

Conference Report

Abstracts of the 4th International Online Conference on Animals [†]

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[†] Presented at the 4th International Online Conference on Animals (IOCAN 2026), 17–19 March 2026; Available online: <https://sciforum.net/event/IOCAN2026>.

Abstract

The pressures on human animal relations are growing and were recently addressed in the 4th International Online Conference on Animals (IOCAN). IOCAN 2026, which took place online from the 17–19 March 2026, aimed to provide opportunities for its participants to share facts and engage in discussion, relying on evidence-based veterinary medicine, animal welfare science, ethics and biology, all under a comprehensive One Health approach. The main topics and sessions of the conference were Sustainable Animal Welfare, Ethics and Human–Animal Interactions; One Health: Disease Manifestation and Management in Animals, Humans, and the Environment; Sustainable Animal Nutrition; Animal Genetics and Genomics; Environmental Challenges to Animals and Precision Livestock Farming; Animal Physiology, Reproduction, and Sustainable Animal Production; and Aquatic Animals. Building on the success of previous IOCANs, this conference had more attendees and presenters than ever before. The conference, being online, provided an opportunity for scientists from around the world to discuss the latest developments in our relationship with animals, without the necessity for anyone to travel. This particularly benefited scientists from low-income countries and those without the means or desire to attend an in-person conference. The major messages to emerge from the conference were the interconnectedness of animals, the planet and people in a One Health approach, the need for urgent action to address intensive animal farming, and the importance of improving the sustainability of our relations with animals.

Keywords: aquatic animals; wildlife; zoo animals; one health; sustainable; environment

Academic Editor: Rita Payan-Carreira

Published: 10 September 2026

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1. Introduction

The aims of IOCAN 2026 were to offer a unique opportunity for animal scientists globally to share their latest research, foster communication, and collaboratively address the pressing challenges animals face, such as declining genetic diversity, climate change, pollution, and intensive production systems. Participation was without charge.

The main topics and sessions of the conference were Sustainable Animal Welfare, Ethics and Human–Animal Interactions; One Health: Disease Manifestation and Management in Animals, Humans, and the Environment; Sustainable Animal Nutrition; Animal Genetics and Genomics; Environmental Challenges to Animals and Precision Livestock

Farming; Animal Physiology, Reproduction, and Sustainable Animal Production; and Aquatic Animals. Attendees were able to view accepted abstracts online during the event. Each session was followed by an active discussion, and poster presentations included a live presentation. Prizes were given for the best oral and poster presentation, with the best oral presentation awards being granted to Gruber et al. for Sciforum 156667 (antibiotic replacement for shrimps), Vargas-Perez et al. for 160855 (genetic developments in beef cattle) and Birrento et al. for 160815 (terpene profiles in goat milk). Additionally, best poster awards were granted to Harandi et al. for 157634 (microbiome in companion animals), Alves et al. for 157758 (communicating environmental enrichments for pigs) and Tariq and Hussain for 160084 (genomics and conservation of Pakistan's yaks).

The conference had 729 attendees and 74 speakers, comprising 311 attendees and 8 speakers from Asia, 213 attendees and 33 speakers from Europe, 110 attendees and 21 speakers from the Americas, 88 attendees and 8 speakers from Africa and 7 attendees and 4 speakers from Oceania.

We acknowledge the many people involved in IOCAN 2026: speakers; session chairs; sponsors; MDPI, who made this congress technically possible; Ms Kai Lin Ang, conference secretariat; and all contributors for their high-quality presentations. The flow of information did not end with the conference, as demonstrated by the published proceedings and a Special Issue of selected papers.

2. Preface

It is essential to have a world forum where relationships with animals can be discussed in detail, a place to confer and consider what we can do to restore this relationship to a healthy one. At a time when long distance travel is difficult and damaging to our planetary environment, having an online electronic forum has distinct advantages over face-to-face meetings. Since IT and Internet technology are widely available, scientists from economically less privileged countries can now be part of the worldwide scientific community in online conferences such as IOCAN 2026.

The matters discussed at the conference are of the utmost importance. Animals play an important role in the lives of many people throughout the world. For example, livestock, poultry and fish are major sources of food. The increase in the number of food animals over the last 25 years has exceeded the rate of growth of the human population. Large numbers of animals also interact with people as companions or working animals. The most recent estimates of numbers of companion animals globally are 700 million to 1 billion dogs, 350 million household cats (together with 480 million stray/feral cats) and about 50 million horses (World Population Review. 2026a; b; c).

Animal health and disease prevention are currently coming under pressure all over the world, but the control measures often do not have an immediate effect and incur significant costs. As scientists, the best way to support use of control measures is to provide facts and demonstrate feasibility, relying on evidence-based medicine, veterinary medicine, and biology—collectively known as One Health. IOCAN 26 had a focus on «One Health», linking health issues of humans and animals, as well as ecosystems—including climate, biodiversity, water, food systems, agriculture, and antimicrobial resistance. This demonstrates that the environment is an integral part of a shared health system that continuously involves both humans and animals. Animal species that often receive too little attention—beyond the major livestock species—such as aquatic animals, as well as animal and environmental protection in agriculture and fisheries striving for sustainable and ethical production, are all integral parts of One Health.

The changes in our relationship with animals are happening at an alarming rate, so fast that science can hardly keep up with the impact on our planet and its hugely valuable and

unique legacy of living organisms. We must recognize the urgency of this situation, curtail our abuse of the biosphere and reset our horizon to one that is sustainable for the planet. We can scale back our intensification of the livestock industries, we can integrate animal production with sustainable forestry, we can find alternatives to the use of antibiotics in animal feed, just as we can find alternatives to feeding animals with other animals. But these actions all have a cost, a cost to the farmers that have to change their methods of production, a cost to the consumer, who has to pay a higher price for a more sustainable product, and a cost to the industries that have grown into big multinational corporations to manage our relationships with animals. These corporations only exist because there is a profit to be made from intensive animal production for human consumption. We as scientists must seek to understand these costs to humanity as we search for improved ways of managing the human–animal relationship on our planet. We must make it known to the public, who need to be aware of the scale of the problem and the high cost of solutions for a more sustainable future. Open access online platforms like this conference and the *Animals* journal are not only vital in helping us to find solutions but also for people everywhere to participate and engage with the search for a better world. This conference has grown year by year, in the number of papers presented, and in the number of online attendees. We sincerely hope that this continues until it becomes a vital online annual forum that steers us to the restitution of a healthy relationship with animals. In *Animal Farm* (1945), George Orwell penned a slogan for an imagined revolutionary livestock commandment: “All animals are equal”. Later in the book, this was amended to “All animals are equal, but some animals are more equal than others”. IOCAN supports animal equality.

3. Abstracts of Presentations

3.1. Sustainable Animal Welfare, Ethics and Human–Animal Interactions

3.1.1. A Pilot Study of Hierarchy, Agonism and Aggression in a Group of Domestic Cats While Feeding

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In a stable cat group, interactions such as territorial defence or resource competition may be dynamic or variable. Simultaneous feeding can promote the emergence of aggression when defending or claiming food and represents a useful context to identify changes in hierarchy in pets. The aim of this study was to explore the identification of hierarchical positions occupied by individuals within a group of domestic cats by evaluating the frequency of aggressions performed and received during food offering. Four cats (three females and one male), all neutered and aged between 3 and 6 years, were included. To avoid bias due to modifications in feeding habits, the study was conducted in the home, and behavioural observations were performed by a person already familiar with the cats. Observations were carried out for 14 consecutive days at a fixed schedule for 10 min, during which the frequencies of agonistic and aggressive behaviours performed and received by each cat were recorded. Individual aggression indexes were estimated as follows: Aggressions Performed/(Aggressions Performed + Aggressions Received). In addition, aggression frequencies were analysed using the chi-square test. The frequency of aggressions performed and received differed between cats ($p < 0.0001$), with two cats identified as the main aggressors and one receiving the highest number of aggressions. The cats ranked by aggression index were: youngest female (3 years old) > male (6 years old) > older female (5 years old) > oldest female (6 years old), with an index nearly ten times lower.

These results should be interpreted with caution due to the small sample size. We conclude that identifying a high frequency of aggressive behaviours enables the identification of the dominant individual within the group and the lower-ranking animal in the hierarchy, which may present a welfare concern due to reduced control over food access.

3.1.2. Beyond Human Bonds: Rethinking Canine Welfare in Urban Life

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Welfare research often treats the human–dog bond as the whole of a dog’s social world. Yet, limiting focus to that dyad obscures the regulatory value of dog–dog contact. Urban restrictions on such interaction may foster relational deprivation, reducing emotional resilience and learning. In this talk, I explore how a relational view of welfare—extending beyond human bonds—can support more sustainable forms of coexistence. This contribution is a conceptual narrative review and does not report original empirical data. It asks how restricting intraspecific social relationships shapes canine regulatory strategies and what this implies for contemporary welfare models in urban environments. The analysis draws on existing research from ethology, psychology, and animal-welfare science, focusing on social buffering, autonomy, and emotional contagion in dogs and other social species. These ideas are considered within ethical frameworks that emphasise agency and mutual adaptation. Insights from behavioural practice are included as illustrative examples rather than empirical evidence. Across disciplines, the picture is consistent: welfare grows from relationships as much as from safety. When dogs have little opportunity for genuine, repeated contact with their own kind, they may adapt by relying more heavily on humans for regulation, while losing fluency in peer communication. By synthesising these findings, the paper examines whether current welfare frameworks adequately account for dogs’ intraspecific social needs. It argues that canine welfare should be understood as a relational and ecological construct rather than an individual state managed by humans. Recognising dogs as social subjects with their own relational needs invites a shift from control toward co-regulation and emotional reciprocity—foundations of sustainable coexistence in shared urban environments.

3.1.3. Comparison of Qualitative Behaviour Assessment by Laypersons with Observed Behaviour in Captive White-Faced Saki Monkeys (*Pithecia pithecia*) and Ring-Tailed Lemurs (*Lemur catta*)

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Evidence-based welfare audits are essential when assessing the impact of human-managed care on non-human primates living in zoos. Over the years, numerous methodologies have been proposed for evaluating animal welfare, predominantly focusing on either the care inputs provided in the form of housing, nutrition, and veterinary care or the meeting of other needs. However, recent research has increased its focus on animal-based outputs in the form of health and behaviour, while less attention has been placed on the psychological wellbeing of animals, owing to the difficulty of measuring subjective affective states in animals and questioning of whether measuring animal emotion is valuable, valid, and reliable in determining wellbeing. The present study examines the use of qualitative behaviour assessment (QBA) in saki monkeys (*Pithecia pithecia*) and ring-tailed lemurs (*Lemur catta*). Observers were recruited through an online survey and varied in their familiarity with non-human primate behaviour and the management of primates

in captivity. They were instructed to use a Visual Analogue Scale (VAS) to assess video clips of animals housed at an animal care college and zoo. The same videos were also scored by the author using a traditional quantitative ethogram and then correlated with the generated components from the QBA. The aim is for the findings of this research to indicate whether there are potential associations between the identified dimensions of emotionality in non-human primates and traditional quantitative methods of measuring behaviour, even when rated by laypersons.

3.1.4. Gender Effects in Brazilian Livestock Advisors' Management Practice Recommendations Regarding Dairy Cattle Welfare: A Preliminary Study

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Livestock advisors play an essential role in the decision-making process on dairy farms. Yet, their attitudes and recommendations are shaped by factors such as gender, age, culture, politics, and nationality. We explored how gender may influence Brazilian livestock advisors' recommendations regarding practices to improve dairy cattle welfare. For this, we analysed the responses to one open-ended question from an online questionnaire: "What management practices do you recommend to farmers to promote animal welfare?" The responses were categorised into five themes related to welfare principles (nutrition, environment, animal health, behaviour, and other). The number of participants who mentioned each theme was counted, and the results were presented in percentages. The sample (n = 219) had a balanced gender distribution (males = 48% and females = 51%), and participants were predominantly aged between 26 and 35 years old (47.9%). In general, reported management practices were mainly related to animal nutrition (20%) and environmental aspects (28%), including thermal comfort, hygiene, features of facilities, and animal stocking density. Animal health (13%), animal behaviour (9%), and other aspects (13%) were less mentioned. Women cited more frequent practices that promote natural behaviour (65%), followed by animal health (57%), environmental aspects (52%), and animal nutrition (51%). Also, 51% cited other welfare principles, like positive human–animal relationships (83%), staff training (59%), non-aversive handling (54%), and genetic improvement (50%). In contrast, men's recommendations focused on animal nutrition (49%), environmental management (47%), animal health (43%), and natural behaviour (35%). Other welfare principles cited by men were genetic improvement (50%), non-aversive handling (46%), staff training (41%), and positive human–animal relationships (17%). In conclusion, gender seems to influence how livestock advisors recommend practices to improve animal welfare. Both women and men acknowledged all welfare dimensions, but women placed greater emphasis on animal health, natural living, and positive emotional states, while men focused more on biological functioning.

