metabolic profile of subclinical mastitis Barbari goats found no significant difference among all the parameters in both the seasons.

12-AAFB-PS-8

Milk metabolic profile of healthy and subclinical mastitis Sahiwal cows during winter season

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This study was carried out on lactating Sahiwal cows reared at dairy farm of College of Veterinary Science, DUVASU Mathura. Total 40 samples of milk were collected and were stored at -20°C prior to analysis. The animals were divided in to two subgroups, namely control/healthy group, subclinical mastitis group on the basis of screening by using somatic cell count (SCC), Electrical Conduction and pH on the milk samples. The Milk samples were collected (Healthy-27 & Subclinical mastitic-13) and analysed for Total Protein, Albumin, Globulin, Albumin/Globulin Ratio, Sodium, Potassium, Aspartate transaminase (AST) Lactate dehydrogenase (LDH) Alkaline phosphatase (ALP) Milk metabolic profile of healthy and subclinical mastitis Sahiwal cows found significant difference in SCC (145.4*±7.61 & 244.3±7.17) pH (6.94*+ 0.04 & 7.33±0.10) & AST (137.4*+ 2.56 & 148.3±1.30) values during winter season the other parameters not found any significant difference.

TECHNICAL SESSION

Development in Alternative Medicine, Pharmaceutical and Nutraceutical Research for Animal Health and Production (AMAH)

LEAD PAPER

AMAH-LP-1

The extended role of ruminant interferon from immunity to physiology

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Interferons (IFN) are cytokines with antiviral activity induced by viral infection. In ruminants, interferons are categorized into three types: type I, type II, and type III. Type I includes IFN-alpha (IFNA), beta (IFNB), delta (IFND), epsilon (IFNE), kappa (IFNK), tau (IFNT), omega (IFNW), and zeta (IFNZ). Type II consists of a single member, IFN-gamma (IFNG). Type III, also referred to as IFN-lambda, comprises three members: IFNL1, IFNL3, and IFNL4. These interferons signal through distinct receptor complexes, with type I and III IFNs initiating innate immune responses and type II IFNs helps to bridge the innate and adaptive immune responses. Type I IFN genes are located on chromosome 2 in sheep, chromosome 3 in buffalo, and chromosome 8 in cattle and goats. Type II IFN is found on chromosome 3 in sheep, chromosome 4 in buffalo, and chromosome 5 in goats and cattle. Type III IFN genes are situated on chromosome 18 in cattle, buffalo, and goats, and on chromosome 14 in sheep. Ruminants commonly exhibit an expansion in the number of innate IFN genes, though they remain located to a single chromosomal location. Gene duplication of IFNs has resulted in copy number variation (CNV), particularly evident in IFNW, IFNT, IFNA, and IFNB in ruminants. For instance, cattle and buffalo have approximately 24 copies of IFNW and 11 copies of IFNB. Unlike non-ruminants, which have a single IFNB copy, cattle possess multiple copies, suggesting reduced constraints on gene duplication or selective pressure for additional genes. Similarly, IFNL genes play a key role at epithelial barriers, with IFN-λ3 and IFN-λ4 present in two copies in buffalo and cattle. In ruminants, the expression of IFNT in embryos is initiated around the time of blastocoel formation and IFNT is a critical regulator of conceptus elongation. IFNE is constitutively expressed by reproductive tract epithelium and regulated by hormones during estrus cycle and reproduction. This expression is regulated by hormonal factors rather than pathogen recognition pathways, which allows for a rapid immune response to potential infections. IFNA, IFNB, IFNL are potent antiviral innate immune genes, their functions are more centered on elimination of viral pathogens. However, the function of IFNL is predominantly modulated by the expression of their unique receptor IFNLR1. Interferons in ruminants play a dual role in immunity and reproduction, highlighting their evolutionary adaptations for survival and reproductive success. Their expansion reflects selective pressure for enhanced immune defense, reproductive support, and epithelial barrier integrity, emphasizing their critical role in ruminant biology.

AMAH-LP-2

Medicinal plant for management of diabetes

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Plants have been used for treatment of various diseases for many years based on experience and folk remedies and continue to draw wide attention. In recent times, focus on plant research has increased all over the world and a large body of evidence has been accumulated to highlight the immense potential of medicinal plants used in various traditional systems of medicine. The use of plants/ natural products in traditional medicines covers a wide range of therapeutic uses to treat the infection as well as many chronic diseases (Fitzgerald *et al.,* 2020). Many people still rely on the traditional medicine and healthcare because of their wider cultural acceptance and affordability (Khanal *et al.,* 2021).

Traditional medicine from plant extracts has proved to be more affordable, clinically effective and has relatively less adverse effects than modern drugs. Plant derived secondary metabolites like steroids, alkaloids, phenolics, lignans, glycosides etc. possess diversified biological properties such as antiallergic, anticancer, antimicrobial, anti-inflammatory, antidiabetic and antioxidant activity. Moreover, during the past few years, some bioactive drugs isolated from plants showed antidiabetic activity with more efficacy than oral hypoglycemic agents used in clinical therapy (Tran *et al.,* 2020).

Diabetes mellitus (DM) is a widespread endocrine disorder that is characterized by elevated levels of glucose in the blood due to insufficiency in insulin production (Type-1 DM) or insulin resistance (Type-2 DM) in the body. According to the ninth edition (2019) of the International Diabetes Federation (IDF), Diabetes Atlas, as of 2019, the total adult population in the age group of 20-79 years, 463 million live with diabetes which is set to increase to 578 million by 2030 (IDF, 2019). Understanding the pathogenesis of diabetes mellitus is extremely important in treatment. Healthy eating, physical activity, and weight control are the center of the therapeutic program for patients with diabetic mellitus (Colberg *et al.,* 2016). The present treatment of diabetes mellitus is focused on controlling and lowering blood glucose levels in the vessels to a normal level (Knight *et al.,* 2005). The mechanism of both modern medicines and traditional medicines to lower blood glucose concentration are: 1) to stimulate beta-cell of the pancreatic islet to release more insulin; 2) inhibit the secretion of hormones which increases blood glucose concentration; 3) increase the sensitivity of insulin receptor site; 4) inhibit glycogen hydrolysis in liver and 5) enhance the use of glucose in tissues and organs (Bhoyar *et al.,* 2011; Wang *et al.,* 2013; Thule, 2012). The use of modern drugs in diabetes mellitus has been associated with a series of adverse effects. For the management of diabetes mellitus, herbal products are better compared to designer drugs because herbal drugs are less pernicious with minimum side effects (Ukwe and Ubaka, 2011). Several ethnopharmacological studies on medicinal plants having beneficial effects on diabetes have been reported (Sani *et al.,* 2012). Plants can be effective herbal medicines because they are natural antioxidants and contain anti-diabetic compounds. The mechanism of action of the antidiabetic activity of plants includes – 1) increased pancreatic secretion of insulin, 2) inhibition of glucose production in the liver, and 3) enhanced glucose uptake in the muscle and adipose tissue. The phytochemicals which have the beneficial effects on diabetic patients include flavonoids, glycosides, terpenoids, saponins, alkaloids, tannins, and steroids (these can reduce high glucose levels in the blood), phenolics and alkaloids (they can improve the performance of pancreatic tissues by increasing insulin secretion or decreasing the intestinal absorption of glucose).Some active principles/ compunds in plants for hypoglycaemic activity include- chlorogenic acid and chicoric acid (these can increase glucose uptake in muscular cells and also increase insulin secretion), Eremanthin (reduces the level of glycosylated hemoglobin (HbA(1c)), serum total cholesterol, triglyceride, LDL-cholesterol and markedly increased plasma insulin, tissue glycogen, HDL-cholesterol and serum protein levels), Phyllodulcin (lower fasting blood glucose and glycosylated hemoglobin (HbA1c) and regulate liver glucose homeostasis), Vanillin (suppressed the levels of blood glucose, serum cholesterol,

triglyceride, low-density lipoprotein cholesterol (LDL-c), Scopoletin (enhances postprandial glucose level by inhibiting the activity of α–glucosiase and α–amylase. It also improves insulin resistance, and enhance glucose uptake by activating the PI3K/ Akt signalling pathway, insulin sensitivity by activating the PPARy/ Akt pathway and restoring the plasma translocation of GLUT2, insulin secretion by the KATP channel-dependent pathway in INS-1 pancreas β–cell.

As the medicinal plants contains the compounds such as flavonoids, phenolic acids, tannins, etc. which have potential antioxidant activity as they have the ability to scavenge free radicals, they have protective effect of the body organs. Thus the use of medicinal plants will bring the level of the blood metabolites, diagnostic enzymes and the *in vivo* antioxidant system of the diabetic patients to the normal level or near to normal level. It will also increase the expression of the genes responsible for glucose transport across the plasma membrane.

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Semen additives: Current updates for quality enhancement in frozen semen production of bulls

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Artificial insemination (AI) contributes to the livestock genetic improvement through rapid dissemination of superior germplasm. Thus, it is considered to be the most important reproductive tool for meeting the demand of improved germplasm and help in faster propagation of elite germplasm.In India, AI was introduced in 1960s in dairy farming enterprise. Currently, the coverage of AI is about 32% of the breedable population with much disparity ranging from 1% in Arunachal Pradesh to 100% in Kerala (BAHS 2022). In comparison, AI coverage in the developed countries is more than 95% since 2000s (Thibier and Wagner 2002). About 779.24 lakh inseminations are performed at 99,410 AI centers in India (BAHS 2022).In buffalo bulls, nearly one third population exhibits poor libido which is an impediment in semen collection. About 50% bulls fail to produce semen of freezable quality. During cryopreservation of bovine semen, the spermatozoa are exposed to variable critical temperature resulting into plasma membrane damage, leakage of vital enzymes and thus there is low survival rate, poor post-thaw thermal resistance and poor fertilizing ability of spermatozoa (Andrabi *et al.,* 2008). The analysis of structural integrity and functional activity of the spermatozoa is very important for sperm metabolism. Attempts should be made to develop optimal semen freezing protocols to address issues related to cryo-injury and capacitation. The number and quality of spermatozoa produced by testis is determined by interaction of two strategies viz. pre-production (before ejaculation) optimization of semen quality through dietary supplementation of nutraceuticals and post-production (after ejaculation) nurturing of spermatozoa which involves *in vitro* treatment of semen with additives (Alvarez 2003). Many studies have demonstrated that addition of additives to extender has beneficial effect on sperm motility and viability in a variety of species including bovine (Bilodeau *et al.,* 2002), equine (Baumber *et al.,* 2002) and ovine (Maia *et al.,* 2007) during cryopreservation. Further, addition of certain additives in the extenders has been found to have beneficial influences to disperse the egg yolk during freezing and prevent sperm membrane from injuries (Bhakat *et al.,* 2011). However, improvement of bovine semen cryopreservation requires a better understanding of the properties of the properties of currently used extenders. Efforts to improve the preservation of bull semen are focused on the modification of extenders (Marti *et al.,* 2003), as well as on the addition of various components to maintain motility, fertilizing capacity and preserve sperm membrane integrity (Riha *et al.,* 2006).The current paper summarizes the use of additives for improvement in frozen semen production of bulls.

Cryoprotectants: The general properties of cryoprotective compounds are that they have low molecular weight, are non-toxic and can permeate cells. These agents achieve their protective effects by increasing the unfrozen fraction at a given temperature and thereby reducing the ionic composition. Most cryoprotective additives (glycerol, propanediol, ethanediol, dimethlysulphoxide, equex, orvus, sucrose, glucose, polyethylene glycol) act in the same manner, have a very similar effect, however, the protective efficiency of these compounds may vary from cell-type to cell-type. Pena *et al.,*. (2003) and Tsutsui *et al.,*. (2000) observed a high rate of post-thaw sperm motility and viability after pre-freezing treatment of semen with Equex Paste and Orvus Paste, respectively.

Enzymatic antioxidants: The enzymes prevent loss of sperm ATP in the extender and improve the antioxidant properties of the extender which is mediated through increased activity of superoxide dismutases and decrease production of malondialdehyde (Bilodeau *et al.,* 2002). They further reported

enhanced sperm motility after addition of 5 U/ml of bovine catalase and 5 mM pyruvate in egg-yolk-tris-glycerol extender.

Calcium: The influx of calcium regulates motility and triggers multiple physiological events in spermatozoa such as hyperactivation, chemotaxis, capacitation and acrosome reaction (Mannowetz *et al.,* 2013). The higher level of seminal calcium facilitates the influx of Ca+2 from extracellular to intracellular environment of sperm which is required to initiate the capacitation and acrosomal reaction and ultimately for gaining motility and sperm-egg interaction (Beigi *et al.,* 2019). Higher intracellular calcium indicates that there could be higher number of calmodulin (Intracellular calcium receptor), Na/K ATPase, inositol and 1, 4, 5 triphosphatase receptor (Intracellular calcium store receptor; Costa *et al.,* 2010). Calmodulin also modulates the protein secretion and upon binding to calcium, it activates different enzymes especially protein kinases, phosphatases and phosphodiesterases (Darszon *et al.,* 2011). Protein kinases are responsible for the phosphorylation of the sperm proteins due to that the spermatozoa gain its motility (Beigi *et al.,* 2019). Experiments conducted on bull semen have shown that addition of calcium in the semen drives hyperactivation of spermatozoa (Mitra and Shivaji 2005).

Manganese: Improvement in sperm quality upon addition of antioxidants to semen indicates indirect evidences for the damaging effects of ROS in sperm function. Radical scavenging activity of manganese related to the rapid quenching of peroxyl radicals has been demonstrated in the biological system including induction of increase in the iron level, which provides direct evidence for Fe-mediated lipid peroxidation in the rat's brain that play an important role in the mechanism of Mn induced neurotoxicology (*Chen et al., 2006*). Supplementation of manganese greatly improved percent post-thaw sperm motility as well as percent viability through a signal transduction pathway (Garbers and Kopf 1980). Tash and Means (1983) suggested that manganese supplementation to sperm cells stimulated the adenylate cyclase activity; which in turn enhanced the level of cAMP. This increase in cAMP through a cascade of events phosphorylated the axonemal proteins which are involved in the sperm moments.Addition of manganese (150 µM) to EYC-G dilutor improved the quality and subsequent fertility of semen (Cheema *et al.,* 2009). Further,supplementation of manganese exhibited positive effects on post-thaw semen quality in buffaloes (Haq *et al.,* 2015).

Ascorbic acid: Vitamin C is a non-enzymatic, water-soluble antioxidant. It is an effective reactive oxygen species with high vitality. Vitamin C as an antioxidant can affect the performance of sperm by reducing sperm cell damage through its radical forager activity. It is important to improve sperm motility and viability or to provide a protective effect against DNA sperm damage (Amini *et al.,* 2015). It has been found to restore fertility by reducing the anti-agglutination factors in membrane from active to inactive form. The mechanism of this process may be through the reaction of vitamin-C with superoxide radicals and hydrogen peroxide, reduction of oxygen radicals, participation in carbohydrate metabolism and maintenance of electron transport chain, neutralization of reactive oxygen species and restoration of superoxide dismutase activity, thus aiding sperm motility (Bansal and Bilaspuri 2011). Singh *et al.,.* (1995) observed that cryopreservation of Murrah buffalo bulls in tris-yolk-glycerol extender with 2.5mM ascorbic acid resulted in significant increase in sperm motility and viability along with decrease in lipid peroxide formation. They further reported that inclusion of ascorbic acid @ 25mM in the diluent resulted in significantly higher post-thaw sperm motility and percentage of live spermatozoa. Further, Hashim *et al.,.* (2017) in breeding bulls also noticed that addition of vitamin C in the semen extender improved the frozen-thawed quality of spermatozoa.

Vitamin-E and α-tocopherol: Moreover, vitamin E is the principal constituent of antioxidant defense system of spermatozoal membrane owing to beneficial effects on semen quality (Yousef *et al.,* 2003; Keskes-Ammar *et al.,* 2003). Vitamin-E protects sperm membrane against oxidative damage and inhibits free radicle induced damage to sensitive cell membrane (Sinclair 2000). It appears to be the first line of defense against the peroxidation of poly unsaturated fatty acids contained in the cellular and subcellular membrane phospholipids (Horton *et al.,* 2002). Likewise, α-tocopherol is a powerful antioxidant because of its ability to break the lipo-peroxidative chain reaction through its interaction with lipid peroxyl and alkoxyl radicals (Donnelly *et al.,* 1999). Addition of vitamin E (1 mg/ml) to cryodiluent showed post-thaw (0 h) increase in sperm motility (60.0±0.0 vs 54.0±2.2%) and acrosome integrity (86.5±5.1 vs 80.2±0.8%; Andrabi *et al.,* 2008). Bansal and Bilaspuri (2009) also reported

that compared to control (3.23±0.36 nmoles/µg protein) vitamin E at all doses (1 mM, 2 mM, 2.5 mM) was effective in reducing the level of MDA (2.94±0.38, 2.77±0.35 and 2.59±0.39 nmoles/µg protein, respectively) in cattle spermatozoa. Addition of vitamin-E (1 mg/ml) to extender prior to cryopreservation of buffalo bull semen protected sperm membrane against oxidative damage and improved the fertilizing potential of spermatozoa (Kumar *et al.,* 2018).

Linolenic acid: Linolenic acid is a long chain omega-3 fatty acid having a molecular mass of 278.436 along with strong polar groups such as oxygen, hydroxyl and carboxyl groups and is the main antioxidant of biological membranes (Kiernan *et al.,* 2013).Linolenic acid present in the plasma membrane provide energy, modulate the structure and composition of lipid rafts, regulate plasma membrane proteins, maintain and normalize plasma membrane function, sustain sperm viability and fertility during chilling and freezing (Shevchenoko and Simons 2010). Preliminary studies have shown evidences of successful use of linolenic acid in cryopreservation of bovine semen (Abavisani *et al.,* 2013). Supplementing 5.0 ng/ml linolenic acid in extender improved the post-thaw quality and *in vivo* fertility of cryopreserved Murrah buffalo bull semen (Singh *et al.,* 2019). In bulls, after freeze motility (63.87±2.1 vs 74.75±1.9%) improved statistically significantly on addition of linolenic acid (5.0 ng/ml) to freezing media (Kaka *et al.,* 2015b). Similar studies (Kaka *et al.,* 2015a) in Brangus-Simmental Cross-breed bulls exhibited significantly higher ($P<0.05$) percentage of motility in linolenic acid treated (5.0 ng/ml) sperm (47.73±0.7%) as compared to their control counterparts (34.53±3.0%).

Cholesterol and cyclodextrins: Apoptosis is a reversible process initiated by different mechanisms including TNF, Fas, caspase 3 and caspase-independent pathways (Balboula *et al.,* 2010; Kim *et al.,* 2010). During vitrificatiAMAon, phosphatidylserine is translocated to the outer leaflet of the plasma membrane (Isachenko *et al.,* 2015). This translocation is likely caused by the disruption of membrane phospholipids asymmetry, which is the key step in apoptosis (Nagata 1997; Glander and Schaller 1999). A membrane-nanodomain-enriched 1-stearoy-2-oleoyl phosphatidylserine can bind cholesterol (Pike *et al.,* 2005), an indispensible component of the plasma membrane that maintains the membrane structural integrity (Maekawa and Fairn 2015). Although cholesterol supplementation improved the quality of spermatozoa in different species (Lee *et al.,* 2015; Moraes *et al.,* 2015), the effect of cholesterol supplementation on cryopreservation-induced DNA damage in mammalian spermatozoa remains unclear.Loss of cholesterol from the plasma membrane during capacitation results in altered concentration of intracellular ions thereby affecting acrosome reaction (Parrish *et al.,* 1999). Incubating and preloading cylcodextrins and cholesterol with bull sperm before cryopreservation leads to recovery of higher percentage of motile and viable sperms after freezing and thawing (Purdy and Graham 2004a). Therefore, cholesterol can be added beneficially to the medium to enhance the cryosurvival in bovine sperms (Purdy and Graham 2004b).

Albumin: Bovine serum albumin (BSA), the most abundant circulating protein in the plasma, has important antioxidant activities through its multiple-binding sites and free radical-trapping properties (Roche *et al.,* 2008). BSA has been shown to improve post thaw quality of bovine (Ashrafi *et al.,* 2013), ovine (Uysal and Bucak, 2007) and caprine (Anghel *et al.,* 2011) semen. Kopf *et al.,* (1999) reported that serum albumin acted as a sink for the removal of cholesterol form the sperm plasma membrane. Addition of serum albumin usually bovine serum albumin removes sperm membrane cholesterol, thereby destabilizing the membrane leading to acrosome reaction of the cryopreserved spermatozoa (Guraya 1999).Rahman *et al.,*. (2015) demonstrated that 0.5% BSA seemed to be beneficial for inclusion inbuffalo semen extender. Further, the protective role of BSA in cryopreservation of buffalo semen has alos been documented by Akhter *et al.,*. (2014).

Cysteine: Cysteine is an analogue of the semi-essential amino acid N-acetyl-cysteine and is a potent antioxidant that plays an important role in improving the production and maintenance of intracellular glutathione and scavenging of free radicals and ROS produced by the metabolism of sperm cells (Tuncer *et al.,* 2010). Cysteine is found in seminal plasma and sperm nucleic acid and during cryopreservation it maintains plasma membrane function, regulates plasma membrane proteins along with energy production (Yin *et al.,* 2016). The impact of cysteine supplementation to freezing media on the quality of frozen-thawed semen in different species is sufficiently comparable. In boars, addition of cysteine in liquid semen increased the vitality of spermatozoa (Kaeoket *et al.,* 2010).

Anghel *et al.,*. (2010) noticed that cysteine supplementation in freezing media resulted in increased motility, acrosome integrity and fertility. *In-vitro* addition of exogenous cysteine to extended semen exhibited greater sperm motility, mitochondrial membrane potential and sperm-zona pellucida binding capability than the control in rabbits (Zhu *et al.,* 2017).

Glutathione: Glutathione, a naturally occurring hydrophilic amino acid having molecular mass 307.33 g/mol is obtained from plants that helps in maintaining plasma membrane integrity along with beneficial antioxidant andhas emerged as a potent additive in improving the quality of post-thaw semen in different species (Bilodeau *et al.,* 2000). Therefore, it is necessary to balance the redox potential in order to maintain the optimal functioning of sperm cells (Buege *et al.,* 2002). Accordingly, glutathione present in plasma membrane provides protection mechanism through maintenance of plasma membrane function, regulation of plasma membrane proteins and energy production during cryopreservation (Baumber *et al.,* 2005).Addition of glutathione in extended semen of Holstein bulls improved post-thaw motility and acrosome integrity (Munsi *et al.,* 2007). Further, Ansari *et al.,*. (2014) also recorded similar observations in Sahiwal bulls that addition of glutathione in Tris yolk diluent improved individual motility. In Labrador,sperm motility was significantly higher in Tris egg yolk extender supplemented with glutathione than in control (untreated; (Cheema and Kaur 2021). Limited studies in buffalo bulls also demonstrated that addition of glutathione in Tris yolk diluent improved individual post-thaw motility of spermatozoa (Wadhwa *et al.,* 2024).

Bicarbonate ion: This is a first messenger in the process of capacitation. The trans-membrane movement of bicarbonate anion could be responsible for an increase in intracellular pH which is observed during capacitation. Supplementation of bicarbonate ion had a positive effect on percent motility, straight-line velocity and curvilinear velocity of the post-thaw spermatozoa (Chaveiro *et al.,* 2006).

Pentoxyfylline (PTX): It is a methylxanthine phosphodiesterase inhibitor which reduces superoxide anions responsible for DNA apoptosis (Maxwell *et al.,* 2002). The use of PTX or related compounds for stimulation better seminal characteristics in cryopreserved spermatozoa viz., motility and curvilinear velocity, capacitation and acrosome reaction has also been demonstrated earlier (Aitken *et al.,* 1997). Similarly, in another study, addition of PTX increased the post-thaw motility as well as sperm fertilizing ability in human semen (Esteves *et al.,* 2007).

Butylated hydroxy toluene (BHT): It is an organic soluble molecule which serves as a scavenger of oxygen free radicals, modifies the properties of the lipid bilayer, minimizes damage to the sperm motility and sperm cell membrane and sustains sperm viability during freezing and thawing (Hammerstedt *et al.,* 1978). BHT readily incorporates into sperm membranes and prevents damage after exposure to cold. Use of spin labels and electron spin resonance techniques suggests that BHT acts on membranes to increase fluidity and to render them less susceptible to cold shock (Anderson *et al.,* 1994). Addition of BHT during liquid storage improved sperm cell function (Bhakat *et al.,* 2011).

Sericin: Sericin a water-soluble globular protein derived from silkworm (Bombyx mori), represents a family of proteins whose molecular mass ranges from 10 to 310 kDa (Wei *et al.,* 2005). It is rich in aspartic acid as well as serine, which have a high content of the hydroxyl group. Sericin plays an important role in suppressing lipid peroxidation, preventing cell death, protection from cryopreservation and various types of stress (Sasaki *et al.,* 2005). Supplementation of 0.25-0.5% sericin in semen extender improved frozen-thawed semen quality through protecting sperm from oxidative stress (Demra *et al.,* 2016). Similar observations were noticed by Kumar *et al.,*. (2015) that addition of sericin improves semen freezability of buffalo bulls by minimizing oxidative stress during cryopreservation.

Sugars: They are a potent source of energy for the spermatozoa; maintain osmotic pressure and electrolyte balance, decrease the equilibration period of freezing and used as a substrate for metabolism. Due to disparity in size, the monosaccharide sugars (fructose, mannose and glucose) than disaccharides (lactose and sucrose) and polysaccharides (raffinose) can penetrate the sperm cell membrane and provide cryoprotection to sperms during freezing. The non-permeable sugars act as cryoprotective in a way similar to glycerol by acting as extracellular dehydrates. Rigau *et al.,*. (2001) found that the protective effect of sugars depend upon the type of diluent used for freezing. They

concluded that maximum protection was provided by a combination of fructose with sucrose and raffinose in Tris extender and xylose in sodium citrate extender.

Kisspeptin: Kisspeptin is a neurohormone encoded by Kiss1 gene and shows its functional activity through G-protein-coupled receptor-54 (Gottsch*et al.,* 2009). A direct effect of kisspeptin at the testicular level has been observed in non-human primates (Tariq and Shabab 2017). Kisspeptin and its receptors are present in the midpiece of spermatozoa that play a vital role in providing energy requirements and Ca^{2+} buffering and subsequently enhancing sperm motility (Pinto *et al.,* 2012). Khan *et al.,.* (2021) observed that adding kisspeptin (40 µM) in extender increased (P<0.05) post-thaw buffalo sperm motility (94.32±1.32% at 40 µM vs 89.27±1.27% in control). Recently, Fayyaz *et al.,.* (2022) also reported that supplementation of kisspeptin in Nili-Ravi semen at 15 µM ameliorated (P<0.05) overall frozen-thawed progressive motility (76.40±3.05 % at 15 µM vs 60.53±3.33 % in control), rapid velocity (79.60±1.60% at 15 µM vs 67.83±0.58% in control group) and other CASA-based kinematics.

Melatonin: In the recent past, melatonin emerged as a potent molecule, owing to beneficial effects on semen quality (Yadav *et al.,* 2023). Melatonin, a N-acetyl-5-methyl tryptamine is an indole derivative that is rhythmically released from pineal gland and plays role in controlling the circadian rhythm of mammals in general and their reproductive processes in particular (Kennaway and Wright 2002). In addition, melatonin possesses antioxidant properties that help in avoiding oxidative stress by scavenging free radicals (Deng *et al.,* 2017). Yadav *et al.,.* (2023) reported that adding melatonin in freezing media improved progressive sperm motility (48.0±0.9% at 2.0 mM vs 42.0±1.3% in control) and live percentage (73.6±0.7 at 2.0 mM vs 61.7±0.8% in control) in Hariana bull semen. Supplementation ofmelatonin in semen at 1.0 mM improved progressive motility (35.87±1.54%) compared with control (29.18±1.72%; Chandra *et al.,* 2020).Alternatively, Chaithra *et al.,.* (2019) observed that incorporation of 0.25 mM melatonin in extender failed to have any effect on post thaw sperm characteristics in Holstein Friesian bulls.

When semen is treated withadditives there is more beneficial effect in terms of semen quality and freezability.Increasing demand for artificial insemination and the aim of sperm banking to preserve more semen with better fertilitycan be achieved by using this strategy.

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Mercury-induced sperm damage: A multifaceted analysis of mechanisms and effects

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Abstract

Mercury exposure is a growing concern in livestock production, affecting reproductive health and fertility. The topic providescomprehensive analysis of the mechanistic, signaling, and kinematic effects of mercury on livestock sperm.Mercury-induced oxidative stress, DNA damage, and mitochondrial dysfunction disrupt normal sperm function, leading to impaired motility, morphology, capacitation, and fertilization ability. Altered calcium, cAMP, and MAPK signaling pathways contribute to these effects. Kinematic analysis reveals decreased sperm velocity, linearity, and beat frequency. Understanding these mechanisms provides valuable insights of mercury-induced reproductive toxicity in livestock. This knowledge can inform strategies to mitigate the effects of mercury exposure on farm animal fertility and productivity.

Introduction:

Spermatozoa must be motile and functionally capable of fertilizing the egg. Environmental toxicants can significantly affect the structure, function, and viability of spermatozoa in livestock, leading to reduced fertility and reproductive performance. These toxicants, which include heavy metals, pesticides, industrial chemicals, and endocrine-disrupting compounds, can harm spermatozoa through direct and indirect mechanisms. Understanding and mitigating the effects of environmental toxicants is crucial for maintaining the reproductive health and productivity of livestock populations.Mercury is a toxic and bio-accumulative heavy metal of global concern. Mercury is extensively utilized in scientific instruments, electrical equipment, and various industrial applications. Its applications include thermometers, barometers, batteries, electrical switches and relays, mercury lamps, solders, semiconductor solar cells, catalysts, preservatives, germicides, electroplating processes, and pharmaceutical production (Clarkson *et al.,* 2006; UNEP 2008; Chan *et al.,* 2011). Mercury is distributed throughout the environment from both natural sources (inorganic form) and human activities (organic form) and thus accumulates in the biosphere. Mercury accumulates in the ovaries, testes, and prostate glands (Creasy *et al.,* 2001). The reproductive toxicity of mercury has been documented in several animal studies, where exposure of mercury led to decreased sperm motility, lower epididymal sperm counts, and reduced normal sperm morphology in rats, mice, and monkeys (Lee *et al.,* 1975; Mohamed *et al.,* 1987; Orisakwe*et al.,* 2001).Below is a detailed breakdown of its effects, focusing on mechanistic understanding, signaling pathways, and alterations in functional dynamics of spermatozoa:

Mechanistic Understanding:

Oxidative stress (OS) has been known as one of the numerous mediators of male sterility through spermatozoon dysfunction. Oxidative stress is a state associated with increased cellular injury triggered by oxygen and oxygen-derived free radicals called reactive oxygen species (ROS). Throughout this process, increased production of ROS overwhelms the body's antioxidant defense. Although tiny amounts of ROS are required for normal sperm functioning but disproportionate levels negatively influence the quality of spermatozoa and impair their overall fertilizing capability and capacity. Oxidative stress has been an area of great attention because ROS and their metabolites attack DNA, lipids, and proteins; alter enzymatic systems, produce irreparable alterations, cause cell death; and ultimately deteriorate semen quality and affect semen attributes which are related to male infertility (Agarwal*et al.,* 2014). Mercury has also been shown to induce oxidative stress,

mitochondrial dysfunction (Gruenwedel*et al.,* 1979; Lund *et al.,* 1993) which can result in alterations in calcium homeostasis and increased lipid peroxidation.

In-vitro treatment of human spermatozoon with mercury concentrations from 10.0 to 160.4 mg/L induced membrane lipid peroxidation and DNA breaks, lowered spermatozoon viability, and decreased the rate of acrosome reaction resulting in spermatozoon dysfunction (Arabi and Heydarnejad, 2007). Hayati *et al.,*. (2019) also reported mercury-induced increase in DNA fragmentation in fish sperms after in-vitro exposure to different concentrations of $HgCl_2$. $HgCl_2$ exposure was correlated with enhanced oxidative stress in reproductive organs, represented not only by augmented lipid peroxidation but also by changes in antioxidant enzymes (SOD and CAT) activity and nonprotein thiol levels (Martinez *et al.,* 2014).The connection between the levels of pituitary hormone and chronic mercury exposure has also been reported as inorganic mercury was found to bind to the luteinizing hormone and impair gonadotropin regulation affecting fertility and reproductive function (Mocevic*et al.,* 2012).At the cellular level, mercury exposure is associated with alterations in membrane permeability, changes in macromolecular structure due to its affinity for sulfhydryl and thiol groups, and DNA damage (Naganuma *et al.,* 2002; Flora *et al.,* 2008).

Signaling Pathways:

Ion channels control the sperm ability to fertilize the egg by regulating sperm maturation in the female reproductive tract and by triggering key sperm physiological responses required for successful fertilization such as hyperactivated motility, chemotaxis, and the acrosome reaction. CatSper, a pH-regulated, calcium-selective ion channel, and KSper (Slo3) are core regulators of sperm tail calcium entry and sperm hyperactivated motility (Lishko*et al.,* 2012). In mammalian sperm, different Aquaporins (AQPs), including AQP3, AQP7, and AQP11, have been identified; their main roles are related to osmoadaptation and sperm motility activation after ejaculation Mercury chloride, an unspecific inhibitor of all AQPs except AQP7 produced an increase in membrane lipid disorder and led to a decrease in sperm motility and kinetics parameters (Delgado-Bermúdez *et al.,* 2021).

cAMP and Ca^{2+} are the important second messengers that play an important role as signaling molecules in modulating mammalian sperm functions (Buffone *et al.,* 2014). Studies have shown that heavy metals like lead and mercury can disrupt calcium signaling in sperm cells. This disruption affects processes such as the acrosome reaction, which is essential for fertilization. Altered calcium homeostasis can lead to compromised sperm function and reduced fertilization potential (Yadav *et al.,* 2024).

Kushawaha*et al.,*. (2020) investigated the effects of mercuric chloride on buck spermatozoa and demonstrated that even low concentrations of mercuric chloride (0.031 µg/mL) significantly inhibited the tyrosine phosphorylation of sperm proteins. This inhibition was associated with decreased sperm motility, reduced membrane integrity, and impaired acrosome reaction, all of which are critical for successful fertilization. Mercury seems to increase levels of cAMP and intracellular calcium without tyrosine phosphorylation, and these alterations result in spontaneous acrosome reaction.

In bovines, a reduction in MAPK signaling is correlated with fertility in bulls. It has been reported that genes associated with oxidative phosphorylation of proteins involve in the MAPK signaling of sperm function are significantly down regulated in low fertile bulls (Paul *et al.,* 2021).

Sperm functional dynamics insights:

Metals accumulation in epididymis, prostate, and seminal fluid may impair progressive sperm motility (Ernst *et al.,* 1991; Hess *et al.,* 1998) and thus reproductive efficiency. Sperm kinematics are often evaluated through computer-assisted semen analysis (CASA) in domestic animals. By using CASA, it is possible to find out the toxic effects of pollutants on spermatozoa including changes in sperm velocity and trajectory of sperm movement.

Mercury affects the different motility parameters (rapid, slow and non-progressive) as well as kinematic motion parameters (VCL, VSL, VAP and LIN) of buck spermatozoa following in vitro exposure (Kushwaha *et al.,* 2021). In-vitro incubation of Holstein bull spermatozoa with $HgCl_2$ resulted in reduction in sperm motility, viability, increase in percent of abnormal sperm, and loss of

sperm membrane integrity indicating the negative effects of mercury on sperm function (Arabi *et al.,* 2006). In-vitro studies have shown that mercury induces DNA breaks in spermatozoa and leads to decreased sperm motility, dysfunction and viability (Mohamed *et al.,* 1986a; Ernst *et al.,* 1991; Rao *et al.,* 1989).

Research on goat spermatozoa revealed that mercury exposure caused the collapse of mitochondrial cristae, leading to reduced ATP production and increased oxidative stress. This mitochondrial damage resulted in decreased sperm motility and viability, highlighting the critical role of intact mitochondrial function in maintaining sperm health. Mercury seems to primarily target sperm mitochondria and plasma membrane and thereby results in spontaneous cell death independent to apoptosis (Kushwaha *et al.,* 2021).

Mercury has a high affinity for binding to the sulfhydryl groups (–SH) of tubulins, which are the main components of sperm axonemal microtubules. This disruption affects the interactions between axonemal microtubular proteins and dynein motors, which are essential for sperm flagellar motility (Mohamed*et al.,* 1986 a, 1986 b; Badr and Ola., 2018). Scanning and transmission electron microscopy images (SEM and TEM) of mercury treated spermatozoa showed loss of plasma membrane integrity, acrosomal membrane damage, damaged outer dense mitochondrial sheath along with collapsed cristae. Defects in mitochondria seem to be responsible for reduced mitochondrial transmembrane potential (MTP) and motility of the buck spermatozoa (Kushwaha *et al.,* 2021).

Conclusions

Mercury, a heavy metal known for its environmental and industrial prevalence,its exposure has devastating effects on sperm, leading to impaired motility, morphology, capacitation, and fertilization ability. Understanding the mechanistic, signaling, and kinematic insights into mercury's impact on sperm can paves the way for targeted interventions, such as antioxidants and chelation therapies, to mitigate mercury-induced reproductive toxicity.

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Homoeopathy in veterinary science: A holistic path to sustainable animal health and production

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Abstract

Homoeopathy has emerged as a promising approach in veterinary science, offering a natural, safe, and holistic method for managing animal health. Homoeopathy addresses a wide range of conditions—from arthritis and allergies to respiratory infections and behavioral challenges like anxiety.

In the realm of livestock farming, homoeopathy is increasingly recognized for its role in promoting sustainable and organic practices. By minimizing dependence on antibiotics and chemical treatments, it helps address the global issue of antimicrobial resistance and ensures residue-free animal products, a critical factor in meeting the rising consumer demand for organic and chemical-free food. Remedies such as *Arnica montana* for injuries and trauma, *Nux vomica* for digestive issues, *Belladonna* for fevers and inflammation, and *Silicea* for wound healing are widely used and valued for their efficacy and safety. These remedies work not only to treat existing conditions but also to enhance the overall immunity and vitality of animals, preventing the onset of diseases.

In an article published on 'Evidence-Based Human Homoeopathy and Veterinary Homoeopathy. Comment on Bergh *et al.,*. A systematic Review of Complementary and Alternative Veterinary' by P Weiermayer, M frass and 2 others, For veterinary homeopathy, the review of 2014 and the meta-analysis of 2015 showed evidence of effectiveness veterinary homoeopathy compared to placebo (p=0.01 for n= 15, pooled OR= 1.69) (CI=1.12-2.26), p=0.02 forn=2, pooled OR=2.62(CI=1.13–6.05).In addition to studies to demonstrate the effectiveness of homeopathy in infections, data from health care research, so-called 'real world data', show the potential for a significant reduction in the use of antibiotics through homeopathic treatments.[i]

Anarticle published by R.T. Mathie *et al.,* titled 'Out comes from Homeopathic Prescribing in Veterinary Practice: A Prospective, Research-Targeted Pilot Study,' reported that 8 practitioners submitted data for 767 individual patients (547dogs, 155cats, 50horses, 5 rabbits, 4 guinea-pigs, 2 birds, 2 goats, 1 cow, and 1 tortoise). Positive outcomes from two or more homeopathic appointments were observed in 539 cases (79.8% showed improvement, 6.1%experienced deterioration, 11.7% showed no change; outcomes were not recorded in 2.4%of follow-ups). Strongly positive results (scores of +2 or +3) were most notable in conditions such as arthritis and epilepsy in dogs, and, to a lesser extent, in atopic dermatitis, gingivitis, and hyperthyroidism in cats[ii].

The rising use of antibiotics in livestock threatens human, animal, and environmental health. Homeopathy offers apotential alternative. In an article published by I. Camerlink and 3 others titled: Homeopathy as replacement to antibiotics in the case of *Escherichia coli* diarrhea in neonatal piglets, 52 sows in their last month of gestation were treated with either the homeopathic agent Coli 30K or a placebo. Piglets from the homeopathic group had significantly less *E. coli* diarrhea compared to the placebo group (P<.0001). Piglets from first-parity sows responded especially well, with shorter duration and less severe symptoms.[iii]

Dr. Petra Weiermayer's case report demonstrated successful management of a 4-year- old horse with antimicrobial-resistant wound healing using homeopathy. These studies underscore homeopathy's role in sustainable and ethical veterinary care.[iv]

Homoeopathy's cost-effectiveness is another significant advantage, especially in large- scale animal farming, where the economic burden of conventional veterinary treatments can be substantial. Its application ranges from improving milk production and managing

mastitisindairyanimalstocontrollinginfectiousdiseaseslikefoot-and-mouth disease in livestock. Additionally, it helps mitigate the stress associated with transportation, environmental changes, and other challenging conditions faced by animals.

The Central Council for Research in Homoeopathy (CCRH) is at the forefront of evidence-based research, knowledge dissemination, and the integration of homoeopathy into veterinary practices. Currently, CCRH has two active studies at DUVASU, Mathura: “Clinical Evaluation of Certain Homoeopathic Medicines Against Mites-Induced Dermatitis in Dogs” and “Management of Otitis Externa in Dogs with Homoeopathic Intervention vs. Standard Treatment-Single Blind, Double Arm, Randomized Control Trial.” Additionally, in collaboration with IVRI, Izzatnagar, Bareilly, CCRH is exploring the “Potential of Homoeopathic Drugs for Treating Neonatal Calf Diarrhoea.” These initiatives demonstrate CCRH’s commitment to advancing veterinary care through innovative homoeopathic research and practice.

With continued research and awareness, homoeopathy has the potential to revolutionize veterinary care, offering a gentle yet powerful alternative that aligns with the principles of sustainable and ethical animal care, contributing to a healthier planet and a global shift towards holistic healthcare systems.

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TECHNICAL SESSION

Development in Alternative Medicine, Pharmaceutical and Nutraceutical Research for Animal Health and Production (AMAH)

ORAL PRESENTATIONS

AMAH-OF-1

Immunomodulant and galactagogue properties of *Ashwagandha*

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Withania somnifera (Ashwagandha) plant was studied for galactagoge and immunomodulant properties. The plant was procured from authentic sources for further exploration from Western and eastern Himalayas. Herbarium specimen was prepared for identification /verification and was submitted to Department of Botany, University of Jammu for identification. *Withania somnifera* was assigned accession no. *HB*JU-17003. Further ethanolic, methanolic and aqueous extracts were prepared for further processing and analysis. The percentage yield of driedethanolic extract was found to be 13.86%,14.12% for methanolic extract and 11.12% foraqueous extract. Total antioxidant activity was also studied. Aqueous extract of *Withania somnifera* showed highest percentage of antioxidant activity (51.34%).Total phenolic content in aqueous extracts were relatively high (6.90 µg GAE/g) when compared with methanol and ethanol extracts. The plants extract in different concentrations were subjected for study on mammary epithelial cells to understand the mechanism of action as galactagogue and immunomodulatory effect using various gene markers for milk production, milk composition and immunomodulation. They were subjected for total RNA isolation for further cDNA synthesis and real time PCR for determination of the quantification of gene of interest. Gene expressions were reported for various candidate genes responsible for milk synthesis and milk composition such as DGAT1, ABCG2, CSN3, GH1, IGF1 etc. and immune related genes such as IL-1, IL-6, TNF-alpha etc.

AMAH-OF-2

Effects of supplementation of *Rubiotic*, a prebiotic fraction of rumen liquor on blood antioxidant profile of broiler chickens challenged with *Escherichia coli*

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Prebiotics augment the growth of beneficial bacteria, while concomitantly reducing pathogen load in intestinal lumen. They are known to reduce systemic intensity and scale of diseases in poultry. This effect is mediated by attenuating pathophysiological effects through various mechanisms including potentiation of anti-oxidant capabilities of the host bird. The author's laboratory isolated and patented an ethanolic fraction of rumen liquor named '*Rubiotic*' that demonstrated significant prebiotic activity *in vivo* and *in vitro*. Avian colibacillosis is an important bacterial infectious disease of poultry caused by *Escherichia coli*. Present study evaluated the efficacy of *Rubiotic* as a prebiotic over blood antioxidant profile of *E. coli* challenged broiler chickens. One hundred and eight unsexed, day-old broiler chicks were divided into three treatment groups, namely CO; EC, and RL (diet supplemented with 0.1% rubiotic). Birds of EC and RL groups were orally challenged on 7 days age with *E. coli* at 1.5 x 10^8 cfu per bird. Each treatment group consisted of six replicates with six birds in each replicate. One bird per replicate was slaughtered on 14, 21 and 28 days of age to collect blood samples. Levels of antioxidant assay did not varied among different treatment groups at 14 days of age, however, significantly higher level of reduced glutathione (GSH) was recorded at 21 days of age in rubiotic supplemented birds as compared to CO and EC groups. At 28 days of age, significantly

higher lipid peroxidation and significantly lower superoxide dismutase and GSH levels were recorded in EC group as compared to CO and RL groups, whereas no significant effect was observed on catalase activity. The antioxidant assay suggests a probable mechanism of protective action of *rubiotic* supplementation over the damaging effect of *E. coli* infection in broiler birds.

AMAH-OF-3

Modulation of Oxidative Stress and Enhancement of Antioxidant Defenses by AI-AgNP Hydrogel Ointment in Wound Healing

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This study evaluated the wound healing efficacy of AI-AgNPs (*Azadirachta indica* silver nanoparticles) hydrogel ointment, focusing on its known concentration and Minimum Inhibitory Concentration (MIC) against *Methicillin-resistant Staphylococcus aureus* (MRSA) and *Escherichia coli*. Significant antimicrobial effects ($P<0.01$) were observed compared to controls. Topical treatments were applied daily until complete wound healing, with assessments of key biochemical parameters in granulation tissue and clinical cases. Hexuronic acid, hexosamine, and hydroxyproline levels, critical indicators of collagen synthesis and tissue repair, were measured on the 7th day post-treatment. AI-AgNPs significantly elevated hexuronic acid (720.54%), hexosamine (128.43%), and hydroxyproline (142.36%) levels compared to control groups ($P<0.001$), demonstrating enhanced wound healing. Protein concentration and DNA quantitation in granulation tissue were also significantly increased ($P<0.001$), indicating promising tissue repair results. In clinical trials, animals with acute and chronic wounds were treated with AI-AgNP hydrogel ointment until complete healing. Bacterial isolates from wounds were identified using Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS), confirming species-level identification of *Escherichia coli*, *Staphylococcus sciuri*, and *Klebsiella pneumonia*. The ointment accelerated wound contraction and healing across various wound types, including lacerations, ulcers, and penetrating wounds, in species such as cattle, dogs, and cats. Serum C-reactive protein (CRP) and interleukin-6 (IL-6) levels remained low, indicating no significant systemic or local inflammation during the treatment. The findings suggest that AI-AgNP hydrogel ointment enhances collagen production, tissue repair, and wound healing while preventing excessive inflammation, making it a promising therapeutic option for managing both acute and chronic wounds.

AMAH-OF-4

Insilico discovery of potential drug componds against Envelope protein of Zika Virus

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Zika virus (ZIKV) is one of important emerged pathogen of the genus flavivirus causes Guillain-Barr´s syndrome and microcephaly in fetus and newborns, which is major concern across the globe. Envelope (E) protein is responsible for viral entry, represents a major target forneutralizing antibodies and, hence, could serve as potential target fordrug screening.As there are no approved drugs available for treatment of Zika virus till today, this study was carried out with objective of discovery of novel drugs against this virus. The envelope protein (E) of the virus and 1050 phytochemicals was retrieved from protein database and pubchem database. The protein ligand interactions were performed through virtual screening by PyRx and Drug Discovery Studio.Further, *in*

silico ADMET and Density function theory (DFT) studies were performed to findout the final hit compounds. Four compounds such as Catechin (-7.6 kCal/mol), Apegenin-7-O-beta-glucopyranoside (-7.5kCal/mol), Baicalin(-7.4 kCal/ kCal/mol) and Madecassic acid (-7.1 kCal/mol) showed highest binding affinity against E protein. Further, cytotoxicity and antiviral effect of these four compounds were tested in invitro; CatechinandApegenin-7-O-beta-glucopyranoside showed highest antiviral effect against Zika virus. Therefore, this study would provide basic information to develop promising antiviral drugs against Zika virus in nearest future.

AMAH-OF-5

Assessment of the anti-proliferative effects of *Euphorbia hirta* L. extract on the MDA-MB-231 human breast cancer cell line.

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Cancer remains a major challenge for humanity and the second leading cause of death worldwide. The incidence of cancer continues to rise steadily and is projected to increase by more than 75% in developed countries and over 90% in developing nations by 2030. The conventional cancer therapeutic regimens include chemotherapy, radiotherapy, and surgery that are used alone or in combination,having several side effects and cost of treatment is very high. The quest for an alternative therapeutic approach that is effective, non-toxic and cost effective in the prevention and treatment of cancer is of utmost importance. The present research was conducted to evaluate the anti-cancerous potential of *E. hirta*on human breast adenocarcinoma (MDA-MB 231) cell line.The whole plant extract demonstrated significant antioxidant activity against DPPH free radicals, hydroxyl radicals, nitric oxide radicals, and superoxide anion radicals, with EC_{50} values of 29.24±3.21 µg/mL, 72.45±1.46 µg/mL, 85.10±1.76 µg/mLand 77.63±1.85 µg/mL, respectively. Additionally, *E.hirta* extract showed promising cytotoxic effects against the MDA-MB-231 cancer cell line, with an IC_{50} value of 89.75 µg/mL.The *E.hirta* extract significantly inhibited the proliferation and migration of MDA-MB-231 cells while inducing apoptotic cell death in a dose-dependent manner. Cell cycle analysis further indicated that the extract disrupted cell progression by arresting the G1 and S phases in MDA-MB-231 cancer cells. In summary, *E. hirta* extract demonstrates *in vitro* antitumor activity by promoting apoptosis and cell cycle arrest in human breast adenocarcinoma (MDA-MB-231) cells, highlighting its potential as a preventive or therapeutic agent for mammary cancer.