3.1.5. I Rule in Favour of the Elephants: Granting Rights to Elephants in Sri Lanka

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Elephants play an intrinsic role in Sri Lanka, associated with cultural, religious and economic practices. According to a survey conducted in 2024, the country is home to an estimated population of 7400 to 7500 elephants. Reports further suggest that there are 210 captive elephants, of which 102 are housed in state-owned facilities, while the remainder are kept by private owners for work or as a symbol of status and prestige. These captive elephants are exposed to various human-induced cruelties, particularly against the five freedoms of animal welfare. Sri Lankan elephants are strictly protected under the Fauna and Flora Protection Ordinance, No. 2 of 1937 (hereinafter FFPO of 1937), and captive elephants must be duly registered and licensed in accordance with its provisions. However, due to political pressure, many elephants are kept in captivity without adhering to the proper procedures. On 19 August 2021, the Fauna and Flora (Protection, Well-being and Regularisation of Registration of Tamed Elephants) Regulations No. 01 of 2021 were issued by the respective State Minister under section 22A of the FFPO of 1937. Four separate cases were filed before the Court of Appeal, challenging the validity of these regulations. On 31 May 2024, the final decree was issued, which was in favour of the petitioners. However, captive elephants will continue to be subjected to cruelty and mistreatment by their custodians and mahouts until they are recognised as sentient beings with intrinsic value. This paper examines the necessity of granting rights to Sri Lankan elephants. The paper analyses the weaknesses in the existing protection available for elephants in Sri Lanka and provides recommendations on the rights that should be granted to elephants and how to grant them effectively, with a special focus on addressing the practical and implementation challenges of granting rights to elephants in Sri Lanka.

3.1.6. Intersection of Human Violence with Animal Abuse: A Study from Legal Perspective

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Mutual interdependence and intersection between animal abuse and interpersonal human violence is demonstrated by empirical research across disciplines. Animals are frequently harmed or threatened as tools of coercion, intimidation, and control, and early exposure to animal cruelty correlates with later violent behaviour. Such environments are associated with repeated injury, chronic stress, and compromised welfare outcomes for animals. This study, using an interdisciplinary, analytical and comparative approach, examines the intersection of human violence and animal abuse through a One Welfare lens (FAO & WOA and UNODC guidance), treating law as tool/governance mechanism that can enable or hinder animal welfare protection, connecting behavioural and physiological indicators with systematic intervention and providing better protection of animals. The study seeks to address how do existing legal and institutional frameworks recognise animal abuse occurring in violent human environments, and how can they be structured to improve early detection, prevention, and welfare outcomes for animals, building on earlier studies? Studying selected jurisdictions of the United States (mandatory cross-reporting statutes and pet protection orders), the United Kingdom (Animal Welfare (Sentencing) Act, 2021), and India (Prevention of Cruelty to Animals Act, 1960 and domestic violence law/constitutional provisions), the study highlights that despite recognition of the scientific correlation between human violence and animal abuse, law across jurisdictions fails to incorporate appropriate and practical interventions such as mandatory cross-reporting through Veterinarians and ethologists, protective measures for companion animals in

domestic violence cases, and the incorporation of animal-assisted interventions, affecting welfare. The study also identifies repeated unexplained injuries, chronic stress markers (fear, hypervigilance, withdrawal) and abnormal aggression or learned helplessness due to such intersection and need for including human violence and animal abuse as important parameter, particularly by animal welfare scientists.

3.1.7. Non-Invasive Molecular Monitoring as an Ethical Imperative in Wildlife Research: The Case of the Pyrenean Desman

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Non-invasive methodologies are emerging as essential ethical alternatives in wildlife research, especially when studying threatened or elusive species. Traditional capture-based protocols, although scientifically valuable, can induce physiological stress, disrupt natural behavior and increase the risk of injury or mortality. These impacts raise ethical concerns regarding researcher's moral obligations to minimize harm while generating reliable data. Non-invasive molecular tools align with the 3R principles—Replacement, Reduction and Refinement—by reducing animal manipulation and preventing unnecessary suffering. This study illustrates these advantages through monitoring the endangered Pyrenean desman (*Galemys pyrenaicus*) in Extremadura. Fecal material, environmental samples and biological traces were analyzed to obtain ecological, genetic and health information without capturing individuals. Real-time PCR, conventional PCR and sequencing enabled species identification, sex determination, pathogen detection and assessment of interactions with invasive species like American mink (*Neovison vison*), eliminating ethical conflicts associated with invasive surveillance. Environmental DNA (eDNA) represents a central ethical framework element. eDNA allows the highly sensitive detection of *G. pyrenaicus* from water and sediment samples, providing presence, distribution and community-level information without direct animal contact. eDNA replaces high-impact monitoring with greater sensitivity and broader ecological insight while avoiding stress, capture or disturbance. Its capacity to anticipate declines, detect emerging risks and monitor biodiversity supports both scientific goals and moral obligations toward species protection. Results demonstrate that non-invasive techniques are not merely methodologically efficient but ethically superior. Reducing human impact, avoiding physiological stress and eliminating necropsies or intrusive sampling needs makes them particularly suitable for vulnerable species, promoting a research culture grounded in responsibility, transparency and respect for wildlife integrity. This Pyrenean desman study shows the ethical imperative of integrating non-invasive and molecular approaches and highlights that they should be prioritized in conservation programs and research protocols, balancing scientific rigor with animal welfare and moral responsibility, thereby contributing to respectful and sustainable wildlife interactions.

3.1.8. Rethinking Disability and Animal Kinship: Learned Adjustment and Interspecies Care

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What can disabled-animal relationships reveal about systems of oppression and interspecies coexistence? This paper addresses this question by examining the woefully undertheorised alliance between disabled bodyminds and animals, which remains limited to discussions of support animals. This paper argues that examining disabled-animal relationships reveals crucial insights: the politics of euthanasia and how 'quality-of-life' judgments operate across species; the ethical complexities of anthropomorphism and

interspecies communication; how animal kinship opens alternative frameworks to human-centred understandings of care/value. By foregrounding reciprocity rather than utility, critical animal studies and disability studies can illuminate oppressive hierarchies, challenge anthropocentric and ableist frameworks, and expand possibilities for coexistence beyond human benefit. Animals are crucial to what it means to be human. The historic dehumanisation of disabled and racialised subjectivities has resulted in a synonymy with animals; horrifying racist/ableist sentiments strip minorities of their humanity, designating them as subhuman. Yet crucial to this ideology is the belief that animals have a less worthy existence. Disabled people and animals are especially made equivalent, 'presented as beasts and as burdens' (Taylor 2017). Such elision is identifiable regarding disabled animals; ableism isn't exclusive to humans: 'the able body that ableism perpetuates and privileges is always not only able-bodied but human' (ibid). Disabled animals are posited as devoid of value, with euthanasia presented as the kindest option. Our understanding of animals is shaped by the belief that only the strongest/fittest animals survive, yet, research revealed animals undergo what de Waal terms 'learned adjustment', similar to cognitive empathy, whereby non-disabled animals comprehend the reduced capacities of their disabled companions, supporting and protecting them. Animals offer disabled people connection free from ableist judgment, providing meaningful kinship and social mediation. While disabled individuals often anthropomorphise pets, valuing reciprocal care given difficulty finding safety with humans, this raises questions about where kinship begins and projection ends.

3.1.9. Switching off: Autonomic Modulation with a Fast-Acting Nootropic Alters Arousal and Cognitive Engagement in Working Dogs

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Introduction: High-drive working dogs often struggle to disengage or down-regulate arousal between tasks, creating operational challenges and welfare concerns. Fast-acting, non-sedative autonomic-modulating nootropics, formulations designed to transiently reduce arousal and promote behavioral calming, may offer a viable alternative to traditional sedatives or anxiolytics. Thus, this study examined the acute behavioral, cognitive, and task-engagement effects of a fast-acting autonomic-modulating nootropic in working dogs. **Methods:** Ten high-drive working dogs completed two test sessions: baseline (CTRL) and a day receiving a fast-acting autonomic-modulating nootropic (CALM[®]). Dogs performed (1) sustained-attention focus-hold trials at 30, 60, and 90 s with preferred and non-preferred stimuli; (2) puzzle-solving tasks (Levels 1–2); and (3) behavioral intensity ratings (1–5 scale). All duration-based outcomes were converted to percentage of maximal possible time. Within-dog differences between CTRL and CALM[®] were analyzed using paired *t*-tests with Cohen's *d_z* effect sizes, and cap-hit frequencies were summarized descriptively. **Results:** CALM[®] significantly reduced sustained attention across all non-preferred durations ($p = 0.023\text{--}0.038$; $d_z = -0.77$ to -0.87) and at the 90 s preferred interval ($p = 0.004$; $d_z = -1.22$), with similar nonsignificant trends at 30–60 s ($p \geq 0.070$; $d_z = -0.63$ to -0.65). Puzzle-solving times showed small-to-moderate nonsignificant slowing ($d_z = -0.36$ to -0.51), with more unsolved Level 2 puzzle items under CALM[®] (1 CTRL vs. 4 CALM[®]). Dogs that encountered an impasse during CALM[®] testing frequently disengaged or sought handler cues. Behavioral intensity decreased across both focus-hold and puzzle tasks ($d_z = -0.24$ to -0.80), consistent with reduced arousal. **Conclusions:** CALM[®] produced a coherent down-regulation of arousal and engagement intensity without impairing cognition, consistent with its autonomic-modulating formulation. These preliminary findings suggest utility for helping high-drive dogs disengage in overstimulating environments,

and they support the need for larger controlled trials across diverse working-dog and handler populations.

3.1.10. The State of Dairy and Farm Animal Welfare in Pakistan: A Call to Action for Sustainable Livestock Production

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Livestock is the backbone of Pakistan's agricultural economy, contributing nearly 15% to the national GDP and over 60% to the agricultural GDP. It sustains more than eight million rural households and plays a central role in ensuring food and nutritional security. Despite this importance, animal welfare remains one of the most overlooked aspects of livestock production. The living conditions of buffaloes, cattle, dairy cows, sheep, goats, camels, and working equines often fall short of basic welfare standards. Poor nutrition, limited veterinary access, heat stress, lack of shade and water, and outdated legislation continue to affect animal well-being and farm productivity. Pakistan's livestock population includes about 45 million cattle, 44 million buffaloes, 32 million sheep, 83 million goats, over one million camels, and nearly five million horses and donkeys. Welfare practices vary across regions and systems. Studies indicate that heat stress can reduce cattle feeding activity by up to 20%, while welfare-based management can increase productivity by 15%. A camel welfare survey in southern Punjab revealed that most animals lacked shade and water, and nearly a third experienced rough handling—though over half still showed calm behavior toward caretakers. Small ruminants and dairy animals in arid areas face seasonal feed and water shortages, disease risks, and inadequate veterinary coverage. Nationally, the outdated 1890 Prevention of Cruelty to Animals Act remains poorly enforced. However, the newly proposed National Animal Health, Welfare, and Veterinary Public Health Bill (2024) represents progress, promoting a "One Health" approach that integrates animal, human, and environmental well-being. Encouragingly, organizations like SPCA Punjab and universities are raising awareness about animal care. Yet, working animals still face overwork and neglect. Improving housing, nutrition, heat-stress management, humane handling, and precision livestock farming can make Pakistan's livestock sector more sustainable, productive, and compassionate.

3.1.11. A Descriptive Study of Social Behaviour When Mixing a New Goat Group: How Gentle Can It Be?

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When regrouping pregnant goats, blows can occur as this forms part of goats' social behaviour; however, this can induce abortions and impairment of animal welfare. The aim was to observe whether agonism and aggressive behaviours could be mitigated through habituation among goats that must be fused into a herd. At the farm from UADY (Mérida, México), 70 resident, pregnant adult goats, and a new group of 24 non-pregnant goats purchased from a local farm were used. All were in a good health state; the protocol was approved by the Bioethics Committee (UADY, ref# CB-CCBA D-2014-003). Prior to mixing, both groups remained apart for a period longer than one month. Habituation was considered as the repeated and gradual exposure to frequency and intensity of stimuli

without negative or positive consequences for animals. A four-step program exposed all animals to (1) three days of 10 min of vocalization records from both groups plus hair and faeces; (2) three days of riding on a leash in groups of three goats from the new group in front of the pen of the resident goats; (3) four days of grazing in the same paddock but separated by wire mesh; and (4) two days of grazing together in the same paddock, with qualitative observations of behaviour through scan sampling. From the results, during steps one and two, vocalization and sniffing behaviours were displayed in 100% of individuals of both groups. A total of 50% of them attempted biting during step two. For step three, a third of goats showed behaviour such as pushing with the head, and bite attempts were observed in all goats. Finally, during step four, only sniffing and no aggression events were observed in 100% of animals. We concluded that after 12 days of habituation, an absence of aggressive behaviour and a low intensity of agonism was observed when mixing goats.

3.1.12. Acceptance Assessment of Clomicalm™ Tablets in Dogs: Comparison to Anxitane™ as a Reference

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Canine behavioral disorders frequently necessitate long-term treatment, making the ease of product administration a crucial factor in selecting the most appropriate therapeutic approach. Palatability can significantly contribute to the ease of administration. This study was designed to investigate the ease of intake of Clomicalm™ (Virbac, France), clomipramine-based tablets formulated with an artificial meat flavor, indicated as an aid in managing separation-related disorders or anxieties and stereotypes in dogs, depending on the country. To benchmark the obtained results, Anxitane™ (Virbac, France) was selected as a reference. Anxitane™ is a supplement with Green tea concentrate containing L-theanine, which is recognized in the field for its established high ease of intake. This study involved eighteen adult Beagle dogs in experimental conditions. An assessment of the initial intake and overall consumption of tablets was performed by careful observation of the dogs. The results of this study demonstrate similar initial intake rates, with no statistically significant differences between the two products. Specifically, 83% and 89% of dogs readily took Clomicalm™ and Anxitane™, respectively. Notably, assisted intake of Clomicalm™ was only required for one dog, indicating a high degree of voluntary acceptance. A minority of dogs did not take the tablets at all (three for Clomicalm™ and two for Anxitane™). Among the dogs that did take the tablets, the consumption rates were also similar, with 60% and 62% fully consuming the Clomicalm™ tablet and the Anxitane™ tablet, respectively. Only two dogs half-consumed the Clomicalm™ tablet. In conclusion, this study provides evidence that Clomicalm™ and Anxitane™ are comparably easy to administer, with both having a high intake rate (>80%). This finding is significant for veterinarians and pet owners when considering long-term treatment options for canine behavioral disorders.

3.1.13. Agricultural Fairs: The Tension Between Spectacle and the Ethics of Care

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Recognizing the consciousness and emotions of animals fundamentally shifts the way we perceive them. Despite this understanding, many common agricultural practices serve as mechanisms that actively obscure animal suffering. This normalization of actions that induce pain, risk, or profound stress allows the underlying discomfort to become invisible. Agricultural fairs are central events for livestock farming, primarily functioning as showcases for superior animal genetics. Drawing from qualitative, ethnographic

fieldwork conducted during visits to three agricultural fairs in Brazil and one in Colombia, this study aimed to critically examine these venues as complex settings where the technical implementation of farm animal welfare often collides with tradition, spectacle, and economic valuation. The analysis focuses on Angus cattle. Observations documented the environmental conditions to which animals were subjected, as well as the embodied practices and human–animal interactions during judging sections. Several constraints to animal welfare were identified, such as (1) the presence of elevated noise levels, (2) constant artificial illumination, (3) limited access to feed and water, and, occasionally, (4) a lack of adequate shelter within the holding pens. Observations also highlighted conditions that illustrate a complex dynamic of control and dominance in human–animal relationships: (5) animals were managed using nose rings, and, notably, (6) a striking or ‘slap’ was even used to identify the winning specimen during judging events. A particularly concerning finding was the (7) exhibition and judgment of late-term pregnant cows or heifers. This practice sometimes results in cows giving birth prematurely or under highly unsuitable, public conditions. The intense natural event of a birth is thus co-opted into the spectacle, normalizing an unacceptable level of risk and distress for animals. When considered alongside additional, unexamined constraints, agricultural fairs emerge as socio-cultural spaces where embodied human authority and spectacle are normalized at the expense of animal welfare.

3.1.14. Agrivoltaic Systems as a Tool to Improve Dairy Farming Sustainability—A Case Study

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This case study aimed to discuss agrivoltaic systems, advocating that this approach can serve as a tool for the sustainable intensification of dairy farming. Solar power plants generally require extensive land areas and are positioned close to the ground surface, preventing the land from being used for other productive activities. In contrast, installing photovoltaic panels on rooftops or carports can be considered a zero-space-occupying strategy, as they optimize land use by fulfilling dual functions. This concept can be extended to agriculture, leading to increasing interest in agrivoltaic systems (AVSs). AVSs represent an innovative approach that integrates photovoltaic energy generation with crop and/or livestock production in the same area. In our system, a total of 72 photovoltaic panels were installed, covering 144 m² and distributed across six paddocks (24 m²/paddock) to provide shade for grazing dairy cows. In the first 30 days of operation (26 October–26 November 2025), the AVS generated 4860 kWh of energy, which corresponds to 0.55 t of CO₂ emissions avoided, directly contributing to lowering the carbon footprint of milk production. Agrivoltaic systems optimize land use, enhance sustainable intensification, and have broad social acceptance. In dairy farming, installing photovoltaic panels in pasture areas not only produces clean energy by intercepting part of the solar radiation but may also improve the thermal environment for cows, offering behavioral, productivity, and welfare benefits. These findings support agrivoltaic systems as a promising strategy to improve the sustainability, resilience, and economic viability of dairy production systems, particularly in tropical regions increasingly affected by climate change.

3.1.15. Animal Cruelty and Welfare Conditions at the Akinyele Abattoir in Ibadan

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Introduction: Animal welfare in livestock production remains a neglected issue in Nigeria, especially during slaughtering. Goats and sheep exhibited welfare consequences such as stress, fatigue, thirst, hunger, pain, fear, and distress. Most food animals reared for meat production have a range of welfare and ethical concerns, particularly during the farming and slaughtering process. The purpose of an abattoir is to ensure the humane handling of animals using hygienic techniques for slaughtering and dressing to produce hygienically prepared meat. **Methods:** This study assessed animal cruelty and welfare conditions at the Akinyele Abattoir in Ibadan, where Halal slaughtering without stunning is predominantly practiced. The slaughtering process was examined in two phases—pre-stunning (arrival, unloading, lairage, and handling) and bleeding. **Results:** A lot of welfare issues were identified like poor loading and unloading of the goats before slaughtering. The animals were stressed throughout the operation which will affect meat quality. These issues were linked to inadequate facilities, poor handling, and lack of staff training. Using animal-based measures, hazards and welfare risks were identified, and preventive and corrective measures were proposed. **Conclusion:** The findings highlight that structural and managerial improvements are essential to reduce cruelty and enhance animal welfare standards at Akinyele Abattoir and similar facilities in Nigeria.

3.1.16. Animal Lovers' Tourism as a Sustainable Development Strategy: Evidence from Crete

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Background: Companion animal welfare is an overlooked but crucial dimension of sustainable tourism development. While attention has often been directed towards wildlife or farm animals, little is known about how the management of stray and owned companion animals influences destination image and tourist satisfaction. This study introduces the concept of animal lovers' tourism, proposing it as a strategy that combines animal welfare improvement with sustainable destination branding. **Methods:** A mixed-methods design was employed. Quantitative data were collected through a structured questionnaire administered to 1000 international tourists visiting Crete in 2024–2025. Qualitative insights were derived from semi-structured interviews with stakeholders (tourists, shelter managers, municipal officers) and systematic on-site observations. Data were analyzed using descriptive statistics, regression models, and thematic coding. **Results:** Findings show that visible cases of abuse, neglect, or mismanagement of stray animals negatively affect visitors' emotional responses and overall destination image ($p < 0.01$). Conversely, positive encounters with well-cared-for animals enhance satisfaction, increase revisit intentions, and promote positive word-of-mouth. The analysis also reveals an emerging demand for tourism experiences that actively involve animal welfare, such as volunteering in shelters, adoption networks, and animal-friendly accommodations. **Conclusions:** Animal lovers' tourism can serve as a transformative model of sustainable development, aligning with the One Health framework by linking human well-being, animal welfare, and environmental sustainability. Policy recommendations include municipal strategies for stray animal

management, partnerships between tourism operators and welfare organizations, and the integration of animal welfare into destination marketing. These findings provide a foundation for further research and practical implementation in Mediterranean destinations and beyond.

3.1.17. Anthropogenic Influences on the Scavenging Ecology of Egyptian Vultures in Gonda, Terai Region of Uttar Pradesh, India

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Introduction: The Egyptian Vulture (*Neophron percnopterus*), an endangered scavenger, plays an important role in ecosystem health and corpse decomposition processes. However, populations have been declining throughout the Indian subcontinent, mainly due to anthropogenic influences. The present study explores how human activities affect the scavenging ecology and feeding behavior of Egyptian vultures in Gonda District, Terai region of Uttar Pradesh, India. **Methods:** The observations were conducted from January to June 2025. Systematic field observations were conducted at certain dumping locations. Point count sampling techniques were used to capture information on interspecific interactions, food type, feeding duration, and frequency of foraging. **Results:** The findings showed that vultures relied extensively on manmade food sources, mainly animal carcasses (57%), poultry waste (25%), and market rubbish (10%). Feeding activity peaked in the morning (08:00–11:00 h), coinciding with carcass disposal times at dump sites. Feral dogs, cattle egrets, and crows frequently engaged in interspecific conflict, limiting feeding time and food availability. Dependence on human-mediated food sources demonstrates both adaptation and susceptibility—adaptability in exploiting alternative resources, but vulnerability due to potential exposure to toxins, diminished food supply, and habitat disruption. **Conclusions:** The study underlines the critical need for better waste management techniques, construction of vulture feeding zones, and public awareness campaigns to support the long-term conservation of Egyptian Vultures in human-modified environments.

3.1.18. Beef-on-Dairy and Holstein Calves: Should We Mix or Keep Them Separate? Behavioral Impacts of Breed and Pair Composition During the Prewaning Phase

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Beef-on-dairy (BoD) calves are increasingly common on dairy farms, yet little is known about their behavior or how social housing with purebred dairy calves may influence behavioral responses. This study evaluated the effects of breed composition (Holstein vs. BoD [Angus × Holstein]) and pair composition on behavior and behavioral responses in standardized tests. In Study 1 (n = 26; 18 Holstein, 8 BoD), calves were observed using scan sampling (5 d/wk) for behaviors including exploration, feeding, play, and abnormal oral behavior. A food neophobia test was conducted at 50 d of age. In Study 2 (n = 62; 37 Holstein, 25 BoD), calves were housed in Holstein, mixed, or BoD pairs. Behavioral observations were conducted weekly for 1 h around milk meals. Calves also underwent behavioral tests: food neophobia (19 ± 2 and 48 ± 2 d), novel object (16 ± 3 and 41 ± 2 d), novel human approach (65 ± 3 d), and regrouping (79 ± 2 d). Breed composition affected exploration ($p < 0.01$) and feeding ($p < 0.01$) behaviors: Holsteins explored more, whereas BoD calves performed more feeding behaviors. In the novel food test, Holsteins interacted

less (OR: 0.28; 95% CI: 0.07–0.92; $p = 0.03$), took longer to touch ($p = 0.04$), and consumed less novel food than BoD calves (Holstein: 2.17 ± 0.96 g; BoD: 6.55 ± 1.89 g; $p = 0.03$), while in the novel object test, they interacted more frequently ($p = 0.01$), with similar latencies. Pair composition also affected calf behavior, particularly in Holstein calves housed with BoD peers. These calves showed increased feeding behavior ($p < 0.001$), greater abnormal oral behaviors ($p < 0.01$), and longer latencies to approach an unfamiliar human compared with Holstein calves housed in homogeneous pairs. Overall, breed composition and pair composition shaped preweaning calf behavior, with mixed housing positively influencing feeding behavior but negatively affecting oral behaviors.