AMAH-OF-6

HADP (Holistic Agriculture Development Programme), a revolution, a challenge, Self-employment and women empowerment

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The Holistic Agriculture Development Programme (HADP) aims to promote sustainable agriculture, self-employment, and women empowerment in Jammu and Kashmir. This comprehensive initiative by the Agricultural Production Department encompasses 29 projects across agriculture and allied sectors, including targeted schemes for the Department of Sheep Husbandry. Key objectives include establishing commercial ewe units and breed-based sheep units with 50% subsidies, creating Common Facilitation Centers for wool, pellet, and mutton value addition, and improving infrastructure in highland pastures.The HADP provides financial and technical support for commercial and breed-based sheep units to boost rural income. Specific projects offer a 50% subsidy

on investments in sheep units, while Common Facilitation Centers are set up to improve product quality and value addition. Infrastructure projects focus on sustaining highland pastures, crucial for sheep rearing.HADP has significantly impacted self-employment rates, with notable participation from women, who are now integral to the sheep husbandry economy in Jammu and Kashmir. Through these initiatives, women gain access to income-generating opportunities, thereby strengthening their economic independence.HADP serves as a model for rural economic growth, effectively addressing challenges in agriculture and livestock development. By fostering self-employment and empowering women, HADP enhances socio-economic development in rural areas. This program underscores the potential of integrated support in transforming agricultural practices and empowering communities, marking substantial progress toward sustainable development in Jammu and Kashmir.

AMAH-OF-7

Effects of dietary incorporation of ethanolic *Curcuma longa* extracts on thenonspecific immunological profiles and resistance against aquaticoomycetes (*Saprolegnia parasitica*) infection of rohu, *Labeo rohita* (Ham.)

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The present study was conducted to elucidate the effect of dietary incorporation of differentlevels of ethanolic *Curcuma longa* (turmeric) extract (ETE) on immune health of *Labeo rohita* (rohu) fingerlings and determining the treatment efficacy of the best immuno-stimulant dose against Saprolegniasis in rohu. Four iso-nitrogenous diets were prepared containing ETE atdifferent inclusion levels viz., @ 0% (control), 0.1% (T1), 1% (T2) and 2% (T3) by mixing the semi-purified ingredients in the required quantity. Fingerlings of rohu (12.34±1.02cm) were fed with these diets for forty-five days and various immune response parameters were measured at the 7th, 15th, 30th and 45th day of sampling. Different immunological parameters like oxygen radical production, serum myelo-peroxidase activity and lysozyme activity showed significant enhancement in fish fed with the 1% ETE diet. However, total globulin content and serum anti-protease activity did not show any significant difference on the 45th day of sampling. The ETE@1% of the diet T2 shown best immuno-stimulating response on the fish health as compared to the other two diet. The rapeutic efficacy studywasconductedbyartificiallychallengingtherohufingerlings with oomycetes *Saprolegnia* spores and feeding the infected fish daily for 15 days with diet T2 @1% bwand relativeper centagesur vival was recorded. The T2 dietenhanced there sistance of rohusubstantially against *Saprolegnia* challenge. Inconclusion,thedietarysupplementationofethanolicturmericextracthadahealthamelioratingeffectinrohu particularly,at1%inclusionlevel.Theresultscollectivelysuggestthepotentialofapplying ETE@1%as immunostimulant havinganti-fungal properties in theaquaculture.

AMAH-OF-8

Antioxidant and antifungal potential of homeopathic medicine: Mechanistic insights using LC-MS/MS, in-silico approach and *in-vivo* studies in systemic candidiasis

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Terminalia chebula (TC) is a widely recognized medicinal plant with significant therapeutic potential. This study investigates the antioxidant and antifungal activities of homeopathic medicine TC against systemic candidiasis, integrating in-vitro, in-vivo, LC-MS/MS, and in-silico approaches. The findings reveal substantial antioxidant, antifungal, and immunomodulatory properties of TC formulations, with the mother tincture and specific potencies (6C and 30C) demonstrating remarkable efficacy in reducing fungal burden, improving hematological parameters, alleviating oxidative stress, and protecting kidney tissues. LC-MS/MS analysis identified a diverse array of bioactive compounds, including hydrolyzable tannins (chebulagic acid, chebulaginic acid, chebulinic acid), phenolic acids (gallic acid, methyl gallate), and flavonoids (quercetin, rutin, isoquercetin), which collectively contribute to its therapeutic efficacy. These compounds reported to exhibit antifungal activity by disrupting fungal cell walls, inhibiting fungal enzymes, and preventing spore formation, while also exerting strong antioxidant effects that reduce oxidative stress and tissue inflammation. Molecular docking studies confirmed key interactions of these phytochemicals with fungal target proteins, such as β-tubulin and sterol 14α-demethylase, elucidating their potential as enzyme inhibitors. This study highlights the multifaceted mechanisms underlying the therapeutic effects of TC formulations and supports further exploration of their clinical applications in systemic infections like candidiasis.

AMAH-OF-9

Black Soldier Fly larval excreta outweighs dried larval meal as feed supplement in cattle and buffalo ration

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Supplementation of animal diets with insect meal (whole insect or larva or fly excreta) is an attractive option due to higher content of proteins, lipids, minerals alongside the economy of production and procurement. To this end, we conducted a pilot study to compare between the nutritive value of black soldier fly (BSF) larval meal with its excreta, both of which were proposed for use as feed supplement in cattle and buffalo rations. Proximate analysis, amino acid and mineral profiling were conducted using standard tools and methods. For a fixed weight of the larvae, the yield of larval excreta was 15 times more than that of dried larval meal indicating better economics of procuring excreta for feed supplementation. The larval excreta contained higher crude protein (CP) (57%), limiting amino acids - lysine (3.8%) and methionine (1.2%), compared to larval meal that had 36% CP, 1.9% lysine and 0.7% methionine. Furthermore, the larval excreta are rich in zinc (143 ppm),

which is an essential element for optimal reproductive performance in cattle. Additionally, the fat content in larval excreta is lower (13%) when compared to dried larval meal (37%), suggesting better suitability for formulating into ruminant ration because high dietary fat interferes with normal rumen metabolism. Collectively, this pilot trial highlights potential of BSF larval excreta over dried larval meal as an alternative feed supplement in dairy and beef rations.

AMAH-OF-10

Effect of probiotics on gut health in Layers

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Inclusion of probiotics in poultry diet has been shown to improve gut health, feed efficiency and dependence on antibiotics. The current study has been conducted to specifically evaluate the histo-pathological effects of feeding probiotics in different segments of the small intestine in commercial layer birds. The trial comprised of 500 birds fed probiotics whereas another 500 birds served as control. Hematoxylin and Eosin staining was used to identify histopathological changes in the tissue sections collected from duodenum, jejenum and ileum. Results showed that feeding probiotics significantly disrupted intestinal epithelium, especially at the apical surface of the villi, suggesting an increase in the turnover rate of intestinal epithelial cells. This may be beneficial as piggybacking the denuded epithelium could eliminate higher bacterial and parasitic infestation. Gut associated lymphoid tissue (GALT) in probiotics fed birds showed notable hyperplasia and increased lymphocyte infiltration into the luminal surface of the intestine. Furthermore, feeding probiotics also caused a significant increase in the length, width, and number of villi in all the three segments of the small intestine. These changes likely increase absorptive surface area for nutrients leading to better feed utilization efficiency as also corroborated by higher feed utilization efficiency and body weight gain in probiotic fed group when compared to the control birds.

AMAH-OS-1

Comparative efficacy of platelet rich plasma and autologous serum in corneal healing in dogs

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The present study was carried out on18 dogs suffering from corneal ulcers to assess the efficacy of autologous PRP and autologous serum in corneal healing. Condition was assessed on the basis of general ophthalmological evaluation and fluorescein staining. On fluorescein dye test the condition was further classified as superficial ulcers, deep ulcers and descemetocele. Initially, all ulcers underwent debridement using a dry, sterile cotton bud after instillation of topical anesthetic eye drop. Based on treatment protocols, dogs were divided into three groups. Group I received medical treatment that included collyria with sterile 0.9% normal saline solution, followed by ophthalmic antibiotic and anti-inflammatory drops. Systemic antibiotic and anti-inflammatory were also given. Group II received subconjunctival injection of autologous PRP at weekly interval along with therapy in group I till healing occurred. Group III received autologous serum (AS) as eye drops @ 1 drop every 3 hours along with treatment protocol of group I. Clinical follow up of cases were done at 3, 5, 7, 10, 14, 21 and 28-days interval. The animals of Group II and III showed a significant faster epithelialization as compared to animals of group I. Thus, autologous PRP as a subconjunctival injection and AS as an eye drop are safe alternative medicine in treating corneal ulcers in clinical

situations. However, PRP therapy exhibited an excellent healing with less healing time over AS in corneal healing. Additionally, less frequent hospital visits and dosing in PRP therapy as compared to AS increased owner compliance in treating corneal ulcers.

AMAH-OS-2

Effect of cow urine distillate fortified with *curcuma amada* extract therapy on subclinical mastitis in cows

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The present study was carried out to evaluate the effect of cow urine distillate fortified *Curcuma amada* extract therapy on subclinical mastitis (SCM) in cows. The diagnostic tools such as California Mastitis Test (CMT) and somatic cell count were utilized for thediagnosing subclinical mastitis. The study was conducted on SCM cows at dairy farms. Thirty cows in the mid-lactation state were randomly selected and divided into five equal-treatment groups viz T0 was control, T1 group was given cow urine distillate(CUD), T2 was given *Curcuma amada* aqueous extract, T3 was given fortified with *Curcuma amada* aqueous extract(@20mg/kg b.w.t), cow urine distillate (@0.15ml/kg b.w.t)diluted in water by orally, for 28 days. Group T4 given standard treatment of Morbofloxacin. The CMT was conducted on total 52 cows out of which 24 animals were found positive for SCM. The SCM prevalence was 46 percent. The study revealed significant alterations in the physical properties of milk samples from subclinical mastitis cows, including increased pH, total protein, albumin, and lactate dehydrogenase enzyme levels, alongside decreased milk fat, density, and viscosity. Hematological parameters also showed significant changes, with increased total white blood cell count, monocytes, and granulocytes, and decreased hemoglobin, packed cell volume, total red blood cell count, and lymphocytes. The anti-mastitic activity of CUD fortified *Curcuma amada* extract was found better than CUD or *C.amada*alone treated groups. However, the group which received standard drug Morbofloxacin showed better anti-mastitic activity in SCM affected cows. The study demonstrates the efficacy of cow urine distillate and *Curcuma amada*extract in treating subclinical mastitis, offering a promising solution to this prevalent issue in the dairy industry.

AMAH-OS-3

Green synthesis of zinc oxide nanoparticles using *Cassia Fistula* leaf extract

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Green synthesis has gained extensive attention as a reliable, sustainable, and ecofriendly protocol for synthesizing a wide range of nano-minerals. In the present work, zinc oxide nanoparticles were synthesized using *Cassia fistula* (*C. fistula*) leaf extract. Ultraviolet-visible spectrophotometer (UV-Vis), dynamic light scattering (DLS), Scanning electron microscopy (SEM), Fourier transform infrared (FTIR) spectroscopy and X-ray diffraction (XRD), techniques were used to analyse the structural and optical characteristics of synthesized nanoparticles. The average diameter of green synthesized ZnO nanoparticles was 82.09 nm. The zeta potential measurements of ZnO nanoparticle was found to be -23.2mV. SEM analysis displayed spherical shaped nanopatricles. FTIR further confirmed the presence of bioactive functional groups involved in the encapsulation and stabilization

of ZnO NPs. The *C. fistula* mediated ZnO NPs showed remarkable antibacterial activity against *Escherichia coliand Staphylococcus aureus*. Thus, the green synthesis of zinc oxide nanoparticles (ZnO NPs) could be employed in pharmaceutical industries and biomedical applications.

AMAH-OS-4

Physico-biochemical, TLC analysis and antimicrobial assay of Gomutra

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The importance of Gomutra has been detailed in Ayurveda since ancient times.Urine analysis plays a pivotal role in disease diagnosis across various veterinary contexts, providing insights into both metabolic and pathological conditions. Urine analysis serves as a non-invasive and versatile diagnostic tool, enabling the identification of metabolic disorders, and evaluation of organ function. The present study was conducted to evaluate the physico-biochemical, TLC analysis and antimicrobial assay of Gomutra. The cow urine samples were collected in a sterile, wide mouth container from Sahiwal breeds reared at Livestock Farm Complex (LFC), DUVASU and evaluated for biochemical examination. The results were obtained by using dipstick method using a Urine analyser, thin layer chromatography, and antimicrobial assay to screen out the presence of amino acids in Gomutra and also to evaluate its antimicrobial potential. The physico-biochemicalparameters were observed as normal, the microscopic examination revealed normal urine. We have been able to identify different amino acids- arginine, glutamine and serine in Gomutra. Antibacterial activity as shown by the zone of inhibition in disc diffusion assay against bacterial pathogens *E. coli* and *Staphylococcus aureus* against Oxytetracycline confirmed Gomutra as a potent antimicrobial agent. The study revealed that Gomutra can be used as an effective antibacterial agent. These findings demonstrate exploration of bioactive compounds with potential medicinal applications. By the use of this analysis, we can comprehend the condition of animals and could also detect the diseases at an early stage.

TECHNICAL SESSION

Development in Alternative Medicine, Pharmaceutical and Nutraceutical Research for Animal Health and Production (AMAH)

POSTER PRESENTATIONS

AMAH-PF-1

Exploration of immunomodulatory properties of Badri Cattle urine in-vitro in Wistar Rats with its comprehensive biochemical profiling

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Nowadays Cowpathy is gaining impetus as an alternative medicine in treating ailments especially concerned with immune system. The present study explored the immunomodulatory effects of Badri cow urine in Wistar rats. The Badri cattle urine collected from the plains, Kumaon, andGarhwal hills of Uttarakhand was subjected to the formation of cow urine distillate (CUD) and fed to Wistar rats for 90 days and its effects on hematological paraments and Cell mediated immune (CMI) response were recorded. Hematological parameterswere measured at baseline and on the 30th, 60th, and 90th days post-treatment along with the biochemical analysis. In addition, cell-mediated immunity was assessed using the lymphocyte transformation test (LST) with Con-A and PHA as stimulants. Also, the biochemical profiling of CUD reveals the presence of minerals, trace elements, and enzymes including Gold and silver. These findings collectively indicate a broad spectrum of potential vital health benefits associated with CUD may have a positive influence on hematopoiesis and immune function in Wistar rats, encompassing protein metabolism, immunological health, bone health, lipid modulation, renal function, metabolic control, and hepatoprotection.

AMAH-PF-2

Ameliorative role of paeoniflorin in testosterone-induced benign prostatic hyperplasia and fibrosis in Wistar rats

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In men aged 50 and older, benign prostatic hyperplasia (BPH) is the fifth most common non-cancerous disorder, affecting approximately 6% of the global population. The economic impact of BPH ranks it as the seventh highest in terms of disease-specific healthcare costs within a one-year period. BPH is characterized by the abnormal proliferation of prostate gland cells, leading to obstructive voiding issues caused by an enlarged prostate. This cellular proliferation occurs in both stromal and epithelial components of the prostate. Developing alternative, natural therapies for BPH has become a key focus in both human and veterinary medicine. In this study, we examined the effects of Paeoniflorin, a terpene glycoside, in countering testosterone-induced BPH in Wistar rats. Our findings indicate that Paeoniflorin significantly mitigated oxidative damage by enhancing antioxidant defenses, including SOD, CAT, GSH, and GPx, while reducing oxidative stress marker MDA. Additionally, Paeoniflorin demonstrated anti-inflammatory benefits by inhibiting NF-κB activation and decreasing inflammatory markers IL-1β, IL-6, and TNF-α in prostate tissue lysates. It also reduced hypoxia-inducible factor-1α (HIF-1α), vascular endothelial growth factor (VEGF), and transforming growth factor β (TGF β1), affirming its anti-angiogenic properties. Furthermore, Paeoniflorin increased the epithelial marker E-Cadherin and decreased the mesenchymal marker vimentin, while also reducing androgen receptor activity, thus blocking AR-androgen binding. Our results suggest that Paeoniflorin exerts its protective effects against BPH by modulating the NF-

κB/AR signaling pathway, demonstrating its potential as an antioxidant, anti-inflammatory, anti-angiogenic, and anti-fibrotic compound. Paeoniflorin shows promise as a prophylactic or therapeutic agent for BPH.

AMAH-PF-3

Phytochemical and antioxidant profiling of *Carica papaya* leaf Extract in various solvents

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Carica papaya, widely regarded for its medicinal properties, is a rich source of phytochemicals and antioxidants, making it highly valuable for promoting human health. With growing interest in identifying natural and safe antioxidants for applications in functional foods, nutraceuticals, and therapeutic agents, this study aimed to evaluate the phytochemical composition, antioxidant activity, total phenolic content, and total flavonoid content of papaya leaf extracts using different solvents. The study identified carbohydrates, phenolic compounds, flavonoids, alkaloids, and tannins in the papaya leaf extracts, showcasing its diverse phytochemical profile. Among the solvents tested, the aqueous extract yielded the highest extraction efficiency (16.3%). Methanol extracts demonstrated the highest total phenolic content (58.6 ±0.18 mg GAE/g) and exhibited potent antioxidant activity with the highest DPPH scavenging activity (48.46 ±0.75%) and the lowest IC50 value (4.464 µg/mL), highlighting its superior radical-scavenging potential. Conversely, the n-Hexane extract displayed the highest total flavonoid content (31.5 ±0.2 mg QE/g). The presence of significant secondary metabolites, coupled with high phenolic and flavonoid content and strong antioxidant activity, underscores the potential of papaya leaf extracts as a natural source of bioactive compounds. These findings support their potential applications in developing natural antioxidants, functional foods, and nutraceuticals, contributing to health and therapeutic advancements. This study highlights Carica papaya leaves as a promising candidate for future research and utilization in natural product-based industries.

AMAH-PF-4

Biogenic synthesis of silver nanoparticles from medicinal plants: innovative solutions for antimicrobial resistance and oxidative stress

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In response to the growing concern of antimicrobial resistance, the exploration of eco-friendly and sustainable therapeutic alternatives has become essential. This study investigates the biosynthesis of silver nanoparticles (AgNPs) using aqueous extracts of four medicinal plants: *Azadirachta indica* (Neem), *Moringa oleifera* (Sajna), *Clitoria ternatea* (Nilkanth), and *Aloe vera*. These plants, known for their rich phytochemical composition, offer a promising source of bioactive compounds for nanoparticle synthesis.The biosynthesized AgNPs were characterized through UV-Vis spectroscopy, FTIR, and FE-SEM analysis. UV-Vis spectroscopy confirmed the successful synthesis with a surface plasmon resonance band observed at 400–450 nm, indicative of AgNP formation. FTIR analysis revealed the dual role of phytoconstituents, such as phenolics, amines, esters, and carboxylic acids, as

reducing and stabilizing agents. Morphological studies using FE-SEM showed the AgNPs were nanosized with predominantly spherical shapes. Antibacterial efficacy was evaluated against methicillin-resistant *Staphylococcus aureus* (MRSA) and *Escherichia coli* using the standard broth dilution method. Among the tested nanoparticles, *A. indica*-derived AgNPs demonstrated superior antibacterial activity with the lowest minimal inhibitory concentrations (MICs). The antioxidant potential of the AgNPs was assessed using the DPPH free radical scavenging assay. *A. indica*-derived AgNPs exhibited the highest scavenging activity, attributed to their high polyphenolic content. Phytochemical analysis identified flavonoids, tannins, phenolics, and other bioactive compounds in the plant extracts, which contribute to the medicinal properties of the synthesized nanoparticles. This study underscores the therapeutic potential of biosynthesized AgNPs as natural antimicrobial and antioxidant agents. The integration of plant-derived nanomaterials offers a sustainable and effective approach to addressing oxidative stress and antimicrobial resistance. These findings highlight the transformative role of phytochemical-based nanotechnology in developing novel treatments for pathological disorders.

AMAH-PS-1

In vitro analysis of *Saccharomyces cerevisiae* probiotic for anthelmintic efficacy

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The extensive use of anthelmintics to combat the helmintic infection has led to an increase in resistance which is now a global problem. The literature suggested a number of probiotics have shown promising anthelmintic activity both *in vitro* and *in vivo*. The adult *Haemonchus* worms were exposed to three forms of *Saccharomyces cerevisiae* such as the live form, the sonicated form and the hydrolysed form in fourteen treatment groups along with controls. The three forms of *Saccharomyces* did not have detrimental effect on the adult *H. contortus* worms upto 48 hours. Egg Hatching, Larval Development and Larval exsheathment inhibition of *Haemonchus contortus* were also performed. We found that live and sonicated extract yeast does not inhibit *in vitro* hatching of *H. contortus* eggs. In contrast, hydrolyzed form of yeast changes the egg morphology of *H. contortus,* such as distortion and disappearance of the egg's cell wall *in vitro*, which were confirmed by scanning electron microscopy. Live and sonicated yeast extract have negligible negative effects on egg development to L1 larvae, whereas the hydrolyzed form of yeast inhibits larval development to a certain extent. Live, sonicated extract and hydrolyzed forms of yeast do not hinder larval exsheathment at all. The spectrofluorometric studies with the three types of yeast on third-stage larvae showed fluorescence in the aromatic amino acid range. The absence of other auto-fluorescent markers showed that all three forms of yeast did not adversely affect the third-stage larvae of *H. contortus*. Thus, only the hydrolyzed form of *Saccharomyces cerevisiae* is effective on *Haemonchus contortus* eggs as well as inhibition of its development into first-stage larvae *in vitro*. Therefore, hydrolyzed *Saccharomyces cerevisiae* may be used to control helminth infections.

AMAH-PS-2

Isolation, purification and immuno-biochemical characterization of serum immunoglobulin G (IgG) of pig

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Immunoglobulins are secreted from B cell and they act as antibodies free in cellular fluids functioning to intercept and eliminate antigenic determinants. Nowadays, pigs are widely used to produce specific antibodies and it is of interest to develop good method for purification of this immunoglobulins for various purposes. The present study involves the method to purify isolate, purify and characterize the immunoglobulins from serum of Ghungroo and Large White Yorkshire breeds. The blood samples of Ghungroo and Large White Yorkshire pigs were collected from Barrackpore and Mohanpur areas of West Bengal and were used for obtaining serum.Dialysis of crude IgG against PBS was done after 50% ammonium sulphate precipitation. Using the Lowry *et al.,*. (1951) method, the crude IgG protein content from pig serum was 38.342 mg/ml. Dialyzed Ammonium sulphate precipitated Ghungroo pigs‘ immunoglobulin (Crude IgG) were applied to Sephacryl S - 200 HR column. Using dialysis the pooled peak-showing fractions were concentrated. Following pure IgG dialysis, 7.23 mg/ml of protein was measured using Lowry's technique. The antibody against crude serum was raised in healthy New Zealand white rabbit. Three protein bands with molecular weights 66.00 kDa, 52.40 kDa, and 20.72 kDa were visible on a 10% SDS-PAGE of crude IgG when compared with marker indicating the molecular weight of the pig immunoglobulin G to be 139.12 kDa. By using the Western blot approach, it was discovered that the purified immunoglobulin was immunoreactive against hyperimmune serum produced in rabbits.

AMAH-PS-3

Effect of poly-herbal formulations on the health and productivity of post-weaned piglets

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Weaning is one of the most crucial and difficult stages of piglet development because it involves separation from the mother and exposure to a new environment. Sudden changes in the overall environment raise physical stress, which causes piglets to develop numerous diseases such as diarrhea. Using phytogenic feed additives instead of antibiotics is safe not only for cattle, butal so for consumers and the environment. Experiments were carried out to determine the effect of Polyherbal formulations (herb extract mixtures) on weanling pigs' growth performance, blood features, blood biochemical profile, phytochemical analysis, and fecal microbial shedding. After 42 days of weaning, 84 Ghoongroo piglets with an average body weight of 7.6±0.6 kg were randomly assigned to three treatments. Treatments used a 2x2 factorial design, with two levels of PHF-I and PHF-II (T1 & T2) at three different concentrations (100 mg/BW, 200 mg/BW, and 250 mg/BW). Piglets were fortified with PHF-supplemented meals during the first several weeks, resulting in decreased (P 0.05) WBC numbers in the T1 group compared to the T2 group. There was no significant difference observed

between the T0 and T1 groups. The biochemical profile of blood parameters showed no significant variation between groups. Finally, adding PHF to the diet of weaning piglets improved their growth performance. However, the good effect on WBC, lymphocyte, and fecal *E. coli* concentrations indicated that these types of PHF have a positive influence on weaning piglet growth performance. Data analysis revealed that there was no significant difference tween the two treatment groups, although a significant difference was found between the doses. Treatment group (T1) demonstrated a lower bacterial burden at various dosage concentrations than treatment group (T2). This data suggests that herbal formulations had a favorable influence on lowering bacterialloads in post-weaned piglets, resulting in a significant reduction in the occurrence of diarrhea.

AMAH-PS-4

Milk metabolic profile of healthy and subclinical mastitis Haryana cows during summer & winter season

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This study was carried out on lactating Haryana cows reared at dairy farm of College of Veterinary Science, DUVASU Mathura. Total 40 samples of milk were collected and were stored at -20°C prior to analysis. The animals were divided in to two subgroups, namely control/healthy group, subclinical mastitis group on the basis of screening by using somatic cell count (SCC), Electrical Conduction and pH on the milk samples. The Milk samples were collected (Healthy-27 & Subclinical mastitic-13) and analysed for Total Protein, Albumin, Globulin, Albumin/Globulin Ratio, Sodium, Potassium, Aspartate transaminase (AST) Lactate dehydrogenase (LDH) Alkaline phosphatase (ALP). Milk metabolic profile of healthy and subclinical mastitis Haryana cows are found significant difference in AST values (138.5*+ 2.50) during summer season & 149.1±1.24 in winter season and found no significant difference in parameters during winter season in healthy and subclinical mastitis Haryana cows.

AMAH-PS-5

Impact of flooring types on total protein and albumin levels in Haryana calves during summer

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This study evaluated the impact of innovative flooring solutions on the Total Protein (g/dl) and Albumin (g/dl) levels in Haryana calves during summer season. The study was conducted on eighteen Haryana calves from December to February 2021 at Livestock Farm Complex DUVASU, Mathura, for 90 days. The calves were housed in a tie-barn shed with a double row tail-to-tail system, receiving individualized care, feeding, and watering. They were divided into three groups based on body weight: the control group (concrete flooring), treatment group 1 (compost/cow dung bed flooring), and treatment group 2 (rubber mat flooring). The mean values of Total Protein (g/dl) and Albumin (g/dl) levels were 6.57±0.16 and 3.11±0.17 in the control group, 6.67±0.17 and 3.54±0.21 in T1, and 6.62±0.12 and 3.40±0.19 in T2, respectively, with no significant differences (P>0.05). This stability suggested that calves' biochemical parameters remain unaffected by the type of flooring, allowing selection of flooring based on other factors such as comfort and cost without compromising calf health. These insights not only contribute to animal biochemistry but also provide practical guidance for enhancing livestock management practices.

AMAH-PS-6

Fatty Acids analysis of *Mallotus phillipensis* leaf extract using Gas Chromatography

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Natural products, particularly plant extracts, either as pure compounds or standardized extracts, offer immense potential for new drug discoveries. One such plant with promising therapeutic potential is *Mallotus philippensis* (MP). This versatile plant is widely distributed across Southeast Asia and has a long history of use in traditional medicine for treating various ailments like liver flukes in cattle, parasitic skin infections, stomach ulcers, and tapeworm infestations. This study presents a comprehensive analysis of the fatty acid content in the ethyl acetate (EA) extract of *MP* leaves, utilizing gas chromatography (GC). The analysis identified 26 distinct compounds, characterized by their retention times (RT) ranging from 11.745 to 54.217 minutes, representing a broad spectrum of volatilities and polarities. The peak area percentages provided a relative quantification of each compound. The predominant compound was methyl butyrate, contributing 58.651% of the total area, highlighting its significant influence on the chemical profile. Other major constituents included methyl octadecenoate (9.436%) and methyl palmitate (7.729%). The identified compounds encompass a diverse array of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs). Among these, notable MUFAs such as methyl myristoleate and methyl octadecenoate were classified as omega-7 and omega-9 fatty acids, respectively, while PUFAs like cis-8,11,14-Eicosatrienoic acid methyl ester and cis-5,8,11,14,17-Eicosapentaenoic acid methyl ester were categorized as omega-6 and omega-3 fatty acids.The study also revealed the presence of trans-isomer fatty acids, such as methyl linolelaidate, a polyunsaturated omega-6 fatty acid, which could have nutritional impacts. The early-eluting compounds, represented by methyl butyrate, were identified as lighter, more volatile molecules, while the late-eluting compounds, such as cis-5, 8, 11, 14, 17-Eicosapentaenoic acid methyl ester**,** represented heavier, less volatile fatty acids.The results underscore the potential applications of MP leaf extract in various settings. Volatile esters like methyl butyrate are widely utilized in flavor and fragrance formulations, whereas the presence of essential fatty acids such as omega-3 and omega-6 PUFAs points to potential nutritional benefits. This study provides valuable insights into the chemical and functional properties of MP leaf extract, supporting its use in pharmaceutical, and nutritional applications.

TECHNICAL SESSION

Contemporary Research in Molecular Diagnostics and Vaccine Production (MDVP)

LEAD PAPERS

MDVP-LP-1

Importance of diagnostic and bio-security measures in emerging and re-emerging livestock diseases of India

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The recent outbreaks of Lumpy Skin Disease (LSD) and African Swine Fever (ASF) in India have led to significant livestock mortality and severe socio-economic consequences. India, home to the world's largest livestock population (535.78 million), faces unique vulnerabilities to emerging and re-emerging diseases due to its geographical location and traditional animal husbandry practices (Yadav *et al.,* 2020). An estimated 60% of emerging diseases are related to the transmission of microorganisms from animals to humans (Skowron *et al.,* 2023), posing potentially debilitating or fatal outcomes if left untreated. However, human and human activities, leading to changes in the overall global climatic conditions, are the principal causes of the diffusion of pathogens, and the increased frequency of these diseases (Leal *et al.,* 2022).

The World Organization for Animal Health (OIE) defines an emerging disease as "a new infection or infestation resulting from the evolution or change of an existing pathogenic agent, a known infection or infestation spreading to a new geographic area or population, or a previously unrecognized pathogenic agent or disease diagnosed for the first time, which has a significant impact on animal or public health." An endemic disease may be considered re-emerging if it shifts its geographic range, expands its host range, or significantly increases in prevalence. The WHO has issued a list of high-priority pathogens that are likely to cause future outbreaks, highlighting the need for research and development (R&D) efforts through priority R&D blueprints (Wang *et al.,* 2024).

In recent years, several zoonotic diseases, including SARS-CoV-2, CCHF, Nipah, Chandipura, and Zika virus infections, have affected human populations in India. In the veterinary sector, LSD and ASF are prominent examples of emerging infections in the country. Antibiotic resistance, which is becoming increasingly common, also plays a significant role in the re-emergence of bacterial infections in both animals and humans (Zumla and Hui, 2019).

LSD primarily affects cattle and buffalo and was initially confined to the African continent. After spreading across continents, the first LSD outbreak in India occurred in Odisha in 2019, and since then, numerous cases have been reported across the country (Sudhakar *et al.,* 2020; Smaraki *et al.,* 2024). During the most devastating disease wave in 2022, the disease affected millions of cattle, resulting in the death of many cattle, with a case fatality rate approaching 5%. Similarly, ASF was first reported in India in early 2020, originating from outbreaks among domestic pigs in two northeastern states, and subsequently spread to several other states, severely impacting pig and pork production. ASF infections in wild boars (*Sus scrofa*) suggest local transmission from domestic pigs, posing a threat to conservation efforts for endangered pygmy hogs (*Porcula salvania*), the world's smallest wild pig (Bora, 2024).

The morbidity, mortality, and socio-economic impact of emerging and re-emerging diseases, as seen with LSD and ASF, underscore the need for robust diagnostic methods and biosecurity measures. Traditional microbiological methods, including culturing the organism and immunological detection of antigens or antibodies in affected body fluids, remain valuable. However, in the face of unprecedented diseases, faster and more specific diagnostic methods, such as nucleic acid amplification techniques like PCR and real-time PCR, are the cornerstone of diagnosis. With the supplementation of genome sequencing, these methods provide reliable diagnosis down the species level. These techniques have become more cost-effective and

accessible for clinical diagnostic facilities after the advent of portable sequencing methods like Oxford Nanopore sequencing (Bagger *et al.,* 2024). MALDI-TOF has revolutionized the identification of bacterial infections. It proves better than PCR-based methods in the cases of unknown or novel infections (Seng *et al.,* 2009). This technique has enabled the early identification of species of *Mycobacterium* from cases of tuberculosis (Alcolea-Medina, *et al.,* 2019) and the identification of novel species of coagulase-negative *Staphylococcus* spp., as causative agents of mastitis.

In addition to diagnostics, mitigating losses requires early identification and rapid response to outbreaks of emerging and re-emerging animal diseases by veterinarians, along with inter-sectoral coordination for diseases with high zoonotic potential. Developing countries often lack sufficient infrastructure, protocols, and techniques to trace the origin and spread of outbreaks promptly and effectively (Saminathan *et al.,* 2016; Meckawy *et al.,* 2022). Vaccination is highly effective in preventing and managing many diseases; however, vaccination campaigns alone are usually insufficient and must be part of a comprehensive control plan (Trovato *et al.,* 2020).

Surprisingly, biosecurity remains an underutilized component of bio-risk management during outbreaks. In non-endemic areas, disinfecting facilities, equipment, and vehicles, along with implementing temporary restrictions on animal movement, are essential measures. These steps not only protect animal health but also reduce zoonotic transmission risks, which holds significant public health importance (Léger *et al.,* 2017; Layton *et al.,* 2017).

India's contiguous and porous borders with Nepal, Bhutan, Pakistan, and Bangladesh, as well as free trade agreements with Nepal and Bhutan, highlight the need for global biosecurity measures to minimize the entry of new pathogens. This requires adequate infrastructure, including check posts, quarantine facilities at seaports and airports, and international land borders, as well as diagnostic facilities with trained personnel and rapid tests to ensure the pathogen-free status of imported livestock and livestock products (Yadav *et al.,* 2020). In conclusion, India must prioritize developing biosecurity infrastructure, fostering inter-sectoral cooperation, and rapidly deploying diagnostic tools to address the risks associated with LSD, ASF, and other emerging and re-emerging infections. Strategic planning and the adoption of comprehensive biosecurity protocols will mitigate the economic and ecological consequences of these diseases, protecting both human and animal health across the region.

Keywords: Lumpy Skin Disease, African Swine Fever, zoonotic diseases, biosecurity, diagnostics, livestock, India, socio-economic impact

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MDVP-LP-3

Pyruvate kinase M2 regulates Japanese encephalitis virus replication in neuronal cells

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Pyruvate kinase isoform M2 (PKM2) is a key modulator of glucose metabolism. While the major role of PKM2 is to facilitate the breakdown of glucose, it is potentially associated with other additional non-glycolytic functions as well. The role of PKM2 in the autoimmune response and inflammatory process is increasingly being acknowledged as a crucial modulator of cellular pathophysiological activity. However, its role in modulating viral replication has not been explored in detail. In the present study, we have shown a significant increase in endogenous PKM2 expression in JEV-infected mouse neuroblastoma cells. Furthermore, overexpression of PKM2 significantly reduced JEV replication, suggesting a negative effect of PKM2 on JEV replication. This was further confirmed by siRNA-mediated downregulation of endogenous PKM2 expression, which resulted in enhanced JEV replication. In silico studies revealed the potential interaction between PKM2 and NS1 protein of JEV. The microscopic studies also showed cellular colocalization of PKM2 and NS1 in the ER of infected cells. The interaction was further validated in vitro by co-immunoprecipitation assay. The present study suggests that PKM2 negatively regulates the JEV replication by its possible interaction with NS1.

MDVP-LP-4

Loop-Mediated Isothermal Amplification (LAMP): An Innovative Approach for On-Site Animal Disease Diagnosis

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The World Health Organization underscores the critical role of early and precise disease diagnosis in maintaining animal health. Since the 1980s, nucleic acid amplification techniques, particularly PCR, have been the benchmark for accurate disease identification. However, PCR's application is hindered in field settings and resource-limited laboratories due to the high cost of equipment. Loop-mediated isothermal amplification (LAMP) emerges as a promising alternative, offering rapid, precise, and cost-effective DNA amplification. Developed by Notomi et al.,. in 2000, LAMP has surpassed other isothermal assays in user adoption due to its exceptional efficiency, specificity, and sensitivity in amplifying target genes under isothermal conditions. Its adaptability allows for the diagnosis of diseases caused by a wide range of microorganisms, including bacteria, viruses, and parasites. LAMP's versatility makes it ideal for testing readily available samples such as saliva, milk, or capillary blood. It is particularly advantageous in scenarios where individuals with minimal expertise can execute the complete diagnostic process from sample collection to result interpretation.
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Introduction

Accurate disease diagnosis is crucial for managing animal health. Early and precise identification of infectious conditions is essential for effective treatment, prognosis, and preventing drug misuse. The World Health Organization outlines that optimal diagnostic tests should exhibit qualities such as sensitivity, specificity, affordability, ease of use, rapidity, adaptability to various climatic conditions, and accessibility of equipment. Molecular diagnostics, particularly nucleic acid amplification techniques, are pivotal in animal health management. These techniques amplify nucleic acid sequences to detectable levels through cyclic enzymatic reactions within minutes to hours. Since its development in the 1980s, the polymerase chain reaction (PCR) has been regarded as the gold standard in nucleic acid amplification. It is extensively utilized in clinical diagnostics, forensic science, and agricultural biotechnology due to its enhanced sensitivity, non-culture-based amplification, and reduced diagnosis times. However, despite its success, PCR faces limitations, particularly the requirement for costly equipment, which restricts its application in resource-limited settings.

The limitations of PCR have driven the development of isothermal amplification assays. Among these, Loop-mediated Isothermal Amplification (LAMP) stands out as a leading technique for disease diagnostics. LAMP has garnered significant attention for its rapid, accurate, and cost-effective nucleic acid amplification capabilities. This method efficiently amplifies minimal DNA quantities into billions of copies with high precision within an hour. Its user-friendly nature makes it a recommended diagnostic tool. LAMP's unique advantages over PCR and other molecular methods fulfill the essential criteria for a definitive diagnostic test.

In 2000, Notomi and colleagues introduced the loop-mediated isothermal amplification (LAMP) technique for DNA detection. Since then, various other isothermal amplification methods have been developed, including helicase-dependent amplification (HDA), recombinase polymerase amplification (RPA), rolling circle amplification (RCA), nicking enzyme amplification reaction (NEAR), and strand displacement amplification (SDA). Despite these advancements, LAMP continues to be the preferred isothermal amplification method among researchers[3].

LAMP boasts a larger user base compared to other isothermal assays due to its publicly available protocols and the wide availability of reagents from various suppliers (Maples*et al.,* 2007). Unlike RPA and NEAR, which are restricted by copyrights, LAMP offers more opportunities for unique adaptations and applications5. Studies have shown that common sample matrix inhibitors, such as those found in blood, urine, saliva, and environmental water, do not hinder LAMP amplification. Additionally, heat lysis of microorganisms can be achieved in standard LAMP settings, allowing users to bypass the typically required steps of lysis, extraction, and purification before nucleic acid amplification. While other isothermal assays may share some of these attributes, LAMP is favored in the scientific community for its quick identification of nucleic acid targets, thanks to its flexibility, accessibility, and resilience. It also demonstrates high efficiency, specificity, and sensitivity in amplifying target genes under isothermal conditions.

The LAMP technique has been proven to be a versatile and flexible method for diagnosing diseases caused by several microorganisms, including bacteria, virus and parasites. Legionella, Giardia, verotoxin-producing E. coli, E. coli O157:H7, Campylobacter, Salmonella, Listeria monocytogenes, and other diseases can all be diagnosed with LAMP-based diagnostic kits (http://loopamp.eiken.co.jp/e/products/index.html). Tokyo's Eiken Chemical Company has made these kits available for purchase[12].

In the industrialized world, diagnostic environments vary from centralized laboratories with advanced automation to on-farm testing in resource-limited settings, facilitating rapid and precise diagnosis. The adoption of diagnostic equipment for home use is increasingly prevalent. The benefits of LAMP are particularly evident in scenarios where individuals with minimal expertise can efficiently execute the complete diagnostic process from sample collection to result interpretation. The method's simplicity and speed align with the nucleic acid amplification technique employed. Unlike traditional samples such as venous whole blood, serum, plasma, or cerebrospinal fluid, LAMP is ideally suited for testing easily obtainable materials like saliva or capillary whole blood. Recent outbreaks, including Ebola, Zika, and SARS-CoV-2, have renewed interest in using urine and saliva as non-invasive diagnostic specimens (Abdullahi*et al.,* 2015; Bonney, *et al.,* 2020 and Wei *et al.,* 2020).

LAMP Principle

LAMP uses four primary primers to target a total of six distinct sequences and a DNA polymerase with a high level of strand displacement activity, such as *Bst polymerase*. The primers are designated as F3, F2, F1, B1, B2 and B3 combined into four primers in a set namely F3, FIP (F1c-F2), BIP (B1c-B2) and B3. Out of these, F3 and B3 are said to be outer primers and required in lesser amount. While FIP and BIP are forward or backward inner primers and are main primers required in higher amount. The stem-loop structures that are generated as a result of LAMP function as initiation sites for future exponential amplification. The amplification process can be sped up even further by the addition of two loop primers (Damhorst,*et al.,* 2015). It is possible to carry out reverse transcription (RT) under conventional LAMP conditions, which enables a one-step reaction to be carried out on DNA and RNA targets(NEB, 2023). RT-LAMP is often carried out using two distinct enzymes, such as *Bst DNA polymerase* and *AMV reverse transcriptase*. However, it has been established that RT-LAMP can be carried out using a single enzyme that possesses both RT and strand-displacing activity.

A typical LAMP reaction consists of two phases: a noncyclical phase followed by the cyclical phase. All four primers are used for amplification in the noncyclic phase; only two outer primers are utilized during the cyclic phase. The result of the noncyclical stage accumulates during the cyclical phase. If six primers are to be utilized, loop primers may be used in the cyclical step.

The LAMP reaction marks with an initial non-cyclical phase, including DNA synthesis by annealing F2 region of FIP and elongation through polymerase action, followed by annealing of BIP in similar manner. The F3 and B3 primer anneals and elongates DNA along with strand displacement to generate single stranded DNA with complimentary regions, which leads to formation of dumbbell

like structure at both the ends. Later in the cyclical phase, the dumbbell shaped, self primed, single stranded DNA undergoes several steps of elongation, eventually generating cauliflower shaped concatamers. For a better understanding of the LAMP reaction mechanism, please refer to the animation available from New England Biolabs.

LAMP reaction components

The essential ingredients to perform a LAMP reaction are as following:

Template: LAMP reactions can use both DNA and RNA as a template. Not requiring sample preparation or DNA extraction is one of LAMP's main advantages over PCR. It functions well with a sample prepared only mildly or as such. Even the use of CSF, heat-treated blood, and serum for LAMP has been reported.

***Enzymes*:** The brains of a LAMP reaction are enzymes. This can be accomplished using any DNA polymerase enzyme with strand displacement activity. *Bst* DNA polymerase is the enzyme that is employed most frequently; *Bsm* DNA polymerase is utilized less often. These enzymes come from *Bacillus smithii* and *Bacillus stearothermophilus*, respectively. These enzymes are inactive exonucleases. The *Bst* and *Bsm* enzymes continue to function as enzymes up to 66°C and 63°C, respectively, even though *Bst* DNA polymerase operates best at 65°C[2].

Primers: The primers are the most important of all the elements. For LAMP amplification to be successful, proper primer design is essential. The target gene's four or six unique regions—F3c, F2c, and F1c on the 3' side and B1, B2, and B3 on the 5' side—have been used to construct four or six primers. These include two optional loop primers (LF and LB), Forward Inner Primer (FIP), Forward Outer Primer (F3), Backward Inner Primer (BIP), and Backward Outer Primer (B3). The F2 region, which is complementary to the F2c area at the 3' end, and the identical sequence as the F1c region at the 5' end make up the Forward Inner Primer (FIP).

The F3 area, analogous to the F3c region, is part of the Forward Outer Primer (F3). The B2 region, which is complementary to the B2c area at the 3' end, and the identical sequence as the B1c region at the 5' end make up the Backward Inner Primer (BIP). The B3 area, a companion to the B3c region, makes up the Backward Outer Primer (B3). An increased number of starting points for DNA synthesis for the LAMP method are provided by the Loop Primers (either Loop Primer B or Loop Primer F), which contain sequences complementary to the single-stranded loop region on the 5' end of the dumbbell-like structure (either between the B1 and B2 regions or between the F1 and F2 regions). The mechanisms by which the inner and loop primers work are distinct. Loop primers boost the sensitivity and efficiency of the LAMP reaction(Goto *et al.,* 2009)by reducing the time needed for positive LAMP amplification. Using available LAMP primer-creating software, such as "Primer Explorer" (Eiken Co.) and "LAMP Designer" (PREMIER Biosoft International), or manually designing the primers are both options.

Reagents:

a) Betaine is employed to stabilize the AT and GC content to guarantee the reaction stability.

b) Deoxynucleoside triphosphate (dNTP), which supplies the necessary nucleotides

c) Magnesium sulphate (MgSO4), which produces pyrophosphate ions and accumulates in the reaction mixture to produce a clear, white precipitate, are additional components[21].

d) Fluorescent DNA dye can be used, to make the pyrophosphate ion precipitate easier to visualize. SYBR Green, HNB, Picogreen, and a metal ion indicator called Calcein are a few DNA dyes employed in LAMP.

e) Buffer solution containing $(NH_4)_2SO_4$, Tris-HCL with a pH of 8.8, $MgSO_4$, and KCl. The minimum incubation time to get positive LAMP amplification may vary from 30 minutes to 180 minutes.

LAMP Methodology

Primer designing

The nucleotide sequence of desired gene can be downloaded from NCBI. LAMP primers are designed using PrimerExplorerV5 online software (https://primerexplorer.jp/e/) with default settings and notepad FASTA format of the desired gene as input sequence.

Sample collection and DNA isolation

The LAMP technique provides flexibility to use variety of samples in the assay *viz.* Biological material (blood, urine, CSF, tissue), food, soil etc. The samples are transported in ice pack to laboratory and processed for genomic DNA isolation. The isolated DNA is checked for output and concentration through 1.5 % gel electrophoresis and Nanodrop.

Optimization of LAMP reaction

To optimize the reaction, F3 and B3 primers from different sets of the designed LAMP primers are used as forward and reverse primers for gene amplification in PCR reaction. The isolated genomic DNA is taken as template. Loop Mediated Isothermal Amplification (LAMP) is optimized with gene specific LAMP primers (F3, FIP, BIP and B3) at several isothermal temperatures (57-67 °C) maintained by thermal cycler in 0.5 ml PCR tubes. Further, the LAMP reaction is optimized for incubation period of the reaction (20-60 min), $MgSO_4$ concentration (6-10 mM), template concentration (5-50 ng/µl). The LAMP reaction is optimized for primer ratio of FIP/BIP to F3/B3 primers. The ratio of 4:1 and 8:1 are commonly used to obtain better results. Betain (0.5-2 M) can be utilized to increase difference between 'Time to Result' of negative and test sample.

Table: Reaction mixture for a typical LAMP amplification

S.No.	Components of LAMP reaction	For 25 µl reaction	Final concentration
1.	10 X ThermoPol Buffer	2.5 µl	1 X
2.	$MgSO_4$ (100 mM)	1.5 µl	6 mM
3.	dNTP (10 mM)	3.5 µl	1.4 mM
4.	FIP (16 µM)	2.5 µl	1.6 µM
5.	BIP (16 µM)	2.5 µl	1.6 µM
6.	F3 (6 µM)	0.85 µl	0.2 µM
7.	B3 (6 µM)	0.85 µl	0.2 µM
8	Betaine (5 M)	0.5 µl	0.5-2 M
9.	Bst DNA polymerase (8,000 units/ml)	1 µl	8 Units
10.	Template	1 µl	10-50 ng

Detection of the LAMP product

Effective genetic analysis using LAMP is dependent on both the success of DNA amplification and the technology used to track the reaction. The LAMP amplified product can be detected using a variety of techniques. Agarose gel electrophoresis on 1.5 to 3% agarose gel, followed by ethidium bromide solution staining and viewing under UV transilluminator, can be used to analyze LAMP reaction products.

In favourable situations, it will result in a ladder-like pattern because stem-loop structures with

various stem lengths emerge. Sequencing or restriction enzyme digestion can both be used to validate the specificity of the amplified product in the LAMP reaction. It is a common practice to directly monitor the LAMP amplicons[2]; in some circumstances, it even serves as the "gold standard" However, the need for UV detection and electrophoresis equipment restricts the usefulness for field applications. Because no post-amplification processing is required, results from LAMP can be seen with the naked eye, which is a considerable benefit over PCR.

The colorimetric endpoint detection of LAMP products is a direct and straightforward readout method that can be used to establish whether or not positive reactions have occurred. Visual turbidity is a reliable sign of a successful LAMP reaction. It is possible to see the outcomes with one's own two eyes and does not need for the use of any specialized equipment. Large numbers of nucleic acids gets amplified during the LAMP process. As the process of amplification continues, LAMP produces both pyrophosphate and protons as by-products of the reaction. which causes an abundance of pyrophosphate ions. Pyrophosphate is a powerful cation binder; When these ions mix with magnesium ions, a white precipitate of magnesium pyrophosphate is produced, which, in the case of a positive reaction, manifests as turbidity. The amount of nucleic acid amplified during the process is exactly proportional to an increase in turbidity. Utilizing a real-time turbidimeter as an OD at 400 nm every 6 seconds, turbidity can be monitored in real-time. Compared to a real-time PCR equipment, it costs less.

Researchers have shown a pH shift at the end point and have utilized pH-sensitive dyes for amplicon detection, such as phenol red, neutral red, cresol red, and m-cresol, to see a visible change in color (Tomita *et al.,* 2006).