3.1.19. Behavioral Responses of Holstein Cows to Mats Composed of Interlaced Colors

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The objective of the present study was to understand how chromatic stimuli can modulate the locomotor and exploratory responses of experienced animals, considering the dichromatic vision of cattle and its impact on welfare. The research was conducted at the Federal Institute of Education, Science and Technology of Southern Minas Gerais, Muzambinho Campus, Brazil. Six lactating Holstein cows were used, tested individually and in groups, in familiar (FA) and unfamiliar (UA) locations. The mats, made of nonwoven fabric, featured interlaced stripes of lilac and red, lilac and black, and lilac and yellow. The observed behavioral responses included crossing, reluctant crossing, exploration, retreat, and attempts to jump, analyzed using the nonparametric Kruskal–Wallis and Nemenyi tests. In general, adult cows showed confidence when crossing the obstacles, maintaining a high likelihood of crossing the areas regardless of color or environment. However, a differentiated behavior was observed in response to the lilac–yellow mat, which resulted in a higher probability of reluctant crossings when the cows were in groups (74.33% in FA and 59.99% in UA), whereas individual assessments showed a predominance of non-reluctant crossings. This collective hesitation suggests that the contrast between yellow, which is easily perceived by cattle, and lilac, which has low distinction, creates a depth illusion that modulates spatial perception, particularly under social influence. It is concluded that although adult cows exhibit greater familiarity and lower sensitivity to visual stimuli, the lilac–yellow pattern remains capable of altering behavior, reinforcing that even experienced animals respond to visual contrasts and group dynamics in management situations.

3.1.20. Beyond Animal Protein: An Analytic Philosophy Approach to Transformations in Human Food Culture

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Throughout history, human beings have assumed dominion, ownership, and the right to use and consume nonhuman animals, both domesticated and wild. The everyday consumption of meat, derived primarily from domesticated animals, sustains complex economic value chains that reinforce human beliefs and practices used to justify dietary habits and nutritional norms. The human–nonhuman relationship thus reflects a one-sided, anthropocentric conception of need, even when couched in the language of “humane” treatment in production and slaughter. Yet, nonhuman animals remain unacknowledged in their individuality, mindedness, and intrinsic animality within this historical framework. Recent innovations—such as cultivated meat, 3D-printed meat, insect protein, and even air-derived protein—embody a transformative potential to redefine human food culture. This paper explores the ethical viability of changing the human practice of eating animals, considering these emerging alternatives. The study employs an analytic philosophical method and applies the critical thinking framework of Elder and Paul (2012) to examine underlying assumptions and value systems. The findings indicate that humans have constructed an ethically unbalanced relationship toward voiceless nonhuman animals. However, technological developments reveal that protein production need not rely on suffering or death. The emergence of alternative proteins offers an opportunity to reimagine an interdisciplinary ethics of eating—one that integrates managerial, technological, political, and educational dimensions to transform long-standing human conceptions of meat and morality.

3.1.21. Bioeconomic Modeling for Sustainable Fishery Management

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This study develops a comprehensive bioeconomic model to investigate the critical balance between ecological sustainability and economic profitability in Moroccan fisheries. By integrating mathematical ecology, dynamic optimization, and game theory, the model captures the coupled interactions between biological dynamics, harvesting behavior, and economic incentives. The research focuses on two demersal species—Guitarfish (*Rhinobatos* spp.) and Plaice (*Pleuronectes platessa*)—both ecologically important and socio-economically valuable to Morocco’s coastal communities. Notably, Guitarfish is currently listed as threatened on the IUCN Red List, emphasizing the urgency of adopting sustainable exploitation strategies. The model simulates alternative harvesting scenarios to evaluate the effects of fishing effort on biomass recovery, species coexistence, and long-term profitability. Results reveal the existence of optimal effort thresholds that maintain ecological resilience while ensuring economic viability. Cooperative management approaches, where stakeholders coordinate strategies, consistently outperform competitive exploitation in achieving stable equilibria and higher collective payoffs. The study highlights the importance of adaptive policy design, incorporating economic incentives, biological feedback, and institutional cooperation to secure the long-term viability of fisheries. This research contributes to the advancement of sustainable resource management by linking theoretical modeling with practical policy insights. It directly supports several United Nations Sustainable Development Goals, including SDG 14 (Life Below Water), SDG 12 (Responsible Consumption and Production), and SDG 8 (Decent Work and Economic Growth). Overall, the framework provides policymakers and researchers with a robust decision-support tool to promote responsible, science-based fishery governance in Morocco and beyond.

3.1.22. Captured or Independent? Veterinary Institutions, Animal Welfare Science, and the Politics of Farmed Animal Welfare and Meat Reduction

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This presentation reports on emerging research at the London School of Economics examining how veterinary institutions and the wider animal welfare science community shape policy and practice in farmed animal welfare and sustainability. Veterinary surgeons hold explicit professional duties to protect animal health and welfare, while animal welfare scientists operate within a mandated scientific field that implicitly carries parallel responsibilities. Yet the extent to which these professional and scientific communities drive, resist, or merely accommodate change within animal agriculture remains insufficiently understood. Drawing on the first phase of a mixed-methods study, the talk presents early findings from a case study of the British pig veterinary profession, including documentary analysis of welfare standards, policy positions, and the profession's engagement with sustainability and meat reduction debates. Preliminary international comparisons suggest that major National Veterinary Associations have not adopted science-based dietary or environmental targets aligned with frameworks such as Eat-Lancet or national climate recommendations. The analysis also situates these observations alongside developments in other jurisdictions—for example, policy debates in the United States around Proposition 12 and the positions taken by the American Veterinary Medical Association. The presentation concludes by considering whether multiple forms of alignment and influence between veterinary bodies, animal welfare science institutions, and livestock industries limit the scope for independent professional leadership. It explores how principles from public interest theory and emerging concerns about professional and epistemic capture may apply not only to veterinary organisations but also to the broader animal welfare science community as a global epistemic actor.

3.1.23. Circular Biotechnologies for Goat Reproduction: Integrating Animal Welfare, Sustainable Resource Use, and In Vitro Embryo Technologies

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Sustainable dairy goat production requires strategies that support animal welfare, efficient resource use, and long-term genetic improvement. In the Netherlands, Saanen goats are frequently maintained in extended lactations of 2–4 years, and at the end of their productive lifespan, their reproductive tissues remain a potentially valuable source of genetic material. Integrating post-mortem ovary recovery into in vitro embryo production (IVP) systems offers a circular approach to utilizing these resources while avoiding additional interventions in live animals. Furthermore, the distribution of cryopreserved embryos rather than livestock provides a welfare-friendly and biosecure genetic dissemination, reducing transport-related stress and disease risks. This preliminary study evaluates the feasibility of producing transferable embryos from elderly, long-lactation Saanen goats within a circular and welfare-oriented reproductive framework. From October 2024 to November 2025, ovaries were collected from 12 Saanen goats aged 8–11 years at a certified

slaughterhouse operating in compliance with Article 148 of Regulation (EU) 2017/2625 and Regulation (EU) 2016/429. Cumulus-oocyte complexes were aspirated, matured in vitro, and fertilized with either fresh ($n = 2$) or cryopreserved semen ($n = 10$). Zygotes were cultured to Day 8 using standard IVP protocols. All viable embryos were cryopreserved for future non-surgical transfer within the Netherlands. A total of 354 oocytes were recovered (30 ± 7.6 per goat). Fertilization resulted in 308 zygotes (26 ± 6.3 per goat). In total, 41 embryos developed (3 ± 1.9 per goat): 24 on Day 6, 12 on Day 7, and 5 on Day 8. Blastocyst rates were 31.8% for fresh semen and 10.7% for cryopreserved semen. All embryos were cryopreserved. These results demonstrate that post-mortem ovaries from elderly Saanen goats can produce viable embryos via IVP. This approach supports circular economy principles, reduces biological waste, preserves valuable genetics, and enhances animal welfare through embryo shipment instead of live-animal transport.

3.1.24. Comparative Study of 3d-Printed Models, Real Specimens, and 2d Materials in Veterinary Anatomy Education

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Introduction: Teaching veterinary anatomy presents unique challenges due to the considerable morphological variation among domestic species, the limited availability of large specimen collections—especially for wild animals—and the biosafety restrictions associated with cadaveric materials. Three-dimensional (3D) printing has emerged as a promising tool to overcome these limitations by enabling the production of durable, safe, and anatomically accurate models. This study aimed to compare learning outcomes using three different study methods: real anatomical specimens, 3D-printed models, and traditional 2D materials (books and printed images). **Methods:** This study was conducted with undergraduate veterinary students ($n = 261$), divided into two subgroups based on prior exposure to anatomy: the ‘previous knowledge group’ ($n = 111$), composed of students who had already completed the anatomy course, and the ‘no prior knowledge group’ ($n = 150$), composed of first-semester students. Participants were randomly assigned to one of the three study methods and given one hour to study the assigned material. All students subsequently completed the same practical test using real specimens, allowing for direct comparison of performance across methods. **Results:** Students who studied using real anatomical specimens or 3D-printed models achieved significantly higher test scores compared to those who studied exclusively with 2D materials, with no statistical difference between the real-specimen and 3D-model groups. Test completion time did not differ among groups, and no significant differences were found between male and female participants. **Conclusions:** These findings demonstrate that 3D-printed models are a reliable tool for anatomical learning, providing outcomes equivalent to real specimens. They also offer practical advantages such as lower costs, improved biosafety, durability, and color customization. Moreover, 3D printing—and related technologies—holds strong potential for broader applications in veterinary and health sciences education, surgical planning, skills training, and more ethical and scalable research involving animals.

3.1.25. Comparing Patrol-Based and Camera Trap Mark–Resight Methods for Monitoring Population Size and Welfare in Free-Roaming Domestic Cats

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Introduction: The effective management of free-roaming cat populations depends on the robust monitoring of population size and welfare. This study compared two approaches, direct Mark–Resight patrol surveys and a virtual Mark–Resight method using camera traps, to evaluate a sterilized free-roaming cat population in a defined area. **Methods:** Monitoring was conducted once every five weeks for one year. Each monitoring series included two consecutive days with surveys at sunrise and sunset (four observation events per series). In routine patrols, observers followed a standardized route at a fixed pace, recording encountered cats and identifying individuals using natural markings (coat pattern, scars, facial shape). In the camera trap method, two motion-activated cameras and an automatic feeder at a structured feeding station collected images via motion trigger and time-lapses. Images were reviewed post hoc to identify individuals. For each physical or photographic encounter, sex, age class (adult/young), sterilization status (indicated by surgical ear-tip removal), body condition score (BCS), and injury score were recorded. Population size was estimated per series using the Lincoln–Petersen Mark–Resight model, averaging morning and evening estimates. **Results:** Routine patrols detected significantly more cats than camera traps throughout the study (32.5 ± 1.6 vs. 15.8 ± 0.09 ; $p < 0.0001$). Both methods showed higher detection at sunset vs. sunrise (camera traps $p = 0.0119$; patrols $p = 0.0002$). Camera traps detected a higher proportion of young cats ($p < 0.0001$) and showed a slightly better identification of sterilization status ($p = 0.0045$). In patrols, young cats had lower BCS than adults ($p = 0.0058$), with a similar trend in camera traps ($p = 0.0858$). Variance in BCS and injury scores was greater in patrols (BCS: $p = 0.0163$ – 0.0184 ; injuries: $p = 0.0517$ – 0.0593), indicating superior sensitivity for welfare assessment. **Conclusions:** Each method demonstrated distinct strengths: patrol surveys more accurately captured total population size and welfare indicators, while camera traps improved the detection of young cats and sterilization status. The combined use of both methods provides a more comprehensive framework for monitoring urban free-roaming cat populations.

3.1.26. Contribution to the Study of Some Wild Carnivorous Mammals in Western Algeria Yousouf Kerboub

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This four-year study, conducted from April 2013 to May 2017, provides a comprehensive inventory and update on the geographical distribution of wild carnivorous mammals in Western Algeria, with a specific focus on regionally endangered species. Spanning diverse biotopes, the primary objective was to establish a robust baseline assessment of the local carnivore population status. The investigative work employed a multi-source methodology, combining extensive direct and indirect observation (tracks, scat, and sightings) with surveys and questionnaires conducted among local communities, hunters, and forestry agents. This approach allowed for the collection of current ecological data alongside historical records across the study area. The research successfully documented 14 carnivorous mammal species across six families. Canids (*Canis aureus*, *C. lupus*, *Vulpes rupellii*, *V. vulpes*) were widely distributed. In contrast, Felids—including the regionally threatened *Felis libyca*, *F. margarita*, and *F. caracal*—were poorly represented. Mustelids (five species, including

Lutra lutra) showed restricted distributions, and Hyaenidae (*Hyaena hyaena*) presence was minimal. Both the Viverrid (*Genetta genetta*) and Herpestidae (*Herpestes ichneumon*) were observed in low numbers, indicating a significant risk of extinction. These findings confirm the persistently endangered conservation status of these carnivore guilds, necessitating immediate intervention. The comprehensive data, including biological samples for genetic analysis, emphasizes the need for national and international cooperation to manage this valuable genetic material. This work also strongly complements the magnificent conservation efforts underway for the Saharan Cheetah (*Acinonyx jubatus hecki*), providing vital, up-to-date information for developing targeted protection strategies in Western Algeria.