For the purpose of detecting amplification, polyethyleneimine (PEI) can be introduced to the reaction tube after amplification. Low molecular weight oligonucleotides cannot mix with PEI; instead, they create an insoluble combination with the high molecular weight amplification product. The addition of PEI causes the precipitate that can be seen to form. PEI cannot, however, be introduced before the reaction begins since it will prevent amplification.

When fluorescent intercalating dyes like SYBR Green I are present, amplified products can also be seen. When SYBR Green I is added to the reaction tube after amplification, a good reaction results in the colour changing from orange to yellow. Using a portable UV torch with a wavelength of 365 nm, fluorescence can be seen visually.

The drawback is that these dyes are said to hinder LAMP amplification, necessitating their addition after amplification by opening the tube and increasing the risk of cross-contamination (Garg *et al.,* 2021).

Several fluorescent dyes have also been utilized for the purpose of colorimetric detection. When calcein is added to a LAMP reaction, the product changes color from orange (indicating a negative reaction) to green (indicating a positive response) (Wastling *et al.,* 2010). Fluorescence increases can be seen visually or by UV light. When added to LAMP processes, Quant-iT Picogreen creates a color change from orange (negative) to green (positive), which is plainly visible with an ultra-violet lamp(Brittain, 1978). This colour change can be attributed to a change in charge. The considerable production of protons that occurs during the LAMP amplification process leads to a decrease in pH that is greater than two pH units.

HNB is an azo dye that alters its hue depending on the pH and/or cation levels of the surrounding environment(Li, *et al.,* 2019). The transformation of HNB from a violet to a sky-blue color, which can be seen by the naked eye, is caused by the synthesis of magnesium pyrophosphate. This transformation is caused because free magnesium levels are reduced during the LAMP reaction as a result of the formation of magnesium pyrophosphate. Eriochrome Black T (EBT) likewise undergoes a colour change from violet to sky blue as Mg^{2+} levels drop during amplification. Similarly, a bright blue-green colour is produced during amplification when malachite green (MG) is present, whereas negative responses appear pale white or colourless.

The colorimetric reading/detection may be variable and subjective to field-settings. As a result, lateral-flow dipsticks (also known as LFD) have been adopted for point-of-care testing because they are generally user friendly and economical. In this step, the biotin-labelled amplicons are hybridized

with FITC, which then causes them to adhere to gold-anti-FITC biotin binding proteins, which ultimately results in the formation of red stripes. This serves as the foundation for biosensors made out of gold nanoparticles(Hashimoto *et al.,* 2017). When compared to colorimetric, turbidimetric, or gel-electrophoresis methods, LFD methods are viewed as being more practical to employ because of their ease of application and speed of detection.

This approach functions well in high-throughput automated systems, where the detection can be interpreted more easily. Fluorescent resonance energy transfer (FRET) based LAMP assay has been demonstrated for target amplicon-specific detection(Ball *et al.,* 2016). In this assay, FRET probes were used to hybridize single stranded LAMP products at the loop region, to provide real-time monitoring of the FRET-bound region.

For real-time detection of LAMP amplicons, colorimetric endpoint analysis can be coupled with fluorescent DNA intercalating dyes. During optimization and reaction kinetic investigations, the addition of real-time fluorescence detection to a colorimetric LAMP assay can be beneficial. The fluorescence signals created by LAMP reactions are generally bright, and the equipment required for fluorescent LAMP are simple: a light source, an emission filter, and a detector. The light source could be a coloured LED or a UV blacklight, and the filter could be low-cost coloured theatre gels. These gels have demonstrated adequate performance for fluorescence photography of LAMP reactions(Tone *et al.,* 2017), and they might be used. Photodiodes, digital cameras (including those on smartphones), and even just using one's own eyes are all common types of detectors.

Real-time monitoring of LAMP, while useful for quantifying targets by correlating time-to-positivity with standard concentration, generally offers less precision compared to qPCR reactions. This is because real-time monitoring of LAMP relies on measuring the amount of time it takes for a sample to become positive. Additionally, RT-LAMP typically exhibits a narrower quantification range than RT-qPCR, with notable variability and deviation from linearity, particularly at the lower end of the range.

However, LAMP is not well suited for applications that require accurate quantification, such as monitoring HIV viral load in response to treatment. LAMP is best useful for situations where a basic qualitative report is sufficient.

Variants of LAMP

The LAMP technology has undergone significant advancements since its inception, resulting in the development of five distinct types: Electric LAMP (eLAMP), Lyophilized LAMP, Lateral Flow Assay LAMP, mini LAMP, and Real-Time Monitoring and Quantification LAMP. These innovations have enhanced the LAMP assay's efficiency, transforming it into a rapid, field-deployable, and user-friendly sample screening tool. By integrating various molecular techniques, including real-time and multiplex detection, along with colorimetric and visual-detection methods, the LAMP assay now offers straightforward identification of positive samples.

The specificity and accuracy of this method are improved thanks to a modification known as multiplex LAMP (mLAMP), which involves the use of more than one gene to identify several target sequences at the same time. Recently, Kim *et al.,*. (2021) developed a mLAMP assay that differentiates between Mycobacterium tuberculosis (MTB) and non-tuberculosis Mycobacterium (NTM) species.

Real-time LAMP was optimizedin a single-tube for specific detection of dengue serotypes using several primer mixes that were exhaustively searched in available viral genome sequences(Papadakis *et al.,* 2022). It demonstrated a high level of sensitivity (95.8%), however it did not cross-detect any additional flaviviruses.

A portable hand-held biomedical device that is capable of performing real-time quantitative colorimetric LAMP has been fabricated(Gadkar *et al.,* 2018). This device can be operated with an application that can be downloaded onto a smartphone. For the purpose of monitoring the color shift that occurs during amplification, a little digital camera that is capable of undergoing digital image analysis was developed. Validation of the device in a clinical setting was performed using SARS-

CoV-2 samples.

The LAMP technique has a number of potential drawbacks, the most significant of which are said to be false positives and non-specific binding. The incorporation of fluorogenic probes that are capable of both self-quenching and dequenching as part of the LAMP primers is yet another strategy for real-time detection and quantitative assessments of amplicons. When it is bound to its target, the fluorescence of a labelled fluorogenic primer-probe that is quenched when it is not bound to its target is produced. Because of this, the enhanced method is now known as the Fluorescence of Loop Primer upon Self Dequenching-LAMP (FLOS-LAMP). It enables the detection of target sequence in real time with a greater degree of specificity. FLOS-LAMP was utilized by Gadkar *et al.,*. (2018) for the purpose of performing quick detection of Varicella-zoster virus utilizing clinical samples. The clinical sensitivity of the target was found to be 96.8%, while its specificity was determined to be 100%. This technique gets rid of non-specific and incorrect results, making the detection approach more sensitive overall. Additionally, Hardinge and Murray(2019) used a variety of quenched fluorescent primer labels to illustrate the robustness, adaptability, and enhanced sensitivity of LAMP.

Another approach for the real-time quantification of the amplification process is called FRET LAMP, which stands for fluorescence resonance energy transfer. It makes use of probes that are laced with a fluorescent dye as well as a quenching dye. After being exposed to light, a fluorescent dye like SYBR Green I will transfer some of its energy to molecules of a quenching dye, which will result in the formation of a substrate that is not fluorescent[40]. FRET LAMP analysis was utilized by Severi *et al.,*. (2020) to detect white spot syndrome virus with a sensitivity of 10^2 copies.

A chip-based LAMP system

For highly contagious infectious diseases, extensive sample testing is essential to control transmission and facilitate contact tracing. This process can be labor-intensive and may lead to delays, exacerbating disease spread. Additionally, the substantial use of reagents increases the cost of sample processing. Insufficient sample material further complicates diagnosis. Advanced microarray systems utilizing microscopic chips have been developed for amplification methods, requiring minimal material and reagents while enabling simultaneous multiple tests. Recent advancements in miniature LAMP techniques, including microfluidic, electrochemical, paper-based, and digital methods[42], have significantly expedited sample processing.

Micro and nanofluidics have emerged as a widely recognized platform for manipulating single molecules and controlling nano-volume fluids. A chip-based microfluidic device has been developed for the direct detection of foodborne pathogens using paper-embedded LAMP reagents and fluorescence detection. This device facilitates LAMP by loading the reaction chambers with essential chemicals and samples, demonstrating sensitivity while maintaining cost-effectiveness. Zhou *et al.,*. have advanced an RT-LAMP microfluidic device for on-field detection of swine coronaviruses, showcasing exceptional sensitivity, specificity, and a rapid reaction time of 40 minutes.

Digital LAMP

In order to do an amplification of DNA digitally, a sample must first be separated into a number of separate chambers. Ma *et al.,*. (2018) were able to create a droplet array microfluidic chip that was capable of running LAMP in an array of multiple uniformly trapped droplets in nanoliters with a detection limit of a single molecule. This chip could be used to execute LAMP in an array. In another line of research, a track-etched polycarbonate membrane was produced in order to facilitate the generation of droplets(Lin *et al.,* 2019). On the membrane, a single-step digital LAMP operation was carried out. This was demonstrated as a straightforward alternative to the intricate chip production process. It provides a benefit for point-of-care detection and analysis in a way that is flexible, straightforward, and expedient.

LAMP vs PCR: Suitability for Field level disease diagnosis

a) *Ease of Use and Cost Efficiency*

The LAMP procedure is straightforward, allowing even those with moderate expertise to complete the experiment efficiently. Its single-step reaction process enhances speed and effectiveness, making it an ideal tool for diagnostic applications. LAMP offers significant advantages over PCR, notably its cost-effectiveness and user-friendliness, as it operates in an isothermal environment like a standard water bath. Consequently, it is a superior method for field-level diagnosis, eliminating the need for expensive equipment such as a thermocycler.

b) Post-amplification processing is unnecessary

Post-amplification processing, essential for PCR-based diagnostics, is unnecessary for LAMP product visualization, offering a notable advantage. LAMP allows for clear, naked-eye observation of amplification.

c) Least or no sample preparation

Molecular diagnosis in the field encounters numerous challenges, with sample preparation being the most critical. The PCR technique may be impeded by inhibitors present in biological samples. Conversely, LAMP offers a versatile solution for direct detection using clinical samples. Its ability to amplify a target gene in unprocessed samples, such as blood, enhances its resilience significantly. This eliminates the need for time-consuming template extraction and purification, making it highly advantageous in healthcare settings where accurate and prompt diagnosis is essential.

d) Exceptional performance

The Loop-mediated Isothermal Amplification (LAMP) technique exhibits exceptional sensitivity, specificity, and amplification efficiency. As noted by Parida *et al.,*. (2008) its remarkable amplification efficiency is attributed to the absence of temporal thermal loss during the isothermal reaction. LAMP can amplify minimal quantities of template DNA to detectable levels. Dhama *et al.,*. highlight that LAMP can detect DNA in samples with degraded copies at concentrations as low as a femtogram. Reports indicate that LAMP's sensitivity is on par with or surpass that of PCR, particularly in outdoor conditions for infection detection. LAMP is demonstrated to be 10–100 times more sensitive than conventional PCR for identifying harmful organisms. However, some claims suggest its sensitivity is comparable to real-time PCR. Notably, LAMP can specifically amplify the 16S rRNA target sequence of *Mycobacterium leprae* amidst *Mycobacterium tuberculosis*, which shares the same gene, thus aiding in distinguishing closely related species and reducing the risk of misdiagnosis or false positives.

e) Fast-paced

The LAMP diagnostic method is exceptionally rapid, requiring just a single straightforward step for successful completion. Utilizing loop primers, a standard LAMP reaction concludes within 30 to 60 minutes. Furthermore, the absence of a need for post-amplification enhances its efficiency.

f) Longevity

LAMP exhibits superior stability compared to PCR and real-time PCR, maintaining its integrity across various elongation times, temperatures, and pH levels. Unlike LAMP, which can be prepared without a cold chain, PCR necessitates the use of a cold chain for the preparation of the master matrix.

Limitation of LAMP

There are still a few significant drawbacks to LAMP, despite the fact that it has a lot of benefits.

Because LAMP primers have to recognize six to eight different portions of the target DNA sequence, the design process for LAMP primers is thought to be complex. Because there are limitations for the free energy of primer binding as well as constraints on the distances between priming sites, it might be difficult to select primer sets that are acceptable for a specific target. There is free software available on the internet that can assist in the construction of LAMP primers; however, the output does not always include loop primers, and human adjustment of the primers is frequently still required. Even with the software, it seems that there is no universal objective function that can predict how well primers will work. Because some candidate sets provide delayed amplification, poor sensitivity, or false-positives for no apparent reason[35], time-consuming empirical testing and redesign of several primer sets are typically required. Both of these processes include multiple primer sets. In addition, LAMP and other isothermal techniques have a notoriety for producing nonspecific amplification as well as false-positive results. The likelihood of primers interacting with one another in a reaction rises proportionately with the number of primers present in the reaction. Primers, when combined with specific auxiliary activities of *Bst* DNA polymerase (terminal transferase, template switching, extension from 3'-mismatches), have the potential to result in non-template amplification[47]. LAMP also has problems with non-specific binding since it forms primer dimers, which can lead to this problem. When numerous primers are used, the likelihood of primer–primer hybridizations occurring, which can lead to template-free amplification and, thus, false-positive results, is significantly increased[47]. Even while primer sets should be checked in advance for potential primer-primer interaction, it is still possible for experiments to produce false-positive results. Carry-over contamination is a common source of LAMP false-positive results[48], particularly when open-tube procedures are utilized for the purpose of analyzing reaction products. During the process of analysis, amplicons have the potential to get aerosolized and disrupt subsequent LAMP experiments. In order to reduce the likelihood of contamination at any stage of the process, the steps of sample preparation, amplification reactions, and post-amplification processing (if necessary) must all take place in separate rooms. Further, by utilizing dyes such as calcein and HNB in a closed-tube LAMP procedure, this problem can be circumvented and avoided. An additional method is the utilization of a UTP/UNG system for the purpose of preventing PCR carry-over contamination. When employing LAMP for clinical diagnostics, one of the most essential things to keep in mind is how to reduce the risk of false-positive amplification. This can be done by the iterative design of primers or through the careful adjustment of reaction conditions. Another drawback is that the LAMP method cannot be utilized for the amplification of sequences that are longer than 300 base pairs. The LAMP method, despite its challenges, is well-suited for pathogen detection across various fields, including clinical diagnostics, agriculture, veterinary medicine, food safety, bioterrorism, and environmental monitoring23.

Conclusion

Accurate identification of illness-causing organisms is essential for proper disease diagnosis and effective management. Since its discovery, LAMP has evolved to meet the complex diagnostic needs of the healthcare system. Enhancements to the original LAMP methodology have been achieved by integrating it with additional molecular techniques, such as reverse transcription, real-time quantification, and multiplex amplification systems. It is compatible with various amplification detection methods, including real-time fluorometry, turbidimetry, and colorimetry. Despite the advanced LAMP technologies facing challenges like complex fabrication, cross-contamination risks, and inconsistent outcomes, ongoing research aims to address these issues. LAMP has proven effective for diagnosing a wide range of pathogens, including allergens, bacteria, viruses, and parasites, and is applicable to allergen detection. The LAMP system has been employed in developing mobile-operated biosensors and integrated on-chip versions. Numerous studies highlight its potential for sensitive, specific, and rapid detection and analysis of infectious pathogens. In summary, LAMP is a promising diagnostic tool due to its resilience, ease of use, and cost-effectiveness, aligning with the WHO's criteria for ideal diagnostics.

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TECHNICAL SESSION

Contemporary Research in Molecular Diagnostics and Vaccine Production (MDVP)

ORAL PRESENTATIONS

MDVP-OF-1

Interaction between BAX, BAD AND Bcl-2 genes with kisspeptin

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Kisspeptin reduces apoptosis by regulating the expression of the BAX, BAD, and Bcl-2 genes in PFs cultured in TCM 199B, TCM 199B + KP, and Standard medium + KP for 3 min, 2, 4, and 6 days, as well as COCs in matured large antral follicles for 24 hr. The expression of the three test genes Bcl-2, BAX, and BAD was examined in preantral, early antral, antral, and large antral follicles, as well as in cumulus-oocyte complexes from *in vivo* grown large antral follicles subjected to *in vitro* maturation for 24 hrs. The control group was *in vivo* cultured follicles. The quantitative expression patterns of test and reference genes (RPLPO, HPRT1, and 18SrRNA) in cumulus cells and oocytes were examined independently at distinct PF developmental stages by qRT-PCR. The Bcl-2, BAX, and BAD gene expression levels in cumulus cells and oocytes were assessed by qRT-PCR in all developmental phases of follicles produced *in vivo* and *in vitro* in TCM 199B, TCM 199B + KP, and Standard medium + KP conditions. The Bcl-2, BAX, and BAD gene expression levels in the cumulus cells and oocytes of PFs cultured in TCM 199B media were significantly different from those at corresponding stages of follicles developed *in vivo*, suggesting that TCM 199B media alone could not replicate *in vivo* conditions. Cumulus cells and oocytes cultured with TCM 199B + KP, the expression levels of the anti-apoptotic gene Bcl-2 and the pro-apoptotic genes BAX and BAD increased and decreased, respectively, mimicking *in vivo* expression patterns. In Standard medium + KP cultured follicles, cumulus cells and oocytes expressed Bcl-2, BAX, and BAD genes at higher and lower levels, respectively, as development progressed from preantral to COC stages after 6 days of cultured PFs and 24 hours of IVM, compared to *in vivo* grown follicles.Kisspeptin supplementation (10 µg/ml) in combination with growth factors and hormones in the culture medium reduced apoptosis by modulating gene expression, enhancing follicle viability, promoting ovarian cell growth, and improving ovine reproduction.

2-MDVP-OF-2

Biosafety and efficacy of immersion vaccine against *Lactococcus garvieae*infection in farmed rainbow trout (*Oncorhynchusmykiss*) of India

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Rainbow trout (*Oncorhynchusmykiss*) is the topmost important coldwater species in fish farming of hills of India. However, due to intensification and change in climate, farmed rainbow trout has been facing the “warm water lactococcosis” disease caused by *Lactococcusgarvieae*. Vaccination by immersion is known to have several advantages over injection, such as reduced stress to fish and mass vaccination within a short time. Therefore, in this study, the efficacy of the formalin-killed autogenous vaccine in rainbow trout (23.45 ±5.87 g)was evaluated by immersion (20 min). Vaccination was done as primary and booster dose at an interval of 7 days, and fish were challenged 7 days post booster dose (pbd). The experiment was conducted in duplicate with two group: treatment group (TG; n=72) -vaccinated and infected by inducer; and control group (CG; n=72) -non-vaccinated and infected by inducer. Blood serum and organs (gill, liver and kidney) for histology was collected 3, 5, 7, 14 and 21 days pbd from TG and CG. Approximately 100% relative percent survival (RPS) was observed in TG in comparison to CG, when exposed to homologous *L. garvieae*strains

(3.56×10^6cfu/ml). The serum lysozyme activity and bactericidal activity increased significantly in TG, in comparison to CG on 21 days pbd. However, no significance difference was found in total protein, albumin, and globulin content of the serum of both the groups. Biosafety evaluation of the vaccine in rainbow trout (n= 10; 28.96 ±18.56 g) indicated no pathological changes at tissue or cellular level till 7 days pbd. No cytotoxicity of the vaccine was observed on EPC cell line. Animal behavior scoring, survival percentage, body weight, gross lesion scoring, and histopathology scoring confirm the biosafety of this vaccine in rainbow trout. The findings of this study suggested that the immersion of rainbow trout to formalin-killed *L. garvieae* vaccine protects against disease caused by *L. garvieae*.

3-MDVP-OF-3

Standardization of a LAMP assay for the detection of *S. agalactiae*

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Molecular disease diagnostic tools are now being widely used for screening infectious diseases. Loop-mediated isothermal amplification (LAMP) assays have attracted a lot of attention as a potentially rapid, accurate, cost-effective and highly suitable field friendly diagnostic tool. Mastitis is the most common economically important health problems in dairy cattle throughout the world. Among the various pathogens causingmastitis, *Streptococcus agalactiae* is of particular importance because of its high infectivity and mainly subclinical nature. The present workwasaimedto standardize the LAMP assay for the detection of *S. agalactiae*. LAMP reaction was standardized forthe*cfb* gene of *S. agalactiae*.For optimization of reagents a range of different components were tested as, without and with (25µM, 20µM and 10µM) loop primers, varying concentrations of internal primers (25 µM and 40 µM), outer primers (25 µM and 5µM) and $MgSO_4$ (2 mM, 4 mM, 6 mM and 8 mM). To determine the optimum reaction conditions, reaction mixtures were incubated at different temperatures using a gradient of 60.0 to 68.4°C and for different periods (30, 60, 90, and 180 minutes).Reaction products were detected by agarose gel electrophoresis as well as by the naked eye using 3mM HNB. In our study no amplification was detected when LAMP assay was performed without loop primers. Further positive LAMP amplification was observed only at the levels of 20µMloop primers, 40µM internal primers, and 5µM outer primers. The optimum ratio of primers for LAMP reaction was found to be 1:8:4 (outer: internal: loop primers). Positive LAMP amplification could only be detected at 64.4°C and 90 minutes.In conclusion, the standardized protocol of LAMP can be used as a rapid, accurate, and low-cost molecular diagnostic technique for the detection of *S. agalactiae*.

MDVP-OF-4

Resistance beyond carbapenemase: Efflux pumps and porin alterations in animal-origin carbapenem resistance in bacterial strains

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Carbapenem-resistant Enterobacteriaceae (CRE) pose a significant challenge due to their resistance to β-lactam antibiotics, including cephalosporins and carbapenems, contributing to the rise of multidrug-resistant (MDR) bacterial strains. Resistance mechanisms, including carbapenemase production, efflux pump activity, and porin modifications, are well-studied in human strains but remain poorly understood in bacteria from animal sources, where carbapenems are infrequently used. This study aimed to investigate the molecular basis of carbapenem resistance in bacterial isolates from

animals.CRE isolates were characterized by morphological, cultural, and biochemical assays. Carbapenemase production was assessed using the Carba NP test and carbapenemase inhibition assays (CIA). Efflux pump activity was evaluated via CCCP-IMP/ETP disc synergy tests, MIC broth microdilution, and EtBr cartwheel assays. Quantitative PCR (qPCR) was used to analyze the expression of outer membrane protein (OMP) and efflux pump genes.Carbapenemase production was detected in 12 isolates using the Carba NP test. PCR confirmed the presence of blaIMP, blaVIM, and blaOXA-48 in these isolates. Among 18 non-susceptible isolates, 5 exhibited efflux pump-mediated resistance, with significant overexpression of acrA and acrB efflux pump genes strongly correlating with imipenem resistance. Exposure to imipenem resulted in the downregulation of porin genes ompF and ompC in most isolates.This study identified multiple mechanisms contributing to carbapenem resistance in animal-origin CRE, including carbapenemase production, efflux pump overactivity, and porin modifications. These findings highlight the complexity of resistance mechanisms in animal strains and underscore the need for monitoring such reservoirs to better understand and manage antibiotic resistance in One Health contexts.

MDVP-OF-5

Prediction of vaccines for lumpy skin disease using Machine Learning (ML) based immune-informatics analysis

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The year of 2022 witnessed the worst hit by the animal disease caused by Lumpy Skin Disease virus (LSDV) and took a toll of 2.4 million cattle. The virus comes under Genus Capripoxvirus. The drawbacks of conventional vaccine can be overcome by use of ML tools to develop a subunit peptide vaccine. In this regard, 156 putative viralgeneswere screened for vaccine-like property to shortlist 51 genes. These were analyzed for MHC-1 and MHC-II reacting epitopes using IEDB server and were subjected to allergenicity analysis and toxicity. A total of 139 T-cell epitopes were found to be immunogenic. Thereafter, 89 T-cell and 48 B-cell predicted epitopes were clustered based on sequence identity. Further,*in silico* cytokine expression is carried out using IFNepitope, IL- 6Pred and IL10Pred webservers. A total of 48 epitopic peptides were selected on the basis of *in silico* cytokine expression studies including 9 B cell epitopes, 27 MHC-I epitopes and 12 MHC-II epitopes. The peptides were joined end-to-end with suitable adjuvant, DIVA sequence and reverse translated to generate three nucleic acid vaccine construct sequences. Further study investigates the potential T cell epitopes of Lumpy Skin Disease Virus (LSDV) evaluates their efficacy in stimulating cellular immune responses. PBMCs from LSDV-exposed animals were found to enhance IFN-γ production significantly on stimulation by a designed peptide. The initial results of the investigation suggest requirement of further *in vivo* studies to confirm the vaccine potency to elicit immune response for LSD infection.

MDVP-OF-6

Deciphering the association betweengrowth traits of Swiss albino mice with microsatellite markers

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Microsatellite markers, also termed as short tandem repeats (STRs), are important for marker-assisted selection to detect genetic diversity, and they are uniformly distributed in eukaryotic genomes. To analyze the relationship between microsatellite loci and growth traits of Swiss albino mice in India, a total of 100 F_7 inbred generation of female mice were genotyped. Genotyping were done by using genomic DNA from tail tissue for 10 microsatellite (MS) loci. All loci showed different degrees of genetic polymorphism. Chi square test revealed that all loci were significantly deviating from Hardy Weinberg equilibrium (HWE) ($P<0.05$). The D2Mit61 and D7Mit323 locus, had significant ($P<0.05$) genotypic associations with adult body weight (ABW). The D9Mit27 locus had significant ($P<0.05$) genotypic associations with body weight at birth time (BWW). In the F_7 inbred mice population, the D1Mit15, D2Mit51, D3Mit55, D5Mit18, D8Mit14, and D10Mit180 locus had non- significant ($P<0.05$) genotypic associations with all growth traits. The microsatellite loci selected in this study showed rich polymorphism in the inbred mice population and were related to the performance and fitness traits, which can be used for the evaluation of genetic quality and early improvement of performance and fitness traits in inbred strain of Swiss albino mice as well as livestock.

MDVP-OS-1

Glycoproteine (gE) gene-based TaqMan Real-time PCR assay for the identification of the Marek's disease virus in poultry

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The aim of the present study was to develop a novel glycoproteine (gE) gene-based TaqMan Real-time PCR assay for the identification of the Marek's disease virus in poultry. A very sensitive TaqMan Real-time PCR approach was developed in this study to detect the Marek's Disease Virus (MDV). Primers intended to amplify the glycoprotein E (*gE*) gene, which remains conserved in the genome of the virus. A consensus area comprising all fifty-seven complete *gE* gene coding domain sequences (CDS) that are publicly available worldwide was used to design the primers. The set of primers amplified 230 bp of the viral genome, while the probe was FAM labelled at 5' end. Sensitivity and specificity was assessed for the assay. The minimum detection limit of the technique was found as 17.6 picogram genome in the clinical sample. It was also shown to be highly specific the detection of field MD virus as itdidn't amplifies the commercial vaccinal strains of HVT (Herpesvirus of Turkey) FC 126 strain and the fowl pox virus (FPV). The applicability of the technique was assessed with the field tissue (liver) samples collected during post-mortem examination of birds suspected of MD and from healthy birds collected from commercial retail poultry outlets (n=22). Ten out of 22 samples were found positive. The developed assay could be used for diagnosis of MD infection in the vaccinated as well as non-vaccinated poultry flocks.

MDVP-OS-2

Cloning of canine perilipin 1 and 2: Insights into lipid metabolism and mammary tumor biology

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Perilipins are lipid droplet-associated proteins that play a crucial role in regulating lipid storage and metabolism. As members of the PAT family (Perilipin, ADRP, TIP47), they are predominantly located on the surface of lipid droplets in adipocytes and other cell types. Perilipins are essential for maintaining lipid droplet stability, regulating lipolysis, and ensuring lipid homeostasis. Among the most extensively studied perilipins, PLIN1 and PLIN2 are integral components of lipid droplets. These proteins are differentially expressed in various cancers, including breast cancer. However, the expression and role of canine perilipins, particularly in canine mammary tumors, remain largely unexplored. This study aims to clone and characterize canine PLIN1 and PLIN2 genes to facilitate an understanding their roles in lipid metabolism and canine mammary tumors.The sequences of canine perilipin 1 (PLIN1) and perilipin 2 (PLIN2) were retrieved from the NCBI database. Based on these sequences, specific primers were designed by introducing restriction enzyme sites, (Bgl II and Not I)for partial canine PLIN1, and (EcoR I and Hind III) for partial canine PLIN2. PCR amplification successfully yielded products of 545 bp for PLIN1 and 794 bp for PLIN2, consistent with the expected fragment sizes. The amplified PCR products were subjected to restriction digestion and subsequently ligated into prokaryotic expression vectors. PLIN1 was cloned into the pRham-SUMO vector (size~2575 bp), while PLIN2 was cloned into the pET32a vector (size ~5900 bp). These vectors were selected for their ability to enhance protein solubility and simplify downstream purification through their fusion tag systems. The ligated constructs were transformed into E. cloni and Escherichia coli DH5α cells, respectively. Colony PCR, restriction digestion analysis, and sequencing confirmed the successful integration of the target genes into the respective vectors.This study provides an efficient approach to produce recombinant PLIN1 and PLIN2 proteins, enabling further investigations into their roles in lipid metabolism and potential biotechnological applications.

MDVP-0S-3

Molecular detection of Verotoxic *E. coli* (VTEC) in milk samples of dairy cattle having subclinical mastitis

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Subclinical mastitis (SCM) has been described as the most difficult issue in dairy production, causing significant financial harm to the dairy sectors in developed as well as developing nations.SCM can be caused by both Gram-positive and Gram-negative bacteria, but Gram-negative bacteria continue to be the most common cause of SCM. VTEC is now emerging pathogen in developed and developing countries responsible for causing HUS & HC in humans. The study was conducted during Feb,2024 to Nov.2024, A total of 250 milk samples were collected from 125 dairy cattle. 44% & 50.40% SCM was detected by CMT & SCC method respectively. All the milk samples were processed for isolation and identification of *E.coli* by cultural and biochemical test. The results revealed 12.00%(30/250) prevalence ofE.coli in 250 milk samples from dairy cattle screened for SCM.All the 30 *E. coli* isolates were processed for detection of VTEC by polymerase chain reaction. 8 VTEC were confirmed by molecular test and found positive for stx2 gene. The presence of VTEC in milk samples revealed its public health importance.

TECHNICAL SESSION

Contemporary Research in Molecular Diagnostics and Vaccine Production (MDVP)

POSTER PRESENTATIONS

MDVP-PF-1

Isolation and molecular detection of selected abortion-causing bacteria in bovines

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Aim of the experiment wasconsidering the important role of bacteria in bovine abortion, primary bacterial abortifacient agents such as*Brucella abortus*, *Listeria monocytogenes*, *Campylobacter fetus,* and*Trueperella pyogenes*were diagnosed through standard bacteriological and PCR-based methods.A total of 85 samples, comprising of vaginal swab (34), placenta (11) & 8 aborted fetuses (40 samples collected from aborted fetuses like stomach contents (8), lung (8), liver (8), heart (8), and intestine (8) of each fetus) were collected from cattle (N=33) and buffaloes (N=11). The presence of bacteria was confirmed through bacterial culture and PCR. Results obtained areOut of the 85 samples collected, 7/34 (20.58%) vaginal samples and 1/6 (16.66%) placental samples were found positive by showing positive growth or PCR. The placental sample showed co-infection of two bacteria. However, none of the aborted fetus contents were found positive, either in culture or PCR. The culture isolation method yielded, 4 samples positive for*Brucella*, 1 for *Listeria* and 1 for *Trueperella* organisms. None of the samples was positive for *Campylobacter*spp. In molecular detection, 4, 2, 2 and 1samples were detected positive for *Brucella abortus*, *Listeria*spp., *Campylobacter* spp., and*Trueperella*spp., respectively. In conclusion, all the target bacteria were found in the study area and the sensitivity of PCR-based methods was found to be better than the culture and isolation methods, especially for *Listeria*spp. *and Campylobacter* spp.

MDVP-PF-2

First report of *Theilerialesto quardi* infection in sheep from Rajasthan and its genotyping

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Tick-borne hemoprotozoan parasitic diseases are rare in sheep and goat. Theileriosis in sheep caused by protozoan parasites of the genus *Theileria*, particularly *Theilerialestoquardi*, *Theileriaovis*, and *Theileriahirci*. *T.lestoquardi* is highly pathogenic species causing high morbidity and mortality in sheep. Reports of this parasite in India are exceedingly rare, with none originating from Rajasthan.This study reports an outbreak of *T. lestoquardi* in a sheep flock in Tonk, Rajasthan. The animals have a history of fever, anorexia, prostration, and laboured breathing. Of the 120 sheep, 18 adult sheep died owing to conditions over a one-month period. The investigation of the flock found weakness, tick infestation, severe jaundice, anaemia, and circling movement. The haemato-biochemical study indicated anaemia, reduced protein levels, and elevated liver enzymes. For molecular diagnosis, unique primers for three distinct genes of *Theileria* were utilised: the merozoite surface protein gene, the cytochrome b gene, and the SSr RNA gene. The product of PCR were cloned and sequenced to identify the causative agent. The sequencing of the three genes verified *T.*

lestoquardi as the etiological agent responsible for mortality in the sheep herd. This is the first report documenting the infection of *T. lestoquardi* in sheep in Rajasthan. The sequencing findings demonstrated around 98-99% similarity with other sequences accessible on the NCBI site. The evolutionary study revealed a close similarity between the current strain and the Hissar strain.The molecular confirmation of *T. lestoquardi* in Rajasthan for the first time will enhance disease surveillance and diagnosis, while underscoring the rising threats to animal health in this region of the nation. The sequencing analysis will aid in comprehending the biology and evolution of this virulent strain of parasite.

MDVP-PF-3

Isolation, molecular characterization, and pathological findings of important bacterialrespiratory infections in goats of Eastern Uttar Pradesh, India

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The small ruminants, particularly goats are susceptible to respiratory diseases due to theirhigh vulnerability, which accounts for roughly 50% of their fatalities. Both humans and animalscan acquire a range of intestinal and extra-intestinal infections from pathogenic Escherichia coli(E. coli) and Pasteurella spp. and other respiratory pathogens. Analogous to intestinal diseases,respiratory diseases pose a significant risk to the goat breeding sector. Pneumonia and associatedmortality incurs huge economic losses to the goat farmers in the eastern plain zone of UttarPradesh, India. Among the respiratory infections in goats, Pasteurella spp. and E. coli infectionsin eastern plain zone of Uttar Pradesh had never been thoroughly studied earlier. To determinethe prevalence of respiratory diseases among goats, the present study was aimed to isolate,identification and molecular characterization of the organisms responsible for respiratoryinfections especially Pasteurella spp. and E. coli. Out of 150 samples collected and processed,40.66% (61/150) were E. coli, 26% (39/150) Pasteurella spp., 20% (30/150) Klebsiella spp., and13.33% (20/150) Staphylococcus spp. were identified in the respiratory tract of goats based ontheir typical colony characteristics, morphology, motility, and bio-chemical properties. In thisstudy, significant percentage of extraintestinal pathogenic E. coli was found in the dead goatswith pneumonia. Based on PCR and phylogenetic analysis targeting 16S rRNA gene, most of theisolatedPasteurella strains were having similarities with *Pasteurellamultocida*. The results ofpresent study confirm the circulation of Pasteurella spp. and E. coli among the population ofgoats in field conditions in eastern plain zone of Uttar Pradesh and necessary control measuresshould be formulated with effective vaccination strategies in small ruminants for the control ofrespiratory infections to reduce the economic losses to goat farmers.

MDVP-PS-1

Virus-Like Particles (VLPs): A promising subunit vaccine approach for eradicating viral diseases

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The livestock and poultry industry isvulnerable to various infectious diseases, resulting in significant economic losses and threats to animal and human health. The development of innovative vaccine strategies is crucial to mitigate these challenges.Conventional animal viral vaccines till date rely on inactivated or attenuated viruses. Researches are going on to explore alternative approaches, including subunit vaccines, to improve vaccine efficacy and safety. Virus-Like Particles (VLPs) have emerged as a promising vaccine platform, offering a safe and effective alternative to traditional vaccines.VLPs are highly organized protein assemblages, formed through spontaneous self-assembly of recombinantly expressed viral structural subunits, but lack the viral genome, rendering them non-infectious. Particulate and multivalent nature of VLPs with retention of native antigenic conformation enable the stimulation of robust humoral and cellular immune responses.Their compatibility with DIVA strategies makes them ideal for developing vaccines against notifiable diseases in animals including Foot and mouth disease, Lumpy skin disease, Classical Swine Fever, Avian Influenza and New castle disease. Despite their potential, only a limited number of veterinary VLP-based vaccines have been licensed. In summary, the development of VLPs as a vaccine strategy in veterinary medicine holds great promise, with far-reaching implications for animal health and welfare.

MDVP-PS-2

Improved detection of Leptospira specific antibodies in dogs using recombinant Loa22 based ELISA

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Leptospirosis is a zoonotic disease affecting various animal species including canines that often require accurate and rapid diagnosis for effective management. This study evaluated the potential of recombinant Loa22 (rLoa22) protein of Leptospira interrogans serovar Canicola for detecting anti-leptospiral antibodies in dogs using an indirect enzyme-linked immunosorbent assay (ELISA). The aim was to develop and assess the sensitivity, specificity, and overall accuracy of this assay compared to the microscopic agglutination test (MAT) which is the gold standard test. The rLoa22 protein was expressed in Escherichia coli and purified using affinity chromatography. An indirect ELISA was developed using purified rLoa22 as the antigen, and its performance was tested on sera from 100 dogs suspected for leptospirosis. The developed ELISA was able to detect all the MAT positive samples demonstrating a sensitivity of 100%. Furthermore, the assay was found to be 96.81% specific and 97.9% accurate with respect to MAT in detecting Leptospira specific antibodies. Interestingly, 03 MAT negative samples were detected as positive by ELISA. In conclusion, rLoa22-based indirect ELISA could be a reliable tool for the diagnosis of canine leptospirosis, offering high sensitivity and specificity. This method offers a practical and reliable alternative to conventional tests, especially in settings where resources for MAT are limited.

MDVP-PS-3

Development of a novel multiplex PCR assay for the molecular diagnosis of hemoparasitic infections

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A novel, rapid, and specific multiplex polymerase chain reaction (mPCR) assay was developed for diagnosing hemoparasitic infections in cattle blood caused by three common pathogens: *Theileria annulata*, *Babesia bigemina*, and *Anaplasma marginale*.Multiplex polymerase chain reaction (PCR) provides a reliable diagnostic tool for the simultaneous detection and differentiation of multiple hemoprotozoan pathogens in a single reaction.Blood samples were collected from the jugular veins of 200 cattle between November 2023 and October 2024 in the Mathura region of Uttar Pradesh, India. The method involves DNA extraction from cattle blood samples, followed by amplification of pathogenusing primers in a single PCR assay. Individual infections were detected in 56, 8, and 19 samples for *T. annulata*, *B. bigemina*, and *A. marginale*, respectively. PCR products are analyzed via agarose gel electrophoresis, where each pathogen is identified by a unique band size, such as ~488 bp for *Babesia spp.*, ~148 bp for *Theileria spp.*, and ~340 bp for *Anaplasma spp.* The technique is validated by ensuring specificity and sensitivity even in cases of low parasitemia.Multiplex PCR significantly enhances diagnostic efficiency, reducing time, cost, and labor compared to singleplex assays. It enables the identification of single and co-infections, in making targeted treatment decisions. In conclusion, multiplex PCR is an advanced, cost-effective tool for diagnosing hemoprotozoan infections in cattle. Its application improves disease monitoring, control, and management, supporting sustainable livestock practices and minimizing the economic impact of these diseases on the cattle industry.

MDVP-PS-4

Impact of lipo-polysaccharides and heat stress on immunological responses in broiler chicken

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The present study was conducted to investigate the pathological effects of lipopolysaccharides (LPS) in broiler chickens. It was designed to examine the impact of LPS on growth, haemato-biochemical, and immunological parameters in broilers. In the current study, a total of 40, day-old broiler chicks (n=40) were equally divided into four treatment groups: C (control), T1 (Heat stress), T2 (LPS), and T3 (Heat stress + LPS), with 10 birds in each group.There was a significant difference in the concentration of serum IgY among the different treatment groups. The T3 group had significantly lower ($P<0.05$) concentration of serum IgY titre compared to the C, T1, and T2 groups, whereas the T1 group had significantly lower ($P<0.05$) concentration of serum IgY titre compared to the C and T2 groups.The T3 group (Heat Stress + LPS) demonstrated significant physiological and

immunological challenges, including reduced serum IgY levels. These findings highlight the compounded adverse effects of combined heat stress and LPS, leading to systemic stress and compromised health compared to the control and other groups. The T1 group (Heat Stress) also exhibited notable effects, such as reduced IgY immunoglobulins. These changes suggest that heat stress alone contributes to immune suppression and inflammation, albeit less severely than the combined stress in T3 (Heat stress + LPS) group.

TECHNICAL SESSION

Advancement in Genomics, Proteomics and Metabolomics Research (GPMR)

LEAD PAPERS

GPMR-LP-1

Toxicoproteomics of arsenicosisfor identification of biosensorgenes with potential diagnostic and therapeutic applications

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Focus of this lecture is on insights in to molecular pathogenesis, and identification of biomarkers (prognostic/diagnostic) and evaluating therapeutic approaches in chronic arsenic toxicity (arsenicosis) through application of cutting-edge proteomics, transcriptomics and related omics technologies.
Keywords: *Arsenic toxicity; Curcumin; Biosensor genes; DEGs; Therapeutic efficacy; Transcriptome, Proteomics and other Omics technologies*

Omics technologies have transformed our knowledge of biology and the life sciences. These technologies include transcriptomics, proteomics, metabolomics, lipidomics, genomics, and phenomics. Wet-lab experiments, databases, in-silico analysis using bioinformatic tools, and data validation through supplementary and complementary wet-lab experiments, such as immunoblotting and qPCR, depending on the validation requirements, are all examples of the powerful biotechnology tools and techniques that omics technologies employ. Proteins are studied in great detail in proteomics. A very potent technique in protein analysis, proteomics adds protein information regarding the location, ratio, and circumstances of protein expression to gene sequence data. The study of proteomics, or functional genomics, focusses on the proteome, which is far more active than the genome.

Proteomic investigations often gather information about the location, abundance/turnover, post-translational modifications (PTMs), activities, and interactions of proteins in a sample. Researchers may utilise these data to draw conclusions or may be directly interested in them, depending on the study design. Both basic and practical research, proteomics finds use in forensics, illness aetiology, and the identification of biomarkers for disease diagnosis. This talk will concentrate on understanding molecular aetiology, identifying prognostic and diagnostic biomarkers, and assessing treatment strategies for chronic arsenic toxicity (arsenicosis) by utilising state-of-the-art proteomics and associated omics technologies.

Chronic Arsenic Toxicity

Arsenic (As) is a significant environmental pollutant and known human carcinogen. Inorganic and organic forms, as well as various valence states, are all present in arsenic, a metalloid. Inorganic arsenic (iAs) is hazardous; its trivalent (As+3) and pentavalent (As+5) forms have the greatest environmental impact, with As+3 having the highest toxicological potency. Long-term consumption of food and water tainted with arsenic causes chronic arsenic toxicity, or arsenicosis, which is a serious public health issue in many regions of the world, including the Indian subcontinent. Hyperkeratosis and pigmentation are cutaneous symptoms of arsenicosis, a multisystem condition that is linked to an increased risk of skin, bladder, and lung cancer as well as diabetes and cardiovascular disease in humans. Because fish is a superfood that is consumed all over the world, the bioaccumulation of As in aquatic animals, and particularly in fish, poses a threat to human health in addition to the organisms' own lives. Along with identifying low-arsenic-accumulating plants and food fish, bioremediation procedures continue to be the top priorities for disease management in this environment, along with prognostic/diagnostic biomarkers for early detection of arsenic exposure and therapeutic interventions.

Pathophysiological changes in carp *Labeorohita* fingerlings following arsenic exposure

In ecotoxicology and biomedical research, fish are frequently used as models to better understand a variety of complicated disorders and provide hints for creating preventative strategies. The early symptoms and pathophysiological alterations in the carp rohu (*Labeorohita*) after exposure to arsenic were examined in an experimental investigation. For 12 days, fish were exposed to arsenic at levels ranging from 0.0 (control) to 15 ppm. The two most noticeable gross alterations after ≥10 ppm arsenic exposure were cataract and skin lesions (patchy discolouration, or dark and white areas on the skin). Histopathological analyses showed renal tubular degeneration, glomerular necrosis, and fatty degeneration and necrosis of the hepatocytes. Immunoblotting study of stress proteins showed that exposed fish's livers overexpressed Hsp90 at doses of ≥10 ppm. The typical skin lesions and cataract in fish resulting from arsenic toxicity were reported for the first time (NatlAcadSci Lett 38: 315-319, 2015).

Immunomodulatory effect of arsenic on cytokine leads to generalized immune suppression

In order for an organism to survive against invasive pathogens, its immune system is essential. An essential component of the cell-mediated immune response are cytokines. Arsenic weakens the host's immune system and is immunotoxic. Growth retardation, cataract development, ulcerations, and other gross pathologies, including skin and eye diseases, have all been linked to arsenic exposure in several fish species. In *Labeorohita*, we examined the impact of arsenic exposure on the expression of the HSP genes HSP47, HSP60, HSP70, HSC71, HSP78, and HSP90 as well as the immune genes IFN-γ, IL-4, IL-10, IL-12, and complement C3a. Fish exposed to arsenic underwent cytokine and HSP gene expression investigations in their kidney and liver tissues, respectively, using RT-PCR, while HSPs were examined using immunoblotting. The study indicated thatarsenic has a widespread immune-suppressive effect that causes both Th1 and Th2 cytokines to be downregulated. In addition, it causes the HSP genes to be upregulated, which is indicative of arsenic-induced cellular stress. Thus, exposure to arsenic impairs *L. rohita*'s immune system and may make it more vulnerable to pathogen attacks(Fish & Shellfish Immunol 44,43-49, 2015).

5. Identification of potential biomarkers of hepatotoxicity by plasma proteome analysis of arsenic-exposed carp

Early identification of arsenic toxicity in patients is need of the hour; however, because arsenicosis must be detected before severe symptoms appear, sensitive and reliable testing technologies are required. We looked into changes in the plasma proteome of *Labeorohita*exposed to arsenic in order to find biomarkers for arsenicosis. Gel-based proteomics technology was used to examine changes in the plasma proteome. After quantitative image analysis of the 2D proteome profiles, MALDI-TOF/TOF MS and/or LC-MS/MS identified 14 distinct spots, including warm-temperature acclimation related 65kDa protein (Wap65), α-2 macroglobulin-like protein (A2ML) (2 spots), transferrin (TF) (3 spots), and apolipoprotein-A1 (Apo-A1) (6 spots). Apolipoprotein-A1 (Apo-A1) (>10-fold), α-2 macroglobulin-like protein (A2ML) (7-fold), transferrin (TF), and warm-temperature acclimation associated 65kDa protein (Wap65 (>2-fold) were among the highly abundant protein spots found in plasma from fish exposed to arsenic, which suggested liver injury. These proteins together may be helpful indicators of hepatotoxicity and chronic liver disease brought on by exposure to arsenic(J Haz Mat 336, 71-80, 2017).

6. Curcumin protects eye lens against arsenic toxicity

Turmeric's bioactive ingredient, curcumin, shows promise as a treatment for a number of acute and chronic inflammatory conditions. We looked into how arsenic affected eye lenses and assessed curcumin's ability to reduce arsenic toxicity. The protective impact of curcumin as a dietary supplement was assessed, along with the gene expression study of α, β, and γ-crystallins and the fatty acid profile of lens tissues of *Labeorohita* exposed to arsenic. Four groups of fish were given a diet supplemented with curcumin (1.5% and 3%), for seven days before they were exposed to arsenic (5 ppm and 15 ppm) for fifteen days. In the ocular lens of arsenic-exposed groups (given a basic diet), gene expression analysis revealed downregulation of α and β-crystallins, while the groups fed a diet supplemented with curcumin showed negligible changes.The concentration of saturated fatty acids and docosahexaenoic acid (DHA) was found to have decreased in the fatty acid fingerprinting of lens

lipids from the arsenic-exposed group. Nonetheless, the fatty acid profile of carps exposed to arsenic and provided a meal supplemented with 3% curcumin stayed constant. In carps exposed to arsenic, the concentration of one antioxidant, non-fatty acid compound (phenol 2,4-bis 1,1 dimethyl; PD), which was found in the GC-MS fingerprinting through the NIST library (version 2.2, 2014) search, dropped; however, in groups supplemented with curcumin, PD returned to normal, demonstrating curcumin's potential as a treatment. Curcumin may protect eye lenses against arsenic toxicity, according to the study's findings(Biol Trace Elem Res 199:3354-3359, 2021).

Identification of Low-Arsenic-Accumulating Food Fishes for Aquaculture

One of the primary ways that humans are exposed to arsenic is through food, such as farmed fish. Arsenic builds up in a variety of tissues in fish raised in contaminated environments. Quantifying the danger associated with various farmed fish species in aquaculture systems contaminated with arsenic is crucial. Twelve fish species gathered from arsenic-contaminated aquaculture systems - *Labeorohita*, *L. catla*, *Cirrhinusmrigala*, *Oreochromisniloticus*, *O. mossambicus*, *Liza tade*, *Puntius javanicus*, *L. calbasu*, *Glossogobiusgiuris*, *Macrobrachiumrosenbergii*, *Ctenopharyngodonidella*, and *Bellamyabengalensis* (gastropod) were tested for arsenic. *C. idella* was found to collect the least amount of arsenic (<0.05 ±0.00 mg kg-1) among the finfishes under study, while *O. mossambicus* accumulated the most (1.0 ±0.18 mg kg-1).

All of the fishes under study, however, had low estimated carcinogenic and non-carcinogenic risks to humans. The target hazard quotient (THQ) value for low-arsenic-accumulating fishes (arsenic conc. < 0.5 mg kg-1) ranged from 0.05 to 0.27 for youngsters, while it ranged from 0.01 to 0.08 for adults. Because raising low-arsenic-accumulating food fishes would also reduce the danger of human exposure through the food chain, *C. mrigala*, *C. idella*, and *M. rosenbergii* could be suggested as candidate species for aquaculture in arsenic-contaminated areas. This strategy would guarantee both food safety and nutritional security (Biol Trace Elem Res 200(6):2923-2936, 2022).

Toxicogenomics of arsenicosis and identification of biosensor genes

Liver transcriptomics was carried out employing NextGen sequencing for deeper investigations ontoxicogenomics of arsenicosis and biomarker discovery. A number of differentially expressed genes (DEGs) were discovered. These genes include those that function as biosensors and can detect/sense, the presence of arsenic and curcumin, simultaneously. Apo variantsand proteoforms were discovered by transcriptomic analysis. We discovered some Apo variants that are elevated in carp exposed to arsenic and that, in a dose-dependent manner, return to their basal expression level in animals fed curcumin supplements, thus indicating promise as diagnostics(communicated).

To conclude, thebiosensor genesidentified employing toxicogenomic and proteomic investigations show promise as potential biomarkers and could be useful in computingdose of curcumin for therapeutic interventions in arsenicosis.

GPMR-LP-2

Post-translational modifications (PTMs) of milk proteins in different goat breeds

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Introduction

Post-translational modifications (PTMs) of milk proteins in geographically diverse goat breeds play a crucial role in modulating the structure, function, and bioactivity of these proteins, influencing both milk quality and its nutritional value. PTMs include a variety of chemical changes that occur after protein synthesis, affecting protein folding, stability, and interactions. In the context of goat breeds, environmental factors, dietary habits, and genetic diversity across different regions can result in distinct PTM profiles in milk proteins. Goat milk is leading in the area for nutraceutical formulation and drug development using goat mammary gland as a bioreactor. Goat milk has unique chemical, biochemical, physical and nutritional characteristics, and has higher digestibility and lower allergenicity over cow milk. Post-translational modifications (PTMs) of milk proteins contributed to their biological functions and their compositional complexity. Post-translational modifications in proteins are crucial for the activity state, localization and protein-protein interactions. Therefore, molecular diversity of goat milk proteins needs to be explored to identify posttranslational modifications for studying potential biological role and protein–protein interaction.

Objectives

The study of PTMs in milk proteins across geographically diverse goat breeds is essential for understanding the variations in milk quality, nutritional value, and processing characteristics. These modifications, shaped by genetic and environmental factors, could provide insights into optimizing dairy production for specific regions, enhancing the bioavailability of essential nutrients, and developing functional foods with targeted health benefits.

Methods

Goat milk samples were collected from 1240 animals belonging to 10 goat breeds/ genotypes during the postpartum days 30–65. Milk samples were collected from natural habitats of the breeds belonging to different geographical and agro-climatic regions of India. Samples were collected between 7:30 and 9:00 hrs. by hand milking. After disinfecting the udder, 30–40 ml of milk was collected directly to the collection tubes, transported to the laboratory at 4ºC within 24 hrs. and stored at -20ºC. Milk subsamples for protein analysis were stored at -40ºC until further analysis. The milk protein variants were analysed by SDS-PAGE. Nano-liquid chromatography mass spectrometry (nLC-MS/MS) was used with minor modifications for PTM analysis. All the identified milk proteins were assigned their gene symbol via the Uniprot knowledgebase (http://www.uniprot.org/). Protein classification of the identified proteome and PTM genes sub-set were performed based on their functional annotations using Gene Ontology (GO) for biological process, subcellular localization and molecular function using the Database for annotation, visualization and integrated discovery (DAVID) version 6.8. The enrichment was performed with Homo sapiens database in background for the identified gene names and measured by fisher exact test in DAVID system.

Results

In the present study, we have identified phosphorylation occurring in S, T, Y, D amino acids and other posttranslational modifications as carboxymethylation, oxidation and acetylation. The phosphosites on Asp residues have been reported for the first time in goat milk. The present study focused on obtaining a comprehensive profile of the PTM sites of goat milk casein and non-casein protein and their interaction. Proteomics as a tool have been employed for the discovery, and

characterization of posttranslational modifications such as phosphorylation and oxidation. Electrospray ionization (ESI) mass spectrometry (MS) is suitable for studying PTM, including phosphorylation and glycosylation, since the technique provides molecular mass determination of native proteins. In this study, we analysed the goat milk proteins to identify both casein and non-casein PTM sites using nLC-MS/MS. We processed distinct protein spots by mass spectrometry for identification of phosphorylation, oxidation, acetylation and caramidomethylation. A peptidome of 201 peptide sequences with posttranslational modifications identified 86 proteins/120 UniprotKB accessions. The phosphorylation site identified on the amino acids serine, threonine, tyrosine and aspartic acid was 128, 70, 42 and 47, respectively. Serine showed highest affinity for phosphate group in the present study and confirming earlier reports. Phosphorylation has been well characterized in the bovine caseins. There are various reports targeting casein fractions in bovine and non-bovine milk by various proteomic approaches. The majority of bovine caseins exist in a phosphorylated form, and the phosphorylated residues vary from individual variants possessing one phosphorylated residue (κ-CN) to 13P for others (αS2 CN). It has been demonstrated that high αS1CN-8P concentration in bovine milk is a great benefit for the production of uncooked curd cheese because αS1CN-8P is hydrolysed more efficiently by chymosin during ripening. Bovine proteome analysis showed more than 30 phosphorylated proteins, which included 5 CSN2, 15 CSN1S1, 10 CSN1S2 and 4 CSN3 casein components. Similarly, donkey milk showed 11 CSN3, 6 CSN1S1 and 3 CSN1S2 casein components.

Goat milk proteins have been analysed using MS/MS. The present study resulted in 105 phosphopeptides from casein and non-casein proteins from all the analysed samples. The conserved peptide sequence of (SSSEE) in casein was also observed in CSN1S1 and CSN1S2 at 1P, 2P and 3P. The phosphosites were identified in casein and whey proteins and on 45 other low abundance proteins. The total number of phosphosites observed in themajor milk proteins associated with S, T, Y and D residues were 32, 18, 11 and 21, respectively. The phosphorylation sites observed for CSN2, CSN1S1, CSN1S2, and CSN3 were 11P, 13P, 17P and 6P, respectively. However, whey proteins BLG showed 19 phosphosites (7D, 3Y, 5T, 4S) and LALBA showed 4 phosphosites (3D, 1Y). The identified phosphopeptides resulted in 12P, 8P, 11P, 5P, 13P and 4P isoforms of CSN1S1, CSN1S2, CSN2, CSN3, BLG and LALBA, respectively. Beta casein showed highest degree of variation in phosphorylation with identification of 17P sites and other PTM such as oxidation in the identified peptides.

Casein and whey proteins are post translationally modified by proteolysis by the milk enzymes, formation of disulphide bond by oxidation of cysteine, differential phosphorylation levels of serine and threonine, and glycosylation of threonine residues. The phosphorylation degree of αS-casein is a prime factor affectingthe technological properties of milk. Therefore, “signature peptides” and “caseome” analysis are being used to investigate adulteration in milk of different species. The identification of cheese from different species has been authenticated by proteolytic peptides. Therefore, proteome analysis of fermented milk products should be carried out due to their nutritional and health economic importance.

The present study reported 45 non-casein phospho proteins assigned to various metabolic pathways. The identified PTM sites varied in milk samples of different goat breeds. Identification of low abundance proteins in milk is difficult as single step analysis fails to detect a large proportion of these proteins. Moreover, to overcome the limited entries in the caprine database, other reference database were used for identification of low abundance proteins. The varying levels and sites of phosphorylation in different breeds may be attributable to various physiological or environmental conditions under the influence of different agro-climatic regions. The other posttranslational modifications such as acetylation, oxidation, and carbamidomethylation have also been reported. In the present study, the protein-protein interaction was analysed to know about the functional properties of proteins. The GO annotations were analysed using DAVID, network and interactions using Cytoscape and STRING and pathways analysis using Reactome database. The keratin proteins interacted with trypsin (PRSS1) and histone proteins interacted with CREB binding protein (CREBBP). The identified phosphoproteins were associated with lactation, response to stress, histone modification, cornification and signalling pathways. It has been reported that caseins are associated with other secreted calcium (phosphate)–binding phosphoproteins, such as osteopontin, in milk.

Protein phosphorylation is vital for the regulation of metabolism, proliferation, inflammation, apoptosis, signalling and other important physiological processes. Autophosphorylation increases the catalytic efficiency of the receptor and provides binding sites for the assembly of downstreamsignalling complexes. Caseins form micelles, which vary from species to species, and when cleaved, generated bioactive peptides, having potential functions making them protein of interest. This gastrointestinal degradation may be the consequence of enzymatic hydrolysis, fermentation and other processes used in dairy production.

The identification and characterization of phosphorylation sites are required to explore signalling networks of milk proteins. Phosphorylation-site provides definitive information on functional relationships between signalling proteins. The peptides released by enzymatic hydrolysis have specific biological functions due to their functional and interactions at cellular level. The identified phospho bioactive peptides were mainly anti-microbial followed by ACE inhibitory, DPP-IV inhibitory, proliferating functions. Anti-oxidative, antioxidant, anxiolytic andhypocholesterolemic peptides were also confirmed from goat milk proteins. Non-bovine milk, for their health potential, economic value and the bioactive components/peptides, the milk protein fractions are being extensively investigated.

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GPMR-LP-3

Understanding brain tumors through proteomics: The evolution of brainprot knowledgebase

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The BrainProt database is a groundbreaking initiative designed to further the Human Brain Proteome Project (HBPP) by integrating transcriptomic and proteomic data with curated literature on brain tumors, Alzheimer's disease, Parkinson's disease, Multiple sclerosis, and other major neurological disorders. With six major domains, BrainProt offers comprehensive data visualization and analysis tools, including the Human Brain Disease Atlas (HBDA), Brain Disease Transcriptome Map (BDTM), and Brain Disease Proteome Map (BDPM). These resources integrate multi-omics data, aiding in the identification of biomarkers and the selection of therapeutic drugs. Additionally, BrainProt's Brain Disease Marker Curator (BDMC) and Brain Disease Drug Finder (BDDF) aggregate over 3000 markers and 1500 clinical trials from public knowledgebases. Leveraging robust backend technologies like Apache and Django, and advanced frontend tools such as React and Visx, BrainProt aims to accelerate the discovery of novel biomarkers and therapeutic strategies, thereby advancing our understanding of human brain diseases.

https://www.brainprot.org/

GPMR-LP-4

Unraveling maternal lineages and genetic diversity in Indian livestock: Insights from mitochondrial DNA Analyses

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In recent years, the erosion of genetic diversity in livestock has become a prominent global issue. This trend is largely driven by the preference for highly specialized breeds, increased mechanization in agriculture, the neglect of native breeds, and various other unforeseen regional factors. To ensure the sustainable preservation of farm animal diversity, a multifaceted strategy is essential. Such a strategy should include identifying and documenting unrecognized populations, defining and recording distinct breeds, identifying breeds at risk, and implementing focused conservation initiatives. Effective management of animal genetic resources requires a detailed molecular analysis of genetic variability within and between breeds. Genetic characterization entails examining DNA sequences to detect polymorphisms, which can then be used to create marker systems and genetic linkage maps in animals. These polymorphisms, reflecting allelic variations, are valuable for inheritance studies and are pivotal in population and phylogenetic research. Microsatellite and mitochondrial markers are frequently utilized in livestock diversity studies.

Mitochondrial DNA (mtDNA) serves as a widely-used genetic marker for investigating genetic variation and evolutionary relationships at both intra- and interspecies levels. Found in all eukaryotes, mitochondria are essential for cellular energy production through oxidative phosphorylation, and they play roles in apoptosis, signaling, metabolic regulation, and the synthesis of key macromolecules. Each cell typically contains numerous copies of mtDNA, averaging about 500. In animals, mtDNA is a circular molecule that includes 22 tRNA genes, 2 rRNA genes, and 13 protein-coding genes. The non-coding regulatory region, commonly known as the D-loop or displacement loop, is crucial for mtDNA replication and transcription. With its highly polymorphic segments, referred to as hypervariable regions (HVI and HVII), the D-loop is instrumental in evolutionary research within vertebrates. Due to its higher level of diversity compared to other parts of the mitochondrial genome, the D-loop is a preferred target for phylogenetic analyses among closely related organisms or species. Polymorphisms in mitochondrial DNA have been invaluable in identifying genetic variations within and between breeds and species, offering profound insights into maternal genetic diversity across various livestock species and revealing polyphyletic origins in species such as cattle, buffalo, goats, sheep, horses, camels, and pigs.

Advantages of mitochondrial DNA markers

Mitochondrial DNA (mtDNA) markers offer several distinct advantages in genetic analysis. Since mitochondrial genes are present as single copies, they eliminate the need to account for paralogous genes, thereby simplifying genetic studies. The haploid nature and maternal inheritance of mtDNA enable clear lineage tracing without the complexity of biparental inheritance. Furthermore, mtDNA exhibits a high mutation rate and lacks recombination, which enriches it as a source of genetic diversity. The substitution rate in mitochondrial genes is generally higher than that in nuclear genes, providing a more rapid evolutionary clock useful for evolutionary studies. This high level of variability within and between species enhances its utility in phylogenetic research. Additionally, mitochondrial gene content is highly conserved across animal species, making it ideal for comparative studies. The absence of introns and minimal intergenic regions in mitochondrial genes further streamline genetic analyses. Lastly, the conserved sequences flanking the control region enable the efficient design of PCR primers, supporting a wide range of genetic investigations.

Insights from genetic studies on Indian livestock using mitochondrial markers

Pigs: Genetic investigations on Indian pigs using mitochondrial markers have revealed significant insights into the evolutionary and domestication history. Globally, mitochondrial genome data from

pigs has identified two primary clades, E and A, representing European and Asian lineages in domestic and wild pig populations, respectively. Studies focused on Indian pig breeds, specifically Agonda Goan, Doom, Ghoongroo, Manipuri Black, Niang Megha, Mali, Banda, Ghurrah, Purnea, Nicobari, Tenyi Vo, WakChambil, Andaman, and the non-descript Ankamali breed from Kerala demonstrate that Indian pigs are represented in both Clades E and A. Indian pig populations exhibit a range of haplogroups and sub-haplogroups, such as A2, A1b, A1a2, D1a1, D1a1a, D1b, D1d, D2, D3, and D4. Importantly, genetic analyses revealed distinct separation between wild and domestic Indian pig populations, with no shared haplotypes, suggesting pronounced genetic differentiation likely due to long-term reproductive isolation shaped by ecological and behavioral differences (Sharma *et al.,* 2023). Extensive phylogeographic analysis of wild boars across global datasets suggested that India may be a pivotal region in pig domestication, as evidenced by identical mitochondrial haplotypes in both wild and domestic pigs. This hypothesis was further strengthened by studies comparing modern and ancient DNA of *Sus scrofa*, which have identified unique ancestral haplotypes in Indian pig populations, highlighting the genetic influence of wild boars on local domestic breeds. Overall, these findings supported the idea of a separate domestication event in India, suggesting that ancient pig lineages may have dispersed from Southeast Asia through the Indian subcontinent, eventually extending into Eurasia and Western Europe.

Donkeys: Research on Indian donkeys using mitochondrial markers has provided valuable insights into their genetic diversity and lineage. A recent study examined complete mitochondrial genomes from four distinct Indian donkey breeds/populations: Halari, Kachchhi, Spiti, andAgra donkeys. Additionally, the genetic relationship between Indian donkeys and international populations was investigated by analyzing a segment of the mtDNA D-loop region (Ahlawat *et al.,* 2023). The haplotype diversity (Hd) and nucleotide diversity (π) indices for Indian donkeys, based on this D-loop fragment, were consistent with diversity levels observed globally, highlighting substantial maternal genetic diversity. Indian donkeys were found to share haplotypes with those from a range of countries, including China, Egypt, Italy, Ethiopia, Nigeria, Peru, the Balkan Peninsula, and Albania, pointing to the historical role of donkeys in global trade and movement. Phylogenetic analyses, utilizing both Neighbor-Joining (NJ) trees and Median-Joining (MJ) networks, situated Indian donkeys within Clades I and II, which are typically linked to African wild asses. The comprehensive mitogenome analysis thus indicated that Indian donkeys likely descended from African wild asses, rather than from Asian wild asses, as their primary ancestral source.

Camels: Camels play an essential role in India's desert ecosystems, with dromedary camels inhabiting the hot deserts and Bactrian camels adapted to the colder regions. The mitochondrial DNA diversity within Indian camel populations has been analyzed and compared with global patterns across the three primary camel species: *Camelus ferus*, *Camelus bactrianus*, and *Camelus dromedarius*. A specific mtDNA segment, encompassing part of the cytochrome b gene, the Thr and Pro tRNA genes, and the beginning of the control region, in 72 dromedary and 8 Bactrian camels from India was analyzed(Satyanarayana *et al.,* 2022). Results showed that Indian dromedaries exhibited higher levels of haplotype and nucleotide diversity (Hd: 0.937, π: 0.00431) than the global averages for both dromedary and Bactrian camels. Signs of population expansion in Indian dromedaries were observed through mismatch distribution and Fu's Fs statistics. Analysis of molecular variance highlighted that 92.15% of genetic variation was between the dromedary, wild Bactrian, and domestic Bactrian camels, indicating distinct maternal lineages. The study further supported the division of Old World camels into three distinct mitochondrial lineages: *C. bactrianus* (Lineage A), *C. ferus* (Lineage B), and *C. dromedarius* (Lineage C).

Goats: A comprehensive analysis was conducted on the genetic diversity and population structure of 28 recognized goat breeds and 5 lesser-known populations across India's four major agro-climatic zones (Diwedi *et al.,* 2020). This study focused on the hypervariable region 1 of the mitochondrial control region in 443 goats, alongside 22 reference sequences, uncovering 341 unique haplotypes that fell into four maternal haplogroups: A, B, C, and D. Haplogroup A was dominant, representing 90% of the sampled animals. High genetic diversity in Indian goats was evidenced by haplotype and nucleotide diversity indices of 0.998 ±0.001 and 0.028 ±0.001, respectively. Demographic analyses, including mismatch distribution, suggested spatial or demographic expansion within Indian goat

populations. Analysis of molecular variance (AMOVA) and the structure of the Median-Joining (MJ) network indicated a lack of phylogeographic structuring among Indian goats, likely due to influences such as unrestricted breeding, reduced grazing areas, and migratory herding. Genetic differentiation among goats from diverse agro-ecological zones appeared to correlate with geographic proximity.

Sheep: The genetic diversity and population structure of 11 indigenous sheep breeds: Rampur Bushair, Poonchi, Changthangi, Bonpala, Tibetan, Shahbadi, Balangir, Kenguri, Bandur, Hassan, and Bellary from three agro-ecological regionshave been examined using mtDNA markers (Sharma *et al.,* 2020). The study analyzed a 1,246 bp fragment of the ovine mitochondrial control region, which includes part of the 12S rRNA coding region and the tRNAPhe gene, across 222 samples. A meta-analysis further included sequences from previous studies to cover all four agro-ecological regions (North temperate, North-western arid and semi-arid, Southern peninsular, and Eastern) and 10 reference sequences for the five global sheep haplogroups (A, B, C, D, and E). The Median-Joining (MJ) network revealed that Indian sheep grouped into three distinct clusters, aligning with haplogroups A, B, and C. Population expansion in Indian sheep breeds was suggested by Fu's Fs, Tajima's D, and goodness-of-fit indices, with mismatch distribution fitting a sudden expansion model across all four agro-ecological zones. AMOVA results showed that only 1.77% of the total genetic diversity was attributed to variation among groups, 6.79% to variation among breeds within groups, and 91.44% to within-breed variation, suggesting minimal genetic structuring based on geographical distribution among Indian sheep.

Buffalo: The genetic diversity and evolutionary origins of India's riverine and swamp buffalo populations have also been explored using complete mitochondrial genome sequencing. Comprehensive sampling was conducted across diverse agro-climatic zones, including 91 riverine buffaloes from 12 distinct breeds and 6 non-descript populations, as well as 16 swamp buffaloes of the Luit breed. By applying next-generation sequencing, the study mapped the mitochondrial genetic landscape of these buffalo subspecies (Ahlawat *et al.,* 2024). Aligning sequences with the buffalo mitochondrial reference genome enabled the identification of mtDNA variations and distinct maternal haplogroups within Indian buffaloes. Phylogenetic analysis clarified the genetic relationships of Indian buffaloes to global haplogroups, classifying Indian swamp buffaloes primarily in the SA haplogroup and identifying the SB2b haplogroup in swamp buffaloes for the first time. Riverine buffaloes clustered into known sub-haplogroups RB1, RB2, and RB3, underscoring northwestern India's significance as a potential domestication center for riverine buffaloes. These results supported the concept of independent domestication events for riverine and swamp buffaloes, emphasizing the importance of genetic analysis in tracing the evolutionary history of domestic animal species.

Horses/ponies: The maternal genetic diversity of six indigenous Indian horse and pony breeds: Bhutia, Kathiawari, Manipuri, Marwari, Spiti, and Zanskarihas been assessed through a detailed mitochondrial genome analysis. DNA samples from 53 horses across various Indian agro-climatic regions were examined, revealing 36 unique haplotypes, with a haplotype diversity of 0.889 and nucleotide diversity of 0.00347, indicating substantial maternal genetic diversity in these equine breeds. A median-joining (MJ) network, constructed from the hypervariable D-loop region alongside Indian equine sequences from NCBI, identified 55 haplotypes, with some shared across 2–5 breeds. Hierarchical AMOVA analysis showed that 95.20% of genetic variation existed within populations, while only 4.80% was distributed among groups, pointing to minimal genetic structuring by geographic region. Phylogenetic analysis incorporating global sequences highlighted notable genetic variability without distinct geographic clustering, suggesting considerable gene flow and interbreeding across regions. The median-joining network based on D-loop sequences positioned Indian horses within seven of the 18 globally recognized haplogroups (A, B, G, J, L, M, and P), with haplogroup A being most prevalent. These findings enhance understanding of equine genetic diversity, reflecting global patterns of high maternal haplotype diversity, and shed light on the complex genetic backgrounds shaped by historical breeding practices.

Conclusion

The use of mitochondrial DNA markers in genetic studies has greatly enhanced our knowledge of the phylogenetic relationships, population dynamics, and evolutionary backgrounds of numerous

livestock species. These markers offer valuable insights into maternal lineages, reveal migration and domestication patterns, and support efforts to conserve genetic diversity. With the continuous advancements and increasing accessibility of sequencing technologies, mitochondrial DNA's role in genetic research is expected to grow, providing deeper and more detailed perspectives on the evolution and domestication of animal genetic resources.

Suggested readings

Ahlawat S, Sharma U, Arora R, Sharma R Chhabra P, Singh KV and Vijh RK (2023) Mitogenomic phylogeny reveals the predominance of the Nubian lineage of African wild ass in Indian donkeys. *Gene* 880: 147627.

Ahlawat S, Sharma U, Chhabra P, Arora R, Sharma R, Singh KV and Vijh RK (2024) Maternal genetic diversity and phylogenetic analysis of Indian riverine and swamp buffaloes: Insights from complete mitochondrial genomes.*Mammalian Genome*https://doi.org/10.1007/s00335-024-10048-1.

Diwedi J, Singh AW, Ahlawat S, Sharma R, Arora R, Sharma H, Raja KN, Verma NK and Tantia MS (2020) Comprehensive analysis of mitochondrial DNA based genetic diversity in Indian goats. *Gene* 756: 14490.

Satyanarayana DS, Ahlawat S, Sharma R, Arora R, Sharma A, Tantia MS and Vijh RK (2022) Mitochondrial DNA diversity divulges high levels of haplotype diversity and lack of genetic structure in the Indian camels. *Gene* 820: 146279.

Satyanarayana DS, Ahlawat S, Sharma R, Arora R, Sharma A, Tantia MS and Vijh RK (2021) Genetic differentiation of Indian dromedary and Bactrian camel populations based on mitochondrial ATP8 and ATP6 genes. *Animal Biotechnology*https://doi.org/10.1080/10495398.2021.1990079.

Sharma A, Ahlawat S, Sharma R, Arora R, Singh KV, Malik D, Banik S, Singh TR and Tantia MS (2023) Tracing the genetic footprints: India's role as a gateway for pig migration and domestication across continents. *Animal Biotechnology* DOI: 10.1080/10495398.2023.2268683.

Sharma R, Ahlawat S, Sharma H, Sharma P, Panchal P, Arora R and Tantia MS (2020) Microsatellite and mitochondrial DNA analyses unveil the genetic structure of native sheep breeds from three major agro-ecological regions of India. *Scientific Reports*10: 20422.

TECHNICAL SESSION

Advancement in Genomics, Proteomics and Metabolomics Research (GPMR)

ORAL PRESENTATIONS

1-GPMR-OF-1

Complete genome sequence analysis of sheep-origin bluetongue virus serotype 4 from India and its comparison with global isolates

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Bluetongue (BT) disease is caused by the bluetongue virus (BTV) which affects both domestic and wild ruminants. The BTV genome is composed of 10 segments, encoding seven structural and four nonstructural proteins, with VP2 and VP5 serving as the primary and secondary serotype-specific structural proteins. The virus isolate WGV103/ABT/HSR was obtained from sheep during an outbreak in Andhra Pradesh in 2008. Viral nucleic acid (dsRNA) was extracted from the virus cultured in BHK-21 cells, and the complete genome was sequenced using, Ion Torrent platform. The genome sequences were submitted to GenBank database with accession numbers KF560417.1 to KF560426.1. Segment 2 showed highest similarity with eastern topotype viruses from India (IND2015/K4, nt/aa: 98.36/98.95%) and China (YTS-4, nt/aa: 91.5/96.8%). Likewise, segment 6 closely matched with eastern viruses from India (IND2015/K4, nt/aa: 98.84/99.62%) and China (YTS-4, nt/aa: 90.8/99.0%). The phylogenetic analysis also revealed similar result. Segment 5, aligned with the western topotype viruses from South Africa, USA and several Indian isolates and showed maximum identity (nt/aa >99/99.8%) with Indian isolates. The remaining segments aligned with the eastern topotype viruses from India, Australia, and other countries. Consequently, WGV103/ABT/HSR was identified as a reassortant BTV4 strain.

GPMR-OF-2

Identification of heme-binding aminoacyl tRNA ligases and prediction of their heme-binding motifs in *Haemonchuscontortus*

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Excess of free heme is toxic. Therefore, hematophagous and hemo-parasites (including *Haemonchuscontortus*, economically the most important Gastrointestinal parasite of small ruminants) must regulate their level of free heme during hematophagy. The toxicity of free heme is mediated by its binding to and subsequent inhibition of various cellular components. Aminoacyl-tRNA ligases (a.k.a. aminoacyl-tRNA synthetases) are ubiquitous enzymes, whose products amino acid-charged tRNAs are essential for protein synthesis. Free-heme binds and inhibits some classes of aminoacyl-tRNA ligases, reducing the efficiency of protein synthesis, in various organisms, including the hemo-protozoan parasite *Plasmodium falciparum*. Furthermore, therapeutics-mediated augmentation of free heme concentration in hemo-parasites, such as *P. falciparum, is* also suggested to inhibit the aminoacyl tRNA ligases and add to the anti-parasitic tool kit, which may also prove a possible therapeutic strategy in *H. contortus*. However, the range of heme-binding tRNA ligases in *H. contortus* (*Hc*-HB-AA-tRNA ligases) is unknown yet. The current work focuses on the first ever identification of *Hc*-HB- AA-tRNA ligases and their heme binding motifs (HBMs) from the putative heme-binding proteins (putative-*Hc*HBPs) in the hematophagous stages of *H. contortus* using a coupled method of isolation of heme binding proteins by hemin-agarose chromatography, and peptide

mass finger printing. Out of the 49 minimum possible isoforms of various aminoacyl tRNA ligases of *H. contortus*(asavailable in the UniProtKB data base), 12 aminoacyl t-RNA ligases of the parasite were found to be heme-binding. Phylogenetic analysis of the *Hc*-HB-AA-t-RNA ligases reveals lack of sequence conservation among them, despite their very similar catalytic function. Estimation of HBMs without considering their solvent accessibility revealed the co-domination of H- based class V (XXXXHXXXX) or the Y-based class VII (XXXXYXXXX) motif, followed by the C-based class I (XXXXCXXXX) in the *Hc*-HB-AA-tRNA ligases. In contrast to the general assessment of all possible HBMs, estimation of the solvent accessible transient HBMs, using the web-based SeqD-HBM algorithm along with Weighted Ensemble Solvent Accessibility (WESA) predictor tool, revealed concealing of most HBMs in the interior of the proteins but the domination of H- based class V (XXXXHXXXX), followed by the Y-based class VII (XXXXYXXXX), and the C-based class I (XXXXCXXXX) motifs for the HBMs remaining on the surface of the *Hc*-HB-AA-tRNA ligases. The presence CP-based class III (XXXXCPXXX) and CP+H-based class IV (XXXXCPXXH) motifs (the so-called heme responsive motifs) on the surface or interior of few of the *Hc*-HB-AA-tRNA ligases indicate them not only to be heme-binding proteins but also to be possible heme-regulated aminoacyl tRNA ligases, which may be involved in heme-responsive gene regulation in the parasite.

GPMR-OF-3

Predicting potential epitopicaminoacid pockets of superoxide dismutase to scavenge reactive oxygen species generated during oxidative stress

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The oxidative stress in both eukaryotic and prokaryotic cells occurs due to the generation of reactive oxygen species like superoxide radicals, hydrogen peroxide radicals and hydroxyl radicals due by miscellaneous agents that cause alteration in mitochondrial functioning. The generated reactive oxygen species damage the vital biomolecules like proteins, lipids, nucleic acids of cells creating aberrations. Superoxide dismutaseare the group of enzymes present universally in all eukaryotic and prokaryotic cells that act as antioxidants to nullify the effect of these reactive oxygen molecules. Three types of superoxide dismutase occur in humans' *viz.,* Cu-Zn superoxide dismutase, Mn superoxide dismutase and superoxide dismutase3. The superoxide dismutase can be administered as drugs for the treatment of oxidative stress. In the present study an extensive computational analysis has been doneto identify B- and T-cell epitopic region of superoxide dismutase molecules of wide range of sources.The nucleotide and amino acid sequences of wide range of eukaryotic and prokaryotic organisms were retrieved from the NCBI server followed by comparative phylogenetic and alignment sequence analysis. I*n silico* analysis was conducted for analysisof variability, relative solvent accessibility, and three-dimensional structuresprediction for all the retrieved sequences and the variations were observed. The epitopic regions consisting of functional aminoacids coded by nucleotides were analysed by Epitope Prediction and Analysis Tools. Top five linear B-cell and T-cell epitopes have been identified.Molecular docking was carried out with the selected epitopic regions with the ligands like superoxide, hydrogen peroxide and hydroxyl radicalsusing HADDOCK and SWISS MODEL server web server and the model quality further validated in PROCHECK server and SPDBV (Swiss PDB Viewer). Then molecular dynamics simulation was carried for the identified targets. The process is helpful in identifying the universal conserved sequence(s) of amino acids reflecting potentially strong free radical scavengingcomponent generated during oxidative stress and can be used as therapeutics in the form of alternative medicine for the same.

GPMR-OF-4

Characterization of mucins associated with the typical fern patterns of buffalo saliva at estrus

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The aim of the present study was to identify the molecules associated with the formation of the typical salivary fern pattern in buffaloes.Saliva samples were collected from 2 Murrah non-pregnant female buffaloes two times a day, before feeding, for the 10 consecutive estrous cycles. Smears were made from saliva and were observed under a compound microscope for salivary fern patterns. Dried buffalo saliva smears containing typical and atypical fern pattern areas were scratched and the scratched powders were subjected to SDS – PAGE, followed by either silver staining or Coomassie brilliant blue staining. To identify the proteins expected to have Post – Translational Modifications (PTMs), MALDI MS/MS analysis was done from the SDS-PAGE gel plugs for two protein bands representing typical fern-like patterns and two protein bands representing atypical fern patterns. Staining the gel revealed that, two band shifts were observed in the SDS - PAGE, one wasat 130 kDa molecular weight and the other was observed at 55-70 kDa molecular weight. The protein associated with the formation of a typical fern pattern was found to have a higher molecular weight as compared to the same proteinassociated with the formation of an atypical fern pattern. Analysis of four protein bands by MALDI MS/MS identified that Mucin – 1 (MW: 58 kDa) was the common protein found in the solutions of scratchedpowder of typical and atypical fern patterns. Considering a band shift at the 58 kDaregion SDS – PAGE gel was stained with ‘stains all’ dye and it was found that the protein associated with the formation of the typical fern pattern was glycosylated in nature as compared to the same protein which was associated with the formation of atypical fern pattern. In conclusion,it was observed that the typical, as well as atypical salivary fern patterns at the estrus stage in buffalo, are formed by different proteins. But Mucin 1 was the common protein involved in the formation of both type of salivary fern patterns. But the Mucin 1 associated with the typical salivary fern patterns were glycosylated whereas, the Mucin 1 associated with the atypical fern patterns were not, which was confirmed by the staining.

GPMR-OF-5

Recombinant expression of canine Perilipin 4: A step toward functional characterization

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Lipid droplet (LD)-associated proteins are critical regulators of lipid metabolism and cellular energy homeostasis. Perilipin 4 (PLIN4), a member of the perilipin protein family, is primarily associated with LD formation and lipid storage in adipose and non-adipose tissues. Despite its biological significance, PLIN4 has been largely unexplored in canines, limiting our understanding of its functional roles in health and disease. This study aimed to clone and express the canine PLIN4 gene to enable further molecular and functional investigations. Total RNA was isolated from canine adipose tissue, and cDNA was synthesized for use as a template in PCR amplification of the canine PLIN4 gene. The canine PLIN4 sequence was retrieved from the NCBI database, and specific primers with restriction enzyme sites for EcoRI and XhoI were designed to amplify a partial coding sequence

(CDS) of 771 bp. Using RT-PCR, the 771 bp amplicon of canine PLIN4 was successfully amplified, cloned into the prokaryotic expression vector pET32a, and transformed into DH5-α cells. The recombinant plasmid was confirmed through sequencing and subsequently introduced into the E. coli expression strain BL21. Protein expression was induced with 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) at 37°C for 6 hours. Following incubation, SDS-PAGE analysis revealed the expression of a recombinant protein with the expected molecular weight of 47 kDa. The identity of the expressed protein was further confirmed through western blotting using anti-His and anti-PLIN4 antibodies. This study successfully cloned and expressed the canine PLIN4 protein, providing the groundwork for future studies on its structure-function relationship and involvement in lipid metabolic disorders.

GPMR-OF-6

Quantitative analysis of P2RX7 transmembrane pore dynamics using molecular dynamics simulations and structural insights

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P2RX7 is a non-selective, cationic transmembrane receptor activated by extracellular ATP (eATP), playing a key role in both acute and chronic inflammatory responses. This receptor is distinctive within the purinergic family due to its requirement for high eATP concentrations for activation and its delayed desensitization. These unique features enable P2RX7 to mediate dual effects, including cytotoxicity and cytoproliferative. Structurally, the transmembrane domains (TMD1-α1 and TMD2-α6) form a pore that facilitates intracellular accumulation of multivalent cations upon eATP activation. Key residues, such as S339 and S342, define the pore's outer and inner boundaries. Previous studies have demonstrated that membrane cholesterol enrichment inhibits P2RX7 activation and pore formation, likely through alterations to membrane properties and receptor dynamics. However, the precise influence of cholesterol on the conformational dynamics and pore behavior of P2RX7 remains underexplored. In this study, we constructed P2RX7-POPC lipid bilayer models with varying cholesterol concentrations and performed all-atom molecular dynamics (MD) simulations. To assess pore dynamics, we analyzed residue distances (S339-S342 across chains A-B, B-C, and A-C) and the radius of gyration of the TMD pore. Our findings revealed that while open forms of P2RX7 exhibited slight changes in S339-S342 distances—particularly at cholesterol concentrations below 30%—closed forms were unaffected. Similarly, the radius of gyration for TMD2 (residues 332-357) and TMD2 (residues 332-345) did not show significant differences in compactness across cholesterol levels. These results suggest that P2RX7 pore dynamics might require longer simulation times to fully capture the conformational changes, as pore dilation occurs on microsecond-to-second timescales. Additionally, the limited conformational sampling inherent in classical MD simulations may restrict exploration of high-energy states. Alternative methods such as enhanced sampling techniques or quantum mechanics-based simulations may provide deeper insights into the cholesterol-dependent modulation of P2RX7 dynamics.

GPMR-OF-7

Role of *fnr* and *narL* genes in the *in vitro* Virulenceof *Salmonella* Typhimurium

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Salmonella Typhimurium (STM) belongs to the *Enterobacteriaceae* family and is a gram-negative bacterium of zoonotic significance. In order to successfully colonize and infect the host cell in a micro-aerophilic environment, it must switch from aerobic to anaerobic respiration. Since poultry, cattle, and pigs are thought to be significant STM reservoirs, the primary mechanism of transmission of salmonellosis is through food products such meat, eggs, and milk. By using the lambda-red recombination method to delete the *fnr* and *narL* genes from its genome, we created a double mutant of STM, which has been examined for a number of virulence and colonization determinants. When it comes to the metabolic transition from aerobic to anaerobic respiration, these two STM genes are crucial. A comparative glucose uptake study by using a fluorescent glucose analog 2-NBDG, revealed that significant decrease was seen mainly in wild type to prevent the phago-lysosomal acidification. In the *in vitro* bacterial burden assay, wild type STM was replicating significantly higher compared to the double mutant. The RT-qPCR assay of many of the glucose metabolism genes (GLUT1, Mondo A and AMPK, aldolase A), immune related genes (IFN-γ, TNFα, IL-1β, IL6, IL10, NF-kβ and MHC-II) showed that the virulence of the double mutant STM got decreased significantly. These results were also supported by ELISA assays at protein level. The SPI-I (*sptP*) and SPI-II (*sspH2* and *srfH*) genes, necessary for entry and replication of the *Salmonella* Typhimurium inside the host, were slightly upregulated in double mutant as compare to wild type for its necessary survival. Hence these findings suggest that *fnr* and *narL* genes are necessary for the survival, replication and mainly virulence of the wild type *Salmonella* Typhimurium.

GPMR-OS-1

The molecular effects of Lipopolysaccharide (LPS) and heat stress on broiler Gut

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The present study was conducted to investigate the molecular effects of lipopolysaccharide (LPS) and heat stress on the broiler gut, focusing on the stimulation of pro-inflammatory cytokines such as TNF-α, IL-1, and IL-6. A total of 40-day-old broiler chicks (n=40) were equally divided into four treatment groups: C (control), T1 (heat stress), T2 (LPS), and T3 (heat stress + LPS), with 10 birds in each group. The study was conducted over 18 days, during which all groups were fed a basal ration. During the last 7 days, the birds were subjected to heat stress, and on the final day, they received an oral LPS dose of 2 mg/kg body weight.Gene expression analysis revealed significant differences in pro-inflammatory response genes compared to the control group. The T3 group had the highest upregulation of TNF-α, IL-1β, and IL-6 mRNA expression across the duodenum, jejunum, and ileum. The T1 group also showed increased TNF-α and IL-1β in the duodenum and ileum, while the T2

group exhibited increased TNF-α and IL-1β in the duodenum. IL-6 expression was significantly higher in the duodenum, jejunum, and ileum for all treatment groups, with the T3 group showing the greatest increase.Thus, it may be concluded that the T3 group (heat stress + LPS), which exhibited the most pronounced adverse effects, including a substantial upregulation of IL-6, IL-1β, and TNF-α across the duodenum, jejunum, and ileum, demonstrated significant immunological challenges, indicating compromised intestinal integrity. This indicates a severe compounded inflammatory response when both stressors are present.

GPMR-OS-2

Protective effect of cineole against tartrazine-induced pancreatic toxicity in male Wistar rats: A biochemical and molecular investigation

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Now days synthetic food dye are being used most commonly as food colorant in food and pharmaceutical industry. The present study, first of its kind in literature, aimed to observe the toxic effects of tartrazine, on the pancreas, and investigate whether this toxicity could be eliminated with co-administration of cineole in male wistar rats for 30 days.For this study, 32 male wistar rats were procured from "Disease Free Small Animal House, LUVAS, Hisar". The rats were divided into 4 equal groups: Group I: control, group II:tartrazine @100mg/kg b.w., group III: cineole @100 mg/kg b.w., and Group IV: tartrazine + cineole. Rat blood samples and pancreas tissue were obtained and biochemical and molecular examinations were conducted on the samples.The results showed that tartrazine administration at dose100 mg/kg body weight, increased level of glucose, pancreas specific enzymes (amylase, lipase), lipid peroxidation,tumor necrosis factor-α and ratio of BAX/BCl2 gene and decreased the level of insulin and antioxidant parameters(glutathione peroxidase) in blood serum and pancreas tissue, leading to inflammation and apoptosis.On the other hand, co-administration of cineole for 30 days at dose 100 mg/kg body weight improved these alterations.Administration of cineole at this dose and time has a protective effect against tartrazine induced pancreatitis in wistar rats. Cineole can be used as a protective agent against tartrazine toxicity.

GPMR-OS-3

Transcriptomic insights into pulmonary adaptation of Indian sheep breeds in diverse environments

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Transcriptome profiling of lung tissues from two genetically diverse sheep breeds—the Changthangi, native to the high-altitude cold deserts of Ladakh, and the Muzaffarnagri, adapted to the low-altitude plains of northern India—was performed to investigate the molecular mechanisms underlying pulmonary adaptation in Indian sheep. Lung samples from four healthy rams of each breed were collected and processed for RNA extraction, sequencing and analysis. High-quality reads (Phred

score ≥ 30) were mapped to the *Ovis aries* reference genome (Oar v4.0), with 98% of the reads aligning successfully. Differential gene expression analysis identified 53 significant genes (adjusted p-value < 0.05; fold change > 1), with 26 upregulated genes in Changthangi sheep and 27 upregulated genes in Muzaffarnagri sheep. Functional annotation revealed that the upregulated genes in Changthangi sheep were predominantly enriched in immune-related processes, including cytokine and chemokine activity.These genes were associated with cellular structures such as inclusion bodies, endocytic vesicles, and F-actin binding protein complexes. Key genes, including *CXCL10*, *CXCL9* and *MMP12*, were identified as pivotal players in NF-kB signaling, protein metabolism, and immune responses. Further gene network analysis revealed a strong interplay between cytokine signaling, the hypoxia-inducible factor (HIF) pathway and NF-kB signaling, suggesting a coordinated response that enables Changthangi sheep to adapt to high-altitude hypoxic conditions. In contrast, upregulated genes in Muzaffarnagri sheep were enriched in processes related to lung structure and function, including collagen biosynthesis and carbohydrate metabolism. Genes such as *COL1A1, NR4A2* and *MYH7* demonstrated involvement in extracellular matrix structure, cell adhesion, and energy metabolism. Structural genes such as *ACTC1, COL1A1* and *MYH7* were central to the network in Muzaffarnagri sheep. Real-time qPCR validation of selected genes (*MEGF6, EPAS1, MMP12, CA3, ACTG2, COL1A1, SPARC, PRDX6*) confirmed RNA-seq findings, with consistent expression patterns. These results provide novel insights into the genetic basis of pulmonary adaptation, emphasizing immune and structural mechanisms unique to high-altitude and low-altitude environments. It improves our understanding of how livestock adapt to different ecological conditions and offers a basis for future research to enhance animal health and performance in challenging environments.

GPMR-OS-4

Validation of identified micro-RNA (miRNA) in NGS study of mammary gland tissue from Indian Goat Breed during different stages of lactation

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Micro-RNAs (miRNAs) are non-coding single-stranded RNA molecules which play important role in mammary gland development and lactation physiology. This study was done with the objective ofvalidation of identified up-regulated micro-RNAs and analysethe expression patterns of these miRNAs in previous study of Next-Generation Sequencing (NGS) data of mammary gland tissue from Indian goat breed maintained at DUVASU Goat Farm at different stagesoflactation. Selection of the five up-regulated miRNAs was done as per standard criteria. Then validation of identified5 up-regulated miRNAswas done using linear poly(A) tailed RT-PCR using SYBR Green and the expression pattern of chosen miRNAs were analyzedusing ΔΔCt quantification method.The results demonstrated the dynamic changes in miRNA expression pattern and two microRNAs chi-miR-29a-5p (logFC=-0.528) andchi-miR-378-5p (logFC=-0.793) out of selected five up-regulated miRNAs from NGS study showed significant downregulation pattern. Bioinformatic predictions and pathway analysis indicated that up-regulated miRNAs might regulate genes involved in casein synthesis, lipid metabolism, and mammary tissue remodelling. Notably, several up-regulated miRNAs were associated with pathways related to milk production, cell proliferation, and immune response.Thus the validation of identified genes associated with lactation and expression profiling of miRNA may highlight new pathways that regulate lactation physiology of goat. It may serve as molecular markers for improving dairy goat productivity through breeding and management practices or can be used in over-expression studies or knockdown studies.

TECHNICAL SESSION

Advancement in Genomics, Proteomics and Metabolomics Research (GPMR)

POSTER PRESENTATIONS

GPMR-PF-1

Trend of circulating leptin hormone and some other biochemical Indices level, polymorphism of LEP and LEPR gene in Hariana cows

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Leptin is 16 kDa polypeptide hormones which plays an important role in regulation of feed intake, energy metabolism, lactogenesis, growth,reproduction and immune functions. The present study was executed to elucidate the trend of plasma leptin hormone and some other metabolitesduring periparturient period and genetic polymorphismby PCR-RFLPassay using *BsaAI and BseGI* restriction enzyme in LEP and LEPR genes, respectively and their associations with production and reproduction traits in Hariana cows. Blood samples collected from 6 pregnant Hariana cows from -30 days prepartum to +90 days postpartum at 15 days interval for biochemical study and from 62 lactating cows for studying genetic polymorphism in LEP and LEPR genes. Biochemical study revealed sharp reduction in plasma leptin and glucose concentration at calving and then gradual increase during lactation whereas plasma urea remains low during pregnancy and then rose after calving. Lipid profile remains normal during prepartum period and increases near term except triglycerides which remains high during pregnancy and become lowest just after calving. The PCR-RFLP analysiscapable of revealing genetic polymorphism in LEP and LEPR gene. LEP/*BsaA*I assay exhibited AA, AB and BB genotypes with 9.67, 54.8 and 35.5 % genotypic frequency, respectively and its association study revealed significant influence of these genotypes on gestation period, dry period, lactation period, total milk yield, milk yield at 300 days, peak yield and days to reach peak yield. Besides, LEPR/*BseG*I assay indicated CC, CT and TT genotypes with 8.06 %, 87.09 % and 4.83 % genotypic frequency, respectively and revealed significant influence of these genotypes on gestation period, lactation period, total milk yield and milk yield at 300 days.

GPMR-PF-2

IGF-1Gene polymorphism and its association to anestrus in Murrah buffaloes

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Many studies reported the role of IGF 1 in reproduction,but not much information is available how the genetic polymorphism of IGF 1 genes play role in regulation of reproduction and anestrus. Present study was carried to investigate the correlation between IGF 1 gene polymorphism and anestrus condition in female Murrah buffaloes. For this study, 100 anestrus buffaloes in three different categories, viz., delayed pubertal (PA), 1stand 2nd lactation (PPA 1), and 3rd lactationand above (PPA 2)were selected from both field and farm (ICAR-CIRB Hisar) conditions. Their anestrus status was confirmed and venous blood was collected for isolation of genomic DNA. The 481bpfragments of theIGF-1 genes present in the genomic DNA were amplified by using the bovine primers designed in-house.RFLP analysis of PCR amplified products was then performed. The genomic DNA samples obtained from all 100 female Murrah buffaloes were of high quality as evident

by their260/280 nm absorbance ratioranged from 1.7 to 1.9. PCR – RFLP analysis shown that restriction enzyme HhaI yielded 481bpsize of amplifiedgene product for IGF 1 gene. Present investigation,through the *in silico* studies,showed a total of ten variations in gene sequence or single nucleotide polymorphism for IGF 1 gene, which included four in PA, three in PPA 1 and three in PPA 2 categories. Our study concluded that there is a correlation between IGF 1 gene polymorphism and anestrus condition in female Murrah buffaloes.

GPMR-PF-3

Gene Ontology (GO) analysis of Bikaneri and Jaisalmeri camel milk proteins

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Camel milk is considered one of the most valuable food sources for nomadic people in arid and semi-arid areas. It has also been consumed as a natural adjuvant for managing various human diseases for centuries due to its high nutritional value and extraordinary medicinal properties. 180 milk samples were collected from Bikaneri and Jaisalmeri camels (90 samples each)from Rajasthan for the proteomic analysis of camel milk using LC-MS/MS.The protein pellets were prepared from 50 mlof pooled raw milk. The trypsinization and clean-up protocol were performed using standard methods.Then, Mass spectrometric analysis of peptide mixtures was performed using the EASY-nLC 1200 system coupled to a Thermo Fisher-Q Exactive equipped with ananoelectrospray ion source. All samples were processed and analyzed with Proteome Discoverer (v2.4) against the UniProt camel reference proteome database. A total of 704 protein groups were identified in the milk of Bikaneri and Jaisalmeri camels onthe UniProt *Camelus dromedarius* Database. There were 21 (3%) and 17 (2.4%) unique protein groups with common 666 (94.6%) proteins in the milk of Bikaneri and Jaisalmeri, respectively.283 biological processes, 329 molecular functions and 170 cellular components of milk proteins were identified by gene ontology (GO) annotation. Translation [GO:0006412]- 23%, carbohydrate metabolic process [GO:0005975]- 20%, cell adhesion [GO:0007155]-12%, complement activation classical pathway [GO:0006958]- 10%, protein folding [GO:0006457]- 10%, glycolytic process [GO:0006096]-7% were the major GO- biological processes in camel milk. ATP binding [GO:0005524]- 16%, calcium ion binding [GO:0005509]- 14%, metal ion binding [GO:0046872]- 11% and serine-type endopeptidase inhibitor activity [GO:0004867]- 11% was identified as significant GO- molecular functions in camel milk. Likewise, the endoplasmic reticulum lumen [GO:0005788]- 20%, keratin filament [GO:0045095]- 17% and cytoplasm [GO:0005737]- 16% were the chief GO- cellular components of the Bikaneri and Jaisalmeri camel milk.