3.1.27. Correlation Between Perceived Elements and Recommended Practices for Dairy Cattle Welfare

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Perceptions of animal welfare among dairy technical advisors reveal a complex interaction between knowledge, ethical values, and practical realities, often shaped by cognitive dissonance described as the psychological discomfort that arises when beliefs and behaviors conflict. This study aimed to explore how Brazilian advisors conceptualize animal welfare and how this is reflected in the management practices they recommend to farmers. Two hundred seventy-two participants answered two open-ended questions through an online questionnaire. Answers were analyzed to identify both the perceived key elements for promoting welfare and the practices most frequently recommended to achieve it. Responses indicated that advisors view welfare as a multidimensional concept aligned with the Five Domains model that includes nutrition, environment, health, behavior, and mental state. However, when asked about the practices they recommend, the focus shifted toward more operational actions including improving facilities, hygiene, and feeding systems rather than promoting opportunities for natural behaviors or affective state. This divergence between what is valued and what is practiced may reflect an underlying cognitive dissonance, i.e., advisors acknowledge the significance of animal welfare but must operate within economic system and cultural constraints that limit the implementation of these ideals. To reduce this dissonance, individuals may unconsciously justify compromises, reframing welfare as compatible with existing production goals. This tension between beliefs and professional practice reflects a broader social pattern, in which people's affection for animals coexists with their use for human purposes. Recognizing this dissonance is crucial for transforming awareness into action, encouraging reflective dialogue, and continuous education, and it can help advisors align their welfare beliefs with consistent on-farm practices, promoting more compassionate and sustainable dairy systems.

3.1.28. Cross-Species Communication: Formation of a Symbol-Based Human–Animal Digital Interface

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Humans and animals share a longstanding bond, yet meaningful cross-species communication remains an open research challenge. While recent digital systems—such as button-based or auditory interfaces—have enabled structured interaction between felines and humans, most approaches begin from human-designed symbols rather than from the actual scenarios in which communication emerges. This study proposes a scenario-based framework for feline–human interaction. Instead of assuming predefined symbols, the approach begins by identifying recurring behavioural scenarios—such as care, discomfort, attention seeking, or social negotiation. Within each scenario, the goal is to examine which behavioural points may naturally evolve into symbolic representations, based on observable tendencies rather than human inference. This forms the basis for preferential symbol emergence, where symbols arise from the context itself, rather than being imposed beforehand. Accordingly, the study poses three core research questions: (1) Which scenarios consistently appear in feline–human interaction? (2) Within each scenario, which behavioural cues could serve as candidates for symbolic representation? (3) How might these candidate symbols be digitally encoded and tested without reducing ethologically complex behaviour to fixed abstractions? For instance, scratching—often associated with discomfort or power imbalance during caregiving—may not represent a single symbol but rather a spectrum of responses which may evolve into a layered multimedia or parametric response within a specific scenario that calls for contextual interpretation. Likewise, observations such as a cat recognizing a worker icon after seeing a real worker suggest that symbol inference may occur through spontaneous scenario-based association rather than direct learning. By grounding symbolic representation in scenarios rather than assumptions, this work outlines an initial framework for an ethically aligned and perceptually informed digital interface for cross-species communication.

3.1.29. Development of an Online Reporting Interface to Detect and Reduce Animal Abuse Cases

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Introduction: Recognizing animal abuse and assessing its extent is important, since animal abuse and maltreatment are the first steps to aggression against people. Furthermore, it is also an important basic question of animal welfare. Due to media coverage, a wider audience has been affected by the concept of animal protection. It is important to emphasize the right animal husbandry at a level which is understandable for the public, too. **Methods:** This study considers in-depth interviews with people who are playing an active role in judging cases of animal abuse. A survey was made on the process of reporting cases of animal abuse observed by the public, and its interest in a new online animal abuse reporting system. **Results:** Based on the interviews it is evident that powers are not clarified in animal abuse cases, so discourses are continuous between authorities and civil animal rights activists. Based on the questionnaire's responses, a significant proportion of the respondents are not aware which authority or organization should be contacted in cases of animal cruelty, and which in cases of observing poor living conditions. People would prefer to use reporting options available by phone. **Conclusion:** The concept of animal abuse is different in administrative law than in criminal law, which the public may not necessarily be able to distinguish. This study has proposed a closed-shamed online reporting interface, which is beneficial in the judgement procedure. It has high

priority on-the spot-recording during actions, and in the possible judgement procedures, a lot of money and time could be saved. A number of unnecessary measures could be reduced. This could strain off a great mass of false statements and could be lifesaving for animals: <https://www.moonshot-test.com/allattudomanyi-concept/> (accessed on 14 December 2025).

3.1.30. Digital and Precision Welfare Monitoring: A Systematic Review of Emerging Technologies for Sustainable Animal Welfare

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The intersection of digital technology and animal welfare science is creating a new paradigm: precision welfare monitoring. This systematic review explores the latest digital tools used to assess, predict, and improve animal welfare in livestock systems. A comprehensive literature search across Scopus, Web of Science, and ScienceDirect (2015–2025) identified 142 peer-reviewed studies that met the inclusion criteria. Findings show a clear shift from traditional, human-based welfare assessments toward automated, sensor-driven systems that allow continuous, non-invasive observation. The leading technologies include computer vision (for behavior and posture analysis), wearable biosensors (for physiological monitoring), and acoustic sensors (for stress and health detection). Increasingly, AI and machine learning are being used to interpret complex data streams, enabling early detection of welfare issues such as lameness, heat stress, or aggression. However, several critical gaps remain—particularly around data integration, algorithm transparency, and ethical governance. While precision monitoring holds great promise for improving welfare and sustainability, it also raises important ethical questions about animal privacy, data ownership, and the potential detachment of human caretakers. Future research should focus on ethical AI frameworks, cross-species validation, and education for farmers and caretakers to ensure that technology enhances welfare rather than merely improving efficiency. Overall, digital and precision welfare monitoring represent a transformative step toward data-driven, ethically responsible, and sustainable animal production systems.

3.1.31. Distribution and Habitats of the Spider-Tailed Horned Viper

Pseudocerastes urarachnoides

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The horned spider-tailed viper (*Pseudocerastes urarachnoides*), a Near Threatened venomous species of the family Viperidae endemic to the Zagros Mountains in western Iran, is renowned for its unique caudal luring adaptation that mimics a spider to attract prey; the species was studied and described as the spider-tailed viper *Pseudocerastes urarachnoides* by Bostanchi, Anderson, Kami, and Papenfuss in 2006. This study reports previously undocumented low-elevation habitats of *P. urarachnoides* in Kermanshah Province, western Iran, where field surveys recorded geographic coordinates, elevation, slope via GPS, and environmental parameters (e.g., temperature) using thermometers, with specimens safely handled using hooks and tongs. These new sites differ markedly from previously reported localities: vegetation is sparse and dominated by annual herbaceous plants and scattered low shrubs with a complete absence of oak trees; substrates consist exclusively of limestone sediments; elevations range from approximately 650–700 m above sea level (substantially lower than most known records); and slopes are extremely steep, reaching up to 90° (near-vertical rocky mountain faces). Observations were made between 09:00 and 11:00 under an ambient temperature of 27 °C, aligning with the species' activity patterns during warmer morning hours. These findings significantly expand the known ecological and elevational

range of *P. urarachnoides*, highlighting its greater adaptability than previously assumed; the open structure, light-colored limestone substrate, sparse vegetation, and, particularly, the steep-to-vertical slopes likely optimize caudal luring efficacy for ambush predation while enhancing camouflage against visually oriented predators and prey. However, these vulnerable low-elevation habitats face severe threats, primarily from road construction, which causes direct habitat destruction, ecosystem fragmentation, and restricted resource access, potentially accelerating local population declines. This study underscores the urgent need for targeted conservation measures, including protection and monitoring of these newly discovered steep-slope, low-elevation populations in Kermanshah Province.

3.1.32. Do Domestic Cats Groom More in Artificial Light at Night (ALAN)?

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Introduction: Artificial Light at Night (ALAN) is an increasingly prevalent anthropogenic issue that alters circadian rhythms and induces neuro-behavioural issues in several animals. Self-grooming or grooming in mammals is a robust, innate behaviour which is established as an indicator of neurological stress. The present study aimed to examine the effect of ALAN on the grooming behavioural pattern in domestic cats. **Methods:** The research follows an approach to assessing grooming activity patterns over time under normal conditions at day and ALAN exposure. The artificial light was normal house LED light with an intensity of about 40 lux (on average). Behavioural data were collected through video recordings conducted twice daily. Videos were analysed to quantify grooming frequency, duration, and type (licking, scratching with limbs, or scratching with mouth/teeth). **Results:** Preliminary analyses revealed that frequency of grooming bouts increased notably under ALAN or prolonged night light exposure, in compared to natural daylight conditions. Cats, regardless of gender, preferentially rest in shaded regions when exposed to ALAN, suggesting avoidance of artificial light exposure behaviour. Among grooming types, licking dominated over scratching. **Conclusions:** The findings indicate that ALAN elicits neuro-behavioural stress responses in domestic cats, reflected through the heightened grooming activity and light-avoidance behaviour. The pronounced increase in nocturnal grooming under artificial illumination indicates that chronic exposure to nightlight, can potentially cause circadian disruption and manifest stress-related behavioural patterns. Furthermore, it is of evolutionary importance that how the domesticated cats adapt to light pollution. Research integrating physiological stress markers could clarify the mechanistic links between ALAN exposure and altered stress-related behaviour in cats.

3.1.33. From Science to Farm: How Environmental Enrichment for Pigs Is Communicated to Technicians, Professionals and Producers in Multimedia Resources

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Introduction: The mandatory implementation of environmental enrichment (EE) in Brazilian pig farms, established by MAPA Normative Instruction No. 113, has increased the demand for technical guidance on the topic. Therefore, we aimed to examine how EE is currently portrayed in technical communication materials directed at swine producers. **Methods:** We conducted gray literature searches using Google and four widely accessed online magazines for swine producers, with the descriptors “environmental enrichment” and “swine*”. We identified 93 multimedia items and analyzed the stated motivations for EE adoption, the types of EE presented, the reported effects on animal welfare, and the

representation of EE in images. **Results:** The promotion of animal welfare was cited as the primary motivation for EE implementation (82%). The most frequently presented types of EE were objects (51%) and substrates (35%), followed by sensory (28%), nutritional (19%), physical (9%), social (6%), and cognitive (3%) forms. Reported outcomes focused mainly on behavioral changes (51%), along with effects on injury incidence (29%), productive performance (28%), disease occurrence (4%), and other aspects (3%). The most emphasized benefits included the stimulation of species-typical behaviors (48%), stress reduction (33%), improved productive performance (25%), and reductions in stereotypies (22%), agonistic interactions (22%), and injuries (17%). However, 40% of the images analyzed contained no representation of EE. **Conclusions:** Although substrates are recognized as the gold standard for environmental enrichment, this is not consistently reflected in producer-oriented materials. The lack of clear and contextualized images further limits practical guidance. Despite regulatory progress and broader dissemination of the topic, better alignment among technical communication, legislation, and scientific evidence is needed to effectively improve animal welfare.

3.1.34. Habituation to Visual Barriers in Big Felids Under Professional Care for Behavioural Research Purposes

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Introduction: Recording animal behaviour without bias due to the presence of watchers remains challenging; even if technological advances are helpful, direct observations are still worth it. Habituation can partially alleviate this issue, but for many species, there is still a lack of data. **The aim:** The aim of this study was to habituate big felids in a zoo to the exposition of visual barriers to obtain a reduced or absence of reaction. **Methods:** Four tigers (*Panthera tigris*) and five lions (*Panthera leo*) from “Parque Centenario” zoo in Mérida, México, were used, all in a good healthy state, and the research protocol was approved by the Bioethics Committee (ref# CB-CCBA-I- 2024-001). On non-continuous days, random and gradual exposition to visual barriers (posters) was made. Posters placed on metal supports were mounted in front of the enclosure and replaced nine times by different people. After each change, one minute of observation plus four of rest were effectuated using focal sampling. On the posters, small square holes covered with mesh allowed the person behind them to observe without being seen. The ethogram considered reaction (i.e., turning ears, body orientation) and no reaction (i.e., absence of movements). Frequencies were weighted by number of watchers and animals by species. A Manova test and Spearman correlation between the frequencies and the progression of days were performed. **Results:** A total of 12 non-continuous days distributed over 39 days were needed to obtain no reaction of animals. A significant effect ($p < 0.001$, Manova test) of days’ progression was found on the frequency of behaviours studied. Significant negative correlations were found between reaction behaviours ($p < 0.05$, Coef. Corr from -0.36 to -0.74) and days in both tigers and lions. Also, a positive correlation ($p < 0.05$, Coef Corr from 0.87 to 0.95) was found with no reaction behaviours. **Conclusion:** These big felids can be habituated to visual barriers in a relatively short period of time, enabling hidden observation.