GPMR-PF-4

Investigation of mutation pattern linked to benzimidazole resistance in *Haemonchus contortus* parasite

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Tick-borne hemoprotozoan parasitic diseases are rare in sheep and goat. Theileriosis in sheep caused by protozoan parasites of the genus *Theileria*, particularly *Theilerialestoquardi*, *Theileriaovis*, and *Theileriahirci*. *T.lestoquardi* is highly pathogenic species causing high morbidity and mortality in sheep. Reports of this parasite in India are exceedingly rare, with none originating

from Rajasthan.This study reports an outbreak of *T. lestoquardi* in a sheep flock in Tonk, Rajasthan. The animals have a history of fever, anorexia, prostration, and laboured breathing. Of the 120 sheep, 18 adult sheep died owing to conditions over a one-month period. The investigation of the flock found weakness, tick infestation, severe jaundice, anaemia, and circling movement. The haematobiochemical study indicated anaemia, reduced protein levels, and elevated liver enzymes. For molecular diagnosis, unique primers for three distinct genes of *Theileria* were utilised: the merozoite surface protein gene, the cytochrome b gene, and the SSr RNA gene. The product of PCR were cloned and sequenced to identify the causative agent. The sequencing of the three genes verified *T. lestoquardi* as the etiological agent responsible for mortality in the sheep herd. This is the first report documenting the infection of *T. lestoquardi* in sheep in Rajasthan. The sequencing findings demonstrated around 98-99% similarity with other sequences accessible on the NCBI site. The evolutionary study revealed a close similarity between the current strain and the Hissar strain.The molecular confirmation of *T. lestoquardi* in Rajasthan for the first time will enhance disease surveillance and diagnosis, while underscoring the rising threats to animal health in this region of the nation. The sequencing analysis will aid in comprehending the biology and evolution of this virulent strain of parasite.

GPMR-PF-5

Absence of C>T polymorphism in exon 6 region of*PIT1*gene in Indian Barbari goats

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The *PIT1* gene, also known as POU class 1 homeobox 1 (*POU1F1*) and *GHF1*, is a pituitary-specific POU-homeodomain transcription factor that plays a crucial role in mammary gland development and milk yield. *PIT1*gene has involvement in a network of transcriptional regulation of hypothalamo-hypophyseal factors, including prolactin and growth hormone.In the present study, investigation of C>T polymorphism in exon 6 region of Pit1 gene was undertaken in 100 Barbari goats, maintained at University Goat farm, DUVASU, Mathura, focusing on the impact of these variations on milk production and composition traits. Amplification of *PIT1*revealed 450 bp product and *Pst*I restriction digestion screening showed monomorphic pattern. Only one type of genotype, namely, TT (450 bp) was observed in population. The frequency of TT genotypes was 100% in all screened samples with T allele (1.0). The results revealed that *PIT1* allele seems to be fixed in screened Barbari goat population. Sequencing confirmed absence of C>Tsubstitution at the *Pst*I site, validating the monomorphism. Consequently, we could not perform the association study of this substitution. These SNP may be usefulindicators in selective breeding initiatives in milch goats, though further research across larger, more diverse populations is recommended to refine selective breeding strategies for improved growth and milk production output in goats.

GPMR-PS-1

Genetic polymorphism of 1-acylglycerol-3-phosphate-O-acyltransferase 6 (*AGPAT6*) gene and their association with milk production traits in Barbari goat

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Goat husbandry is vital for sustainable agriculture, providing milk, meat, fiber, and manure. The present study investigated the genetic polymorphism within the 1-Acylglycerol-3-phosphate O-acyltransferase 6 (*AGPAT6*) gene in Barbari goats, maintained at University Goat farm, DUVASU, Mathura, focusing on the impact of these variations on milk production and composition traits. *AGPAT6* plays a critical role in lipid biosynthesis,was amplified and analysed using PCR-RFLP techniques, producing a 372 bp fragment that was electrophoresed and documented. Digestion of the*AGPAT6* gene with*Eco*RII enzyme revealed three types of genotypes including AA, GA, and GGshowing polymorphic patterns with allele frequencies of 0.315 and 0.685 for A for G, respectively. Among the 100 samples, the heterozygous GA genotype was most frequent (49%), followed by GG(44%) andAA (7%). Sequencing also confirmed the G→A substitution at the*Eco*RII site, validating the polymorphism. Hardy-Weinberg equilibrium was analysed indicated studied population was in HW equilibrium (χ^2 = 1.836, P<0.05).Although AGPAT6/*Eco*RII polymorphisms showed significant (P<0.05) association with milk yield and composition traits, AA genotype has significant lower milk yield/day and fat% than GA and GG genotypes. These insights support the potential application of marker-assisted selection in goat breeding, though further research across larger, more diverse populations is recommended to refine selective breeding strategies for improved milk production and composition traits in milchgoats.

GPMR-PS-2

Identification of selection signature contributing to high altitude adaptation in Changthangi goat

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The Changthangi goat, native to the high-altitude regions of the Ladakh Plateau in northern India, has evolved to thrive in oxygen-deprived environments typical of elevations exceeding 4,000 meters. To study the genetic basis of high-altitude adaptation in the Changthangi goat, we performed a comparative genomic analysis with the Jamnapari goat, a milch breed from tropical climates, using whole-genome resequencing data from 15 animals of each breed. We employed selection sweep analyses using interpopulation approaches (Cross-Population Extended Haplotype Homozygosity (XP-EHH) and FST). Several genomic regions exhibiting strong selection signatures were detected, suggesting a potential role in high-altitude adaptation.Functional enrichment analysis of the candidate genes revealed significant involvement in processes such aspositive regulation of angiogenesis, cardiac muscle cell development, regulation of vasoconstriction, keratinocyte proliferation.Further pathway analysis indicated enrichment in biological processes related to heart development, blood

vessel development, inflammatory response regulation, calcium ion homeostasis, and calcium ion transport via high-voltage-gated calcium channels.In addition, genes encoding keratins (KRTs) and keratin-associated proteins (KRTAPs) were identified, highlighting their crucial role in cashmere fiber production and contributing to the high-altitude adaptation of the Changthangigoat.The genes identified, including BCL2,CACNG6, MAP3K9, SHISA9, ANGPTL3, NOS2, MAPK10, BCL7C, PIK3CA, ACVR2B,ADGRB3, ADGRG6, are likely integral to the adaptation of the Changthangi goat to high-altitude environments.These findings suggest that genetic adaptations in Changthangi goats are primarily associated with mechanisms that alleviate hypoxic stress and environmental challenges at high altitudes, providing key insights into the biological pathways critical for survival in extreme climates.

TECHNICAL SESSION

Innovations in Biotechnology (Food, Animal, Fish, Environmental and Nano-technology) (IBT)

LEAD PAPERS

IBT-LP-1

Gene-edited zebrafish as a biotechnological tool for advancing food and nutritional security

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CRISPR/Cas9 is a full-proof editing tool for targeted gene modification at desired chromosomal locations. It disrupts a specific gene through the non-homologous end joining (NHEJ) DNA repair pathway, a mechanism prone to errors that typically result in nucleotide deletions, insertions, or substitutions, without the incorporation of exogenous genetic material. Contrary to farmed animals and carps, zebrafish can be bred year-round within laboratory containment facilities and hence serve as an effective model organism having the advantage of rapidly generating gene-edited data for basic and applied biology research. Understanding the molecular mechanisms associated with growth, disease resistance, and reproduction is crucial for improving farmed animals and rohu carp (*Labeorohita*). Myostatin, being a member ofa large TGF-beta family, acts as a negative regulator of muscle growth.There is limited information available regarding the role of negative regulators, such as gonadotropin-inhibitory hormone (GnIH), in the process of gonadal development and maturation in teleosts.It is anticipated that the inhibition of GnIH through gene editing may promote early gonadal maturation. Our objective is to develop myostatin and GnIHedited rohu carp and zebrafish using CRISPR/Cas9 technology to investigate their fundamental physiological functions. We have successfully generated stable lines of GnIH-edited zebrafishes, providing clues on its associated reproductive and growth functions. We have also edited perforin and toll-like receptor 22 (TLR22) to highlight their immunological functions. Additionally, the myostatin gene was targeted aiming towards increased skeletal muscle growth. The detailed results associated with a specific beneficial trait shall be highlighted and discussed

IBT-LP-2

Host-microbiome interaction in acquisition of thermo-tolerance in ruminant

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In the scenario of climate change, increase in surface temperature and humidity has resulted in stressful environment beyond physiological limits of acclimatization leading to heat stress (HS) in ruminant. The global economic loss due to heat stress (HS) is estimated to be $39·94 billion per year by the end of the century. The losses in most tropical and subtropical regions are projected to be far greater than in temperate regions. In India, it is estimated that the losses only in the milk production due to HS will be INR 11.93 billion during 2020-2029. Considering production losses due to HS, increasing population and burgeoning demand of livestock products, a novel and sustainable solution is vital for ensuring food and nutritional security in the changing climate. Several physiological, biochemical and molecular markers of heat tolerance have been identified and have been utilized in manipulating feeding, breeding and management practices to improve productivity of livestock but still more precision is required. Rumen acts as a bioreactor and contributes 70% of energy source as volatile fatty acid and 50% of protein in ruminants by anaerobic fermentation mainly by bacteria and protozoa. HS changes the motility, saliva input, retention time of digesta, temperature, pH and epithelial function in the rumen besides host neuroendocrine and cytokine responses which affect the rumen function. It has been reported that HS alters the microbial population and tends to change volatile fatty acid concentration in buffaloes, and digestibility and methane emission in crossbred cattle. It has been also reported several physiological biomarkers of HS and their thresholds temperature humidity indices (THIs) in different livestock species to identify differential heat tolerance among the species and among the breeds. In recent times study of microbiome and metabolome in heat sensitive and heat tolerant zebu cattle and buffalo phenotypes at different HS conditions have been initiated for identification of microbiome and metabolome-based markers of heat tolerance in cattle and buffalo; and the result may be used to formulate short- and long-term strategies to ameliorate HS. This paper aims to underline the studies carried out in this area.

Climate change and Livestock

The Intergovernment Panel on Climate Change (IPCC) has predicted a rise in the global surface temperature of earth in the range of 1.8–4 °C by the end of 21st century. Over the period of 1880-2012 the global temperature has sown a warming trend of 0.85 °C and a similar trend has also been recorded in India. In many parts of the world, especially in tropical areas, the viability of ruminant production systems is seen as being seriously threatened by climate change. Increase in earth surface temperature and humidity has resulted in a stressful environment beyond the physiological limits of acclimatization leading to heat stress (HS). In recent years increase in incidences of heat waves and future prediction that heat waves will be more in number with increased intensity has exacerbated the adverse effects. The trend of difference in maximum temperature and minimum temperature shows that it is decreasing with time which will certainly affect the thermal gradient between animal and environment and the livestock will suffer with more heat stress issues. Similarly, predictions about the humidity also show that relative humidity will also increase which will worsen the situation by increasing the number of both pathological microorganism and ecto-parasite population. Future trends also suggest that climate change will also affect both the feed and fodder quality and quantity. The global economic losses due to HS is estimated to be $39·94 billion per year by the end of the century and the losses only in the milk production due to HS in India would be INR 11.93 billion during 2020-2029. Livestock provides direct employment to 19.4 million persons and supports the livelihood of two third Indian populations. Therefore, HS indirectly affects the employment generation, livelihood and food security in India. The animal scientists have recommended HS amelioration

strategies including physical protection, feeding regime, and genetic improvement. Supported by the increased animal management costs and innovative molecular genetics technologies, breeding heat-tolerant cows seems to be the best solution and cost-benefit option to alleviate heat stress that would improve animal productivity and welfare.

Physiological Markers of Heat stress

Animals need to be genetically compatible and produce optimally in order to thrive in a variety of adverse conditions in a climate challenging environment.Livestock may adapt by wide range of morphological, behavioural, physiological, biochemical, endocrine, cellular, and molecular responses. The body size, extremity, skin colour, sweat gland and hair coat. The primary response exhibited by an animal to heat stress that assist in homeostasis and heat balance in stressed animals are respiration rate, pulse rate, skin temperature, and rectal temperature. Blood-biochemical (non-esterified fatty acids, hemoglobin, hematocrit, packed cell volume)and endocrine markers (tri-iodothyronine, thyroxine, cortisol) and many more along with cellular responses like heat shock proteins, cytokines, toll-like receptors and apoptotic genes help in maintaining homeostasis in animals (Kumar *et al.,* 2018, Yadav *et al.,*, 2021).

Effect of heat stress and rumen ecosystem

HS reduces dry matter intake, rumination, rumen activity, and reticulo-rumen motility, thus affecting the fractional passage rate of digesta in the gastrointestinal tract. Moderately higher level of HS causes alteration in cortisol, epinephrine, insulin and inflammatory cytokine levels which are reported to change the colonization pattern of microbes. Severe HS causes increase in body temperature which leads increase in rumen temperature (Rt).HS caused corneal damage and detachment of the cornea, increased the space between the granulosa and spinosum cells, and caused significant separation of the layers of the rumen papillae. Furthermore, HS tended to increase the thickness of the cornea and granulosum, but did not affect the thickness of all papillae of the rumen. On the contrary, Yazdi *et al.,.* (2016) reported that papilla height increased dramatically by HS due to an increase in selective dietary intake of cereal grains. It is also reported that expression of Na-$HCO3^-$ co-transport system which is responsible for absorption of volatile fatty acid (VFA) in the rumen papilla is decreased and causing less secretion of $HCO3^-$ in the rumen. Accumulation of VFAs and reduced biocarbonate influx from saliva and rumen papilla causes decreased ruminalpH. Thus, HS affects rumen function directly by reducing DMI, disturbing the rumen motility pattern and passage rate of digesta, decreasing pH and increasing the rumen temperature, and indirectly by perturbing electrolyte imbalance, hormone secretion, antioxidant defense system, metabolism and inflammatory cytokines. Our group has reported that during moderate HS digestibility increases and methane emission decreases whereas an opposite trend was reported during extreme HS in both crossbred cattle and buffalo (Yadav *et al.,* 2016b; Wankar *et al.,* 2021). Kim *et al.,.* (2022) has reviewed the literature related to microbial basis of HS mediated changes in fermentation pattern and metabolome, and correlated with different physiological responses of the host. Different studies show that heat stress has differential effects on both volatile fatty acid production and microbial population which may be a key to a thought that there may be a variation in the microbial population in the heat tolerant and heat sensitive animals.

Rumen metabolome and microbiome as marker of thermotolerance

Fluctuations in the number and composition of rumen bacteria reflect not only changes in the nutrient levels but also changes in the physiological environment of the rumen. Alteration in rumen bacteria in cows may be useful for identifying cows with differential heat resistance. Wang *et al.,.* (2022) reported that the generic taxonomic composition of the rumen microbiota is altered in the light of the differences in heat tolerance. Regarding the differential abundance comparison analysis at the phylum level, it was reported that the bacteria were mainly composed of Firmicutes and Bacteroidetes (the relative abundance > 5%) both in the HT and HS cows. Tenericutes phylum contributes to the food consumption and digestion, and the genera including Rikenellaceae. Ruminococcaceae_UCG-014 and Ruminococcaceae_NK4A214_group and Rikenellaceae can degrade structural carbohydrates and starch in the rumen of dairy cows. These bacteria were found to have elevated abundances in the HT cows may play a crucial role in the basic digestion and metabolic processes in the rumen. In particular, Ruminococcaceae taxa plays an important role in cellulose and hemicellulose degradation

as well as rumen biohydrogenation pathways, since the enhancement of hemicellulose degradation is used to degrade proteins, can improve feed utilization and ultimately improve animal performance. Succiniclasticum and Rikenellaceae_RC9_gut_group were found to be more abundant in the HT cows compared to the HS cows. It is well known fact that propionate production is often associated with lower methane emission. In Chinese sheep a negative correlation between Succiniclasticum and methane emissions was reported. Importantly, Succiniclasticum can produce propionic acid by deacidifying succinic acid, competing with methanogens for hydrogen, and contributing to the attenuation of methanogenesis. Not only Succiniclasticum but also Rikenellaceae_RC9_gut_group, which belong to Rikenellaceae, may contribute to reduce the methanogenesis rate by participating in the VFAs production and hydrogen scavenging. In agreement with this finding, Succiniclasticum and Rikenellaceae_RC9_gut_group were much more abundant in the HT cows with a high propionic content. Taken together, these bacteria likely contribute to yielding propionate in the HT cows, and propionate as a major product in HT cows may underlie the inhibition of methane Production. Previous studies on cattle have revealed that Prevotellaceae were usually dominant in the rumen and associated with propionic acid production. Prevotellaceae and Prevotella_1 were found to be abundant in the HS than HT cows and they were not significantly correlated with propionic acid production. The correlation between heat tolerance, production efficiency of cows and methane emissions on needs to be further explored.

The studies on Rikenellaceae_RC9_gut_group, which belong to the Bacteroidetes phylum, reported acorrelation withthyroxine metabolism (Khakisahneh *et al.,* 2021). Both Rikenellaceae and Rikenellaceae_RC9_gut_group were negatively associated correlated with RR and RT and positively correlated to the production of propionate which may be involved in providing a more effective energy recovery system in the HT cows compared with the HS cows. Propionic acid, a substrate for gluconeogenesis, activated gluconeogenic gene expression to maintain energy homeostasis in HT cows. The studies on dairy goats reported thatChristensenellaceae were linked to immunomodulation and homeostasis. It was also reported that the increased abundance of Christensenellaceae_R-7_group in the HT cows may improve the stability of ruminal ecosystem under heat stress conditions.

Metabolites reflect alterations in the metabolism of dairy cows, which enable a comprehensive understanding of an organism's physiological and biochemical status. Wang *et al.,.* (2022) found that maltose, glycerol, and mannitol contents in the HT cows increased compared with the HS cows. It is likely that the HT cows may minimize the adipose tissue triglyceride mobilization allowing for a stronger ability to alleviate the negative energy balance they suffer from during heat stress. Glycerol intake contributes to improved thermoregulation and heat tolerance ability when humans are exposed to hot environments. Similarly, Kim *et al.,.* (2022) reported that Holstein cows had higher RT and RR than Jersey cows under the heat stress condition of THI of 87.5, providing that the last breed is less susceptible to heat stress, and has more carbohydrate-related metabolic pathway genes. Consistent with previous reports, the current data demonstrate that differential metabolites (glycerol, mannitol, and maltose) were negatively correlated to respiratory rate, since lower respiratory rate indicated to the thermoregulatory ability of the HT cows, which were more advantageous than the HS cows in terms of body heat production and heat dissipation as well as showed better adaptability to harsh environments. Due to the limited information available on the role of specific metabolites in the regulation of heat tolerance, the physiological parameters and differential metabolites registered for the HT cows in the present study are relevant to better understand mechanisms of heat tolerance in dairy cows. It is well accepted that rumen metabolites are associated with changes in bacterial flora. Furthermore, maltose and mannitol were positively correlated with Rikenellaceae_RC9_gut_group. It is worth noting that Rikenellaceae_RC9_gut_group can degrade structural carbohydrates and starch in the rumen of dairy cows, which play a key role in carbohydrate metabolism. This would imply that a shift in the metabolic pathways of the rumen microbes may affect carbohydrate degradation. Under the same dietary level and feeding conditions, the HT cows compared to HS cows may efficiently convert microorganisms in the rumen into VFAs through carbohydrate metabolism to obtain the carbon skeleton of gluconeogenesis and alleviate heat stress by providing the metabolic energy required for rumen microbiota.

Cows with higher rumen temperatures had an altered rumen microbial population (Yadav *et al.,* 2013; Correia *et al.,* 2021, Yadav *et al.,* 2022). This includes the reduction of Fibrobacter succinogenes, Flavoni fractori, Prevotella ruminicola, Rumin-ococcus flavefaciens, and Treponema bacteria. The reduction of these rumen bacteria increased the population of lactic acid bacteria as a result of the amount of substrate suitable for their metabolism. These bacteria produce large amounts of lactate in the rumen, which causes a sharp drop in the pH of the rumen (Correia Sales *et al.,* 2021). In response, the animals consumed minimal amounts of feed, resulting in reduced rumen motility and rumination and changes in the microbial population of the rumen and pH between 5.82 and 6.03. Specifically, the ratio of acetate to propionate decreases during heat stress, leading to individual differences in rumen pH, transit rate, and digestive retention time. Furthermore, reduced rumen function reduces VFA production in the rumen. Meanwhile, previous studies found that plasma lactate levels in dairy cows increase under heat stress conditions. This indicates that higher lactate concentrations in the rumen can lead to increased transport to the blood, negatively affecting animal health. Additionally, HS significantly reduced rumen acetate levels. In this context, lactate and acetate are known to be the main metabolites of fiber and soluble carbohydrates. HS significantly changed feed, and especially the animal consumes more concentrate (rather than forage). Subsequently, a higher percentage of concentrate consumption in the diet may be the reason for changes in lactate and acetate in the rumen of heat-stressed dairy cows. Wang *et al.,.* (2022) reported that the levels of nine metabolites were significantly higher in heat-exposed buffaloes than those under non heat conditions, while the levels of 23 metabolites were significantly lower in the rumen of HS-exposed buffaloes exposed to HS than those of buffaloes exposed to HS. The pH and concentration of acetic acid, propionic acid, butyric acid, and TVFA were decreased in heat-stressed buffalo compared to NHS animals. Most studies observed a decrease in total VFA and acetate concentrations and an increase in butyrate/isobutyrate levels during heat stress in various ruminants. However, heat stress has not been reported to affect the VFA content in cattle in some studies. A change in VFA concentration with varying levels of heat stress has also been reported. Contrary to previous reports, Yadav *et al.,.* (2022) reported that acetate and propionate levels tended to increase and butyrate levels decreased during heat stress in buffaloes, which was also confirmed by the corresponding changes in the abundance of different bacterial populations. It can be hypothesized that the microbial population of buffalo rumen bacteria showed resistance to heat stress and the abundance of fiber-degrading bacteria increased to optimize the production of enzymes for fermentation in the unfavorable rumen environment. Acetate production is directly proportional to methane production; however, Yadav *et al.,.* (2022) reported that despite a minimal increase in acetate production, a decrease in Methanobrevibacter was observed, indicating that the Methanobrevibacter population in the rumen depends not only on substrates but also on environmental stress. Yadav *et al.,.* (2022) concluded that the adaptive responses of the rumen ecosystem cannot cause detectable harm to the fermentation pattern.

Weng *et al.,.* (2024) studied the effect of heat stress on the rumen microbiome and other physiological parameters in lactating Montbéliarde × Holstein and Holstein cows and reported that during heat stress, milk yield, milk fat yield, milk protein yield, milk protein, and milk lactose in Montbéliarde × Holstein cows were higher than those in Holstein cows, whereas milk yield variation and somatic cell counts were lower than those in Holstein cows. The sequencing results indicated that the rumen of Montbéliarde × Holstein cows was significantly enriched with beneficial bacteria, such as Rikenellaceae, Allobaculum, and YRC22. In addition, correlations were observed between specific ruminal bacteria and lactation performance. Ruminal metabolites related to antioxidant and anti-inflammatory properties were higher in Montbéliarde × Holstein cows than in Holstein cows.

Role of microbiome in adaptation in harsh climatic condition

Nguyen *et al.,.* (2023) reported that the ruminalmicrobiome in beef heifers display higher levels of dissimilatory nitrate reduction, whereas the bison rumen microbiome seemed to suppress this pathway. The bison ruminalmicrobiome had higher expression of GDH2, a critical enzyme in central metabolism that diverts carbon skeletons away from nitrogen metabolism toward energy-generating carbon metabolism and volatile fatty acid synthesis. GDH expression was found to be tightly regulated in beef heifersruminal contents, and bison rumen content inoculation had no effect on its expression. It showsthe crucial role of ruminalmicrobiome in the adaptation of ruminants to their

natural diet and environmental factors. Zhang *et al.,.* (2022) collected fecal samples of cattle from four representative climatic regions of China, namely, the mesotemperate (HLJ), warm temperate (SD), subtropical (HK), and tropical (SS) regions and reported that with increasing climatic temperature from HLJ to SS, the abundance of Firmicutes increased, accompanied by an increasing Firmicutes to Bacteroidota ratio. Proteobacteria showed a trend of reduction from HLJ to SS. Patescibacteria, Chloroflexi, and Actinobacteriota were particularly highest in SS for adapting to the tropical environment. The microbial phenotype in the tropics was characterized by an increase in Gram-positive bacteria and a decrease in Gram-negative bacteria, aerobic bacteria, and the forming of biofilms. Consistently, the functional abundances of organismal systems and metabolism were decreased to reduce the material and energy demands in a hot environment. Genetic information processing and information storage and processing may be how gut flora deals with hot conditions. Huang *et al.,.* (2021) studied the rumen microbiome of the ruminants in the harsh high-altitude environment and reported that bacteria was significantly different among ruminant species yak (*Bosgrunniens*), cattle (*Bostaurus*) and sheep (*Ovisaries*), but there were no differences between the indigenous and introduced sheep breeds. At the genus level, *Fibrobacter*, *Lachnospira* and *Pseudobutyrivibrio* were more abundant in the rumen of yak, while *Prevotella* was significantly more abundant in cattle than in the other ruminants; enterotypes affiliated with the uncultured Ruminococcaceae and *Prevotella* was more dominant in the indigenous and introduced ruminants respectively.The results demonstrated thatyak which has evolved as a distinctive species with specialised physiological and anatomical adaptations, has a rumen bacterial population that favours its survival in this extreme environment.Pan *et al.,.* (2024) revealed notable modifications in the control of the rumen transcriptome and rumen microbiota in plateau-acclimated Xizang goats at high altitudes and found that both genes that enhance plateau resistance and those associated with antioxidant activity were upregulated. Furthermore,alterations in the *Papillibacter*, *Quinella*, and *Saccharofermentans* microbiomes were noted, potentially aiding Xizang goats in adapting to the harsh climate conditions on the plateau.

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Sugar code: An introduction to third alphabet of life

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The characteristics of a code are determined by the symbols that are used to create combinations that store information (such as polymers and oligomers). Similar to proteins and nucleic acids, oligo- and polysaccharides are widely distributed and serve as a biochemical substrate for the formation of molecular communications. Notably, the letters of the sugar code system (the third alphabet of life) are exceptionally versatile in their ability to code words due to variations in the anomery and linkage location of the glycosidic bond, ring size, and branching. This immense potential for vocabulary growth is realized by the enzyme machinery for glycan biosynthesis (writers). As seen with proteins and nucleic acids, it offers the potential for dynamic editing and erasure. Based on more than a dozen folds, a sizable panel of sugar receptors (lectins) has evolved to match the diversity of glycomes. The information encoded in glycan is "read" by lectins. Glycan-lectin recognition is selective and particular due to a combination of hydrogen/coordination bonding, ionic pairing, stacking, C-H/π-interactions, and spatial glycan presentation patterns. Glycan display, the nature of the cognate glycoconjugate, and the modular design of lectins all contribute to the high number of post-binding events. They provide a functional, frequently context-dependent meaning or meanings to an entry in the glycan vocabulary, creating the sugar code dictionary.

Introduction

The genetic code is typically viewed as the exclusive embodiment of biological information translated into cellular effects. The molecular foundation for correctly replicating templates and initiating the transfer of biological information is the complementarity between base pairs of nucleotides (Fig. 1). The 'code words' made up of the 'letters' from both nucleotides and amino acids dictate the genetic code, directly linking the two alphabets of life. The order of building blocks in nucleic acids and proteins fully determines the 'message' in each type of oligo- or polymer. It can be inferred from Fig. 2a, b that there are repetitive molecular processes that allow the biopolymer chain to increase, through phosphodiester bonds or peptide bonds.

The circumstances surrounding coding on the cell surface are different, and taking into account its fundamental requirements explains why Fig. 1 needs to be expanded, changing the appearance of the right portion of Fig. 2. When it comes to genetic coding, the density of information given must be high enough for signals on the cell surface because there isn't much room for the wide variety of messages that can be communicated. A distinct class of biomolecule is needed to achieve this. The third alphabet of life must possess the ability to synthesize a far greater number of isomers, or "words," from its structural constituents than are possible with nucleotides or amino acids. As Fig. 2 illustrates, monosaccharides are perfect for this purpose, a basic realization that led "to one of the last great frontiers of biochemistry" (Hart 1992; Cook 1995). The presence of chemically analogous hydroxyl groups makes it easier for different links to create glycosidic bonds, which allow monosaccharides from the anomeric capacity to be bridged.

As a result, the glycome is extremely complex (Laine 1997; Rüdiger and Gabius 2009). Consequently, glycosylation/glycans had to be included to the paradigm of the flow of biological information (Fig. 1, bottom). DNA codes for the proteins (like glycosyl transferases) that assemble the glycans from the monosaccharides, the third alphabet of life.Glycans are present ubiquitously on proteins and sphingolipids. With the contributions to the first part of this special issue, principles of protein and lipid glycosylation are depicted and illustrated by instructive examples of how glycan synthesis shapes distinct aspects of cellular (re) activity (Reuter and Gabius 1999; Kopitz 2009; Zuber and Roth 2009; Corfield and Berry 2015; Gabius 2015; Hennet and Cabalzar 2015; Ledeen and Wu 2015).

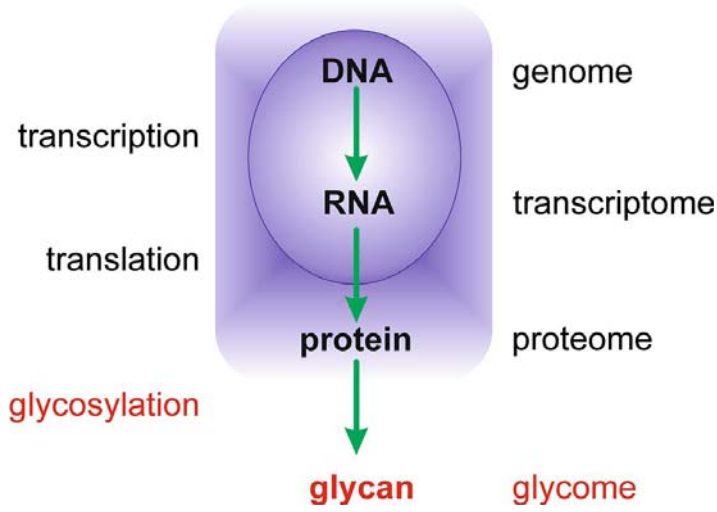

Fig. 1. Flow of biological information was initially confined to the realm of the genetic code. The growing realization of the functional significance of glycans as platform for the sugar code accounts for adding carbohydrate-based coding to the scheme; (from Kaltner and Gabius 2012)

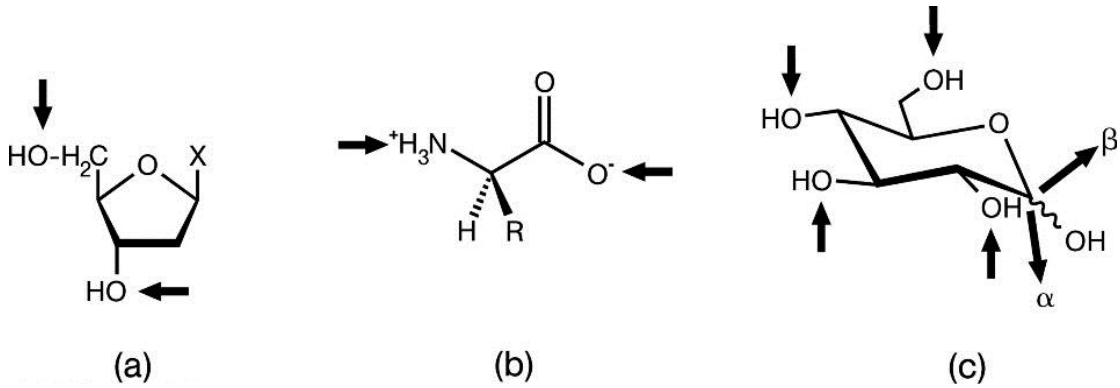

Fig. 2 Illustration of the linkage points of oligomer formation of nucleic acids (a), proteins (b) and glycans (c). In contrast to the letters of the first and second alphabets of life, each carbohydrate unit can engage any hydroxyl group for letting a chain grow or introduce branches, and the configuration at the anomeric center can vary (from Rüdiger and Gabius 2009)

Molecular foundation of sugar code

Glycan chains are made up of units of carbohydrates. This phrase was first used to describe the findings of a simple hexose study. The stoichiometry of Cn(H2O)m with n ≥ m was obtained from them. The common hexose glucose ($C_6(H_2O)_6$) is initially structurally represented by the (Fischer) projection formula of the open chain (Fig. 3a). Energy-wise, a cyclic structure—the hexopyranose, which is a ring with five C atoms and one O atom—is significantly more desirable in solution than the linear form (Fig. 3b). The two substituents of each carbon atom—the H atom and the hydroxyl or hydroxymethyl group—are then shown in both axial and equatorial orientations (Fig. 3c). A new "letter" (epimer) is created when the two locations at carbon atoms C2-C5 are switched, resulting in an isomerisation. This is shown in Fig. 3c,d by illustrating the difference between glucose (Fig. 3c) and galactose, its 4'-epimer (Fig. 3d).

The hydroxyl groups are interchangeable chemically. Thus, each hydroxyl group has the potential to participate in both chain start and elongation. The glycosidic linkage can join a reactive centre with various hydroxyl groups (Fig. 2c), unlike nucleotides and amino acids, whose chains are constructed either by 5', 3'-phosphodiester or by peptide bonds (Fig. 2a,b). The anomeric position (at C1 in Fig. 1) loaded by the activation reaction with an appropriate leaving group is the reactive centreutilised in chemical synthesis and physiological processes (Paulson and Colley, 1989; Schmidt, 1997; Oscarson, 2009; Hennet and Cabalzar, 2015). Notably, each diglycoside can be synthesized to yield its α- or β-anomers by presenting it in two spatial orientations, i.e., in α- or β-forms (Fig. 2c).As a result, there are far more sequence permutations with carbohydrates as building blocks than with

nucleic acids and proteins (Laine, 1997), allowing for variety at anomer and linkage locations. As a result, information may be encoded in the language of sugars at a density that is significantly higher than that of proteins or nucleic acids, and carbohydrates provide more.

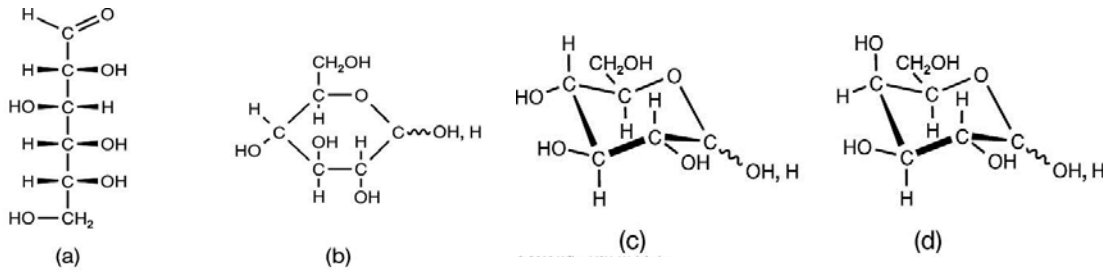

Fig. 3 Illustration of the two types of projection formula and the chair-like conformation of D-glucose. The open-chain (Fischer) (a) and hexopyranose (Haworth) projection formulas (b) as well as the 4C1 low-energy chair-like pyranose conformation (c) are presented. Structural variability at the anomeric center (α or β) is symbolized by a wavy line. Epimer formation from D-glucose (c) to D-galactose (d) leads to the axial positioning of the 4-hydroxyl group in D-galactose and changes in the topological signature of hydroxyl and polarized C-H groups (from Rüdiger and Gabius, 2009).

Considering the aspect of coding capacities, Fig. 3c is highly instructive and deserves a second look. In addition to illustrating sources of sequence variability in chains, it furthermore tells us that glycans cannot only produce linear oligo- and polymers (the first dimension of the sugar code). By bringing in more than two hydroxyl groups of a sugar unit into glycosidic bonding, branched structures are possible (the second dimension of the sugar code). This mode of structural versatility ensures to make oligomers available with high-coding capacity, ideal as signals on the cell surface, and these linear and branched structures are endowed with a special virtue: their spatial flexibility (the third dimension of the sugar code) is often restricted to few energetically privileged conformers (Carver, 1993; Gabius, 1998; von der Lieth *et al.,* 1998; Bush *et al.,* 1999).

Process of encoding of information in sugar code (Writers)

A variety of proteins with distinct functions are needed to create signals based on carbohydrates. Initially, the letters are created, either from food or through activation with the correct nucleotide triphosphate. The resulting product, like CMP-N-acetylneuraminic acid made in the nucleus, typically requires transportation to the location where glycan assembly takes place. During this series of reactions, glycosyl transferases transfer the sugar molecule from a donor, which is usually bound to a nucleotide like CMP, GDP, or UDP, to an acceptor, such as a protein or sphingolipid scaffold, to start the formation of glycoconjugates or to elongate a sugar chain. Evidently, the set of enzymes and transport proteins required for preparing and utilizing activated substrates for glycan production are known as glycogenes, essential for creating sugar-based signals. Furthermore, enzymes that add position-specific substitutions like sulphate groups, as demonstrated in the three structures of Fig. 4bd, are included in this category.

The extent to which scaffolds are used for proteins provides insight into the widespread prevalence of protein glycosylation. The Golgi apparatus, originally described as an "internal reticular apparatus" by Camillo Golgi in Pavia on April 19, 1898, is a key location for glycosylation (Dröscher, 1998). The final result is determined by the combination of enzyme presence and localization, substrate concentrations, availability of sugars and transporters, as well as fluxes along the assembly line (Roth, 1996; Pavelka, 1997; Bard and Chia, 2016). Of course, this system provides numerous opportunities for making dynamic changes to the product profile. Thus, the genetic code does not directly determine the glycome profile. However, transcription can be modulated to create an impact.

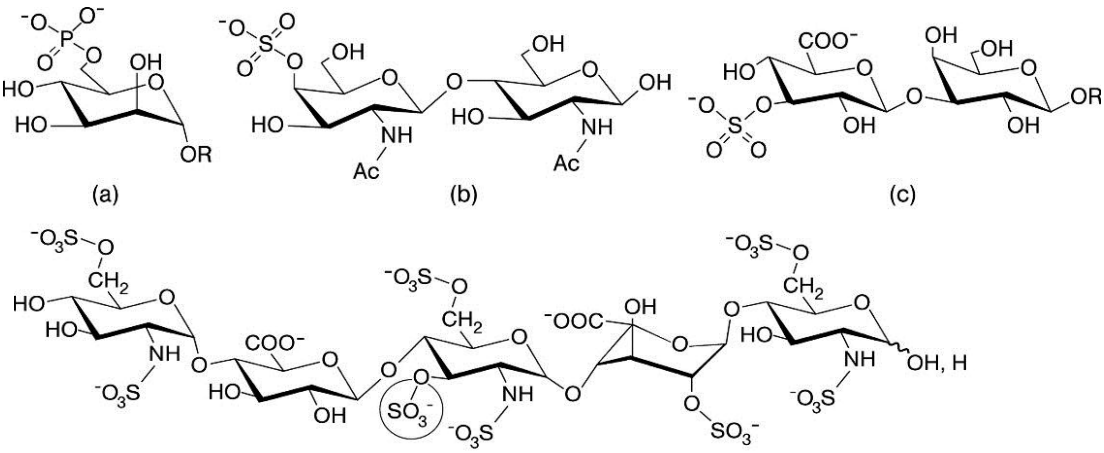

Fig. 4 Illustration of phosphorylated (phosphated) and sulfated (sulfurylated) glycan "words". 6- Phosphorylation of a mannose moiety (in the context of a mannose-rich pentasaccharide) is the key section of a routing signal in lysosomal enzymes (a), 4-sulfation of the GalNAcβ1,4GlcNAc (LacdiNAc) epitope forms the "postal code" for clearance from circulation by hepatic endothelial cells of pituitary glycoprotein hormones labeled in such a way (b), the HNK-1 (human natural killer-1) epitope (3-sulfated GlcAβ1,3Galβ1,4GlcNAc) is involved in cell adhesion/migration in the nervous system (c) and the encircled 3- O-sulfation in the pentasaccharide's center is essential for heparin's anti-coagulant activity (d). All sugars are in their pyranose form. Please note that the central glucosamine unit has N,O-trisulfation and that the 2- sulfated IdoA, given in the 1C4 conformation, can also adopt the hinge-like $^{2}S_{O}$ skew-boat structure (please see Fig. 5; about 60% or more for the 2SO form in equilibrium depending on the structural context) when present within glycosaminoglycan chains of the proteoglycan heparin. 2-Sulfation of IdoA serves two purposes: favoring the hinge-like $^{2}S_{O}$ conformation and precluding re-conversion to GlcA (from Rüdiger and Gabius, 2009).

In the natural world, researchers have discovered 41 connections between eight amino acids and 13 various monosaccharides, forming links in a variety of glycoproteins (Spiro, 2002). After glycoprotein synthesis begins, each stage of chain elongation is performed by a particular glycosyltransferase. Different groups of proteins are encompassed by each family of glycosyltransferases based on their specificity to a particular sugar. Every group shares the ability to speed up the incorporation of the specific sugar in a particular physiological linkage and anomeric sites (Hennet, 2002; Harduin-Lepers *et al.,* 2005; Ma *et al.,* 2006; Takashima and Tsuji, 2011; Aplin and Jones, 2012; Togayachi and Narimatsu, 2012). Total 13 fucosyltransferases have been identified for attaching this moiety to protein (in O-fucosylation) and to glycans in α1,2, α1,3/4 and α1,6 connections. The process of mucin-type O-glycosylation begins with 20 enzymes known as UDP-N-acetyl-α-D-galactosamine: polypeptide N-acetylgalactosaminyltransferases in humans (Bennett *et al.,* 2012; Raman *et al.,* 2012). Carbohydrates' unique benefits for compact coding come from the intricate glycan biosynthesis machinery. To create the array of mammalian glycans, which is expected to consist of more than 7000 structures, around 500-700 proteins are predicted to collaborate (Moremen *et al.,* 2012; Cummings and Pierce, 2014; Neelamegham and Mahal, 2016). The varying structural complexity of the sugar component in glycoproteins and -lipids spans from a single carbohydrate unit (found in O-GlcNAcylated proteins) to penta-antennary (complex-type) N-glycans with branch extensions through N-acetyllactosamine repeats.

In the routes of mucin N- and O -type glycosylations, a series of branch points facilitates to let synthesis go through various pathways (Brockhausen and Schachter, 1997; Zuber and Roth, 2009; Gabius *et al.,* 2016). Which route is taken to what extent can rapidly be (re)directed by the molecular switches mentioned above, that is from transcriptional regulation to substrate fluxes, and switches also affect the glycan chains after the 'writing' process has ended. If a sugar code is operative, then we can expect glycan signals to be dynamically processed by erasers.

Biological alteration of sugar code (erasers)

One puzzling aspect of protein glycosylation is the shared source of diverse N-glycans, derived from the lipid-linked Glc3Man9GlcNAc2 14-mer glycan. Deleting a large part of it raised the query: "Is Nature inefficient?" (Gahmberg and Tolvanen, 1996). During entry into the endoplasmic reticulum

(ER), N-glycan is attached cotranslationally to nascent polypeptides at asparagine amide's nitrogen in a specific sequon, and then progressively trimmed by glucosidases and mannosidases while traveling to the Golgi apparatus (Roth, 2002; Zuber and Roth, 2009). The initial glucose residue linked at α1,2 is quickly removed (half-life less than 2 minutes) to advance the process and prevent the oligosaccharyltransferase reaction from being reversible. The subsequent trimming process creates a monoglucosylated signal checked by molecular chaperones, and the subsequent shortening of the glycan is necessary for the proper transportation of glycoproteins for their redesign in Golgi cisternae (Brockhausen and Schachter, 1997; Roth, 2002; Roth and Zuber, 2017). Removing branch extensions gradually until reaching a core heptasaccharide allows for the creation of complex- and hybrid-type N-glycans. This process demonstrates eraser functionality, which affects all N-glycans of glycoproteins before they mature in the Golgi apparatus. Therefore, a significant amount of the N-glycan that was initially moved serves its (temporary) purpose only while passing from the ER to the Golgi. When the stable core successfully completes its quality control mission, erasers create space for new signals and functions of glycans (Varki, 2017).

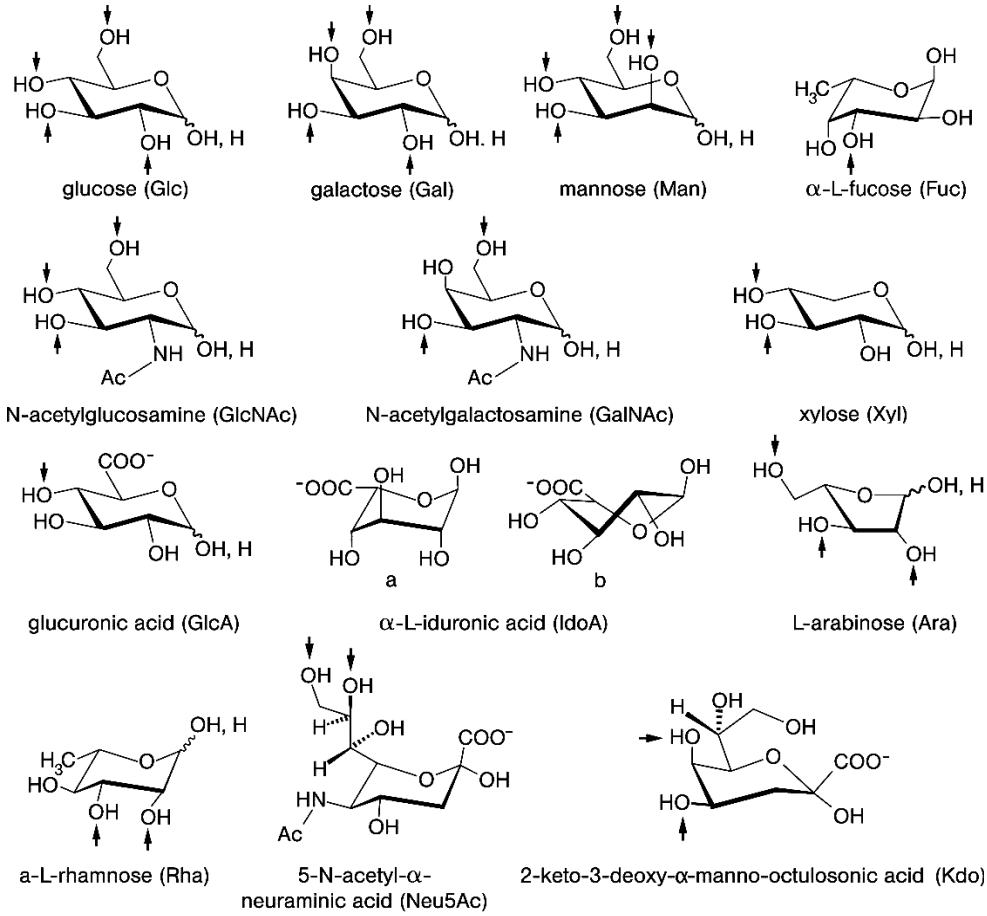

Fig. 5 Illustration of the alphabet of the sugar language. Structural representation, name and symbol as well as the set of known acceptor positions (arrows) in glycoconjugates are given for each letter. Four sugars have L-configuration: fucose (6-deoxy-L-galactose), rhamnose (6-deoxy-L-mannose) and arabinose are introduced during chain elongation, whereas L-iduronic acid (IdoA) results from post-synthetic epimerization of glucuronic acid at C-5. The 1C4 conformation of IdoA (a) is in equilibrium with the 2SO form (b) in glycosaminoglycan chains, where this uronic acid can be 2-sulfated. All other "letters" are D-sugars. Neu5Ac, one of the more than 50 sialic acids, often terminates sugar chains in animal glycoconjugates. Kdo is a constituent of lipopolysaccharides in the cell walls of Gram-negative bacteria and is also found in cell wall polysaccharides of green algae and higher plants. Foreign to mammalian glycobiochemistry, microbial polysaccharides contain the furanose ring form of D-galactose and also D/L-arabinose indicated by an italic "f" derived from the heterocyclus furan. The α-anomer is prevalent for the pentose arabinose, e.g. in mycobacterial cell wall arabinogalactan and lipoarabinomannan. β1-5/6-Linked galactofuranoside is present in the arabinogalactan and the β1-3/6 linkage in lipopolysaccharides (from Rüdiger and Gabius, 2009).

Mature glycans can also serve as targets for erasers, including the full de-N-glycosylation by N-glycanases as demonstrated by Suzuki *et al.,*. (1997) and Harada *et al.,*. (2015). Removing a specific section (‘letters’) from a glycan ‘word’ eliminates its original significance and can result in more than just deactivating a signal, revealing a different meaning in the process. Examination of these forms of activity showed a complex level of glycan reconstruction with indication of actions operating in specific manners in organs, tissues, and cells. Sugar units are involved in individual processing and can also operate at the substitution level. Sialic acids provide a useful case study regarding their O-acetylation, as shown in Figure 5 at the center of the bottom row (Reuter and Gabius, 1996). The acetylation of hydroxyl groups at positions C4 and C7-9 by transferases is reminiscent of N-acetylation of Arg/Lys residues in histones. Some esterases can use O-acetylated sialic acids as substrates (Langereis *et al.,* 2015). Enzymatically hindered de-O-acetylation of sialic acids halts mouse development at the 2-cell stage, indicating the importance of O-acetyl groups for differentiation, serving as a molecular clue for proper development (Varki *et al.,* 1991; Mandal *et al.,* 2015).

Interpretation of sugar code (Readers)

At a molecular level, the process of 'reading' begins with the bonding of the glycan and its receptor. Essentially, Fig.3 Insights have already been given regarding the origins of enabling intermolecular Hydrogen bonding recognition. The alignment of polarized C-H linkages through its directionality is a key aspect for complementarity, along with the stacking of patches. Aromatic amino acids (Trp, Tyr, Phe) with π-electrons play a key role in establishing contact (Gabius and colleagues, 2011). Proteins use binding sites for sugars to help with the delivery of Ca2+, serving as a way to aid in the process extending the range of interactions through coordination bonding (Gabius, 2011b).

To address the question on fold numbers, several structural patterns have been discovered through crystallography that can hold carbohydrates (refer to Solís *et al.,* 2015; Manning *et al.,* 2017 for structural representations). The origin of the generic term for these proteins can be traced back to likening them to 'reading' a message, specifically the cell surface glycans on red blood cells. Specifically, a traditional method to test glycan-binding proteins involves measuring the clumping of red blood cells dependent on carbohydrates. Clear preference for how proteins from plants react with ABH glycans on red blood cells. The term phytohaemagglutinins was inspired by blood groups, distinguishing them from blood group-specific antibodies. These can result in deadly cell aggregation by combining incompatible blood groups of recipient and donor. The word lectin originates from the Latin term lego, which means to select or read (Boyd and Shapleigh, 1954; for more information refer to Boyd, 1954, 1963). Lectins are currently classified as glycan-binding (glyco)proteins, unlike antibodies and enzymes, they do not act on the bound ligands like writers and erasers. Lectins, like glycans, are found in organisms across all branches of the evolutionary tree.

This modular puzzle reveals a way to combine various active centers for a complex task, as demonstrated by a slime mold lectin in coordinating cell movement (Gabius *et al.,* 1985). Even though the interpretation of a glycan-encoded message involves a lectin site making direct contact with the ligand, its resulting effects are influenced, at least partially, by the structure of the lectin. Structural parameters of lectin and the glycan at various levels on specifically (its structure, presentation, and the glycoconjugate's nature as carrier) are therefore very important. For instance, this interaction allows lectins in host defence to easily detect foreign glycan patterns are depicted in Figure 6. This same figure also shows instances of variation in lectin families, with galectins specifically highlighted in the left side.

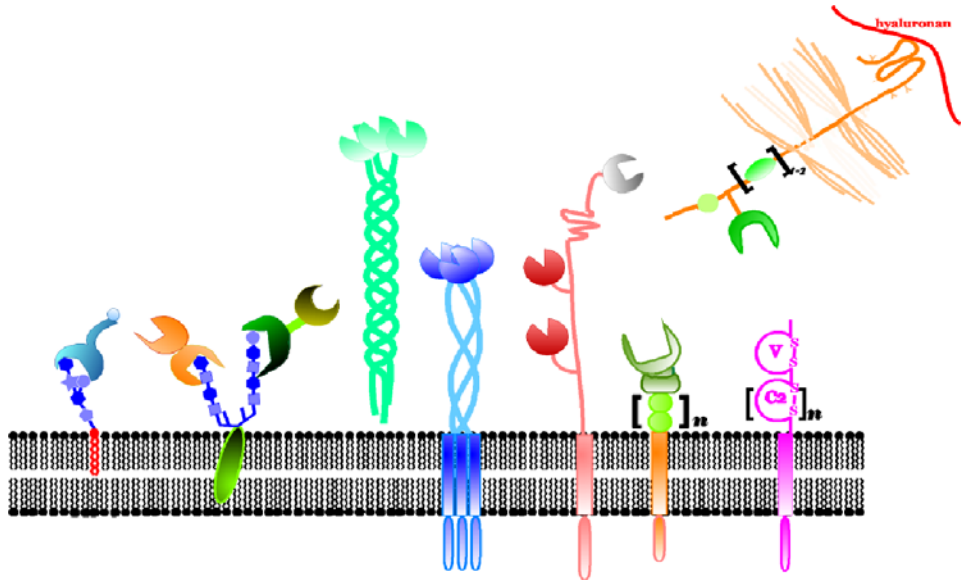

Fig. 6 Illustration of the strategic ways how carbohydrate recognition domains (CRDs) in animal lectins are positioned to reach optimal ligand selection (for example to separate self from non-self glycan profiles in innate immunity) and topological complementarity. From left to right, the CRD display in the three subtypes within the galectin family (chimeric, proto-type and tandem-repeat-type arrangements binding to a ganglioside or a branched complex-type N-glycan without or with terminal α 2,3-sialylation), the presentation of CRDs (C-type or fibrinogen-like domain) in serum and surfactant collectins or ficolins connected to their collagenous stalks and the non-covalent association of binding sites in transmembrane C-type lectins by - helical coiled-coil stalks (for example asialoglycoprotein and Kupffer cell receptors, the scavenger receptor C-type lectin, CD23, DC-SIGN or DC-SIGNR) are given.

By highlighting the importance of illustrating a change in a sugar molecule like 4-O-sulfation of LacdiNAc in Figure 4b, it demonstrates its ability to target a specific audience by acting like a postal code (refer to the legend in Figure 4 for more information). Moving the sulfate group to the 3'-position and incorporating galactose in sulfatides with long-chain fatty acids creates a signal recognized by another galectin (Delacour *et al.,* 2005; Stechly *et al.,* 2009; Velasco *et al.,* 2013). Tiny modifications in the glycan structure can be detected by lectins through thorough examination. In this way, writers, erasers, and readers work together, focusing on the biological significance of sugar coding. The present research obstacle is to decrypt the sugar code, unraveling the complexities of glycan creation, modification, and display, along with how lectins recognize glycans and the structural aspects influencing response specificity.

Conclusions

An in-depth examination of sugar properties clearly demonstrates their suitability as code symbols. Collaboration among writers, editors, and proofreaders builds an extensive lexicon through the utilization of these symbols. The reading is based on the molecular complementarity achieved through coordination, hydrogen and ionic bonding, C-H/π-interaction, and stacking. Similar to glycans, sugar receptors (lectins) exist in various forms, with over twelve protein folds providing sugar binding capabilities to the proteins within the lectin superfamily. The large scale of sequence variations in CRDs, along with a wide range of quaternary structures and modular designs, gives the lectin toolbox a plethora of opportunities to interact selectively with cellular components. Glycoconjugates are important for triggering significant events after binding, similar to decoding a message. In this process, the vocabulary is transformed into a sugar code dictionary. A glycan 'term' may have varying interpretations based on the context, as there is some uncertainty in the language.

The new discoveries, in summary, will lead us to innovative hypotheses and a deeper comprehension of cellular systems. For instance, through experimenting with glycan or lectin characteristics driven by hypotheses, rational engineering has the potential to create innovative tools for various purposes, such as developing biologically active lectin variations with unconventional structures to serve as a basis for CRD presentation. This information also shows us how the three

essential components of life are working together in the transmission of biological data. Every code system is designed to fulfill specific life requirements. Convincing proof exists to make the term 'sugar code' widely known. Returning to the opening remarks of N. Sharon1975, he wrapped up his talk by expressing his wish that he had successfully conveyed the significance and excitement of this field.

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Codon usage bias: A viable tool for bacterial attenuation

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Introduction

The most effective strategy to control infectious diseases is through vaccination. The different vaccines such as killed, sub unit and live vaccines have been developed to control bacterial and viral infections Amongst different types the live vaccine has the potential to induce long term immunity and is thus receiving increased attention. The traditional approach of making live attenuated bacterial vaccines is to delete toxin or virulence specific genes. There are many methods for identifying such genes. Virulence factor for bacteria can be identified by signature tagged mutagenesis (Saenz *et al.,* 2005) or *in vivo* expression technologies (Angelichio *et al.,*, 2002). Other methods of choosing a gene are based on their known key regulatory role like making a bacterium dependent on a particular nutrient which is not normally available *in vivo* (Aro$^-$ mutant (Hoiseth *et al.,*, 1981), crp$^-$ mutant (Tacket *et al.,*, 1992). Most of the knockout live attenuated vaccines are highly attenuated and are not able to induce appropriate immune response. Currently many new strategies are being tried for making live attenuated vaccine. One of the novel approach is to reduce expression of virulence/toxin genes in a graded manner so that bacteria with the requisite virulence can be generated for use as a vaccine (Runco *et al.,*, 2014). This is possible recently by recoding a gene/genome by changing existing codon usage with biased codons or biased codon pairs (CPB) (Coleman *et al.,*, 2008; Mueller *et al.,,* 2010; Martrus*et al.,*, 2013, Cheng *et al.,* 2015).

Description

There are 20 amino acids encoded by 64 codons including three stop codons. So, though multiple codons exist for most of amino acids, all are not used equally instead they are used selectively. Changes in the DNA sequence of a protein between two synonymous codons are often assumed to have no effect and are thus called synonymous changes or even silent changes. However, even though synonymous codons encode the same amino acids, it has been shown for a wide variety of organisms that different synonymous codons are used with different frequencies (Wilson *et al.,*, 1980). This phenomenon has been termed codon bias (Ruth *et al.,*, 2008). Codon-usage patterns is related to the relative abundance of tRNA isoacceptors (Post *et al.,*, 1980) and genes encoding proteins of high versus low expression show differences in their codon preferences (Gouy*et al.,*, 1982). This is the fact which is used in recoding for reducing expression and has been shown to attenuate virus pathogenicity in case of many viruses (Coleman *et al.,*, 2008; Mueller *et al.,,* 2010; Martrus *et al.,*, 2013, Cheng *et al.,* 2015).There are two published reports of its use in bacteria in *Streptococcus pneumoniae* (Coleman *et al.,*, 2011) and in *Salmonella Typhimurium* (Behera *et al.,* 2015, Nikhil *et al.,* 2022). Coleman *et al.,* had shown that recoding toxin gene of *Streptococcus pneumonia* reduces its expression and pathogenicity while Behera *et al.,* had shown recoding a key transcription factor also reduces pathogenicity. Cloning of toxin/virulence gene is permitted only in high bio safety labs having high containment facilities. However there is every chance of escape of the toxin gene from lab to outside. Hence an alternate approach is required to achieve the same effect. Assuming that any change which may alter the ability of a pathogen to efficiently utilize available resources may affect its survival in vivo, we had earlier shown that recoding a key transcriptional regulator reduces its pathogenicity. Taking the argument further, we hypothesized that instead of taking a key regulator which have multiple effects, why not take a key metabolite which have similar effect on whole spectrum of metabolism including the genes responsible for pathogenicity. So, to test this concept we had recoded methionine t-RNA synthetase (MetG) gene of *Salmonella Typhimurium* which codes for methionine tRNA synthetase, an enzyme which charges methionine to its corresponding tRNA to

initiate protein synthesis. Further, nucleotide synthetic enzymes are important for the survival both *in-vitro* and *in-vivo*. Among which we chose PurA coding for adenylosuccinate synthatase and deoptimized it. We also tried this concept in *Pasteurella multocida*. The *fis* gene of *Pasteurella multocida* was deoptimized by changing the codon usage pattern of wild gene with synonymous rare codons. Further, we hypothesized that decreasing expression of key anaerobic regulator (*fnr*) which also has an important role in pathogenesis of *Salmonella Typhimurium* will compromise its anaerobic survival mechanism and will also affects its pathogenicity. As a proof of principle recently we recoded *fnr*gene of *Salmonella Typhimurium* and studied its effect on different parameters of pathogenicity.

We had used RNA chaperone *hfq*, a global post-transcriptional regulator of bacterial gene expression that regulates about 20 % genes in *Salmonella*, as the target of recoding. Recoding decreased the expression of Hfq protein about two-fold in the mutant as compared to the parent strain. Recoding did not affect growth kinetics, but in growth competition the mutant strain was outcompeted by the parent strain. There was significant decrease in survivability of mutant strain in macrophage as compared to the parent strain. The biofilm formation was significantly impaired in case of recoded mutant. The mutants were also less motile as compared to the parent strain. Intraperitoneal infection of mice with the mutant strain had shown better survival as compared to parent strain. The results showed that recoding is an effective method of reducing virulence. We had partially recoded the protein coding sequence of methionine tRNA synthetase by changing the existing codons with the synonymous rare codons. Methionine tRNAsynthetase is a key metabolic regulator and is a survival gene of *Salmonella Typhimurium* but has no known role in virulence or pathogenicity. The results also indicateed that reducing the expression of a key metabolite instead of virulence or toxin gene can also attenuate a pathogen. We had chosen purA gene for decreasing its expression and instead of recoding the gene using synonymous rare codons or codon pairs, we have attempted to lowering the strength of its promoter. Both *in vitro* and *in vivo* pathogenicity of the purA mutant was reduced as compared to the wild one. Changing the codon usage of *fis*gene with synonymous rare codons also reduced pathogenicity of *Pasteurella multocida* in mice. In *Salmonella Typhimurium* the adaptation to low oxygen tension is mediated by key regulatory protein FNR. FNR is a global anaerobic regulator and regulates more than three hundred genes including metabolic and virulence genes. Our study showed that recoding the anaerobic regulator *fnr and Arc A* of STM significantly compromised its growth, decreased motility, biofilm forming ability and survival within macrophages. Further, the recoded *fnr*strain showed reduced colonisation ability and faecal shedding in mice.

Conclusion

Codon bias is being widely used to attenuate the pathogenicity of virus however there are only few studies in bacteria on recoding the genome using codon/codon pair bias. The bacteria have very large genome, theoretically it is possible to recode complete genome, but that's an expensive affair. Thus, choosing an appropriate target gene for recoding requires careful assessment. However, we feel that genome recoding can be exploited to attenuate bacterial pathogenicity to a level appropriate for the use as vaccine, and thus it provides a new avenue for live attenuated bacterial vaccine development.

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IBT-LP-5

Copy number variation and Genomic evolution, with a focus on the human salivary agglutinin gene (DMBT1)

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Salivary agglutinin, encoded by the gene DMBT1, acts as a pattern recognition receptor (PRRs) in innate immunity and mediates epithelial differentiationin human. The central region of the protein contains copy number variable tandemly-repeated scavenger receptor cysteine-rich (SRCR) domains which bind with bacteria and viruses.The paralogue ratio test (PRT) was used to estimate the diploid copy number of two regions of DMBT1 gene (CNV1 and CNV2) and results were validated with other CNV estimation assays. Both CNV1 and CNV2 at DMBT1 were multiple allelic CNVs and diploid copy number varied in different populations.To study the evolutionary basis of CNV at DMBT1, 971 samples from 52 populations from the Human Genome Diversity Panel (HGDP-CEPH) were used. The subsistence history of human populations was found to have an impact on the frequency distribution of both CNVs at DMBT1. The current study indicates that the shift to a starch-rich diet after the development of agriculture has led to an increase in dental caries by *Streptococcus mutans*. This has favoured CNV1 and CNV2 alleles at DMBT1 with more S. mutans-binding SRCR domains in agricultural populations.

Introduction

Human genetic variation is the genetic diversity or variation in alleles of genes of humans and represents the total amount of genetic diversity within the human genome at both the individual and the population level (Conrad *et al.,* 2010). Copy number variants (CNVs) account for a major proportion of human genetic polymorphism and contribute to the differences between individual humans (Craddock *et al.,* 2010). CNVs play an important role in genetic susceptibility to common disease. Deletions, insertions and duplications of DNA segments ranging from several kilobases (kb) to megabases (Mb) in size at variable number, in comparison with a reference genome are collectively referred to as copy number variants (CNV) (Conrad *et al.,* 2010). A CNV can be simple tandem duplication, or may involve complex gains or losses of homologous sequences at multiple sites in the genome. Recent studies show that up to 12% of the human genome is subject to CNV (Conrad *et al.,* 2010). Genes involved in the development and activity of both the immune system and brain tend to be enriched in CNVs (Zhang *et al.,* 2009).

The simplest type of copy number variation in the human genome may occur due to deletion or duplication of a gene. A diploid genome contains two copies of a particular gene, one on each chromosome. Copy number can be categorized into diallelic and multiallelic groups. Diallelic CNVs have two alleles and could produce three different genotypes in both deletion and duplication events. A simple deletion event could change the diploid copy number of particular gene and therefore could result in diploid copy number of two, one or zero. Similarly, a diploid genome could therefore contain two, three, or four copies of gene after simple duplication event in genome. But the pattern of deletion or duplication events in the genome is not always simple and could result complex copy number variation, known as multiallelic copy number variants (Wain *et al.,* 2009). A diploid genome after successive rounds of duplication could produce multiallelic copy number variants in diploid copy genome. Multiallelic CNVs have more than two alleles and could produce more than three genotypes. Generally, the size of genomic segments of deletion and duplication regions can vary from a few hundred to several million base pairs and could contain an entire gene, part of a gene, a region outside of a gene, or several genes in case of larger variants.

Based on the mutational origin and molecular mechanism of their formationCNVs can be classified into two classes; frequently termed “recurrent” and “non-recurrent” CNVs.Recurrent CNVs exist in regions containing large segmental duplications and are mainly generated by a non-allelic homologous recombination mechanism of CNV formation. 20-40% of normal polymorphic CNVs can be classified as recurrent CNVs (Conrad *et al.,*. 2010). Non-recurrent CNVs involve large genomic regions and break-point analysis shows minimal or no-homology is required for non-recurrent CNV formation (Conrad *et al.,* 2010). Non-recurrent CNVs can be generated by non-homologous end joining (NHEJ) or fork-stalling and template switching (FoSTeS) mechanisms (Zhang *et al.,* 2009).

The functional importance of many CNVs is relatively clear; reduced copy number of a gene can be correlated with reduced expression level, while duplicated copies of a gene can lead to increase expression level (Stranger *et al.,* 2007). 85%-95% of CNVs in human and mice were reported to be associated with a change in expression of the affected genes (Stranger *et al.,* 2007). CNVs are thought to be a major driving force in evolution and adaptation (Zhang *et al.,* 2009). Additional copies of genes provide redundancy in sequence, so that some copies maintain the original function while extra copies are free to evolve new or modified functions. The copy number variation of specific genes can offer selective advantage in human adaptation and evolution. But much variation in copy number of specific genes is disadvantageous and leads to a group of pathological conditions known as genomic disorders (Lupski& Ph, 2007). CNVs have been reported to confer risk of complex disease including susceptibility to autism (Sebat *et al.,* 2007), schizophrenia (Stefansson *et al.,* 2009), Crohn’s disease (Mccarroll *et al.,* 2009), psoriasis (Hollox *et al.,* 2008), systemic lupus erythematosus (Aitman *et al.,* 2006).

Analysis of copy number variation using paralogue ratio test (PRT)

The detection of accurate copy number of a particular gene is more challenging than SNP genotyping. CNV typing measures a quantitative difference rather than a qualitative difference. In recent years different methods have been developed to study copy number variants (CNVs) with greater accuracy and precision.The accuracy and precision of CNV typing method also depends on adequate availability of well-characterized copy number reference controls to allow for comparison of results. However, at present, no single existing methodology has the scope for accurately genotyping all CNV classes for large case-control studies and that power comes from combining methods and repeat typing.

The paralogue ratio test (PRT) is a comparative PCR–based method where identical primer pairs are used to co-amplify a copy number variable ‘‘test’’ region and a non-variable ‘‘reference’’ region using PCR (Hollox *et al.,*. 2008). The identical primer pairs amplify the copy number variable region of interest and one non-copy number variable reference region of the genome.The PCR amplicons can be distinguished easily by capillary electrophoresis based on their small size difference.

PRTs can be divided into two types, depending on the location of the reference target compared to the test target. Trans-PRTs have the reference amplicon on a different chromosome, or greater than 500 kb away from the test amplicon, and cis-PRTs have the test and reference amplicons closer together. PRT has the advantage of high-throughput analysis for CNV typing of large cohort of samples in a cost-effective way using small amount (5 ng) of DNA.

Deleted in malignant brain tumours 1 (DMBT1)

DMBT1 encodes the pattern-recognition glycoprotein DMBT1, also known as SALSA, gp340, or salivary agglutinin. In humans, salivary agglutinin protein mediates two possible functions in regenerative processes and pathogen defense (Mollenhauer *et al.,* 2007; Müller *et al.,* 2012).DMBT1 binds to a variety of pathogens through a tandemly arranged scavenger receptor cysteine-rich (SRCR) domain, with the number of domains polymorphic in humans.The DMBT1gene spans a genomic region of about 80 kb and consists of 55 exons (Mollenhauer *et al.,* 2007). DMBT1 is composed of tandemly arranged scavenger receptor cysteine-rich (SRCR) domains and interspersed glycosylated Ser/Thr-rich motif (SID) domains for microbe and host-ligand binding and CUB (complement C1r/C1s, Uegf, Bmp1) and ZP (zona pellucida-like) domains for cell polarization and polymerization (Mollenhauer *et al.,* 2007).

The major part of the genomic sequence of DMBT1 is composed of 13 highly similar scavenger receptor cysteine rich (SRCR) domains separated by SIDs (SRCR-interspersed domains).DMBT1 alleles with an altered number of SRCR domains and SIDs due to copy number variation in the tandem repeated units (Mollenhauer *et al.,*. 2007). The *DMBT1* gene shows extensive multiallelic copy-number variation (CNV) across all populations, with the tandemly repeated CNV leading to different alleles with between 7 and 21 SRCR domains (Polley *et al.,* 2015).

Estimationand distribution of copy number variation at DMBT1

The Wellcome Trust Case Control Consortium (WTCCC) used Agilent 210k aCGH chip for CNV-typing and a total 28 DMBT1 specific probes covered 40kb genomic region of DMBT1 gene were analyzed in four HapMap phase I populations (CEU, YRI, CHB and JPT) (Conrad *et al.,* 2010). Previous studies had also characterized deletion polymorphism at DMBT1 using Long-range PCR, southern blotting and SSCP (Sasaki *et al.,* 2002).The Database of Genomic variants shows that the middle portion of DMBT1 is copy number variable region.

Two regions (CNV1 and CNV2) of DMBT1 were genotyped using different PCR-based PRT assays (Polley *et al.,* 2015, Polley *et al.,* 2016a, Polley *et al.,* 2016b, Hains *et al.,* 2021). The study genotyped higher copy number individuals accurately without any copy number bias for both CNVs at DMBT1 using PRT based assay. The present study showed positive correlation between the aCGH and PRT assays of CNV1 but better clustering was noticed for PRT assays without any overlapping even for higher copies. The aCGHassay did not call CNV2 copy number whereas PRT assay called CNV2 copy number but the clustering was not as good as CNV1 assays for higher copies. The limitation of long PCR for both CNVs was not found in PRT assays.

The present study found that both CNV1 and CNV2 were multiallelic CNV in all HapMap populations. The modal copy number was 4 for CNV1 in all populations but copy number distribution was different in different HapMap populations. The African individuals showed higher frequency (48%) of higher copy (copy number 5 and 6) CNV1 compared to European population (0%). The opposite trend was noticed for lower copies (2 and 3) of CNV1 in two populations where higher frequency of low copy CNV1 was noticed for European samples (19%) compared to African samples (2%) (Polley *et al.,* 2015). The mean CNV2 copy number was different for different HapMap populations. The mean CNV2 copy number was higher (5.33) in the HapMap CEU population compared to HapMap JPT and CHB (mean CNV2 number 4.03) and HapMap YRI (mean CNV2 number 3.85). The African samples showed higher frequency of lower copy number class (4 or less) but European sample showed higher frequency of higher copy number classes (5 or more). The present study shows that PRT assays are robust in accurately calling both CNVs of DMBT1 and can be used to estimate both CNVs of DMBT1 in large case-control study(Polley *et al.,* 2015).

Analysis of copy number variation of DMBT1 using physical mapping approaches

Analysis and validation of DMBT1 copy number was performed using two physical mapping approaches; Fibre-FISH (Fluorescence in situ hybridization) and Pulse field gel electrophoresis (PFGE). Both methods estimated DNA size difference and allelic status at the chromosomal level which were similar to that predicted from DNA sequence analysis and long PCR. The sequence analysis and long PCR results showed the predicted size difference was approx. 12.7 kb which covered 4 tandem-repeated SRCR domains. The Fibre-FISH technique estimated a 4.6 to 4.7kb size difference for one-unit CNV2, indicating a single SRCR domain was involved in one-unit CNV2 change. Due to the small size difference (4.2kb) for one unit of CNV2, PFGE did not resolve the DNA fragment very accurately for single unit CNV2 changes. PFGE was used to estimate the size difference for the CNV1 locus only, which covered 12.7 kb at the genomic level. The present study indicates there are a lot of advantages in both the Fibre-FISH and PFGE approaches. Both approaches successfully estimated the allelic status of both CNVs at DMBT1, which was not possible by PRT or aCGH approaches. Fibre-FISH and PFGE also estimated almost exactly the size of DNA fragments at the chromosomal level depending on the copy number of DMBT1. Both techniques were labour intensive, low throughput and required high molecular weight DNA or agarose-embedded DNA, which is not always available from archived samples(Polley *et al.,* 2015).

Extent of *dmbt1* copy number diversity in global populations

The global diversity of DMBT1 was determined based on diploid copy number of CNV1 and CNV2 on the CEPH-Human Genome Diversity Project (HGDP) panel of 971 individuals from 52 populations worldwide. A similar range was observed for CNV1 (2-7 copies per diploid genome) and for CNV2 (0-11 copies per diploid genome) as in the HapMap samples. Although, at the individual level, there was no detectable relationship between diploid copy number at CNV1 and CNV2 (r2=0.01 for all HGDP), there was a clear negative relationship at the population level (r2=0.11) and at the continental level (r2=0.43)(Polley *et al.,* 2015).

Evolutionary basis of dmbt1 copy number variation in global populations

It has been reported that human adaptation to diet and different life styles had an important effect on the human genome. Pathogen-driven selection has also been identified as a selective pressure throughout the human genome (Fumagalli *et al.,* 2011). So, pathogen diversity might be a selective pressure influencing the frequency distributions of CNV1 and CNV2. The Kendall correlation, without considering distance from Africa, found a strong positive relationship between mean CNV1 and both virus and protozoa richness, but a strong negative relationship between mean CNV2 and both virus and protozoa richness in HGDP-CEPH populations. However partial Mantel tests considering distance from Africa found marginal correlation between mean CNV2 and bacteria richness data (Polley *et al.,* 2015). The earlier study found a significant negative relationship between agricultural populations the mean CNV1 copy number whereas a significant positive relation was found in hunter gather populations for this locus. A significant positive relationship was found between agricultural populations and mean CNV2 but the opposite trend was noticed for the same locus in hunter-gather populations.Agricultural populations, as opposed to hunter-gatherer and prehistoric hominid communities, typically had a high copy number of CNV2 and a low copy number of CNV1. It was proposed that SRCR domain units with suitable binding patterns evolved to modify $DMBT1^{SAG}$ binding to the tooth surfaceor *S. mutans*, or both. Finally, it was determined that a population history of agriculture correlated with DMBT1 CNV (Polley *et al.,* 2015).

Another recent study reported a strong signature of balancing selection spanning the 5′ region of the *DMBT1* gene. The signal of balancing selection across populations is determined by one haplotype containing a short SRCR domain repeat copy-number allele.The partial decrease in caries-associated S. mutans binding may be caused by the short protein isoform of DMBT1 (Alharbi *et al.,* 2022).

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TECHNICAL SESSION

Innovations in Biotechnology (Food, Animal, Fish, Environmental and Nano-technology) (IBT)

ORAL PRESENTATIONS

IBT-OF-1

Triploidy induction in rainbow trout (*Oncorhynchus Mykiss*) by pressure shock

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Rainbow trout is a major candidate fish species for aquaculture in coldwater regions of India. Being a potential species in hills, its mass production has become a priority concern with maintaining the ecological biodiversity management in coldwater. Field experiments were conducted at different geographical places of hill states. Healthy brooders were selected in the ratio of 1:1 male and female to obtain the eggs. Pressure shock was applied to retain the second polar body in developing embryo. Time Temperature Unit (TTU); delay timing after fertilization to retain the second polar body and exposure time of pressure shock treatment were standardized for better triploid induction rate (TR). Pressure shock at 9000, 9500 and 10000 psi for three levels of time exposure 3, 5 and 7 minutes was applied to green eggs at 9.5^{0}C water temperature. In all the operations, pressure at 10000 psi and exposure time of 7 min resulted in all dead eggs. Viable eggs were obtained with the pressure of 9000 and 9500 for exposure time of 3 & 5 mins. At pressure of 9000 psi for exposure time of 5 min triploidy induction rate was recorded as 20-26%, while at 9500 psi for 5 min induction rate was recorded highest as almost 100%. Study reveals that successful triploidy induction is feasible with pressure shock for the prime coldwater aquaculture fish species, rainbow trout. Karyotyping of the fry after yolk absorption and erythrocytes measurements was used for verification of triploidy. In the treated group, three sets of chromosomes (88-90) were observed in chromosome plates. Erythrocytes measurement (µm) reflects the 13.2% larger cell size and 12.5% larger nucleus in triploids over the diploids. In triploids, the average size of the erythrocytes is 16.36 µm with 6.88 µm nucleus size, while it is 14.48 µm and 6.16 µm in the case of diploids. Accuracy of TTU, exposure time of pressure shock and better rearing conditions are required for success of triploids production. The extra-genetic material in triploid rainbow trout influence to be more heterozygous and exhibits enhanced growth and survival than its diploid counterpart. The sterile triploids of the carnivore rainbow trout fish are also useful for reducing the environmental risk due to farm escape fish. The technology has been certified and disseminated for promotion of aquaculture production of trout for livelihood support to the people dwelling in hills.

IBT-OF-2

Commercial exploitation of pig excreta for small scale bioethanol and physical characterization of its ash

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It has been demonstrated that livestock waste products can be a reliable source of biofuel. There are few publications on the use of pig (Sus scrofa) excreta for bioethanol synthesis, despite studies suggesting that animal excreta may be utilized to make bioethanol. The aim of this study was to use a

traditional anaerobic fermentation method with Saccharomyces cervices to manufacture bioethanol on a small scale from pig excreta. The single distilled solvent produced from the pretreatment (with 4% H_2SO_4) and fermented pig excreta was assessed using gas chromatography (GC), and the results showed 89.59 percent purity and a retention time of 4.104 minutes that was closer to absolute ethanol values. Proton nuclear magnetic resonance (1 H NMR) analysis of the fermented distilled colorless from pig excrement was used to identify the signal peaks indicating the CH3, OH, and CH2 groups of ethyl alcohol. The minimum inhibitory concentration (MIC) of bioethanol was determined to be between 11 and 25 µg/mL after its antibacterial activity was assessed against a number of gram-positive and gram-negative pathogens. Further the left over excreta after bioethanol extraction was exposed for ash preparation and Mineral and scanning electron microscopy investigation. Suggesting that minerals like Zn, Mn and Fe, 160PPM, 230PPM and 753PPM accordingly also Aluminium 3679 PPM.Its commercial use as a fly ash substitute is suggested by SEM structural analysis. This study may result in a more creative and clever way to dispose of pig excrement and waste with less harm to the environment.

IBT-OF-3

Cloning, expression and partial characterization of recombinant buffalo cysteine-rich secretory protein 1

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Cysteine-rich secretory proteins (CRISPs) belong to the CAP super family having roles in various physiological processes including sperm functions. CRISP1 is an acidic glycoprotein of epididymis origin, known to attach to the surface of sperm and hinder capacitation. In this study, buffalo CRISP1 protein was expressed in *E. coli*, purified and subjected to partial characterization. The molecular cloning of 686 bp fragment (G41-T726) of buffalo CRISP1 mRNA(NCBI acc. No. XM_006050714.4) was carried out in pET22b(+) expression vector by using RNA isolated from buffalo cauda epididymis and following standard cloning procedure. Expression of recombinant protein was optimized by using different strains of *E. coli,* levels of IPTG and induction temperatures. Higher expression of the recombinant protein was observed in BL21(DE3)-codon plus *E. coli* strain after induction with 0.25 mM IPTG at 23 °C for 14 h. However, the protein was expressed as inclusion bodies which was solubilised by urea and purified by using Ni-NTA affinity chromatography. The purified protein was refolded by dialysing slowly over 48 h. Functional characterization of the recombinant protein involved assessing its effect on sperm motility and capacitation. Treatment of Percoll washed buffalo sperm with 20 µg/ml recombinant CRISP1 protein caused a significant decrease in progressive motility and the number of capacitated sperm in the presence of capacitating media. The effect of pH and temperature on the activity of the protein was also studied. The activity of the protein was unaffected when treated up to 60 °C, while the activity was decreased beyond this temperature. The protein was found active between pH 6 and 9 with the highest activity observed at pH 8. In addition, the effect of CRISP1 protein on protein tyrosine phosphorylation during sperm capacitation was investigated. Western blot analysis showed a significant reduction in tyrosine phosphorylated p72 and p47 proteins in the presence of CRISP1 protein. In conclusion, the recombinant buffalo CRISP1 protein was successfully produced by using E. coli in functional form demonstrating strong inhibitory activity on sperm motility, capacitation and protein tyrosine phosphorylation.

IBT-OF-4

BMP4 tailors embryonic stem cell differentiation towards germline age

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Differentiation of embryonic stem cells could be tailored towards a specific lineage under specific culture conditions, the most special case of which is the differentiation towards germ cells-transcending the generations. A number of molecules have been elucidated to play critical role in this differentiation. Bone Morphogenetic Proteins (BMP) have also been postulated to exert their effect towards differentiation of the pluripotent/totipotent cells into germline cells as well as into other somaticlineages during early embryonic development. The present study was undertaken to exploit this effect of BMP4 stimulation on differentiation of buffalo embryonic stem (ES) cells into germ lineage cells. For this purpose, ES cells were subjected to *in vitro* differentiation in floating (3-dimensional) and adherent cultures, under graded/different BMP4 concentrations and culture intervals, so as to identify the optimal conditions for such process. ES cells used for the study were from the bubaline ES cell lines (48+XX), developed specifically for the study. The progress towards directed differentiation was examined through quantification of the genes and proteins associated with germ cell lineage, but otherwise absent in ES cell state, as well as the specific expression states of the germ cells vis-à-vis, meiosis, sperm and oocytes. Gene expression data analysis revealed that BMP4 induced expression of primordial germ cell genes like like DAZL, and VASA; as well as meiotic genes like SYCP3, MLH1, TNP1/2 and PRM3. Gene expression specific to mature gametes like BOULE, TEKT1 (Spermatocyte markers) and GDF9, ZP2 and ZP3 (oocyte markers) was also studied. The maximum induction of the genes was observed at 50ng/ml concentration for a culture period of 14 days in both floating (3D) and adherent cultures. Immunocytochemical analysis of embryoid bodies (EBs) and monolayer adherent cultures revealed expression of PGC- (c-KIT, DAZL and VASA); Meiotic- (SYCP3, MLH1 and PROTAMINE1); Spermatocyte- (ACROSIN and HAPRIN); and Oocyte- markers (GDF9 and ZP4). The differentiation cultures showed embryonic development and progressed through 2-cell, 4-cell, 8-cell and blastocyst-like structures. Global DNA methylation analysis showed significantly ($p < 0.05$) decreased levels of 5-methyl-2- deoxycytidine in EBs obtained in optimum differentiation medium. This epigenetic event together with expression of PGC, meiotic and germ cell markers indicate development of successful strategy for development of in vitro germ cells from embryonic stem cells. Further refinement of the technique shall ensure production of germ cells under in vitro conditions, marking a great leap in the area of assisted Reproductivity.

IBT-OF-5

Synthetic Genome recoding *vis-a-vis* synergistic effect in pathogenicity attenuationof *Salmonella* Typhimurium

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To develop a genome recoded mutant of *Salmonella* Typhimurium by changing the codon usage with synonomous rare codons and evaluation of its pathogenicity. Recoding the genome with synomomous rare codons is being used as a method to attenuate virulence mostly in viruses. In this study we had recoded *hfq* an important transcription regulator and generated a single mutant (*hfq* recoded) and a double mutant (*hfq* and methionine t RNA synthetase *G(metG)* recoded) of *Salmonella* Typhimurium by homologous recombination. The recoded *hfq*was made by replacing the codon usage of *hfq*gene with rare codons used in *Salmonella* Typhimurium. For the recoded double mutant, recoded *hfq* gene was introduced to a recoded *metG* (methionine tRNA synthetase) mutant of

*Salmonella.*We did not find significant difference in *in vitro* growth kinetics between mutant and parent strains. In mixed culture wild strain outcompeted mutants *(hfq, met G, hfq+met G*). Intracellular survival of parent and mutant strains tested in macrophage cell line RAW 264.7 showed significant decrease ($P < 0.05$) in the survivability of the mutant strain as compared to the parent strain. Biofilm formation was impaired ($P < 0.05$) in case of recoded mutants (*hfq*, *hfq+met G*). The mutants were also less motile than the parent strains when checked on 0.3 % LB agar. *In vivo* pathogenicity of mutants were checked after intraperitoneal infection of BALB/c mice. The percent survival of mice infected with *hfq* and *metG* mutants was 14.28 % and 21.42% respectively, while in case of double mutant (*hfq + met G*), 28.57% of mice survived the intraperitoneal infection. From this study it is concluded that genome recoding with rare codons has a synergistc effect in pathogenicity attenuation.

IBT-OF-6

Development and characterization of Nano-composite bio-based, active edible functionalized packaging material over meat food model

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The study aimed to develop whey protein concentrate bio-based Nano-composite active film reinforced with Zno Nanoparticle, functionalized with citronella grass oil (EO) and to assess the packaging potential of chicken nuggets wrapped with the developed film at refrigeration storage (4±1ºC). Based on the MIC of citronella grass oil against *B. cereus, E. coli, S. typhimurium, and S. aureus*. 0.5, 1, and 1.5% EO concentrations were selected as base concentration for incorporation. The incorporation of EOs in the active film resulted in significant ($P<0.05$) decrease in tensile strength and significant ($P<0.05$) increase in elongation at break. The X-ray and FT-IR differentiation images showed the reduced crystalline structure of the film. Differential scanning calorimeter outputs displayed smoothness. The antimicrobial activity against food pathogens showed significantly ($P<0.05$) increased zone of inhibition. Finally, chicken nuggets overwrapped with the a forementioned films were stored aerobically at refrigeration temperature (4±1ºC) to evaluate antimicrobial, antioxidant, and sensory characteristics. The result indicated that pH, peroxide, FFA, and TBA values of treatments were significantly ($P<0.05$) lower than controls however significantly ($P<0.05$) higher DPPH activity was observed in all treatments. The total plate count, psychrophilic count, and, yeast &mold count were also significantly ($P<0.01$) lower in treatment groups and were within the permissible limits. The treated samples were well acceptable throughout the storage period of 15 days. The application of Nano-composite, active bio-based film was found proficient in confining product quality attributes throughout storage. The study can be further recommended for additional research for a future scale-up to an industrial scale.

IBT-OF-7

Hydroponics: A technological intervention into crop improvement research

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Hydroponics, a soilless cultivation system, has become a pivotal tool in crop improvement research, enabling precise control over environmental and nutritional factors. This technology facilitates high-throughput phenotyping by allowing rapid and uniform growth conditions, critical for evaluating large populations of plants under controlled stress scenarios. Researchers can assess

morphological, physiological, and biochemical traits at the seedling stage, providing early insights into plant performance. A significant advantage of hydroponics is its role in studying root architecture, which is difficult to observe in soil. By offering a clear view of root systems, hydroponics aids in characterizing traits such as root length, density, and branching patterns, essential for understanding nutrient uptake and stress resilience. Moreover, it enables the identification of elite germplasm with tolerance to abiotic stresses like salinity, drought, and nutrient deficiencies. Hydroponics also supports biochemical and physiological analyses, such as water content, water deficit, membrane leakage, chlorophyll content, proline accumulation, protein content and enzyme assays. These data are instrumental in identifying genetic markers and pathways linked to stress tolerance and quality traits. Furthermore, the uniformity provided by hydroponics enhances reproducibility in experiments, ensuring reliable results. Through its integration with advanced tools like genomics, imaging, and automation, hydroponics accelerates the identification and development of resilient and high-quality crop varieties. As a versatile platform, hydroponics is transforming crop improvement research, paving the way for sustainable agricultural solutions in the face of global challenges

IBT-OF-8

Role of cryopreservation in conservation of endangered plants

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The biological diversity and variability of life on Earth, or biodiversity, must be preserved for the benefit of both the current and future generations. Unfortunately, a number of tropical and subtropical species face extinction due to (a)biotic stressors and climate change. The germplasms of these species must be preserved for the current and upcoming genetic enhancement initiatives. Maintaining tissues at the extremely low temperature of liquid nitrogen, or cryopreservation, is a viable long-term preservation method that can be used for both vegetatively and generatively (by means of seeds) propagated crops, including those with resistant seeds. In breeding projects and the development of new cultivars, maintaining genetic and ecological variety is essential. There are now many plant species, wild variants, and regional decorative and fruit plant forms that is in danger of going extinct. Cryopreservation, which involves keeping biological samples in tanks with liquid nitrogen, is thought to be the best way to preserve plant genetic resources over the long term. However, the development of effective cryogenic processes is a challenging undertaking that necessitates taking into account multiple aspects. Special attention should be paid to how cryopreservation affects the consistency and stability of the samples that are kept. The process of cryopreservation involves cooling materials to extremely low temperatures in order to preserve organelles, cells, tissues, or any other biological architecture. Both practically and conceptually, the live cell's response to ice formation is fascinating. Simple freezing or cooling for extended periods of time may not preserve other viable tissues and stem cells, which are crucial for use in basic research and medical applications, because osmotic shock, membrane damage, and ice crystal formation during freezing and thawing will cause cell death. Successful cryopreservation of cells and tissues has increased recently with the use of cryoprotective agents (CPAs) and temperature control technology.

IBT-OF-9

Transgenic crop: currentstatus and future prospects

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With the global population projected to exceed 9 billion by 2050, contemporary agriculture facesimmense challenges, necessitating crops with higher yields, improved quality, and reduced inputs (Zhang *et al.,* 2018). Single-gene modifications are in adequate to meet this dem and, highlighting the need for crops with complextraits, such asstresstolerance, nutrient-useefficiency, and combination sofmultipletraits (Kamthanetal, 2016). The transfer of genes (transgenes) orgene elements with known functions into elite crop varieties has enabled the development of genetically modified (GM) crops with desirable traits. These crops serve as a potent supplement to the slow and labor-intensive traditional breeding methods, meeting the demand forquality foods (Kumar *et al.,* 2020). Genetic modification, a specialized technology that alters an organism's genetic makeup, of teninvolves combining genes from different organisms through recombinant DNA technology. The resulting organisms, termed genetically modified (GM), genetically engineered, ortransgenic, are transforming agriculture by improving food and crop quality cost-effectively (Bawa *et al.,*2012). Future advancements include developing nutrient-rich orphan crops like cassava and finger millet,which are nutritionally inferior to staple crops, and addressing post-harvest losses in fruits and vegetables through delayed ripening genes. Additionally, efforts are under way to improve the nutritional quality of crops. In India, significant progressis being made with field trials for transgeniccrops and experiments in volving 85 plant species for various traits (Shukla *et al.,* 2018). GM crops offer a promising solution to the challenges of global food security and sustainable agriculture.

IBT-OS-1

Forecasting future production: A study on Hariana cattle

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This study aimed to develop statistical models to predict a cow's total lifetime milk production based on early life performance data. Multiple linear regression technique. Data on 702 Hariana cows from two farms were analyzed. To build models, we considered several factors: age at first calving (AFC), first dry period (FDP), first calving interval (FCI), and first lactation total milk yield (FLTMY). However, due to high correlations between FDP and FCI, FDP was excluded from the final analysis. Using a stepwise regression technique, various combinations of the remaining traits were tested to find the best predictors of lifetime milk yield. The model with the highest R-squared value (32.95%) and the lowest error metrics (AIC, BIC, and RMSE) was selected as the optimal model. This model incorporated all three traits: AFC, FCI, and FLTMY.The study provides a valuable tool for predicting the lifetime milk yield of Hariana cows based on their early life performance, aiding in breeding decisions and herd management.

IBT-OS-2

A study on lifetime milk yield and reproductive traits in Hariana cattle

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This research aimed to analyse several key reproductive and productive traits of Hariana cattle: first lactation total milk yield (FLTMY), first lactation length (FLL), productive life (PL), herd life (HL), and total life (TL). Standard statistical analysis data on Hariana cattle from two farms were collected. The average FLTMY, FLL, PL, HL, and TL were estimated to be 971.2±16.9 liters, 328.2±3.8 days, 2019.1±42.1 days, 2251.8±43.9 days, and 3915.7±44.2 days, respectively. Heritability estimates for FLTMY and FLL were moderate to high, suggesting a genetic component to these traits. Statistical analysis revealed that the season of calving significantly affected FLL, while the period of calving influenced FLTMY. Farm, birth period, and age at first calving (AFC) were found to significantly impact PL, HL, and TL.

IBT-OS-3

Comparative hepatic transcriptomics of Aseel and Kadaknath chickens reveals genetic and metabolic diversity

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Aseel and Kadaknath are two popular chicken breeds of India. Aseel is well known for an aggressive behaviour and fighting ability and Kadaknath is popular for its black meat and eggs having proven medicinal and added nutritional values. The liver is the primary organ responsible for lipid metabolism, including the synthesis and breakdown of fatty acids. This impacts the intramuscular fat content, a critical determinant of meat tenderness, juiciness and flavour. The metabolism of liver cells in fighter chickens, meat and black chickens would differ based on their genetics, productivity and activity levels. To explore the genetic basis of these differences a comparative transcriptomic analysis of the hepatic tissue in Aseel and Kadaknath chickens was conducted using RNA sequencing. The differential expression analysis of the hepatic transcriptome of Kadaknath and Aseel chicken identified of 435 significantly differentially expressed genes (Padj and FDR < 0.05). The total up-regulated genes in Kadaknath were 203 while 232 genes were up-regulated in Aseel. Functional annotation revealed that the upregulated genes in Kadaknath were predominantly enriched in various biological processes such as positive regulation of triglyceride catabolic process, positive regulation of protein tyrosine kinase activity, glycerol catabolic process, negative regulation of interferon-gamma production and peptide cross-linking. The enriched pathway associated with these genes was the adipocytokine signalling pathway. In contrast, significantly enriched KEGG pathways in Aseel included oxidative phosphorylation, with higher expression of genes associated with cellular response to estrogen stimulus, regulation of lipid biosynthetic process, innervation, complement receptor mediated signalling pathway and tetrahydrobiopterin biosynthetic. These findings indicate that the identified pathways and processes influence key aspects of lipid metabolism, energy production and immune regulation. These metabolic distinctions between Aseel and Kadaknath breeds reflect their functional adaptations, shaped by the specific traits they have been selected for over time.

TECHNICAL SESSION

Innovations in Biotechnology (Food, Animal, Fish, Environmental and Nano-technology) (IBT)

POSTER PRESENTATIONS

IBT-PF-1

Methionine sulfoxide reductases are important virulence determinants in *Salmonella* Typhimurium

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Being an intracellular pathogen, *Salmonella* encounters but survives host oxidative stress response. Because of sulphur atom, methionine (Met) residues are highly prone to oxidation and convert into methionine sulfoxide (Met-SO). The Met-SO formation results in compromised protein function and affects cellular survival. Methionine sulfoxide reductases (Msrs) repair Met-SO to Met, reactivate damaged proteins and augment bacterial survival under oxidative stress conditions. *Salmonella* harbours five Msrs which are specific for free/protein bound, and 'R'/'S' types of Met-SO. By deleting all*msr*s, we created a pan *msr* gene deletion (Δ5*msr*) strain in *S.* Typhimurium. The Δ5*msr* mutant strain has been highly sensitive to hypochlorous acid, chloramine T and paraquat. Further, the Δ5*msr* mutant strain accumulated higher levels of malondialdehyde, protein carbonyls, and aggregated protein. However, the Δ5 *msr* mutant strain exhibited lower levels of free amines. Further, the Δ5*msr* mutant strain has been highly susceptible to neutrophils and showed defective fitness in the mice spleen and liver. In conclusion, *msr*s prevent macromolecular damage and contribute to virulence of *S. Typhimurium*.

IBT-PF-2

One health approach study on prevalence and risk factor associated with Shigatoxin producing *E.coli* in pet animals, pet handlers & their surrounding environment

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The One Health approach is a collaborative effort to balance and optimize the health of people, animals, and the environment. One Health approach also recognizes that the health of people is closely connected to the health of animals and our shared environment. Strains of both VTEC O157 and non-O157 VTEC can be highly infectious, with a significant risk of infection on exposure to a single cell. The rates of VTEC infection, hospitalisation and death from VTEC illness varies. The aim of this study was to examine Shigatox in producing *E.coli* (STEC) in dogs and cats, pet handlers and their surrounding environment to know the source and risk factors associated with them. A total of 310 samples comprising of 180 dog faeces (60 healthy, 60 diarrhoeic and 60 diseased), 30 cat faeces (25 healthy and 05 diarrhoeic) and 100 samples from environmental sources (10 hand swabs of dog owner,10 hand swabs of veterinarian, 10 commercial dog food,10 home-made food, 20 dog drinking water, 20 surface swabs of dog kennel and veterinary hospital, 20 flies around dog kennel and veterinary hospital) were processed to screen *E.coli* for STEC.

Out of total 310 samples, 142 *E.coli* isolates were obtained. The overall percent of *E.coli* from dogs, cats, pet handlers and environmental sources were found 61.66 %, 60.00 %, 13.00%, respectively. A total of 69 STEC were confirmed, which is 48.59% of the total *E.coli* and 22.25% of

the total sample collected. The overall percent of STEC from dogs, cats, pet handlers and environmental sources were found 32.22% (26.66% in healthy, 40.00% in diarrheic and 30.00% in diseased), 16.66% (16.00% in healthy and 20.00% in diarrheic) and 6.00% (15.00% in fly, 10.00% in surface swabs and 10.00% in hand swabs of dog owner), respectively. Out of 69 STEC, total 44 samples were found positive for stx1 (38 from dogs, 3 from cats, and 3 from environmental sources),8 for both stx1 and stx2 (6 from dogs, 1 from cats and 1 from environmental samples), 3 for both stx1 and hylA (2 from dogs, 1 from cats), 12 for both stx1 and eaeA (11 from dogs,1 from environmental samples), 2 for eaeA, stx1 and hlyA (1 from dogs and 1 from environmental sources).

Prevalence of STEC was higher in non-descript breed,0-3 month age group of pups, female, coprophagic dog and dog having co-habitation with other dogs. Our results demonstrate that dogs and cats may have a role in the infection of humans by STEC, STEC infections can occur through consuming contaminated food or water, contact with infected animals or their fecal matter, and contact with an infected person, probably serving as a vehicle for human infection, and thus emphasize the health risks for owners and their families and a good example of one health approach to control STEC infection in humans.