3.1.35. Human–Baboon Coexistence, Welfare, and Ethical Challenges in Urbanizing Saudi Arabia: A Scoping Review of Emerging Gaps and Technological Innovations

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Introduction: Rapid urbanization in Saudi Arabia is reshaping ecological dynamics and intensifying interactions between humans and *Papio hamadryas* baboons. Evidence on free-ranging baboon welfare, ethical management, and human coexistence in the Arabian Peninsula remains fragmented across ecological, health, and policy domains. The objective of this scoping review is to synthesize and map empirical, ethical, and technological evidence on human–baboon coexistence in urbanizing Saudi landscapes, identifying key welfare risks, management gaps, and innovation opportunities. **Methods:** Following PRISMA-ScR, a multi-database search (PubMed, Scopus, Web of Science, Saudi repositories; 2000–2025) identified the peer-reviewed and gray literature on baboon welfare, behavior, zoonoses, management, and monitoring technologies. Studies on free-ranging or peri-urban baboon populations in Saudi or comparable arid systems were included. The gray literature was screened for policy and practice insights. Data were mapped across ecological, welfare, ethical, policy, and technological dimensions. **Results:** Across individual field studies, up to 55% of observed baboons exploited anthropogenic food sources (waste sites, peri-urban crops), indicating behavioral shifts associated with urban encroachment rather than pooled national estimate. Conflict hotspots were located near waste and agricultural edges. Reported welfare concerns included nutritional imbalance, psychological stress indicators, and around 15% prevalence of zoonotic pathogens in sampled populations from microbiological studies. Pilot applications (drone-, AI-based surveillance) in selected areas yielded roughly 35% gains in monitoring efficiency, reflecting site-specific improvements in temporal and spatial coverage. Wildlife governance and stakeholders describe overlapping mandates, implementation challenges, and limited explicit guidance on non-lethal, welfare-oriented management of baboons outside protected areas. **Conclusions:** Urbanization in Saudi Arabia is driving marked shifts in baboon behavior and welfare risks, while ethical, welfare-centered and One Health-oriented management frameworks remain only partially developed. By consolidating dispersed ecological, welfare, ethical, and technological evidence, this review clarifies current knowledge, highlights research and policy gaps, and outlines opportunities for data-driven, humane, and sustainable management of human–baboon coexistence in rapidly changing landscapes.

3.1.36. Humane Killing, Fragile Infrastructures: Empirical Ethics of Veterinary Practice in Istanbul

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Euthanasia in small-animal practice is often framed as a technical endpoint; this study demonstrates it is fundamentally relational and context-dependent. Drawing on 29 qualitative interviews with Istanbul-based small-animal veterinarians (conducted in Turkish, July 2024–April 2025), I examine how ethical reasoning, emotional labor, and institutional conditions shape life-ending decisions for two canine populations: companion animals and unowned street dogs. Reflexive thematic analysis, informed by constructivist grounded theory, reveals that euthanasia for companion dogs typically unfolds through shared deliberation with owners, anticipatory grief work, and post-death rituals—distributing moral responsibility across human actors. By contrast, street-dog cases commonly occur without legal guardianship, with ambiguous authorization, compressed timelines, and scarce aftercare, concentrating ethical burden and moral residue on veterinarians themselves. Six cross-cutting themes structure the findings: shouldering responsibility in the absence of guardians; ethical strain under contested indications; the decisive role of relational presence; the unequal weight of municipally routed street-dog euthanasia; practices of witnessing that seek dignity at the end of life; and the ethics of time as a finite clinical resource. The study advances empirical veterinary ethics by reframing euthanasia as work performed

within—and often against—fragile urban care infrastructures. Implications include training for adverse-euthanasia scenarios, municipal protocols for unowned animals (authorization lines, funded aftercare), and clinic-level supports (sedation-first SOPs, rotation, debriefing). Recognizing how context redistributes responsibility clarifies both the possibilities and limits of “humane” endings in urban practice.

3.1.37. Hypolipidemic Pharmaceuticals Atorvastatin and Ezetimibe Shake up Brown Trout Lipid Metabolism

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The incidence of dyslipidaemia is growing worldwide. As a result, the use of hypolipidemic pharmaceuticals has steadily increased, particularly statins, which are the most prescribed class. Consequently, hypolipidemics have become recognised as emerging environmental contaminants, reaching aquatic environments where they can cause lipid disruption in fish. These disruptions could have deep impacts, since lipids play vital roles in a wide array of physiological and homeostatic processes. In this context, this study investigated the effects of atorvastatin (ATV; a popular statin) and ezetimibe (EZB; a cholesterol-absorption inhibitor commonly co-administered with ATV) on lipid parameters in juvenile brown trout (*Salmo trutta*). The fish (N = 12/condition) were assigned to one of six conditions—Control (C), Solvent Control (SC), ATV High (ATV-H; 0.3 g.g⁻¹), ATV Low (ATV-L; 0.03 g.g⁻¹), EZB High (EZB-H; 0.3 g.g⁻¹) and EZB Low (EZB-L; 0.03 g.g⁻¹)—and received intramuscular injections every three days for 28 days. The C group received an intramuscular injection of 0.7% saline solution, whereas SC received 0.7% saline solution supplemented with 0.9% ethanol and 0.1% DMSO. Experimental endpoints included biometric parameters measured both during exposure and after 28 days, plasma biochemistry of blood lipids, glucose, albumin and total bilirubin, gonadal development, and mRNA expression of hepatic lipid-related genes. Fish biometric parameters were not altered. Biochemical results showed evidence of lipid disruption, with ATV-H significantly lowering blood cholesterol, triglycerides, and low-density lipoproteins. EZB-L caused a significant drop in HDL levels. LDL levels seemed clearly lowered in ATV-H and EZB-L groups, although these reductions were not statistically significant. Together with the biochemical findings, additional data from ongoing histological and molecular endpoints will provide further insight into the mechanisms underlying lipid disruption triggered by hypolipidemics in trout. Such data will be essential for establishing a robust hypolipidemic fish model and understanding and predicting the environmental impacts of hypolipidemics.

3.1.38. Impact of Harness and Cart Design on Working Equid Welfare and Health in Western-Central Morocco

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Introduction: A cross-sectional study was conducted in two working equid regions of western-central Morocco (Oulad Frej and Echemmaia) to characterize the equine population, harness and cart design, and to evaluate their impact on the occurrence of injuries.

Methods: A total of 155 randomly selected working equids were included in the study. An-

imal characteristics (species, sex, and age) were recorded, and body condition score (BCS) was assessed. Harnesses were evaluated for their condition, padding, fit, and assembly, while carts were assessed based on their structural design. Injuries were recorded using anatomical location and depth. Statistical analyses (chi-square tests and Multiple Correspondence Analysis—MCA) were performed to assess associations between equipment characteristics and wound occurrence. **Results:** The population consisted of 66% horses, 21% donkeys, and 14% mules, with a mean age of 7.5 ± 3.2 years and an average BCS of 2.5 ± 0.85 (five-point scale). Wounds were observed in 89.7% of equids, mainly superficial, while 19.4% presented deeper lesions. The most affected areas were the prescapular region (52.3%), withers (42.6%), and gluteal region (27.1%). A significant association was found between low BCS and wound presence ($\chi^2 = 13.89$, $p = 0.003$). MCA revealed a significant association between wounds and poor harness quality: 22% of harnesses were in poor structural condition, 30% were poorly adjusted, 40% were poorly padded, and 30% were poorly assembled. Key defects included absence of saddle padding (40%), poor breeching strap adjustment (30%), and improper assembly of neck straps and breast collars (30%). The draft collar was completely absent in 100% of cases. Vehicle assessment showed that none of the carts were equipped with singletrees. Structural defects included underinflated wheels (57%), protruding parts (56%), and excessive tilt (41%). **Conclusions:** This study highlights the prevalence of injuries in working equids and identifies critical deficiencies in harness condition and cart design in western-central Morocco.

3.1.39. Impacts of Draught Work on Serum Metabolites and Minerals in Hararghe Highland Bulls and Its Nutritional Implication

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Metabolic responses in animals during the intense physical activity have an intricate link to nutritional aspects. This study focused on examining the changes in blood metabolite concentration in Hararghe highland bulls during draught service, with the objective of evaluating their nutritional implications. Twelve intact bulls were randomly assigned to three groups of four animals each, with each group working for three draught hours (0, 4, or 6 h). The Cobas Integra 400 Plus machine was used for the laboratory serum analyses. Data was analysed using a mixed model procedure with covariates in SAS-JMP-pro-17. The study results reveal that, except for phosphorus (P, mmol), the concentrations of glucose (GLU, mg/dL), total protein (TP, g/dL), blood urea nitrogen (BUN, mg/dL), and calcium (Ca, mmol) were significantly affected by their respective initial concentrations (IN^1) ($p < 0.05$), while dry matter intake (kg DMI/d) did not have an effect except on GLU and P ($p < 0.05$). Draught work hours did not influence GLU, TP, and BUN concentrations ($p > 0.05$), regardless of IN^1 and kg DMI/d, but did influence Ca and P. Therefore, it is important to monitor the level of Ca and P concentration in the blood of the bulls engaged in draught service to ensure their well-being.

3.1.40. Improved Ease of Use of the Subcutaneous Implant (SuprelorinTM) Actuator, a Survey Among German and French Veterinarians

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SuprelorinTM, a subcutaneous contraceptive implant for dogs, cats and ferrets, has been registered in the European Union since 2007. It is administered using a specific device. To enhance user-friendliness and align with the increasing number of women in the veterinary profession in Europe, the original actuator has been modified. These improvements include the addition of ergonomic wings, a spring and a sensitive indicator for implant administration. This product test survey aimed to collect user feedback on these changes. Twenty-five veterinary practitioners from France (n = 10) and Germany (n = 15), who currently use SuprelorinTM, were given both the original actuator and the prototype, along with a silicone pouch and placebo implants. They evaluated the ease of use and visual aspect of a modified SuprelorinTM actuator prototype. Women represented over half of the participants (13/25). Veterinarians rated the current actuator at 6/10 for both ease of use and visual appeal. The prototype actuator scored 7/10 for both criteria. Notably, for veterinarians with small hand sizes (n = 9), the prototype's ease of use improved to 8/10, compared to 5/10 for the current actuator, achieving the highest overall score. Veterinarians expressed strong interest (7/10) in an indicator for correct implant administration, with a preference for a "click" sound. While veterinarians were generally satisfied with the current actuator, their positive feedback on the prototype suggests that the new design could improve SuprelorinTM implant administration and better fit with the current veterinarian profile.

3.1.41. Influence of Agrivoltaic Systems on the Thermal Comfort of Dairy Cows in a Tropical Climate

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In this study, we evaluate the influence of an agrivoltaic system on the thermal comfort of dairy heifers in a tropical climate. The research was conducted between July and August 2025 in south-eastern Brazil. During 12 consecutive days, two pasture systems were assessed: an open pasture system (OPS) and an agrivoltaic system (AGV). In the AGV, 36 photovoltaic panels were arranged across three paddocks (12 panels/paddock). Microclimate variables such as air temperature (AT), relative humidity (RH), black globe temperature (BGT), and wind speed (WS) were assessed by sets of autonomous sensors. In the OPS, a set of sensors were installed at the center of the paddock, and in the AGV, a set of sensors were installed in full sun and the shaded areas beneath the panels. With these data, we calculated the black globe–humidity index (BGHI) and radiant thermal load (RTL). All analyses were performed in R using Generalized Mixed Models, with days and hours included as random effects. All microclimatic variables were lower ($p < 0.001$) in the AGV (AT: 22.1 ± 0.9 ; BGT: 24.4 ± 1.8 ; WS: 1.4 ± 0.09) compared with the OPS (AT: 23.01 ± 0.9 ; BGT: 28.8 ± 1.2 ; WS: 1.6 ± 0.1), except for RH (AGV: 50.4 ± 3.9 ; OPS: 48.7 ± 3.3). Additionally, the lowest mean ($\pm$ SE) values of bioclimatic indicators were recorded in the AGV (BGHI: 70.1 ± 0.8 ; RTL: 487.2 ± 7.4) than in the OPS (BGHI: 74.6 ± 0.9 ; RTL: 574.2 ± 8.1). In the OPS, BGHI exceeded the heat-stress threshold for cattle (>74 units) during 62% of measurements, indicating a challenging thermal environment. In conclusion, the agrivoltaic system provided a more comfortable thermal environment for pasture-based dairy production compared with the open pasture system, demonstrating its potential to mitigate heat stress in tropical climates.