IBT-PF-3

Assessment of CuO nanoparticles induced hepatic injury in male Wistar rats

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The rapidly expanding discipline of nanotechnology enables the creation of materials with new dimensions, unique qualities, and a wider range of uses. Due to their broad range of applications and widespread use in numerous industries, copper oxide nanoparticles (CuO-NPs) have accumulated in a variety of environmental ecological niches. The present study aimed to evaluate the effect of Copper Oxide (CuO) Nanoparticles (NP) on liver following 45 days oral exposure in rats. For this purpose, a total of seventy-two rats were randomly divided into 4 groups. Rats of Group I was fed with basal feed and water and served as control rats. Rats of Group II, III, IV were given a daily dosage of CuO NP 1/10th, 1/20th, and 1/40th of the LD-50, were used to assess the amount of hepatic damage in the toxicity. Results showed that on administration of CuO nanoparticle enzymatic activities of aspartate aminotransferase (AST), alanine (ALT) and alkaline phosphatase (ALP) in plasma were significantly increased due to CuO-NPs administration. The supernatants of rat Liver tissue homogenates were showed the significant decrease in GSH, Catalase (CAT) and SOD activity, whereas the lipid peroxidation product (MDA) levels were increased. Further, light microscope investigation revealed that CuO-NPs exposure induced histopathological alterations in the liver tissue. Oral exposure of CuO nanoparticles to rats causes significant toxicity to the liver and it might be due to oxidative stress.

IBT-PF-4

Biological activity of phyto fabricated bimetallic (Ag-Au) nanoparticles

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Noble metal-based nanoparticles, particularly bimetallic nanoparticles (BNPs), are becoming more and more popular in nanoscience research due to their exceptional properties and promising applications in fields such as biomedicine, optics, electrochemistry, and green catalysis. While chemically synthesized BNPs possess catalytic properties, they can also pose environmental risks. To address this concern, biogenic Au-Ag BNP synthesis has emerged as a more eco-friendly alternative. These nanoparticles not only exhibit antimicrobial properties but also demonstrate potential for catalyzing the reduction or degradation of environmentally harmful organic dyes.In this study, aqueous Bael leaf (BL) extract was employed as both a reducing and capping agent in the biogenic synthesis of gold-silver bimetallic nanoparticles (Au-Ag BNPs) via microwave-assistance. Co-reduction of Silver nitrate ($AgNO_3$) and chloroauric acid ($HAuCl_4$) aqueous solutions with the BL extract under microwave irradiation, resulted in the formation of stable BNPs with predominantly spherical shapes.The synthesized BNPs were characterized by UV-Vis spectroscopy, confirming surface plasmon resonance (SPR) with an absorbance peak centered at 535 nm. A potent photocatalytic activity was observed for the synthesized BNP, in terms of reduction and degradation of methylene blue dye in the presence of $NaBH_4$.The dye was degraded less than 10 minutes instead of more than 40 min without catalyst, highlighting the BNPs' potential as an innovative approach for the removal of industrial dye effluents. Furthermore, the antibacterial activity of the BNPs was assessed using agar well diffusion against MRSA, Gram-positive Staphylococcus aureus and Gram-negative Escherichia coli, showing strong bactericidal effects. These findings suggest that the biogenic BNPs could serve as potent antimicrobial agents, capable of inhibiting the growth of a wide range of microbes.

IBT-PS-1

Effect of supplementation of nano zinc oxide on blood biochemical parameters of broiler chicken

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Nanotechnology is an emerging technology that has potential to transform the various sectors including poultry sector around the world. Nanoparticles of zinc oxide has attracted attention as an alternative feed supplement for poultry due to its high bioavailability, antibacterial and immuno-stimulant characteristics. This study delves into the transformative potential of nanotechnology in influencing the blood biochemistry of poultry birds. The aim of the present research was to study the effect of nano zinc oxide on blood biochemical parameters.300 day old (Vencobb 430Y) straight run commercial broiler chicksreared for a period for 42 days on deep litter system. Chicks were distributed randomly on equal body weight basis into four treatment groups (T_1, T_2, T_3, T_4) and one control group (T_0)having four replicates of 15 chicks in each group. Birds were fed standard broilerdiet as per BIS, 2007 without supplementation of Zn (T_0), basal diet supplemented with ZnO @ 100 mg/kg of feed (T_1),nZnO @ 20 mg/kg of feed (T_2), nZnO @ 30 mg/kg of feed (T_3) and Zn-Met. @ 40 mg/kg of feed (T_4). At the end of sixth week, blood samples of two birds from each replicate were collected for biochemical estimations. The blood biochemical parameters viz., blood glucose,

HDL, Albumin, A:G ratio were not affected. The value of cholesterol, LDL was significantly lower in T_3 group. Total protein, globulin were significantly higher in T_3 group and lowest in T_0 group. ALT and AST were found significant in all treatment groups. Blood biochemical parameters viz., total protein, globulin, ALT, AST were increased whereas cholesterol, LDL were decreased due to supplementation of nano zinc oxide @ 30mg/kg of diet.

IBT-PS-2

Transport induced stress in chickens and potential effectiveness of zinc nanoparticles and ascorbic acid on meat quality

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The study assessed the efficacy of phytofabricated zinc oxide nanoparticles (CF-ZnONPs) and L-ascorbic acid in alleviating pre-slaughter transport induced stress in broiler birds of sonali breed. A total of 120 birds were divided into four groups: negative control (T_1) which were loaded in vehicle but not transported, Positive control (T_2) birds were transported without any prior supplementation, T_3 and T_4, birds were transported with prior supplementation of CF-ZnONPs and L-ascorbic acid. Meat quality parameters and metabolomic analysis was conducted. Results obtained observed significant ($p<0.01$) reduction in live weight, meat colour and pH but increased driploss percentage in broilers of T_2 group. Significant ($p<0.01$) elevated H/L ratio was recorded in all transported groups. Research in lipidomics and flavor metabolomics sufficiently identified biomarkers that control meat quality. However, supplementation with CF-ZnONPs and L- ascorbic acid notably reversed these adverse effects, restoring meat quality and other metabolic profiles. Overall, these findings highlight the importance of antioxidant supplementation in alleviating transport induced stress in broiler birds.

IBT-PS-3

Factors influencing Hariana cattle lifetime milk yield

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This research investigated the lifetime milk production of Hariana cattle in organized farms by Least-squares analysis. The study examined the impact of various non-genetic factors such as calving period, season, age at first calving (AFC), and farm on different milk yield parameters. Statistical analysis revealed that farm, calving season, and period significantly influenced the first dry period. Farm and AFC class were found to be significant factors affecting ALT. Additionally, birth period significantly impacted milk yields in the LT2MY, LT3MY, LT4MY, and LT5MY, as well as ALT. Calving season had a significant effect on milk yields in the LT3MY, LT4MY, and LT5MY. The study found that animals born in autumn had the shortest first dry period and the highest milk yields in the LT2MY, LT3MY, LT4MY, and LT5MY lactations, as well as the highest ALT.

TECHNICAL SESSION

Innovations in Aquaculture, Wildlife and Veterinary Research (AWVR)

LEAD PAPERS

AWVR-LP-1

Application of biotechnology for ploidy manipulation in fishes

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Aquaculture is prided as the fastest-growing animal food-producing sector in the world. This advancement has been made possible by the collection of knowledge on the biology of many fish species and its applications in the development of advanced modern technology. For example, in animals, the ejection of two polar bodies is caused by the degeneration of three of four meiotic products during female gamete development. The discovery of this pathway has allowed for the intentional induction of polyploidy by simply blocking the polar bodies from escaping and so suppressing the first or second meiotic division. This has piqued scientists' interest in the idea of experimentally producing polyploidy in cultured farm animals. Artificial chromosome manipulation techniques were initially created for amphibians, but have since proven to be suitable for other aquatic animals. Today, there are archives of research on the application of chromosome manipulation techniques in many finfish species using diverse shock treatments. However, among all chromosome modification approaches, inducing triploidy is one of the most effective methods for producing sterile fish. The advantages of sterility in triploid fish become increasingly apparent as the animal's culture time continues beyond sexual development. This is due to sterility, which allows the energy required for gamete production to be channe led into somatic growth. Consequently, this will improve the fish’s flesh quality, reduce mortality and prevent fish reproduction thereby minimizing the possible impact of genetic and ecological disorder linked to the interactions between wild and cultured fishes.There are various ways for inducing triploids, some of which have been well described in many earlier researches. The various means for suppressing the second meiotic division may include temperature shock (heat and cold), pressure shock, chemical shock, and some anesthetics as well as electric shock.Pressure shock, on the other hand, has been shown to provide more exact findings, 100% triploidy induction success, and lower larvae mortality; nevertheless, it requires more expensive equipment to perform induction.

Triploidy induction

Triploids can be produced via a variety of techniques, including temperature shock (hot and cold), pressure shock, and chemical shock, certain anaesthetics, and electric shock. Because of its simplicity, low cost, and scalability for mass production, temperature appears to be the most frequently used shock for chromosomal manipulation, however, it is less reliable to provide consistent and exact outcomes, likely as a result of the challenge in applying a controlled temperature uniformly to an egg batch. It is possible to cause triploidy in prawns by preventing the extrusion of PBI or PBII using a shock such as a temperature shock, pressure shock, or chemical shock.

On the other hand, pressure shock has been documented to produce several accurate findings, 100% success in inducing triploidy, and a reduction in larval mortality; nevertheless, it necessitates more expensive equipment to effectuate induction. According to previous studies, many fish species were successfully induced using hydrostatic or temperature shock methods, including *Larimichthyscrocea* Sander vitreus, *Salvelinus fontinalis*, Paralichthysolivaceus and Oncorhynchus mykiss.

Triploidy identification

Diploid and triploid fish are morphologically equivalent during their entire life cycle but they are cytologically different. Hence, there are many direct or indirect methods to identify the ploidy of a fish. Triploid fish have been identified by various indirect methods such as Electrophoresis of protein and examination of morphology, counting of nucleoli, measurement of nuclear and cellular size of

erythrocyte, Chromosomal counting by karyotyping analysis, DNA content determination by Flow Cytometry and Microsatellite markers (Kang et.al. 2013).The most accurate method for determining polyploidy is chromosomal preparation because it gives precise information on chromosome counts and other essential information for cytogenetic study. Among direct method, karyotyping is the only irrefutable technique to determine ploidy. However, it is also the most time-consuming and frustrating one, which limits its usefulness in fish mass screening. DNA loops that extend into the nucleoli of interphase nuclei are known as nucleolar organiserregions (AgNOR). The NORs contain tandem repeats of DNA that encode for ribosomal RNA. The NORs are transcribed to rRNA under the influence of RNA polymerase. The NOR counting method is useful and the least expensive approach for determining the ploidy status. In the AgNOR technique proteins that provide the silver staining are known as AgNOR proteins. The nucleoli staining with silver nitrate ($AgNO_3$) proved to be a useful and effective method to assess the ploidy of the treated animals.

The other approach for identifying ploidy is erythrocyte size from a blood smear. As the number of chromosomes increased, it was generally anticipated that the measured or computed erythrocyte properties would significantly rise in the triploid progenies. The triploid have larger erythrocyte cellular and nuclear sizes. Higher haemoglobin content per cell was observed in triploid fish after an increase in erythrocyte size.

Growth performances of triploids

Aquaculture is interested in triploid fish because they are believed to be functionally sterile, which suggests that as adults, they shift metabolizable energy from gamete formation to somatic growth. Growth rate is a crucial factor in improving the genetics of fish for industrial production. It has been discovered that the comparison of growth between triploid and diploid organisms is also influenced by the environment. Hence, Triploids have several advantages, depending on the species and culture conditions. Growth improvement associated with triploidy has been observed in adult fish, including plaice hybrids Pleuronectes spp. (Lincoln, 1981), channel catfish, *Ictalurus punctatus* (Rafinesque), rainbow trout, *Oncorhynchus mykiss* and Asian catfish, *Clarias macrocephalus*.

Successful triploidy induction in rainbow trout for better growth

Rainbow trout is a highly priced exotic fish of the Coldwater aquaculture sector. Early sexual maturation and slow growth due to inbreeding depression are major problems in the commercial rainbow trout farming. Both male and female rainbow trout commence gonadal development well before they reach marketable size (500g). Sexual maturation is accompanied by a significant reduction in somatic growth as energy resources are diverted to meet the costs of developing gonads and secondary sexual characteristics. Decline in flesh quality and appearance associated with sexual maturation also decreases market value. If farm fish escape from any culture system, then it can reproduce and destroy the wild stock. Sterilization is helpful for reducing detrimental effects of sexual maturation on normal growth of fish. Triploids are sterile and can be released to the wild without danger of becoming established (exotic introductions) or of contaminating local gene pools. Due to extra genetic material, triploids remain more heterozygous resulting in better growth. ICAR-DCFR has developed technology for 100 % triploidy induction in rainbow trout by pressure shock treatment using aqua pressure vessel. However, heat shock is also applicable with success of 80% triploidy induction in rainbow trout, but results in genetic abnormality and less survival. Karyotyping and erythrocytes measurement are direct methods for conformity of the triploidy induction. However, AgNOR is an applicable indirect method for triploidy detection in field condition. Growth of the triploids stock is better in comparison to diploids. Mass scale production of triploids of rainbow trout is feasible by pressure shock. Sterile triploids are also helpful for reducing environmental risk in wild condition. This technique is applicable for increasing 19-20% growth in rainbow trout in farm condition and potential increase in trout production in the country. Field studies reflect 30% higher growth at early stage and 20.2% better growth and 19.0% better yield of triploids over diploids in captive rearing. 107.2 percent return on variable cost and higher benefit cost ratio in triploids indicates the economic feasibility of triploids culture. Triploids also reflect 20% better growth and rudimentary gonads in wild condition, which is helpful for environmental management. Sterile triploids of rainbow trout can be released into the wild without the danger of becoming established (exotic introductions)

or of contaminating local gene pools. The triploid fish is characterised with a significantly higher heart to somatic index and lower liver to somatic index.

Conclusion

A viable strategy that could overcome the fish farming constraints in large scale operation is the production of triploid fish that are sterile and more heterozygous. Among the various approaches that have been assessed or proposed for producing reproductively sterile populations of fish, induced triploidy is the only currently feasible for use in commercial fish farming. The best way of triploidy induction is duplication of maternal genome by blocking completion of the second meiotic division shortly after fertilization. This can be achieved through a number of techniques including chemical, thermal or mechanical methods. Due to extra genetic material, triploid remain more heterozygous and will have superior performance traits as compared to their diploid counterparts. Moreover, sterile triploids will negate production losses due to early sexual maturation and related reduction in somatic growth, as it prevents energy resources being diverted to meet the costs of developing gonads and secondary sexual characteristics. Triploid fish will also help in better management of the exotic species in natural aquatic ecosystems, without the risk of contaminating existing gene pools and disrupting local fish diversity. Overall, the characteristics of the envisaged triploid fish will augur to enhance the overall aquaculture productivity in the country. In India, there are pioneering studies on triploidy and tetraploidy induction in Indian major carps Labeorohita and Catlacatla by using thermal shocks and colchicines. First time, ICAR-DCFR has achieved 100 % triploidy induction in rainbow trout by pressure shock treatment using aqua pressure vessel.

AWVR-LP-2

Antimicrobial resistance and one health approach

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Global human health is seriously threatened by the emergence and spread of antibiotic resistance in human, animal, and zoonotic infections. Antibiotic-resistant bacteria are thought to have emerged and spread in the modern era as a result of the use of antibiotics in both human and veterinary medicine, particularly when large doses of antibiotics are given to livestock in order to promote animal growth. The primary pathways of antibiotic resistance are covered in this article. One of the major global health threats to human and animal health is antimicrobial resistance (AMR). The ancient trait of bacterial resistance to antimicrobial substances allows for bacterial survival in a changing environment and across time. Additionally, bacteria employ their evolutionary machinery to adjust to the selection pressure that antibiotic treatments apply, which lowers the therapeutic intervention's efficacy against infections in humans and animals.

Introduction

Antibiotics were considered "miracle drugs" when they were discovered in 1928, and their discovery changed modern medicine. Antibiotics are used to treat bacterial infections, but they have also made significant advancements in medicine possible. Major surgeries, organ transplants, and chemotherapy for cancer would not be possible without them (Shaw-Taylor, 2020). Animal health and wellbeing significantly improved as a result of the availability of antibiotics (Economou *et al.,* 20015). Additionally, the early use of antibiotics in animals raised for food coincided with the shift in livestock production systems from extensive to intensive industrialized (Steinfeld 2004). However, "the use of antimicrobials can, and will, lead to resistance," as Sir Alexander Fleming cautioned. Antimicrobial resistance (AMR) is a naturally occurring phenomenon that arises when a pathogen or commensal bacterium that was previously sensitive to an antibiotic develops acquired resistance and becomes resistant to the antibiotic. Bacteria are hardy, uncomplicated microorganisms that can adapt to their surroundings and change when favourable conditions arise, such as when antibiotics are present. Antimicrobial resistance (AMR) must thus be viewed as a natural phenomenon that is facilitated by all antibiotics. The primary cause of the AMR danger is the overuse and misuse of antibiotics, which frequently occurs needlessly or without a prescription. Additionally, the selection and spread of resistant bacteria have been greatly aided by the use of antibiotics in animals, whether they be food-producing, companion, or exotic animals (Varela *et al.,* 2021).

Antibiotic resistance has been linked to the use of antibiotics in agriculture, animal care, and human health as well as its global emergence. The creation and spread of bacteria with antibiotic resistance characteristics is thought to be mostly caused by the industrial usage of antibiotics for the purpose of promoting animal growth. Antimicrobial-resistant bacteria frequently spread to humans through incredibly complicated and unpredictable pathways. In general, there are two main ways to acquire antibiotic resistance: (i) directly through contact with animals that produce food or human carriers; and (ii) indirectly through the food chain or by exposure to areas with high levels of antimicrobial resistance pollution (e.g., hospitals, nosocomial acquisition, manure, waste water, and agriculture land). Antimicrobial resistant bacteria are highly prevalent among people who have direct contact with animals, particularly farm workers and veterinarians (Nadimpalli *et al.,* 2018; Jackson and Villarroel, 2012) Numerous research that have looked into the transmission of antimicrobial resistant bacteria from animals to humans have revealed this.

Antibiotic-resistant bacteria may arise as a result of the widespread use of antibiotics in agriculture, particularly for the purpose of promoting growth. Due to the high conductivity of such environments (e.g., high animal densities in the confide areas [feedlots and barns]), once antibiotic-resistant bacteria emerged among the food-producing animals, this phenotypic trait will be preserved by the same selective pressure and quickly spread to other animals and humans. When opposed to direct acquisition, the process of acquiring antimicrobial-resistant microorganisms indirectly is typically more complicated. Antibiotics used in human, animal, and agricultural medicine are known to release a significant amount of their active forms into the environment (Thanner *et al.,* 2016). Consequently, a selection pressure resulting from the existence of active antibiotic compounds in the environment could lead to the formation of antibiotic-resistant phenotypes in a variety of microbial species that naturally fill this niche. Antibiotic resistance phenotypes can spread horizontally to human, animal, or zoonotic pathogens if they arise on a mobile genetic element. This could be extremely dangerous for public health. Another significant way that antimicrobial resistant bacteria can be indirectly transferred to people, animals, and the environment at large is through aerosols produced from high antimicrobial resistance pollution sites.

Mechanism

Antibiotics cause microbial cell death or the halting of growth by attacking vital bacterial physiology and biochemistry. The bacterial cell wall, the cell membrane, protein synthesis, DNA and RNA synthesis, and the metabolism of folic acid (vitamin B9) are the five main targets of antibiotics. Since eukaryotic cells, which include human cells, lack or differ from these bacterial targets, antibiotics are comparatively safe medications. Penicillins, cephalosporins, and carbapenems are examples of β-lactam antibiotics that inhibit the formation of the bacterial cell wall. Higher species lack this structure, which is necessary for bacterial life. Tetracycline, aminoglycoside, macrolide, and other antibiotics target the bacterial ribosome because it differs from the eukaryotic ribosome enough to prevent cross-inhibition. Four general mechanisms—target modification, efflux, immunity and bypass, and enzyme- catalyzed destruction—lead to antibiotic resistance. The development of enzymes that alter the targets of antibiotics, as in the case of ribosomal methylation, or the mutation of the targets themselves, such as the topoisomerases that the fluoroquinolone antibiotics target, are two ways that target modification can happen. Target change that involves the engagement of new biosynthetic machinery to modify the structure of the cell wall is known as vancomycin resistance. Antibiotics are expelled from cells by a vast family of protein pumps known as efflux. Proteins that attach to antibiotics or their targets during immunity stop the antibiotic from binding to the target. For example, β-lactamases hydrolytically cleave the core β-lactam ring that is characteristic of the class and essential to antibiotic action.

Limiting drug uptake

As was previously indicated, bacteria naturally differ in their capacity to restrict the absorption of antimicrobial drugs. Certain types of chemicals are blocked from entering gram negative bacteria due to the composition and functions of the LPS layer. As a result, such bacteria have an intrinsic resistance to some classes of potent antibiotics (Blair *et al.,* 2015). An excellent example of how efficient this natural barrier is the fact that glycopeptide antibiotics, such as vancomycin, are ineffective against Gram-negative bacteria due to their inability to get through the outer membrane. Tetracyclines, some fluoroquinolones, and β -lactams are among the hydrophilic compounds that are most impacted by changes in the outer membrane's permeability (Blair *et al.,* 2015). Gram-positive bacteria do not possess an outer membrane, and restricting drug access is not as prevalent. In the enterococci, the fact that polar molecules have difficulty penetrating the cell wall gives intrinsic resistance to aminoglycosides. Biofilm formation is another mechanism which helps in the colonization of bacteria (Pang *et al.,*2019). The biofilm matrix includes polysaccharides, proteins, and DNA, making antimicrobial agents difficult to enter the bacteria and thereby providing defense (Hall *et al.,* 2017).

Drug target modification

One frequent process by which bacteria develop antibiotic resistance is the alteration of the drug's target (Reygaert 2018). One mechanism of resistance to β-lactam antibiotics is changes in the quantity and/or organization of penicillin-binding proteins (PBPs). PBPs are transpeptidases that help

the cell wall's peptidoglycan to form. Variations in the PBP count have an impact on the quantity of medication that can attach to the target (Blázquez *et al.,* 2012). A structural change, such as the emergence of the mecA gene in *S. aureus*, will lessen or stop drug binding altogether (Foster, 2017). The erythromycin ribosome methylase (erm) gene family is an additional example. It methylates 16S rRNA and modifies the drug-binding site, preventing the binding of macrolides, streptogramins, and lincosamines (Peterson and Kaur, 2018). Mutations in DNA gyrase or topoisomerase IV mediate resistance to medications that block nucleic acid synthesis, such as fluoroquinolones. The alterations in gyrase and topoisomerase composition result in a decrease or elimination of the drug's capacity to bind to these constituents (Nainu *et al.,* 2021).

Numerous parts of the bacterial cell might be targets for antimicrobial treatments, and an equal number of targets could be altered by the bacteria to make them resistant to those medications. Changes in the number and/or structure of PBPs (penicillin-binding proteins) are one way that gram positive bacteria develop resistance to β-lactam antibiotics, which are used almost exclusively by them. PBPs are transpeptidases that help the cell wall's peptidoglycan to form. The quantity of drug that can bind to that target is affected by a change in the number of PBPs (an increase in PBPs with a decreased drug binding ability, or a decrease in PBPs with normal drug binding). A structural alteration (e.g., PBP2a in S. aureus due to acquisition of the mecA gene) may reduce or completely prevent the medication from binding (Reygaert, 2009; Beceiro *et al.,* 2013). Glycopeptides, like vancomycin, function by obstructing the formation of cell walls, while lipopeptides, like daptomycin, function by depolarizing the cell membrane. These medications are intrinsically resistant to gram negative bacteria (thick LPS coating) (Randall *et al.,* 2013). Vancomycin resistance has emerged as a significant problem in Staphylococcus aureus (MRSA) and enterococci (VRE—vancomycin-resistant enterococci). The mechanism of resistance is the acquisition of van genes, which alters the structure of peptidoglycan precursors and reduces vancomycin's binding capacity (Cox and Wright, 2013; Randall *et al.,* 2013).

Resistance to medications that target nucleic acid synthesis (such as fluoroquinolones) arises from changes in DNA gyrase (found in gram-negative bacteria, such as gyrA) or topoisomerase IV (found in gram-positive bacteria, such as grlA). The drug's capacity to bind to gyrase and topoisomerase is reduced or eliminated as a result of these alterations, which alter their structural makeup (Hawkey, 2003; Redgrave *et al.,* 2014). Resistance to medications that block metabolic pathways arises from either overproduction of resistant DHPS and DHFR enzymes (sulfonamides—DHPS, trimethoprim—DHFR) or mutations in the enzymes (DHPS, dihydropteroate synthase, and DHFR, dihydrofolate reductase) involved in the folate biosynthesis pathway. Because they are structural analogues of the natural substrates (trimethoprim—dihydrofolate, and sulfonamides—p-amino-benzoic acid), the sulfonamides and trimethoprim bind to their corresponding enzymes. These medications work by attaching to the enzymes' active sites and causing competitive inhibition. The active site of these enzymes is typically where mutations occur, and the ensuing structural alterations in the enzyme prevent drug binding while preserving the ability of the natural substrate to bind (Huovinen *et al.,* 1995; Vedantam *et al.,* 1998).

Drug inactivation

Antibiotics can be rendered inactive by bacteria in one of two ways: either by destroying the medication or by changing its chemical composition (Blair *et al.,* 2015).

Chemical modification of the drug

Enzymes produced by bacteria have the ability to attach different chemical groups to pharmaceuticals. As a result, the antibiotic is unable to attach to the bacterial cell's target. The most efficient way of medication inactivation by chemical group transfer is the addition of phosphoryl, acetyl, and adenyl groups to the molecule (Lin *et al.,* 2015). The most versatile method, acetylation, has been shown to work against aminoglycosides, fluoroquinolones, streptogramins, and chloramphenicol. It is thought that phosphorylation and adenylation target the aminoglycosides. When aminoglycoside modifying enzymes (AMEs) are involved, the aminoglycoside molecule's hydroxyl or amino groups are covalently changed, rendering it inert. It is among the best illustrations of drug modification-induced resistance (Roberts, 2004).

Destroying the drug

The most widely used antibacterial drugs are β-lactam medications, like cephalosporins and penicillin (Bevan *et al.,* 2017). All members of this pharmacological class share a four-sided - lactam loop, which forms its core structure. The primary mechanism of -lactam resistance is the destruction of the -lactam loop by the action of -lactamases. By hydrolyzing the synthesis of the -lactam ring, the -lactamases prevent penicillin-binding proteins (PBP) from binding to the compound (Blázquez *et al.,* 2012).

Drug efflux

The inherent resistance of Gram-negative bacteria is largely attributed to the active export of several antibiotics from the cell by bacterial efflux pumps. Most bacteria have a variety of efflux pump configurations. The main job of the efflux pumps is to remove harmful substances from the bacterial cell; several pumps are capable of transporting a wide range of substances. The five main families of efflux pumps are the ATP-binding cassette (ABC) family, large facilitator superfamily (MFS), resistance-nodulation cell division (RND) family, multidrug and toxic compound extrusion (MATE) family, and small multidrug resistance (SMR) family. These families are grouped based on their energy supply and structure (Reygaert, 2009). All other efflux pump families are singular pumps that transfer substrates across the cytoplasmic membrane, with the exception of the RND family, which consists of multiple parts pumps that efflux substrate across the cell envelope. Genes for efflux pumps are chromosomally encoded in bacteria. Certain cues in the environment or the presence of a suitable substrate can cause some to express constitutively, while others are induced or overexpressed (high-level resistance is typically caused by a mutation that changes the transport channel).

Tetracycline resistance is a textbook example of efflux-mediated resistance, in which Tet efflux pumps (of the MFS family) use proton exchange as a source of energy to extrude tetra cyclines. Several MDR efflux pumps, such as MexAB-OprM in P. aeruginosa and AcrAB-TolC in Enterobacterales (of the RND family) can extrude tetracyclines as part of their contribution to MDR (Zhanel *et al.,* 2013). Resistance to macrolides is another clinically relevant phenotype induced by the efflux mechanism. The mef genes, which extrude the macrolide class of antibiotics, encode the most well-characterized efflux pumps (e.g., erythromycin). MacB, an ABC family member, acts as a tripartite pump (MacAB-TolC) for extruding macrolide drugs (Roberts, 2004).

EmrB, an MFS component, functions in E. Coli as a tripartite pump (EmrAB-TolC) to extrude nalidixic acid (Tanabe*et al.,* 2009; Jo *et al.,* 2017). Due to their chromosomal encoding, efflux pumps present in gram-positive bacteria may confer intrinsic resistance. Fluoroquinolone efflux pumps and members of the MATE and MFS families are examples of these pumps. Gram negative bacteria contain efflux pumps that are extensively distributed and can originate from all five of the families; the RND family contains the majority of the clinically important pumps (Kourtesi *et al.,* 2013).

One Health approach to combat AMR

In practice, One Health is a multidisciplinary and multi-sectorial approach to preventing emergent and resurgent infectious diseases, emphasizing the necessity for combined action from the public, private, non-profit, and academic sectors. This concept recognizes the inextricable link between the environment, plant, animal, and human health and, as such, is paramount in the fight against antimicrobial resistance (AMR). The concept is defined as an integrated, unifying approach that aims to sustainably balance and optimize the health of people, animals, and ecosystems.

Among the many challenges to be overcome are the conflicting interests of various economic sectors and organizations involved in human, animal, and environmental health. One Health is defined as “the collaborative effort of multiple health science professions, together with their related disciplines and institutions—working locally, nationally, and globally—to attain optimal health for people, domestic animals, wildlife, plants, and our environment”. The concept has long been established, stemming from the understanding that humans and animals are mutually dependent on one another and that they share many infectious diseases in addition to their shared environment. Up to 75% of infectious diseases that affect humans and have resurfaced in the past several decades are thought to be zoonotic, meaning they have their origins in animals. The public health and animal health organizations' health promotion and health protection initiatives, consumer education

campaigns for the general public and animal owners, farmer outreach programs, veterinary consultations, farm industry publications for farmers, veterinary curricula, and professional development programs for veterinarians and physicians are all opportunities to increase knowledge and comprehension of the One Health dimensions of antimicrobial resistance. Key strategies for addressing AMR from the One Health approach are described (Velazquez- Meza *et al.,* 2022):

1. Launch a global public awareness effort to inform people about the dangers of using antibiotics excessively and inappropriately. Reducing the amount of antibiotic prescriptions can be achieved by implementing successful public campaigns.
2. Minimize the needless use of antimicrobials in agriculture and the environmental contamination they cause. Agricultural and aquacultural practices account for the majority of antimicrobial consumption worldwide. Prophylactic and growth-promoting use of antimicrobials should be regarded as risky and unnecessary. Additionally, research indicates that animals excrete a considerable percentage of antimicrobials (75%–90%) that are not metabolized and end up in the environment.
3. Enhance global drug resistance surveillance. To better understand three areas—antibiotic consumption in humans and animals, understanding the molecular basis of AMR—the medical and scientific community needs a clear understanding of both historical and current data on AMR. This will help them to clarify new mechanisms of resistance acquisition, know cases conclusively, and anticipate future threats.
4. Encourage fresh, quick clinical diagnoses. Inappropriate antibiotic prescriptions are the result of misdiagnoses made in both public and private hospitals. The creation of quick and precise diagnostic tools will enable medical professionals to give antimicrobials to people who require them.
5. Encourage the creation and application of vaccinations and substitutes. The number of people with infections requiring antimicrobial therapy will decline with the introduction of vaccinations targeted against antibiotic-resistant bacteria that cause serious infections. To develop new vaccines and antimicrobial alternatives like phage treatment, probiotics, antibodies, and lysins, among others, more funding is currently required.
6. Acknowledge and expand the pool of individuals handling infectious diseases. Experts in infectious disease, infection control, nursing, microbiology, pharmacy, veterinary medicine, and epidemiology are needed to address antimicrobial resistance (AMR). Countries must spend money on this human resource's training in order to do this.
7. The development of new antimicrobials is unattractive to pharmaceutical companies because there are still relatively effective antimicrobials on the market. It is difficult to predict exactly how and when AMR will develop, creating uncertainty for pharmaceutical companies when making business decisions. Improved incentives should be created to encourage investment in both new and improved drugs.
8. Form an international alliance to combat AMR with genuine action. For the fight against AMR to make meaningful progress, global action is required. To bring about change, it iscritical to place AMR on the global political agenda and tackle it using a One Health approach.

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Significance of gut microbiome-neuroimmune interactions in health and neurological disease

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Introduction

From the ancient times, it is known that gut homeostasis significantly affects the mood, behaviour and other physiological activities of the human beings. The gut microbiotais one of the key factor of gastrointestinal tract (GIT) that known to influence different physiological activities and behaviour of the host, including gastrointestinal homeostasis, energy metabolism, immunological homeostasis, and specifically neuronal activity and immunity of the host. This change in physiology and behaviour in the host may be influenced by the bidirectional communication between the immune system and microbiotaof the gut. The imbalances in this conversation may result into immune dysfunction and immune-mediated disorders in central nervous system.

The term "gut microbiota" refers to the billions of different microorganisms that are found in the human gastrointestinal tract, including bacteria, viruses, and fungi. The relevance of the gut microbiome–neuroimmune axis as a regulator of central nervous system homeostasis is highlighted by the fact that dysbiosis of the gut microbiome results in dysregulatedneuroimmune responses, which may be exhibited as co-morbidities of neurological, neuropsychiatric, and neuro-developmental diseases.In this review, we focus on the latest facts highlighting the significance of the gut microbiomein regulation of the neuro-immune homeostasisof the CNS in health and diseases.

Segments of Neuroimmune System

The neuro-immune system of the CNS can be divided into two important physiological segments that consist of anatomical barriers surrounding brain tissues and immune cells residing in the CNS for the physiological protection of the brain.

1. Barrier Tissues around CNS

This is one of the key segments of the neuro-immune system of the CNS comprising meninges, anatomical tissue barrier surrounding brain and blood brain barrier, physiological barrier around CNS.

a) Anatomical barrier: The meninges

The CNS is lined with immunologically active barrier tissues called meninges, which play important roles in immunological surveillance, neuroinflammatory reactions, and damage repair (1). The recent studied exhibited existence of various types of immune cells in the menegial compartment which is composed of subtypes of macrophages, and also includes innate and adaptive immune cells including innate lymphoid cells (ILCs), dendritic cells (DCs), neutrophils, NK cells, T cells, and B cells. The meningeal immune cells, which reside in close proximity to the brain parenchyma, have the ability to secrete a range of pro- and anti-inflammatory cytokines that compatible to receptors on neurons and glia (1). It has been evident from the studies the function of brain parenchymal cells is directly influenced by the resident and transient meningeal immune cells through the peripheral immune response.

According to a recent study, the GIT is the source of meningeal IFN-γ-producing NK cells, which are programmed by signals coming from the microbiota (2). By triggering T cell death during EAE, these gut-licensed meningeal IFN-γ+ NK cells reduced CNS inflammation and enhanced the anti-inflammatory actions of astrocytes. Some scientific studies suggest that gut microbes also influences meningeal humoral immune responses in addition to the cell-mediated immune responses

in the meninges. In a study it has been observed that the intestinal microbiome guides the development of resident IgA positive plasma cells (3) in the dura mater adjacent to dural venous sinuses of the meninges that protectthe CNS from intravenous pathogens which are also influenced by the gut microbiome.

b) Physiological barrier: Blood Brain Barrier

The BBB is another barrier tissue that controls neuroimmunehomeostasis in the CNS. The cerebral microvascular endothelium, pericytes, neurons, astrocytes, and extracellular matrix make up the dynamic and tightly controlled BBB (4).

The endothelium in the BBB contains a range of tight junction proteins, solute transporters, and receptors to restrict paracellular diffusion of water-soluble compounds while promoting selective transport of nutrients and metabolites from the blood into the brain (5). The BBB plays a crucial role during healthy condition by acting as a physical barrier againstthe influx of peripheral immune cells and components and limit theneuroimmune cross-talks between the periphery and the brain. Thepermeability of BBB is compromised during diseased conditions which lead to increased barrier permeability resulting in infiltration of immune cells and proinflammatorysignaling molecules that lead to neuroinflammation in CNS.

Furthermore the development and function of intestinal epithelial barrier is found to be regulated by the gut microbiota. In an investigation, the brain parenchyma of GF mice showed higher levels of intravenously administered probes due to evaluated BBB permeability in GF vs SPF animals. It was found to be associated with lower expression of the tight junction proteins occludin and claudin-5 in the brain parenchyma of GF mice. Likewise,reduction in integrity of BBB by administration of antibiotics in mice (bacitracin, neomycin, natamycin, meropenem, and vancomycin)and rhesusmonkeys (amoxicillin and clavulanic acid) was found to be associated with increase in the phylum *Proteobacteria* and decrease in SCFA-producing bacteria (*Phascolarctobacterium, Subdoligranulum, Faecalibacterium, Blautia, Roseburia, Ruminococcus, Coprococcus, Dorea,* and *Anaerostipes*) of the phylum *Firmicutes* . It is evident from the findings of these studies that altering the gut microbiomeinfluences the characteristics of the BBB (6).

2. Brain resident immune cells

This segment of brain harbours two kinds of important immune cells, microglial and astrocyte cells in CNS.

a) The microglial cells

These are myeloid cells of the CNS that plays a crucial role in neurogenesis, neurotransmission, synaptic remodeling, neuro-inflammation, and damage repair in the central nervous system during homeostasis (7). The microglia and tissue-resident macrophages share numerous transcriptional programsand both can react to peripherally generated factors and local CNS-derived cues. The microglial dysfunction is reported to be linked with a number of neurological disorders, revealing important functional regulators and underlying cellular processes that may help to ascertain new molecular targets for addressing disease symptoms.

According to recent preclinical studies, the gut microbiota is crucial for regulating microglial physiology and addressing enteric dysfunction, which is observed in a number of neurological illnesses, could re-establish microglial function. In a preliminary comparison analysis, microglia from germ-free (GF) mice displayed an immature phenotype that was characterized by transcriptional programs coupled with reduced immune function (pathogen recognition, antigen presentation and type 1 IFN signalling) and increase proliferation (Ki67 and Ddit4) and survival (Csf1r and Pu.1) of microglia (8), compared to conventionally colonized [specific-pathogen-free (SPF)] control mice. Interestingly, the key transcription factor Mafb, which is responsible for guiding the microglial transition from early to adult phenotype, was up-regulated in microglia from GF mice despitetheir immature state (8), further suggesting that the absence of the gut microbiota leads to abnormalities in microglial development.

b) Astrocytes

The proper growth, development andactivity of the brain are regulated by tissue-resident stromal cells of the CNS called astrocytes which provide structural support, synthesize metabolites, modulate neurotransmission,andcontributein immune-related functions of brain (9). Though astrocytes are not the major immune cells of the brain, however they regulatemicroglial activity in response to immunomodulatory cytokines and they further secrete additional cytokines that encourage pro- and anti-inflammatoryprograms. Inflammation observed in many neurological diseases is contributed by the reactive astrocytes together with microglialactivation .The astrocytes readily react to the changes in the microbiome-regulated central and peripheral processessuch as AHRsignaling, microglia function, and peripheral immunity (9).

The crucial role of microglia-specific AHR signaling was demonstrated by a mechanism that blocked astrocyte reactivity by increasing the ratio of transforming growth factor alpha (TGF-α) to vascular endothelial growth factor B (VEGF-B) engagement on astrocytic receptors ERBB1 and FLT1, respectively.

How gut microbiome influences neuro-immunity of CNS

Several studies has reported that the gut microbiota influence CNS homeostasis by affecting the microglial synaptic pruning and by releasing some of the immune-modulators.

a) Influence of gut microbiome on microglial synaptic pruning

In addition to the impact of microbiomeon microglial gene expression programs, the gut microbiota is also involved in regulatingone of the important microglial processes such as synaptic pruning. Microglia by activity dependent synaptic pruning eliminates the extra synaptic connections between neurons that are created during neurogenesis and synaptogenesis. In an investigation, mice treated with antibiotic(ampicillin, gentamicin, metronidazole, neomycin, and vancomycin) for two weeks showed microglia with enhanced expression of synapse organization and assembly pathways. This suggests that the gut microbiota can influence microglia-mediated synaptic pruning (10).

The complement system, an innate immunological mechanism, is necessary for microglia to prune synapses.It is observed that high amounts of the complement proteins C1qa and C3 are expressed by microglia in the central nervous system (CNS) including exclusive expression of the C3 receptor, CD11b during homeostasis (11). In an experiment,microglia isolated fromneonate GF mice, showed expression of high levels of C1qa and low levels of CD11b compared to control animals,whereas opposite trends in these complements was reported in adult microglia, corroborating a association between the gut microbiomeand the microglialcomplement system (12). In addition up-regulation in expression of complement proteins observed in the brain in response to CNS damage and neurodegenerative illnessresulted in microglia-mediated synaptic loss (13). Furthermore peripheral immune cells, which function as intermediary sensors of microbial compounds, temporarily dwell in the brain parenchyma,cerebral spinal fluid (CSF), and meninges (14), influence microglia development and synaptic pruning.

Microbiome derived immunomodulator

The gut microbiome produces and releases some of the chemical factors that affect the neuro-immuno homeostasis of the CNS. The important microbial factors include short chain fatty acids (SCFA), lipopolysaccharides (LPS) and aryl hydrocarbon receptor (AHR) ligands.

a) Short Chain Fatty Acids (SCFAs):

Microbial fermentation of dietary fibers produces SCFAs, which are powerful modulators of host physiology. SCFA levels are significantly lower in the host when the gut microbiota is absent, which may explain the abnormalities in microglial maturation observed in GF and antibiotic-treated mice (15). In addition SCFAs have the ability to modify microglial function via modifying epigenetic programming. The SCFA butyrate is reported to be a histone deacetylase inhibitor (HDACi) that inhibits HDAC3 to increase antimicrobial activity of macrophages. Besides inhibiting HDAC activity and expression, acetate, the most prevalent SCFA in the brain, can also enhance histone acetylation by acting as a substrate for histone acetyltransferases. This is evident from an experiment in which supplementation of acetate in cultured microglia reversed LPS-induced H3K9 hypoacetylation and

nonhistone protein acetylation.Long-term acetate administration in an investigation restored H3K9 hypoacetylation and decreased the production of the proinflammatory cytokine IL-1β (6)

b) Lipopolysaccharides (LPS)

The TLRs are expressed in CNS by neural stem cells, neurons, oligodendrocytes, astrocytes, and microglia which are found to regulate pathways concerned with neurodevelopment, neuroplasticity and neurodegeneration in CNS (6). The microglia of CNS expresses all range of TLRs (1-9) that recognize diverse range of molecular patterns associated with gut microbes, including lipoproteins (TLR2/6), peptidoglycans (TLR2/6), double-stranded RNA (TLR3), LPS (TLR4), CpG-DNA (TLR9), and peptidoglycans (TLR2/6). However, in an experiment, mice raised without gut microorganisms showed functional abnormalities in their microglia, which hindered their ability to respond to TLR signals. In contrast to SPF mice, GF mice that received intracerebral or systemic injections of LPS showed microglia with diminished innate immune responses, as seen by reductions in cytokine and chemokine output (15).

c) AHR: Aryl Hydrocarbon Receptors (AHR) Ligands

An important amino acid, tryptophan is mostly obtained by animals from their food. In addition to controlling key pathways for the production of tryptophan derivatives, such as kynurenine, serotonin, and indole precursors, as well as ligands of the aryl hydrocarbon receptor (AHR), the gut microbiota is crucial for intestinal tryptophan metabolism.

Dietary tryptophan is converted to indole by enteric tryptophanase-expressing bacteria. The host utilizes this indole as a precursor for the manufacture of AHR agonists such indoxylsulfate and indole-3-propionic acid (IPA) .

Numerous CNS-resident cells and peripheral immune cells express AHR, which is found to be up-regulated in a variety of brain-resident cells, including microglia, in mouse models of experimental autoimmune encephalitis (EAE), ischemic stroke, intracerebral hemorrhage and LPS-induced neuro-inflammation (6).

Evidence of significance of microbiome in Multiple Sclerosis (MS)

The pathological hallmarks of MS include gliosis, T cell-dependent demyelination, peripheral immunological activation and infiltration, and loss of BBB integrity. MS is a diverse illness linked to over 100 genetic predisposing variations and environmental variables, including viral infections, vitamin D insufficiency, circadian disturbance, and, more recently, gut microbiomedysbiosis (16).

Specifically, compared to healthy controls, MS patients had greater relative abundances of *Methano brevibacter* and *Akkermansia muciniphila* and reduced relative abundances of *Prevotella, Faecalibacterium prausnitzii, Bacteroides coprophilus, and Bacteroides fragilis* (17).

The gut microbiota may play a part in the development of MS symptoms, as evidenced by the fact that transplanting the fecalmicrobiota of MS patients into GF mice increased MS-associated pathology in response to EAE. These negative microbiome effects were linked to a decrease in Treg IL-10 production, suggesting that malfunctioning Tregs had a diminished ability to prevent pathogenic neuroinflammation brought on by proinflammatory T helper type 1 (Th1) and Th17 cells.

Restoring the gut microbiome composition may help alleviate MS-related symptoms and transplanting SPF microbiota into the EAE mice model showed reduction in EAE symptoms. This suggests that persistent dysbiosis may be the cause of clinical symptoms (6).

Reduced propionic acid (PA) in serum and stool samples was found in MS patients in a recent study, and this was linked to a decrease in SCFA-producing *Butyricimonas*. After 14 days of PA supplementation, Treg and Th17 balance of blood changed to a more regulatory phenotype, and isolated Tregs' ability to suppress in vitro was improved. In MS patients, long-term PA supplementation significantly slowed the overall course of the illness as measured by the subcortical gray matter volume and relapse rate. Together these results suggest that the gut microbiota and chemicals produced from them regulate neuroimmune mechanisms that affect MS risk (6).

Conclusion

The gut microbiome influences the central nervous system through the gut-neuroimmune axis. The microbiomeaffects the core processes of brain, such as neurogenesis and neurotransmission by affecting the maturation and function of the resident immune cells in the brain, which influence a variety of host behaviours. In addition, the gut microbiomeinfluence the neuro-immuno homeostasis of brain not only by regulating the integrity of the BBB but also byaffecting the repertoire of leukocytesin the meningeal immune compartment, which together serve as the interface between the CNSand systemic immunity. Further the gut microbiome is vital for developmentand function of immune homeostasis, not only locally in the intestine but systemically in the periphery, as well. It can be concluded from these findings that the gut microbiomeacts as a crucial regulator of neuroimmunehomeostasis within thebrain and between the brain and periphery.

Further extensive studies are required to delineate the interaction of gut microbes and associated products with host cells along with their signal transduction pathway that eventuallyestablishthe neuroimmune functions that are associatedto brain activity and behavior. Theinvestigation ofmicrobiome-neuroimmuneconnections willdisclosethe cross-talk between the gut and brain and can be crucial for the development of novelstrategies as well as therapeutics for the management of a variety of neurological disorders.

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Acute phase proteins as biomarkers of uterine health in domestic animals: An update

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Among the chemical mediators released in response to tissue damage are the serum proteins called acute-phase proteins(APPs). Acute phase proteins (APPs) refer to a group of glycoproteins, mainly synthesized by liver parenchyma cells as a part of acute phase response (APR) to stimuli like infection, inflammation and tissue damage in animals and released into the bloodstream (Marinkovic *et al.,* 1989), and are unrelated to immunoglobulins. These are regarded as "biomarkers of inflammation, infection and trauma" and are a group of proteins produced by the liver in the initial stages of the inflammatory response. An ideal biomarker should be easily detectable and effective in both identifying the onset as well as monitoring the progress/outcome of the disease.Hence, have been suggested as possible indicatorsformonitoringinflammation and recovery in human and veterinary medicine.

APPs are categorized as "positively reacting" or "negatively reacting" as per variations in their circulatory concentrations. Positive APPs, such as haptoglobin (Hpt), serum amyloid A (SAA), C-reactive protein (CRP), and fibrinogen, show a "major", "moderate", and "minor" increase in their serum level upon initiation of APR. Major APPs, such as SAA, Hpt, and CRP, are characterized by a low basal serum concentration (usually below 1.0 µg/mL) in healthy animals, but may increases up to 1000 folds on stimulation, reaching a peak 24-48 hours after the insult and falling rapidly during recovery. Moderate APPs as alpha1 acid glycoprotein (AGP) increase from 5-10 folds following an inflammatory stimulus, reaching a peak after 2 or 3 days and then decrease more slowly than other major APPs. Minor APPs, whose utility is still debated in veterinary medicine, show a more gradual increase of 50-100% from the normal resting levels. The negative APPs, such as albumin and transferrin, decrease during the inflammatory response and, apart from albumin, have limited use in clinical pathology (Petersen *et al.,*2004).

There is evidence of local cellular expression of genes for acute phase proteins in peripheral tissues but the liver is the principal site of their generation (Eckersall and Bell 2010, Turner *et al.,*2012). They have a number of functions as part of the immune response including opsonisation of microbes (to enhance destruction by phagocytosis), recruitment of inflammatory cells to sites of inflammation and regulation of the inflammatory response.Reproductive failure is one of the most significant factors that limit the productivity of domestic animal production systems; hence the aim of this paper is limited to provide updates on use of important APPs in relevant animal reproductive diseases.

Acute phase response

Acute phase response (APR) is a dynamic process to minimize tissue damage while enhancing the repair process to provide an early protection against tissue insults (Petersen *et al.,* 2004). During APR, specific plasma proteins, known as acute-phase proteins (APPs), are mainly secreted by the liver. The defence mechanisms of innate immunity are numerous, APR is most important. Acute phase response is designed to check the infection until the adaptive, highly specialized immune response is initiated, which is followed by repairing processes to terminate the episode of inflammation. They have multiple functions as part of the immune response including opsonisation of microbes (to enhance destruction by phagocytosis), recruitment of inflammatory cells to site of inflammation and regulation of the inflammatory response. Hence, APPs promote immunoglobulin production and tissue repair alongwith recycling useful molecules and debris. The protective function of APPs against damaging effects of enzymes formed during inflammatory response is also of significant importance.