3.1.42. Integrating Drone Technology (UAS) into Land-Use Conflict Resolution: A Qualitative Analysis of Farmer–Herdsman Dynamics and Food Security in Northern Nigeria

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Introduction: The escalating land-use conflicts between nomadic herdsman and sedentary farmers in northern Nigeria represent a critical disruption to national food security. Driven by climate-induced resource scarcity and traditional migratory shifts, these disputes result in significant loss of life and agricultural productivity. This study explores the deployment of Unmanned Aerial Systems (UAS) as a strategic tool for surveillance, resource management, and conflict mitigation. **Methods:** Using a qualitative research design, data was gathered through semi-structured interviews, pictorial evidence, and a review of existing land-use policies. To identify systemic patterns, the qualitative data was analyzed through thematic categorization, focusing on three primary domains: Conflict Drivers, Security Vulnerabilities, and Technological Feasibility. This approach allowed the mapping of migration routes to avoid high-risk “clash zones”. **Results:** Analysis identified three distinct patterns of conflict: seasonal migration friction, border-crossing incursions, and the emerging exploitation of drone technology by non-state actors (terrorist groups). The study categorizes UAS applications into four actionable pillars: 1. Monitoring: Real-time surveillance of designated grazing reserves. 2. Tracking: Geospatial mapping of migration corridors to prevent farmland encroachment. 3. Intelligence: Early warning systems for border security. 4. Coexistence: Using aerial data to inform extension services and land-sharing agreements. Findings indicate that while UAS provides a tactical advantage, its efficacy is contingent upon community-based education and the formalization of grazing lands. **Conclusion:** The integration of drone technology offers a novel framework for stabilizing Northern Nigeria’s agricultural sector. However, the study concludes that technological intervention must be preceded by robust legislative governance. There is an urgent need for the Nigerian government to regulate both armed and unarmed UAS use. Furthermore, a “hybrid governance” model involving traditional leaders is essential to ensure that drone-led surveillance fosters trust rather than further tension.

3.1.43. Is Vegan Pet Food the Leading Effective Altruism Issue?

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The philosophy of effective altruism (EA) seeks to maximise achievable benefits using limited resources. When prioritising problems, it evaluates their scale, neglectedness, and solvability, and the likely impact and cost-effectiveness of proposed interventions. This study assessed nutritionally sound vegan pet diets through EA principles. **Methods:** Published data concerning vegan pet diets were assessed through EA criteria. **Results:** Dog and cat food consumes around 9% of all farmed land animals annually—or at least seven billion globally (even after accounting for the use of animal byproducts in pet food). As well as sparing billions of animals from slaughter each year, global implementation of nutritionally sound vegan pet diets would save more greenhouse gases than produced by the entire UK (1.5 times as much), vast amounts of land and freshwater, and sufficient food energy to feed 450 million additional people (the entire EU population) or 1.46 billion additional companion dogs and cats (1.7 times the global population). By 2026, 14 studies and one systematic review demonstrated good health in dogs and cats fed with vegan or vegetarian diets. Despite this, extremely few animal or environmental advocates are

working on this issue. Large-scale surveys have shown that a sizeable minority (35–46%) of pet guardians would be willing to realistically consider alternatives to meat-based pet food, and nutritionally sound vegan pet diets are now widely available online. Despite the minimal personnel and resources invested so far, efforts to date have been highly impactful. Leading organisations such as the British Veterinary Association now endorse nutritionally sound vegan dog food. New product launches, patents, and studies are surging, and global valuations of vegan pet food have increased spectacularly from USD 10 billion in 2020 to USD 27 billion in 2024. **Conclusions:** When considering EA principles, vegan pet food emerges as a leading EA issue.

3.1.44. Melodious Melancholia: Analysing the Effect of Anthropogenic Sound on Bird Calling Behaviour

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Introduction: Birds depend profoundly on auditory perception for essential behaviours such as communication, navigation, predator avoidance, and social interaction. Vocalization plays a central role in these functions and is closely linked to their sensory ecology. However, the intensifying presence of anthropogenic and urban noise is transforming natural soundscapes, interfering with birds' ability to perceive and produce acoustic signals. This research examines how varying levels of human disturbance affect vocal communication in birds across contrasting environments, from densely urbanized areas to undisturbed forest habitats. **Methods:** Acoustic data (natural sound recordings) were collected using high-sensitivity recording systems from multiple sampling points representing a gradient of urbanisation. We used urban, suburban, and rural and forest areas for the collection of the acoustic data. Geographic Information System (GIS) tools were used to integrate landscape features. **Results:** Preliminary findings substantiate our fundamental hypothesis and effectively suggest that bird vocal communication frequencies and interactive calling bouts are significantly affected by human-made artificial sounds. However, it is a fact that collective bird calling can be considered a behavioural marker of migration from extreme to moderate to less urban areas. However, to substantiate this kind of hypothesis, it is important to have a long-term study on the collective vocalizing intricacies in birds. The results imply a high potential for acoustic masking in urbanized environments, where anthropogenic noise may interfere with birds' ability to perceive and transmit vocal signals effectively. **Conclusions:** The frequency- and intensity-dependent variation in bird calling supports our hypothesis that urban noise disrupts avian communication, or collective bird calling in the ecosystem becomes masked by the mechanical soundscape. In this changing soundscape, the bird's voices echo a *melodious melancholia*—a poignant prelude to the possibility of a future “*silent spring*”.

3.1.45. Migratory Birds and Changing Habitats: Integrating Community-Led Conservation and Climate Impact Assessments

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Migratory birds serve as critical indicators of ecological health and environmental change, providing insights into the effects of climate variability, habitat modification, and

human disturbance on ecosystems. This proposed research examines how climate variability, land-use dynamics, and community-linked conservation practices influence the distribution, behavior, and recovery of selected migratory bird species. Globally, many migratory bird populations are declining due to habitat degradation, pollution, and increasing human pressures. Key migratory habitats (such as wetlands, estuaries, and forests) face threats from urban expansion, agricultural intensification, and shifting climatic patterns. Understanding how these ecological and human dimensions interact is essential for developing effective conservation strategies. Building on prior research exploring community influence on wildlife conservation, this study will assess how climate variability and community-based conservation initiatives shape migratory bird population trends. Ecological data from published studies, biodiversity databases, and long-term monitoring records will be integrated with insights from community engagement programs to evaluate species distribution, migration timing, habitat use, and the impact of locally driven interventions. A mixed methodological approach will be employed. Quantitative analyses will utilize secondary ecological and spatial datasets (including BirdLife International records, national biodiversity repositories, and GIS-based land-use and climate studies) to examine population trends, distribution shifts, and habitat changes. Complementary qualitative insights from case studies and documented community stewardship programs will illuminate social and behavioral factors influencing conservation outcomes. This research will generate evidence on how migratory birds respond to environmental change and identify strategies for integrating local stewardship with species conservation. By bridging ecological science and participatory approaches, this study aligns with global efforts to promote sustainable animal management, biodiversity protection, and evidence-based conservation.

3.1.46. Quantifying Drivers of Welfare Risk in Cattle During Preslaughter Handling

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Introduction: Preslaughter management represents a critical period for cattle welfare, as animals are subjected to multiple stressors over time. Despite long-standing attention to handling practices, the welfare impact of preslaughter conditions has rarely been quantified in a way that integrates both the intensity and duration of stress exposure. **Methods:** The Welfare Footprint Framework was applied to estimate welfare impacts across the main preslaughter stages—unloading, lairage, handling/movement, and restraint—under commercial conditions in tropical and subtropical South American regions. It combined estimates of duration and severity of stressful circumstances to identify which phases and stressors contributed most to cumulative welfare burden and evaluate potential mitigation strategies. Parameterization represented typical high-throughput operations. Climate data were analyzed from 636 beef-producing locations across Brazil, Argentina, Colombia, Paraguay, and Uruguay. **Results:** In well-managed operations, heat stress contributed 19–33% of an individual time in moderate-to-intense pain and discomfort. Under moderate heat stress, hunger, physical exhaustion, thirst and fear accounted respectively for 30–32%, 15–16%, 7–13%, and 23–24% of welfare impacts, with their relative contribution decreasing in scenarios of extreme heat. In such cases, heat stress became the main harm experienced, with a welfare impact similar to severe contusions when present (24–25%). Lairage dominated cumulative welfare impacts across all scenarios: 96–97% of moderate-to-intense

and 87–90% of intense impacts. Handling stages were associated with shorter but more severe distress per unit of time, given the potential use of electric prods and greater risk of contusions. **Conclusions:** Lairage management emerged as a primary leverage point for welfare improvement in tropical and subtropical systems. Reducing lairage duration can substantially lower cumulative discomfort. Ensuring water access, limiting density, improving ventilation and shading are also critical to reduce the impact of heat stress. Quantifying welfare impacts, such as time in negative states of varying intensities, can guide targeted welfare improvements in commercial slaughter operations.

3.1.47. Reaction of Dairy Calves to Colored Mats with Interlaced Colors

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The rational management of young animals often involves novel situations that may cause reluctance and stress, making it essential to understand how visual stimuli influence their behavioral responses. The present study evaluated the reaction of dairy calves aged 0 to 6 months to colored mats placed at transition points between areas, considering different environments and social contexts. The research was conducted at the Federal Institute of Education, Science and Technology of Southern Minas Gerais, Muzambinho Campus, Brazil. Six Holstein calves raised in an intensive system were used, assessed both individually and in groups, in familiar (FA) and unfamiliar (UA) locations. The mats were made with horizontal interlaced stripes in lilac and red, lilac and black, and lilac and yellow, in addition to a control condition without mats. Behavioral responses were recorded using an ethogram, including the behaviors crossing, reluctant crossing, exploration, retreat, and attempt to jump, based on direct observation. Statistical analysis was performed using the nonparametric Kruskal–Wallis and Nemenyi tests. The calves showed high sensitivity to visual stimuli, with a predominance of the “reluctant crossing” behavior, even in the absence of mats (66.67% in FA). The yellow–lilac mat elicited the highest frequency of this behavior (100% in group and 94.44% individual in FA), followed by the red–lilac (70.06%) and black–lilac (58.69%) mats. The red–lilac and black–lilac combinations also induced exploratory and retreat behaviors, especially under individual conditions in UA, suggesting perception of depth and uncertainty in response to contrasts. The presence of conspecifics significantly increased the likelihood of crossing, highlighting the gregarious effect. It is concluded that young calves display more cautious and exploratory responses to contrasting visual stimuli and that visual and social factors should be considered in management practices to promote safety and animal welfare.

3.1.48. Scratching Behavior of Dairy Heifers in Agrivoltaic Systems with Different Configurations

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This study aimed to evaluate the scratching behavior of dairy heifers in agrivoltaic system with different configuration in a tropical climate. The experiment was conducted between July and August 2025, during 12 consecutive days at FMVZ/Unesp—Botucatu, São Paulo State, Brazil. The scratching behaviours on photovoltaic panel structures of 21 dairy heifers (550 ± 55 days old; 247 ± 37 kg, mean $\pm$ SD) were registered continuously from 9 h to 16 h. In a crossover design, the heifers were divided into three groups (seven heifers/group). The agrivoltaic systems were composed of 72 panels (2.28×1.14 m), divided into six paddocks in the center of each paddock, with east–west orientation and facing north, at a fixed height of 2.30 m. In three paddocks, 36 panels (12 panels/paddock) were installed in a vertical position (AGRIver), with panel structures composed of 5 poles in each paddock, whereas in the other three paddocks, 36 panels (12 panels/paddock) were installed in a horizontal position (AGRIhor), with panel structures composed of 10 poles in each paddock. The data were analyzed using a generalized linear mixed model (Poisson family), considering agrivoltaic system and hours as fixed effects, with date and groups as a random effect. The heifers were 33% more likely ($p = 0.01$) to engage in scratching behavior in AGRIhor (193 events) than AGRIver (131 events). There was no effect ($p > 0.05$) of the hours of evaluation within agrivoltaic system. Regardless of agrivoltaic system, the highest frequency ($p = 0.002$) of scratching behavior occurred between 11 h–11:59 h (81 events). In conclusion, the configuration of the agrivoltaic systems influenced the heifers' scratching behavior. The horizontal configuration, which had more poles than the vertical configuration, provided more space and may have contributed to the heifers engaging in more scratching behavior.