APPs play a significant role in different stages of the inflammatory response, and thus may serve as markers of various types of diseases in cattle (Madenet *al.,* 2012, Tothovaet *al.,* 2014). There is a large species difference in the production of APPs as what might be a positive APP in one species may be non-existent in another species. Ruminants are significantly different from other species in their acute phase response. A positive correlation between APPs levels and severity of disease and the extent of the tissue damage was also reported by Baumann and Gauldie (1994). The production of APPs in response to increased secretion of pro-inflammatory cytokines is among the systemic consequences to endotoxemia. Several previous reports linked increased concentrations of APPs with the presence of uterine disease. Uterine involution was associated with a decrease in the concentrations of APPs but bacterial contamination increases the APPs irrespective of the involution status (Sheldon *et al.,* 2001).InAPR, cytokines act as messengers between the site of disorder/disease and the hepatocytes that synthetize the APPs.Pro-inflammatory cytokines are potent stimulators for the hepatic production of APPs such as Hpt, SAA, acid glycoprotein or ceruloplasmin (Tothovaet *al.,* 2008).

A. Major acute phase proteins

Aomngt the various APPs occurring in bovines, haptoglobin (Hpt) and serum amyloid-A (SAA) are the primary positive APPs and are diagnostically, also the most important in ruminants (Tothovaet *al.,* 2014). Hpt and SAA have been studied as major APPs in domestic ruminants for various production and reproductive diseasesmajorly during the last few decades only.Hpt and SAA were said to play an important role in the reproductive processes by intensifying phagocytosis processes of pathogens in uterus and aiding reconstruction of the endometrium (Krakowski and Zdzisińska 2007).The serum levels of Hp, SAA, Cp, IL-6, IL-10, TNF-α, NO and MDA were significantly (P<0.05) increased, along with reduction of CAT, GPx, SOD and TAC in buffalo–cows with endometritis compared to healthy ones(El-Sayed *et al.,* 2024).

Haptoglobin profiles in relation to reproductive health

Haptoglobinis a plasma protein synthesized by hepatocytes that binds to free hemoglobin to prevent oxidative damage. Once bound to Hpt, haemoglobin loses its oxidizing ability. Haptoglobin-haemoglobin complex is removed from the circulation through endocytosis after recognition by the receptor CD163 expressed by monocytes and macrophages. Production of Hpt is strongly increased by pro-inflammatory cytokines such as IL-6. In ruminants, Hpt is a major APP and has been suggested as a diagnostic marker for diseases such as mastitis and respiratory diseases (Hiss *et al.,* 2004). Haptoglobin's role on the immune system is still unclear, but it is speculated that Hpt acts to regulate the immune responses with an anti-inflammatory activity (Huntoonet *al.,*2008).

In healthy cattle, the serum haptoglobin concentration is <20 mg/L and increased upto>2.0g/L within two days of infection. Hpt concentration is effective in the diagnosis and prognosis of mastitis, peritonitis, pneumonia and endometritis in cattle (Petersen *et al.,* 2004). In last few years, studies regarding the uterine APPs profiles in cows have emerged. Many studies have indicated Hpt as a clinically useful parameter for measuring the occurrence and severity of inflammatory responses particularly in cattle with endometritis and other natural or experimental infectious conditions (Hirvonenet *al.,* 1999).

Serum haptoglobin concentration in cows suffering from postpartum reproductive disorders was significantly greater than in the healthy heifers and postpartum cows. Uterine infections increase the serum Hpt profiles as observed during sub-clinical endometritis and acute metritis in postpartum dairy cows (Hirvonen *et al.,*1999, Galvaoet *al.,* 2010). Acute phase protein concentration has important role in the establishment and resistance of endometritis in dairy cows as evidenced by significant decrease in elevatedHpt concentration in cows with subclinical endometritis (SCE) and clinical endometritis (CE) (compared to healthy counterparts) after successful treatment (Heidarpouret *al.,* 2012). Hence, assessment of serum Hpt concentrations at different points of time could serve as a reliable biomarker for the diagnosis and monitoring of treatment following different therapeutic modalities.

Studies regarding the APPs profiles in uterus of cows have emerged in the last few years. Similar type of acute phase response has also been recorded in uterine fluids as in the general circulation which was indicated by significantly higher Hptconcentrations in serum and uterine flush of cows

with SCE group compared to healthy ones on days 22 and 40 postpartum, with an overall declining trend with the increased postpartum interval(Brodzki*et al.,* 2015). However, the generally higher serum than the uterine concentrations on each sampling has been reported (De-Jun *et al.,* 2010) in postpartum cows with SCE.

Only a few studies regarding APPs profiles in endometriticbuffaloes have been reported.Inflammatory condition of the endometriumindicated by an acute phase response has also been reported in buffaloes with SCE as significantly higher Haptoglobin (Hpt) concentrations in uterine lavage and serum Indian water buffaloes affected with SCE group were recorded compared to the healthy counterparts (Singh, 2017, Singh *et al.,* 2022). Concentrations of Hpt were significantly higher in serum than in uterine flush of SCE buffaloes, whereas within healthy buffaloes group, uterine flush and serum profiles were statistically similar($p>0.05$). Significantly greater Hptconcentrations in the serum and uterine lavage of buffaloes with SCE compared to their healthy counterparts on day 21 as well as 28 postpartum indicates the plausible diagnostic and prognostic role of APPs in postpartum bovine uterine health (Singh *et al.,* 2024). APPs, particularly haptoglobin, can be indicative of the severity of acute uterine infection or trauma and predicting subsequent fertility.

Serum amyloid-A profiles in relation to reproductive health

SAA belongs to a family of apolipoproteins. Serum amyloid A (SAA) is both an amyloidogenic protein of amyloid-A amyloidosis and an acute phase protein in most animal species. SAA is an apolipoprotein of the first line of acute phase response and its secretion is dependent on IL-1 and/or TNF-α (Tothova *et al.,* 2014). Although multiple isoforms of SAA (SAA-1, -2, -3, and -4etc)have been identified through amino acid sequence analysis, and SAA-1 and SAA-2 are predominantly produced in the liver and represent the main circulating isoforms in the plasma (Uhlar and Whitehead, 1999). In the dairy cow, seven isoforms have been recognized in the blood, but SAA-1 and SAA-2 are mainly expressed in liverand starts to increase within the first 4 h with a pick at 24–48 h after tissue trauma (Cray *et al.,* 2009).

Although there is a possibility that the synthesis of APPs takes place outside the liver, their presence in the uterine endometrium cells of cows was not confirmed in earlier *in-vitro* study (Davies *et al.,* 2008). However, Chapwanya*et al.,* (2013) suggested the plausibility of production of serum amyloid-A by bovine endometrium. Both the protein and mRNA of SAA were increased in endometritis or in LPS-stimulated cells, and the increases were positively correlated with the severity of endometritis*in-vivo* or LPS stimulation strength *in-vitro* study (Zhang *et al.,*.2018).

SAA serum concentration in healthy cows is not related with the physiological estrus phase. Thus, higher concentrations of SAA in the blood and cervico-vaginal mucus of healthy animals in late postpartum may be important to modulate physiological inflammation and prevent tissue damage caused by severe inflammation (Adnane *et al.,* 2017).High values of SAA have been found in cows with ovarian cysts such as luteal (>35 mg/L) and follicular cysts (>50 mg/L) compared to the healthy (<10 mg/L) dairy cows (Brodzki *et al.*,, 2015).

SAA is generally present at quite low levels in the blood of healthy animals. Significantly higher serum level of SAA in cows with endometritis compared to healthy has been reported and differential serum SAA concentrations in healthy cows (14.24 mg/L); cows with low level endometritis (20.25 mg/L); mild endometritis (28.1 mg/L); and severe endometritis (34.62 mg/L) were also reported (Kaya *et al.,* 2016). Higher SAA concentrations in serum of SCE affected postpartum cows (on 22 as well as 60 days postpartum) were reported (Brodzki *et al.,* 2015a, b). Likewise, within each group, the serum concentrations were also comparatively higher than the uterine in these two studies. The significantly higher serum SAA values in endometritic (ranging from 33.97±2.14 to 35.42±0.58 µg/ml) compared to healthy cows was reported by Biswal*et al.,* (2014). Complementing these results, Wen Chan *et al.,* (2010) reported significantly lower serum SAA levels (16 µg/ml) in healthy compared to metritic (decreasing serially from 85 to 33 µg/ml over 3 months postpartum) postpartum cows.SAA concentrations in uterine flush were significantly higher in waterbuffaloes with SCE compared to healthy ones (76.29±9.78 vs. 43.34±7.10µg/ml), whereas, the concentrations in serum samples were statistically similar(Singh 2017). Postpartum buffaloes diagnosed with SCE on day 21 postpartum recorded significantly higher SAA concentrations in uterine flush and serum on day 21 as well as day 28 postpartum. A non-significant decline was observed in serum and uterine profiles over

the postpartum time within each group indicating resolution of endometrial inflammation (Singh *et al.,* 2024). Chapwanya*et al.,* (2009) also reported that expression of SAA3 mRNA was increased in early postpartum (2 weeks) compared to late postpartum (9 weeks) cows and that SAA profiles reflected the severity of inflammation.In addition, reports suggest that serum SAA could be employedbefore calvingto screen a dairy herd for the potential occurrence of uterine infections (Dervishi *et al.,* 2016).

B. Minor Acute Phase Proteins

The fibrinogen and ceruloplasmin as considered as minor APPs in domestic ruminants and their profiles has been studied in a few studies in domestic bovines.

Fibrinogen profiles in relation to reproductive health

Fibrinogen is a major soluble plasma glycoprotein that plays a key role in haemostasis.The fibrinogen molecule consists of pairs of three polypeptide chains (α, β, and γ) connected by disulphide bridges. Fibrinogen also participates in the response to infection or injury by acting as a positive APPs and its concentration may increase 2-10 times upon inflammation and infection (Ali *et al.,* 2018).The plasma fibrinogen levels greatly increased in sick cows appeared to be related to inflammation and tissue destruction. The metritic cows presented higher serum concentrations of fibrinogen compared to healthy counterparts (Paiano *et al.,* 2023). However, on the contrary, serum fibrinogen profileswere reported not be a good marker for diagnosing or monitoring uterine infection or inflammation in postpartum dairy cows(Jeremejeva*et al.,* 2012, Bazzazan *et al.,* 2022). However, plasma fibrinogen concentrations did not by different phases of estrous cycle (Samimi *et al.,* 2020).

Ceruloplasmin profiles in relation to reproductive health

Ceruloplasmin is a plasma α-2 glycoprotein and plays an important role in copper transport in the blood stream (95% of copper in animals) and iron metabolism (ferroxidase) (Floris *et al.,* 2000). Ceruloplasmin play a role in tissue repair by mediating the transport of copper by lysyl oxidase and Cu-Zn superoxide dismutase enzymes. Blood ceruloplasmin profiles declines gradually and reached the minimum level at complete uterine involution, indicating it's useful to assess uterine involution in buffaloes (Rao *et al.,*. 2008).Serum ceruloplasmin levels increase significantly in the presence of endometritis and proportionate to the severity of endometritis (Kaya *et al.,* 2016).Clinical health problems related to inflammation such as clinical mastitis, metritis and retained placentawere associated with increase in ceruloplasmin concentrations in dairy cows cows (Mayasari *et al.,* 2017). Serum ceruloplasmin values were greater in repeat breeding animals than in the healthy cows (Azizipour *et al.,* 2024).It was also indicated that ceruloplasmin levels could be used in the diagnosis of endometritis as an alternative to Hpt and SAA levels.

In spite of the varied APPs concentrations reported during various postpartum uterine disturbances, high APPs concentrations during postpartum period in bovines are generally associated with poor uterine health(Bazzano *et al.,* 2022). However, APPs profiles need to be interpreted in the full clinical context as they possess poor diagnostic specificity.

Conclusion

The acute phase proteins concentrations increase in proportion to the severity of inflammation. APPs could be employed for diagnosis and monitoring thetreatment outcomes of reproductive problemsin domestic animals.There arestill challenges in determination of APPsprofiles for different uterine diseases due to unspecified basal values, variations inthe method of analysis, geographical location, nutrition,species differences and climate etc.

TECHNICAL SESSION

Innovations in Aquaculture,Wildlife and Veterinary Research (AWVR)

ORAL PRESENTATIONS

AWVR-OF-1

Biochemical and molecular characterization of *Lachnospira pectinoschiza* isolated from Goat rumen and its effect on *in vitro* fermentation parameters

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Isolation of pectinophile bacteria *Lachnospirapectinoschiza* from goat rumen was carried out. Their molecular characterization was done after amplification of 16s rDNA on the basis of gene sequence using Ez Bio Cloud database. Isolated culture was evaluated in *In vitro* gas production test for their effect on gas production, methane production, *in vitro* true digestibility and volatile acid fraction on high (100%gram straw) and medium (50% Gram straw and 50% concentrate mixture) roughage ration. There was no significant effect of *Lachnospirapectinoschiza* culture on the *in vitro* gas production, methane production as well as *in vitro* true digestibility. Total volatile fatty acids and their fractions were also similar among all the groups. The effect was similar on both type of ration. However lower methane production and higher digestibility was reported with medium roughage ration. The present study concluded that *Lachnospirapectinoschiza* isolated from rumen of goat has no significant effect ofon*in vitro* rumen fermentation metabolites on high and medium roughage ration.

AWVR-OF-2

Isolation, identification and culture sensitivity of bacteria associated with pyometra in dogs

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Medical management can be an alternative in breeding dogs with open cervix pyometra. Hence, an appropriate antibiotic treatment needs to be initiated and a culture sensitivity test of the isolated bacterial spp.should be carried out for better outcome.The present study was undertaken to isolate and identify bacteria in vaginal swabs of pyometra affected dogs. Seventy five dogs presented at Multi Speciality Veterinary Hospital, Guru AngadDev Veterinary and Animal Sciences University, Ludhiana and diagnosed with open cervix pyometra were included in this study. Vaginal swab samples from all dogs were collected and processed for isolation and identification of bacteria. The samples were inoculated on basal, differential and selective media including Eosin Methylene Blue, Manittol Salt Agar, MacConkey Lactose Agar and Nutrient Agar and were identified. All the isolates obtained were tested for sensitivity to various antibiotics by using Kirby Bauer disk diffusion method. *E. coli* (61.3%) was found to be the most predominant bacteria responsible for pyometra in dogs followed by*Staphylococcus*spp. (24.0%), *Klebsiella* spp. (9.3%) and *Pseudomonas* spp. (5.3%). Culture sensitivity of Gram negative bacteria indicated highest sensitivity toward enrofloxacin (73.3%) followed by ciprofloxacin (68.0%). Organisms were highly resistant to cefotaxime (65.3%) followed by ampicillin (60.0%). Intermediate resistance was seen towards chloramphenicol (58.7%), while no intermediate resistance was seen against ampicillin. Gram positive bacteria were highly sensitive to tetracycline (84.0%) followed by doxycycline (82.7%) and amikacin (70.7%). The

isolates were most resistant to enrofloxacin (76%) followed by neomycin (69.3%) and ofloxacin (64.0%). Highest intermediate resistance was recorded against gentamicin (21.3%). None of the isolates showed intermediate resistance against ampicillin, enrofloxacin, neomycin and tetracycline. Therefore, use of suitable antibiotic based on susceptibility results alongwith supportive treatments is essential for clinical management of pyometra. In conclusion, *E. coli*appeared to be the main bacteria responsible for pyometra in dogs. Further, Gram negative bacteria were highly sensitive to enrofloxacin and gram positive to tetracycline antibiotics.

AWVR-OF-3

Exploring the presence of *Campylobacter* in wild animals and development of probe-based qPCR assay for its detection

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In recent decades, *Campylobacter* has gained importance in developed and developing countries as a leading cause ofhuman diarrhoea across the globe. The foodborne zoonosis caused by *Campylobacter* spreads through food of animal origin and contact with the feces of animals, including wild animals. The present study examines the prevalence of thermophilic *Campylobacter*in wild animals and birds from zoos/sanctuaries/national parks of Uttarakhand, Uttar Pradesh, and Chhattisgarh. A total of 521 samples were analyzed and *Campylobacter* was isolated and characterized by biochemical tests, PCR, and nucleotide sequencing. It was found that the highest prevalence was found in wild ruminants followed by non-ruminants and birds. Culturing *Campylobacter* is considered difficult due to strict environmental needs, slow growth rate, susceptibility to stress and need for specialized media which can lead to false negative results in standard culture methods. Therefore, looking into the utmost need for a rapid, sensitive, and specific assay to detect and quantify *Campylobacter*in environmental and clinical fecal samples, a fluorescent probe-based real-time PCR assay is also developed in the current study which can detect even a single copy of bacteria in the sample. The study will help to pave the way toward exploring the wild side of campylobacter which is essential to ensure the one-health.

AWVR-OF-4

Developing a cost-effective and safe alternative to Tricaine methane-sulfonatefor fish anesthesia: Introducing AQUA-FSD

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Fish anesthesia is vital for minimizing stress during various handling procedures, including experimental microbial challenges, blood collection, breeding activities such as egg and sperm collection, and tissue sampling. Tricaine methane-sulfonate (MS-222)is a widely used fish anesthetic; however, its costliness, poor solubility in hard water, and narrow safety margin necessitate precise dosing to prevent inadvertent euthanasia. Additionally, MS222's efficacy is reduced in warm, calcium-rich water, limiting its utility in certain environments like hilly regions. Furthermore, the lack of high-precision weighing balances in field conditions and its high cost make MS-222 impractical for farmers.To address these challenges, a safe and cost-effective liquid anesthetic formulation was developed as an alternative to MS-222. This formulation is approximately 20 times cheaper, has a shelf life of 2.5 years at 4°C, and can be conveniently administered using an ordinary dropper. It exhibits no known toxicity to fish of any age or species, with no recorded fish mortalities during

experimental trials. Moreover, it maintains potency in warm, hard water conditions and ensures a quick recovery time (average of 30-60 seconds under aeration).This technology, termed AQUA-FSD, is intended for use in state fisheries colleges, fishery universities, research laboratories, and fish farms to minimize stress and injury during fish handling. AQUA-FSD facilitates routine fish sampling for research, academic, and diagnostic purposes, offering a versatile solution for the aquaculture industry.

AWVR-OF-5

Parasitic infestation and severe anaemia in goats: A case study of high mortality in a subtropical region

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In subtropical regions like India, parasitic infections pose a significant risk to livestock health, especially during rainy and winter seasons when temperature and humidity levels favor parasite proliferation. In a recent case, a goat farmer had a herd of 40 goats, aged 1.5 to 2 years, of which 23 succumbed, leaving 17 alive. This high mortality rate raised concerns about an underlying parasitic etiology. The affected goats showed symptoms of diarrhea 2–3 days prior to their death. A routine blood examination of the remaining flock revealed severe anemia with hemoglobin levels as low as 3.8 g/dL, indicating a compromised health status with a significant impact. Further fecal examination revealed the presence of strongyle and Trichuris eggs, signifying a gastrointestinal parasitic burden. Consequently, we promptly administered anti-parasitic treatment to the remaining 17 animals to prevent further losses. The outbreak resulted in a substantial economic burden to the farmer, highlighting the role of livestock in supporting rural livelihood. The study also highlights the importance of awareness among farmers in mitigating parasitic infections and regular health monitoring, including fecal screening and blood tests, as well as timely deworming schedules to prevent similar outbreaks.

AWVR-OF-6

Eco friendly gravity driven water circulatory comparative design of recirculating aquaculture system (RAS) with efficient use of energy

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This study investigates the hydrodynamic performance, hydraulic gradients, and hydrostatic pressure in a low-cost, gravity-driven Recirculating Aquaculture System (RAS) developed to optimize water recirculation without relying on pumps. Utilizing gravitational force, water was channeled through PVC pipelines from high-elevation production tanks to lower filtration units, ensuring continuous flow. The system's design prioritized effective water flow management to facilitate efficient circulation, bottom cleaning, and water quality maintenance. Flow rates were monitored weekly over a four-week period, with recorded maximum, medium, and minimum flow rates of 14–16, 7–9, and 3–4 liters per minute, respectively. This translated into daily recirculation volumes of 21,600, 11,520, and 4,795 liters per day and supported daily recirculation cycles of 14.4,

4.8, and 2 times at each flow level. The study also calculated hydrostatic pressure, resulting in 108,549 Pa based on a water depth of 29 inches and a density of 1000 kg/m³. The system's hydraulic gradient was found to be near-zero (0.1305), confirming that the gravity-driven setup effectively maintains water flow rates between filtration units without the need for mechanical pumping. The findings affirm the sustainability and cost-effectiveness of low-cost RAS configurations, which, by efficiently managing ammonia and nitrite levels, support a stable and healthy aquatic environment essential for aquaculture productivity. It also demonstrates the feasibility of implementing scalable, gravity-based RAS technology, which aligns with the broader goal of achieving resource-efficient aquaculture systems that benefit from minimal energy demands and operational simplicity.

AWVR-OS-1

Advancing diagnosis of urinary tract infections in dogs through urinalysis and bacterial culturing

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This study aimed to assess urinary tract infections (UTIs) in dogs brought to TVCC and Private clinics in Mathura. A total of 76 urine samples were collected from dogs, following aseptic procedures to minimize contamination. Urinalysis, a vital diagnostic tool, was employed to analyze the physical, biochemical, and microscopic components of urine. Key parameters, including color, odor, pH, specific gravity, glucose, protein, and the presence of leukocytes, erythrocytes, and crystals, were evaluated using urine dipsticks and automatic analyzers. Common urine collection methods included free capture, catheterization, and cystocentesis. After cleaning the urinary tract area with 70% ethanol swabs, samples were transported to the Department of Veterinary Microbiology for laboratory analysis. Urine samples were cultured in Nutrient broth and incubated at 37°C for 24 hours. Turbid samples were streaked on Nutrient Agar plates, and Gram staining was performed to differentiate between gram-positive and gram-negative bacteria. Gram-negative cultures were further analyzed on EMB Agar plates, where *Escherichia coli* colonies exhibited a characteristic green metallic sheen, while gram-positive cultures were cultured on Mannitol Salt Agar (MSA) and other selective media to isolate *Staphylococcus* species. Biochemical tests, including IMViC, catalase, oxidase, coagulase, and hemolysis on sheep agar, were used for further bacterial identification.The results highlighted the importance of urinalysis in diagnosing UTIs and assessing kidney function, contributing to early detection and prevention of renal failure. Despite its critical role, urinalysis remains underutilized in veterinary medicine. This study emphasizes its value in diagnosing UTIs and its potential for enhancing the diagnosis and treatment of urinary tract diseases in dogs. The findings support the need for more comprehensive use of urinalysis in clinical veterinary practices.

AWVR-OS-2

Unraveling the lifespan puzzle: A study on Hariana cattle

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Investigating the lifespan of Hariana cattle in organized farms. Standard statistical analysis Data from two farms, DUVASU Mathura and BabugarhHapur, was collected on cows born between 1962 and 2013. The average productive life, herd life, and total life were calculated as 2019.1±42.1, 2251.8±43.9, and 3915.7±44.2 days, respectively. Factors like birth period, season, age at first calving (AFC), and farm were analysed to understand their impact on lifespan. The study found that farm,

birth period, and AFC class significantly influenced all three lifespan measures. Cows born in autumn had the longest productive life, while those in lower AFC classes had longer herd and productive lives.

AWVR-OS-3

Impact of non-genetic factors on Hariana cattle productivity

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This research aimed to analyse factors influencing crucial reproductive and productive traits in Hariana cattle viz age at first calving (AFC), and daily milk yield over productive, herd, and total lifespans by Least-squares analysis. Data from two farms were analysed to identify the impact of non-genetic factors like birth period and season, AFC age group, and farm. Statistical analysis revealed that farm and birth period significantly affected AFC, daily milk yield during herd and total life. Birth period also influenced daily milk yield during productive life, while AFC class impacted daily milk yield during total life. The average AFC was found to be 1668.3±16.6 days, with average daily milk yields of 1.878±0.04, 1.7350.05, and 1.029±0.03 liters during productive, herd, and total life, respectively. Farm and Period of birth had significant effect on AFC, ALT/HL & ALT/TL; Period of birth had significant effect on ALT/PL; AFC class had significant effect on ALT/TL.

AWVR-OS-4

Optimizing bovine oocyte maturation: The impact of estradiol-enriched *in-vitro* media on ovum pick-up success

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The Present study evaluated the effects of 17-β estradiol supplementation at three concentrations (0.5 µg/ml, 1.0 µg/ml, and 1.5µg/ml) in *in-vitro* maturation (IVM) media on bovine oocyte maturation. Oocytes were collected from Hariana and Sahiwal donor cows subjected to ovum pick-up (OPU) protocols at weekly and bi-weekly intervals. Maturation rates were assessed based on cumulus cell expansion, categorized as no expansion, first-degree, or second-degree expansion. Results showed that estradiol supplementation significantly enhanced oocyte maturation, with 1.0 µg/ml and 1.5 µg/ml yielding higher rates compared to 0.5 µg/ml. Weekly OPU protocols yielded maturation rates of 72.48% (1.0 µg/ml) and 80.44% (1.5 µg/ml), while bi-weekly protocols achieved 73.88% and 66.25% for the same concentrations.The highest degree of cumulus expansion (second-degree) was associated with 1.0 µg/ml and 1.5 µg/ml estradiol, indicating superior maturation. Weekly OPU protocols showed slightly higher maturation rates than bi-weekly protocols, but the differences were not statistically significant. These findings highlight the critical role of estradiol-enriched IVM media in optimizing oocyte maturation, with 1.0 µg/ml and 1.5 µg/ml identified as the most effective concentrations. This study underscores the potential of estradiol supplementation in improving outcomes in bovine reproductive biotechnology, providing valuable insights for enhancing *in-vitro* fertilization and embryo production processes.

AWVR-OS-5

Evaluation of anti-inflammatory effects of lactoferrin and dexamethasone sodium on acetic acid induced ulcerative colitis in Wistar rats

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The current study aims to examine the anti-inflammatory activity of lactoferrin in acetic acid-induced colitis in Wistar rats. The rats were separated into five groups, with Group 1 being sham. Group 2 represents the positive control. Group 3 received dexamethasone sodium at 2 mg/kg body weight i.p. from days 1 to 14. Group 4 received lactoferrin at a dose of 200 mg/kg body weight per oral from days 1 to 14. Group 5 received dexamethasone sodium at a dose of 1 mg/kg body weight i/p and lactoferrin at a dose of 100 mg/kg body weight p/o from days 1-14. On day 1, groups 2, 3, 4, and 5 received an intra-colonic dose of 2 ml of acetic acid (4% v/v). On day 14 of the experiment, the rats were slaughtered, and the spleens were rapidly collected and their weights measured. The toxic group increased the weight of the spleen, whereas the lactoferrin-treated group had the opposite effect. Tissue samples from the colon, liver, and spleen were also obtained for anti-inflammatory marker quantification. The toxic group showed higher levels of TNF-α and MPO activity, and decreased levels of IL-10, while the lactoferrin treated group demonstrated the reverse effect. The findings indicate that lactoferrin has an anti-inflammatory impact on acetic acid-induced colitis in rats. As a result, lactoferrin has the potential to be used as a therapeutic agent for colitis.

AWVR-OS-6

Effects of manger height on plasma glucose in black bengal goats under stall-feeding conditions

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This study of 90 days explored the impact of manger height on plasma cholesterol levels in stall fed Black Bengal goats at Goat Farm and Research Centre of DUVASU, Mathura, with the objective of determining how varying the height of feed mangers influences cholesterol levels, an important biochemical parameter linked to metabolic health conducted. Eighteen Black Bengal goats were selected and divided into three groups with six animals in each group, based on different manger heights: the control group with a manger height of 20 cm, Treatment 1 (T1) group with a manger height of 10 cm, and Treatment 2 (T2) group with a manger height of 30 cm. The mean values of blood cholesterol (mg/dl) concentration were found 148.22, 141.42 and 140.35 in Control, T1 and T2 groups, respectively, with no significant differences ($P>0.05$). These findings indicated that the height of the manger does not significantly affect plasma cholesterol levels in Black Bengal goats, suggesting that plasma cholesterol concentrations remain relatively stable regardless of feeder height. These insights contribute to the field of animal biochemistry and provide practical guidance for optimizing livestock management practices, ensuring that feeder height is not a critical variable in cholesterol management for goat.

TECHNICAL SESSION

Innovations in Aquaculture,Wildlife and Veterinary Research (AWVR)

POSTER PRESENTATIONS

AWVR-PF-1

Conservation strategies for wild population for fishery resource management

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Effective conservation strategies are imperative for the sustainable management of fishery resources, especially as wild fish populations increasingly face anthropogenic pressures such as overexploitation, habitat degradation, climate change, and pollution. These strategic approaches focus on maintaining and rehabilitating healthy fish stocks and aquatic ecosystems. Primarily, the designation and stringent enforcement of protected areas are essential for preserving critical habitats, providing safe zones for spawning and juvenile development. Furthermore, adopting ecosystem-based management practices ensures that interspecies ecological interactions and entire trophic structures are considered, thereby supporting the long-term ecological integrity of fisheries.Adaptive management frameworks, rely on continuous environmental monitoring and enable dynamic regulatory adjustments in response to evolving ecological and population data. Community-based co-management frameworks further engage local stakeholders, ensuring that resource utilization aligns with both socio-economic needs and conservation imperatives. Additionally, the integration of cutting-edge technologies, such as satellite telemetry and genetic analysis, enhances the precision of resource monitoring and population assessments. Collectively, these methodologies are designed to foster the resilience and sustainability of wild fish populations, thereby securing the ecological and economic viability of fishery resources for future generations.

AWVR-PS-1

Development of models for prediction of lifetime milk production in Hariana cattle

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To predict future milk production (LT2MY, LT3MY, LT4MY, and LT5MY) based on early life traits like age at first calving (AFC), first calving interval (FCI), and first lactation total milk yield (FLTMY). Multiple linear regression. This research analysed the performance records of 648 Hariana cattle over several decades from two farms. Using a stepwise regression technique, the researchers developed statistical models to predict future milk yields. The best models were selected based on various statistical criteria, including AIC, BIC, Mallow's Cp, and variance inflation factor. The optimal models achieved high levels of accuracy, with R-squared values of 75.46%, 66.63%, 59.4%, and 55.03% for LT2MY, LT3MY, LT4MY, and LT5MY, respectively.The model with FLTMY was selected as optimum model for prediction of lifetime milk production in Hariana cattle.

AWVR-PS-2

Effect of captive environment on behavior and physiological stress in tigers (*Panthera tigris*)

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Human-induced disturbances significantly affect Indian wildlife, especially wide-ranging species liketigers (Panthera tigris). Zoos play a crucial role in their conservation through captive breeding, research, and education, but the artificial environments can lead to abnormal behaviors, such as pacing and head bobbing, due to reduced natural activities. This study evaluates how biological and environmental factors influence stereotypic behavior and stress markers (cortisol, T3, and T4) in captive tigers.Findings indicate that tigers spend most of their time exploring (average 55%) and resting (average 31%). Enclosure design is critical; smaller enclosures correlate with significantly higher levels of faecal cortisol and thyroid hormones ($P<0.001$). A positive correlation (0.34) between cortisol levels and stereotypic behavior suggests that stress increases as enclosure size decreases. In contrast, environments rich in enrichment activities lead to significantly lower levels of stereotypic behavior ($P<0.001$).The study highlights the importance of providing multiple exploring and resting sites to maximize the use of available space, especially for older and more timid animals. Outdoor enclosures should incorporate natural elements like tree cover, water bodies, and opportunities for socialization to promote natural behaviors and reduce stress. Additionally, keeper attitudes play a vital role in tiger welfare, underscoring the need for scientifically trained staff to enhance the care of captive tigers. Overall, effective management and environmental enrichment are essential for improving the well-being of captive tigers.

AWVR-PS-3

Molecular detection of LSDV in clinical samples and immune-histochemical validation in embryonated chicken eggs

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Lumpy Skin Disease (LSD), caused by a highly contagious poxvirus, poses a significant threat to the livestock sector. Recent outbreaks of LSD virus (LSDV) in India and neighbouring countries such as China, Bangladesh, Pakistan, Myanmar, Vietnam, and Thailand have resulted in substantial economic losses, particularly for the dairy industry. In India, the first reported outbreak occurred in 2019, followed by a more severe wave in 2022, which caused widespread morbidity and mortality among cattle. This study aimed to detect and characterize LSDV in clinical samples (Tissue sample ($n=72$), nasal swab ($n=42$), Whole blood ($n=78$) and Serum ($n=82$) from symptomatic animals received at Lumpy Skin Disease diagnostic laboratory, Department of Veterinary Microbiology, COVSc& AH, DUVASU January during 2023 to September 2023. Detection methods included conventional PCR, real-time PCR (RT-PCR), and double antigen ELISA. Virus isolation was conducted using the chorioallantoic membrane (CAM) of chicken embryos, with viral adaptation and multiplication confirmed via immunohistochemical analysis. Conventional PCR identified the presence of the LSDV genome, producing a characteristic 192 bp amplicon in all the tissue samples, while none of the whole blood and nasal swab found positive. Real-time PCR with a TaqMan probe

detected LSDV DNA in all 72 extracted DNA samples from tissue/tissue fluids, with cycle threshold (Ct) values ranging from 14.82 to 23.25. Notably, real-time PCR detecting LSDV in 7.69 % whole blood (*n=6*) and 11.9% nasal swab (*n=5*). These findings highlight the low detection rates of LSDV in whole blood and nasal swabs using conventional PCR methods and highlights qPCR method for sensitive detection. Double antigen ELISA successfully identified LSDV-specific antibodies in serum samples 5.36 % (*n=29*) symptomatic animals. Furthermore, selected clinical samples were propagated in chicken embryos. Post-inoculation examination of the CAM revealed thickening, congestion, hemorrhages, and pock lesions indicative of viral multiplication. Immunohistochemical analysis of CAM tissue using from the second and third viral passages confirmed virus multiplication indicating successful adaptation of LSDV in chicken embryo. This study underscores the utility of molecular diagnostics and immunohistochemical techniques in identifying and propagating LSDV. These findings contribute to the understanding of LSDV pathogenesis and enhance diagnostic capabilities for better management and control of the disease.

AWVR-PS-4

Diagnostic and prognostic value of serum and urinary biomarkers in chronic kidney disease in dogs

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Urinary biomarkers provide valuable insights into renal health by reflecting early tubular and glomerular dysfunction, even before substantial decreases in GFR are evident. The urinary BUN/urinary creatinine ratio and urinary plasma/urinary creatinine ratio highlight renal concentrating ability and tubular damage, offering sensitive indicators of disease severity and progression. Chronic kidney disease (CKD) is a prevalent and progressive disorder in dogs, characterized by significant alterations in renal function and associated biochemical markers. The aim of this study was to retrospectively evaluate key biochemical parameters for their diagnostic and prognostic significance across different CKD stages as per the IRIS classification. A total of 24 dogs, categorized into Control, stage 2, Stage 3, and Stage 4 CKD groups, were included in the study. Blood and urine samples were analysed for blood urea nitrogen (BUN), serum creatinine, urinary creatinine, urinary BUN/urinary creatinine ratio, and urinary plasma/urinary creatinine ratio. The results revealed highly significant increases ($p < 0.01$) in BUN, serum creatinine, urinary creatinine, and the urinary BUN/urinary creatinine ratio across CKD stages, reflecting the progressive decline in renal filtration capacity. BUN levels increased from 17.99±3.8 mg/dL in the control group to 172.12±19.58 mg/dL in Stage 4, while serum creatinine rose from 0.67±0.11 mg/dL to 11.13±1.54 mg/dL, underscoring their diagnostic value in assessing glomerular filtration rate (GFR) reduction. Urinary creatinine and the urinary BUN/urinary creatinine ratio showed significant changes, further emphasizing their utility in detecting renal dysfunction. The urinary plasma/urinary creatinine ratio demonstrated a moderate but significant increase ($p < 0.05$), indicating its relevance as an auxiliary marker of disease progression. This study highlights the importance of integrating urinary and serum biomarkers for a comprehensive evaluation of CKD, facilitating early diagnosis, accurate staging, and informed therapeutic interventions, ultimately improving outcomes in affected dogs.

AWVR-PS-5

Drug resistance and virulence in Enterobacteriaceae: A correlation study and analternate treatment plan

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Drug resistance entero bacteriaceae and virulence factor are differently correlated to conduct this study a total of 79 drug-resistant *Enterobacteriaceae* including 38 *Escherichia coli* and 19 *Klebsiellapneumoniae* were isolated and characterized from 393 samples collected from various stray dogs which were found sickorbrought to the veterinary clinics/hospitals in and around Kolkata. Of 79 *Enterobacteriaceae,* 27 were extended-spectrum β-lactamase, 53 were Amp Ctype β-lactamase producers and 9 were confirmed as Metallo β-lactamase producers phenotypically. To establishment the correlation between the drug resistance and their virulence amultiplex PCR was deployed for phylogrouping and Phylogrouping categorized the majority of *Ecoli* inphylo groups A (21) and B1(13) followed by Phylogroups-B2(5), F(3), and D(2) and five was un-typable. ERIC PCR differentiated the 38 MDR *E. coli*s trains into five clades A to E, Clade C (n:11) contained the majority of the isolates, followed by E (n:7), A (n:5), clade D (n:6), and clade B (n:4). 19 MDR *Klebsiellapneumoniae* isolates were divided among 4 clades A-D. A, B, and D clades consistedof 3 isolates each and clade C contained 6 isolates. Most of the MDR isolates werenon-pathogenic (commensal) and very few were pathogenic. The management of *E.coli* infections was successfully intervened by the possible the rapeuticre mediation employing eucalyptusoil. When applied to CRE isolates, eucalyptus oil had potent antibacterial properties. To determine the oil's efficacy inclinicals ituations, more research mustbed one. Therefore, it is possible that stray dogs, who have not received any prior antibiotic medication, could actasa powerfulreservoir for AMR infections, and it is not impossible for these pathogens to spread across the ecosystem. The study concludes by restating oremphasizing theadage, "Resistance any where is resistance very where."

AWVR-PS-6

Risk factor analysis of Antibiotic-resistant *E. coli* and *Salmonella* spp. in chicken and duck populations from six agroclimatic zones in West Bengal, a molecular approach

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The study focused on understanding the resistance patterns of *E. coli* in chicks and ducks across six agroclimatic zones in West Bengal, India. Cloacal swabs (n=525) were collected from birds in backyard and commercial farms for antimicrobial resistance profiling, gene screening, and phylotyping. The isolates showed high resistance to certain antibiotics like ampicillin (47.1%), ceftriaxone (27.0%), aztreonam (25.2%), tetracycline (20.0%), enrofloxacin (19.6%), and nalidixic acid (18.8%). The majority of the isolates were susceptible to amoxicillin-clavulanic acid (97%), chloramphenicol (97.5%), and imipenem (>98%). In general, duck isolates exhibited higher levels of antibiotic resistance, whereas resistance was minimal in isolates from the hill and red laterite zones. The investigation detected both extended-spectrum (120 isolates) and AmpC type β-lactamases (95 isolates). Regardless of phylotype, the majority of the isolates were susceptible to imipenem,

chloramphenicol, and amoxicillin-clavulanic acid, but resistant to ampicillin, cefpodoxime, cefotaxime, and ceftriaxone. Resistance patterns varied by phylotype, with phylotype A being the most prevalent and resistant to piperacillin-tazobactam compared to other phylotypes. Logistic regression study showed that isolates from the ancient and alluvial zone (OR: 2.63, p<001), ducks (OR: 0.02, p<001), and phylotype C (OR: 2.10, P<0.005) were more likely to produce ESBL. The research highlighted the risk of *Salmonella* spp. infection in poultry across different agroclimatic zones. Tetracycline had the highest resistance (91.2%), followed by nalidixic acid (66.6%), cefotaxime (35.0%), and ampicillin (62.2%). Salmonella enterica isolates showed limited association with ESBL production, with layer isolates showing a marginal connection. Notably, isolates from red and laterite zones displayed a stronger association with ESBL production than those from coastal saline zones. The study emphasizes the need for a comprehensive approach to address antimicrobial resistance, considering factors beyond antibiotic use, such as biosecurity and vulnerability to resistant elements spreading. The findings shed light on the complexity of AMR and the importance of multifaceted strategies to combat it effectively.

AWVR-PS-7

Histopathological evaluation of the efficacy of lactoferrin and dexamethasone sodium on acetic acid induced ulcerative colitis in wistar rats

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The current study aimed to determine the protective impact of lactoferrin in acetic acid-induced colitis in wistar rats. The rats were separated into five groups, with Group 1 representing sham. Group 2 represents the positive control. Group 3 received dexamethasone sodium at 2 mg/kg body weight i.p. from days 1 to 14. Group 4 received lactoferrin at a dose of 200 mg/kg body weight per oral from days 1 to 14. Group 5 received dexamethasone sodium at a dose of 1 mg/kg body weight i/p and lactoferrin at a dose of 100 mg/kg body weight p/o from days 1-14. On day 1, groups 2, 3, 4, and 5 received an intra-colonic dose of 2 ml of acetic acid (4% v/v). Tissue samples of colon, liver, and kidney were collected and processed for histopathological examination, which showed congestion, necrosis of epithelium and crypts, infiltration of inflammatory cells, depletion of goblet cells, and ulceration of mucosa in colon, mild congestion and mild sinusoidal dilation in liver and minimum tubular damage, mild degenerative changes, mild vacuolations in glomeruli of kidney in acetic acid induced groups. This improvement was seen in group 5, followed by groups 4 and 3. The findings indicate that Lf, in combination with dexamethasone sodium, has an ameliorative and preventive effect against pathological alterations in acetic acid-induced colitis in rats. As a result, Lf could be a possible therapeutic agent against acetic acid-induced colitis in rats, as well as a promising nutraceutical chemical for the treatment of colitis.

AWVR-PS-8

Impact of flooring on plasma creatinine of Sahiwal heifers

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This study evaluated the impact of different flooring types on the Plasma Creatinine (mg/dl)of Sahiwal heifers. Twenty four healthy Sahiwal heifers maintained at LFC of DUVASU, Mathura were randomly distributed into four groups on the basis of body weight and age. Heifers of firstgroup were reared on concrete flooring (T1) which served as control group, the heifers of second group were reared on Sand flooring (T2), the heifers of third group were reared on Cowdungbed flooring (T3) and the heifers of fourth group were reared on Rubber mat installed flooring (T4). The animals were exposed to their respective floorings round the clock, for which they were kept in tethered conditions. The values ranged from 1.25 to 1.31 mg/dl in T1 (Control), 1.18 to 1.27 mg/dl in T2, 1.10 to 1.31 mg/dl in T3 and 1.08 to 1.31 (mg/dl) in T4 treatment groups. The pooled mean values of creatinine concentration were not significantly different (P>0.05) in all treatment groups.The stable creatinine concentrations observed across all flooring types suggested that variations in flooring did not impact the metabolic health of the heifers. Therefore, flooring can be selected based on other practical considerations such as cost, comfort, and maintenance without worrying about adverse effects on plasma creatinine levels. These findings provide valuable insights for livestock management and contribute to the broader field of animal biochemistry.

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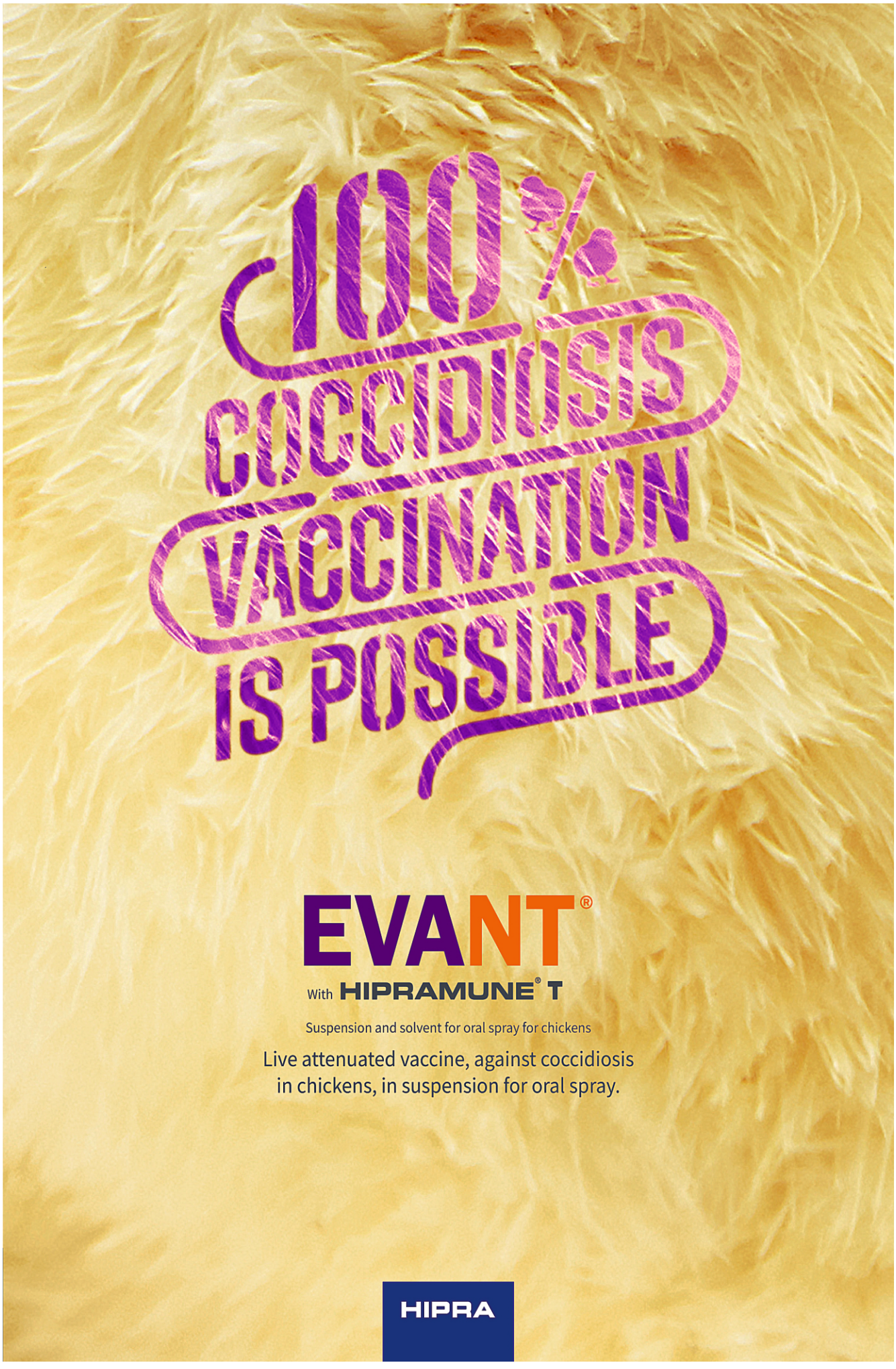
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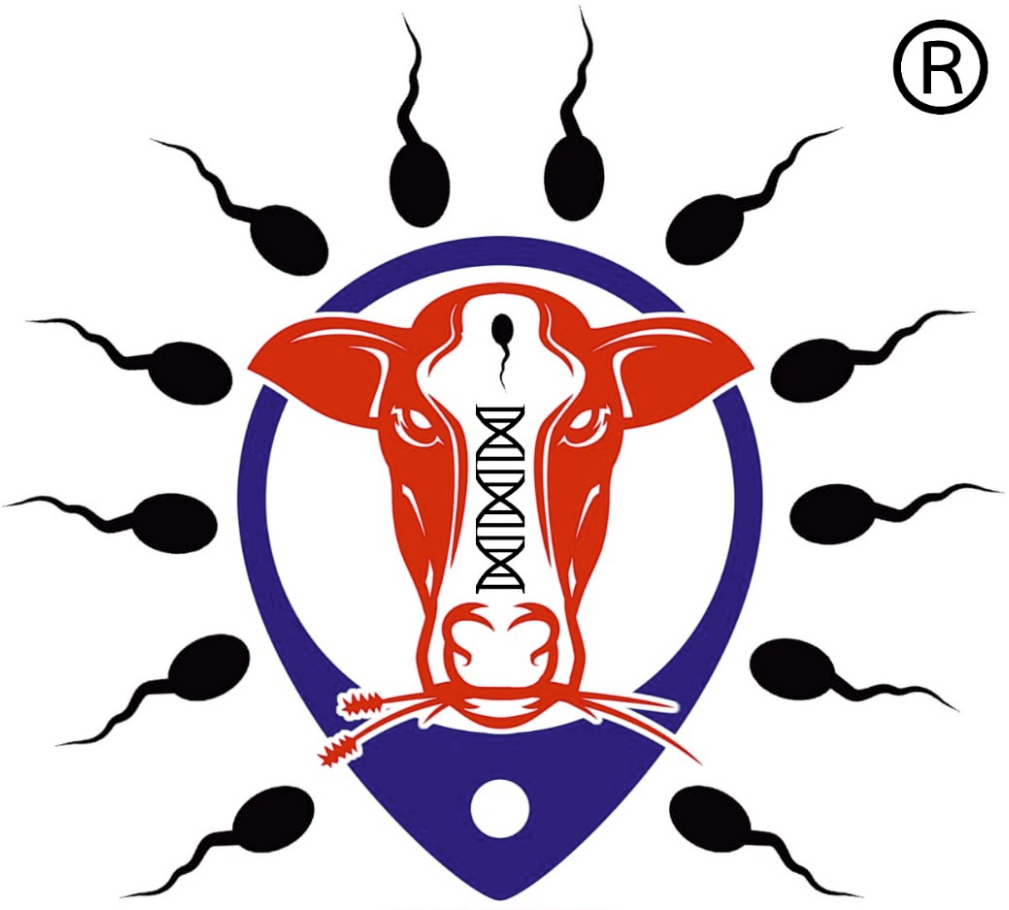

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