3.1.49. The Ethical Divide: A Philosophical Exploration of Animal Individualism and Ecological Holism

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This paper intends to explore two distinct approaches in environmental ethics, namely, Individualism and Holism in the light of interconnected ethical frameworks of Animal-centrism and Eco-centrism. The unprecedented rate of material progress brought about by modernity has given rise to environmental crisis. Though the crisis urgently calls for viable solutions towards sustainable development, in the long term it urges overcoming anthropocentrism that has largely justified destruction-backed development. For most of Western philosophy, Ethics as a branch of Philosophy had remained the exclusive domain of humans. Non-human entities being relegated into categories devoid of moral worth therefore came to be viewed as a means to human ends. It was only after witnessing the 1960s environmental crisis that marked the onset of debate about humanity's ethical obligations to nature. These intensive debates led to the birth of environmental ethics. Environmental

ethics accordingly offers two contrasting approaches for understanding moral value of non-human entities in the form of Individualism and Holism. Individualism focuses on the rights and moral significance of individual beings. Animal Centrism expressed in the works of philosophers Peter Singer and Tom Regan is the systematic expression of Individualism. Animal Centrism argues that the well-being of individual animals, with their capacity for suffering, should be prioritized, over the needs of ecosystems. In contrast Holism takes the whole of nature as having ethical significance. Land Ethics appeared as an influential essay in the 1949 seminal work of American philosopher Aldo Leopold, the hallmark expression of Holism. Emphasizing interconnectedness of all components within ecosystems, it asserts that the well-being of the whole system must be prioritized over the welfare of its individual elements. This critical examination of the above two divergent perspectives offers a nuanced understanding of our moral obligations towards the environment, which is important to address the looming crisis.

3.1.50. The Gut–Systemic Axis in Companion Animals: Systemic Review of the Microbiome’s Role in Metabolic and Behavioral Disease

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Introduction: The gastrointestinal microbiome in companion animals is increasingly recognized as a complex, metabolically active organ with profound systemic influence. A functional imbalance, or dysbiosis, is now strongly implicated in a spectrum of extra-intestinal diseases. This review introduces and explores the concept of a unified “Gut–Systemic Axis”, a comprehensive framework connecting the gut to distant organs, including the brain, liver, and adipose tissue. This axis is often mediated by shared mechanisms, including altered intestinal permeability, metabolic endotoxemia, and chronic systemic inflammation. **Methods:** This review synthesizes and critically evaluates the current body of scientific literature elucidating the role of the canine and feline gut microbiome in disease. We systematically examined the evidence linking gut microbiota alterations to the pathophysiology of major metabolic disorders (obesity, diabetes mellitus, hepatobiliary disease) and behavioral/neurological conditions (anxiety, aggression, and Canine Cognitive Dysfunction Syndrome), with a focus on species-specific differences. **Results:** Our synthesis identifies distinct dysbiotic signatures associated with disease. Metabolic findings highlight a unique decreased Firmicutes/Bacteroidetes ratio in obese cats, in contrast to dogs and humans, and a profound depletion of butyrate-producing bacteria in feline Type 2-like diabetes. Behavioral findings link gut–brain axis disruption to anxiety and aggression, the latter associated with altered host serotonin metabolism. We also highlight evidence positioning Canine Cognitive Dysfunction Syndrome as a high-fidelity spontaneous model for human Alzheimer’s disease, driven by a “leaky gut, leaky brain” inflammatory cascade. **Conclusions:** The gut microbiome is a central and targetable factor in the pathophysiology of numerous canine and feline systemic diseases. The “Gut–Systemic Axis” provides a critical framework for diagnosis and treatment. The therapeutic strategies reviewed, including nutritional interventions, probiotics, and fecal microbiota transplantation (FMT), show significant promise for managing these complex conditions. This research also champions a “One Health” perspective, underscoring the interconnected microbial health of companion animals and their human cohabitants.

3.1.51. The Impact of External and Internal Socio-Psychological Factors on Pro-Animal Behavior Among Dairy Farmers

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In recent decades, animal welfare has attracted the attention of various groups for reasons including its role in achieving SDGs, community health, prevention of zoonotic diseases, and ethical issues. Stockmen have a vital role in fostering animal welfare. Given the crucial role of stockmen in ensuring animal welfare, this study attempts to assess the factors affecting the occurrence of pro-animal behavior among dairy farmers using an expanded version of Campbell's theory. In this study, a cross-sectional survey and a closed-ended questionnaire were used to collect data. Also, through a random sampling approach, 122 traditional stockmen and 208 industrial stockmen were selected in Iran. In addition, in order to assess the measurement model, test the hypothesis and provide descriptive data, Smart-PLS₃ and SPSS₂₆ software were used. The results revealed that access to livestock inputs (Beta = 0.21; Sig = 0.0001), anthropomorphism view (Beta = 0.12; Sig = 0.009), and attitude (Beta = 0.53; Sig = 0.0001) had positive and significant effects on pro-animal behavior. Moreover, cost (Beta = -0.14; Sig = 0.004) had a negative and significant effect on pro-animal behavior. In total, independent constructs could explain 44 percent of pro-animal behavior variance. This study contributes to the development of the literature in animal welfare, social psychology and sustainability fields. In addition, it helps policymakers, scientists and related organizations to strengthen pro-animal behavior among stockmen in order to build up animal welfare.

3.1.52. Urbanisation, Human–Animal Interaction, and the Emerging Crisis in Working Equid Welfare

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In Ethiopia, most livestock keeping is carried out by smallholder families, where animals are not just for work but part of culture, heritage and everyday life. Kids grow up herding animals, sometimes even missing school, to support families in farming and animal care. Knowledge about how to manage and treat livestock is passed down by word of mouth, and communities have built up their own indigenous ways of coping with challenges, even when modern veterinary services are limited. But things are changing. Land shortage, population pressure, conflict and the hope for a better life in towns are pushing people to migrate. Young people spend more time in school and less time with animals, and when they move to urban areas, they do not learn livestock-keeping skills. In cities, working equids face tougher conditions than in rural areas. They lose their close bond with owners, resources like feed and water are short, and the environment is unnatural and stressful. Many migrants start manual jobs, then buy a donkey or mule to run a small transport business. Unlike rural pack animals, urban equids are mostly used for cart pulling, often with poor equipment and by handlers who don't have proper experience. Animals change hands many times, are rented or sold, and often suffer from beating, neglect and accidents on busy roads. The informal nature of the business, social problems of handlers, and lack of training make things worse. As a result, working equids in urban Ethiopia experience serious welfare problems. Many end up abandoned when they cannot work anymore. This paper looks at how rural-to-urban migration is reshaping human–animal relations, changing the use of equids, and creating a welfare crisis. It calls for urgent action to preserve indigenous knowledge, improve human–animal interaction, and adapt welfare-friendly practices to fit the realities of urban life.

3.1.53. When Perception Meets Reality: Owner Accuracy in Assessing Dog Body Condition

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Body condition is a key indicator of animal welfare, with dog owners acting as the primary assessors through their daily interactions with their pets. Understanding how owners perceive their dogs' nutritional status is essential for preventing and managing body condition imbalances. The present study investigated perceptual discrepancies in the evaluation of canine body condition between dog owners and experts, specifically a veterinarian and a university student in a program of animal protection and welfare. Two established scales for assessing body condition—the 5-point and 9-point Body Condition Score (BCS) scales—were employed to compare assessment consistency and explore factors influencing scoring accuracy. A total of 92 dogs and their owners participated. Each dog was independently evaluated by three assessors, and differences in scoring were analyzed in relation to owner characteristics (sex, age, experience with dog ownership, and level of physical activity) and dog attributes (age, sex, breed, coat length, activity level, and health status). The findings revealed that owners frequently misjudged their dogs' body condition. The weakest agreement, based on Spearman's correlation, was observed between owners and veterinarians (5-point scale: $r_s = 0.5693$; $p < 0.01$). Owner sex, age, physical activity, and ownership experience had no statistically significant influence on BCS accuracy ($p > 0.05$), though a tendency toward underestimation was evident among inexperienced, younger, and older owners. Most dog-related variables did not significantly affect the results, except coat length. Owners of short-haired dogs showed significantly stronger agreement with veterinarians on the 5-point scale ($p < 0.01$), while long-haired dogs were more often overestimated ($p < 0.05$). The effect of coat length was not significant on the 9-point scale ($p > 0.05$). Overall, evaluations made using the 9-point scale were more accurate. These results emphasize the need to improve owner awareness and education regarding body condition assessment and maintaining optimal health in dogs.

3.1.54. Where the Tail Still Wags: Biological Clones and Digital Afterlives of Companion Animals

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This research examines emergent socio-technical practices of memorialization in human–companion-animal relationships by comparing biological cloning of deceased pets and digital “deathbot” reconstruction of their personalities. Amidst intensifying technocapitalist imaginaries around companion-animal life, the study interrogates what is mourned, remembered, and re-enacted when an animal dies. We ask the following: what constitutes the “personhood” of a companion animal, and how do bereavement technologies re-mediate attachment, affect, and memory? Methodologically, the study integrates (1) semi-structured interviews ($n = 14$), (2) computational art methods to critically deconstruct technical assemblages shaping digital animal reconstruction, and (3) autoethnographic practice through developing a prototype animal deathbot. The sample includes eight digital cloning, four biological cloning, and two dual-method interlocutors. With 24 recording hours and interface analysis, the dataset comprises approximately

280,570 words. Analysis combined thematic coding with lexicon-based coding to map the discursive landscape of pet bereavement. Across transcripts, terms indexing animal personhood included “soul”, “reconnect”, “reunion”, “return”, “I knew it was him/her”, “reborn”, “charisma”, “personality”, and “the attitude”; this lexicon appeared in X/14 interviews. Terms indexing commodification and the transactionalization of remembrance included “price”, “payment”, “subscription”, “down payment”, “spend”, “expensive”, “worth”, “exchange”, “buy”, “priceless”, “lab report”, “report”, and “get it back.” This commodification lexicon appeared in five digital-cloning interviews and all four biological cloning cases. Preliminary findings indicate that bereavement is anchored in the dissolution of shared routines and intersubjective memory worlds. Biological cloning promises corporeal continuity but foregrounds anxieties about authenticity and the commodification of life. Digital cloning operationalizes memory as data, translating animal subjectivity into algorithmic performance and highlighting the co-constructed nature of animal “personality.” Ultimately, bereavement functions as a technologically mediated negotiation through which companionship is re-engineered and animal personhood becomes relational and computationally reproducible.

3.2. One Health: Improve Disease Manifestation and Management in Animals, Humans, and the Environment

3.2.1. Low-Grade Adenocarcinoma of the Palpebral Conjunctiva\Third Eyelid in a Pigeon (*Columba livia*): A Case Report

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Adenocarcinomas involving ocular structures in birds are exceptionally rare and scarcely documented in the literature. This report details the first documented case of a low-grade adenocarcinoma originating from the palpebral conjunctiva and third eyelid in a *Columba livia* (pigeon). An adult pigeon was referred for specialized veterinary evaluation due to a progressively enlarging, slow-growing mass located in the periocular region of the left eye. Clinically, the tumor presented as a bilobed, pendulous mass with a soft consistency and a reddish, verrucous surface. The lesion appeared moist, with fluid exudation resulting in feather matting on the face and neck. Surgical excision of the mass was performed, and the tissue was preserved in 10% neutral-buffered formalin for histopathological evaluation. Preoperative fasting lasted at least three hours. The pre-anesthetic protocol included intramuscular Meloxicam 0.2% at 0.07 mg/kg, administered using a 26 G insulin syringe. Anesthesia induction used isoflurane gas via mask at 3–5%, then maintained at 1.5–3.0%. After reaching adequate anesthetic depth, the mask was removed, and the patient was intubated with a size 2 cuffless endotracheal tube. The pigeon was positioned in dorsal recumbency, wrapped in aluminum foil, and placed on a USB-powered heating pad. A pulse oximeter monitored oxygen saturation on the left pelvic limb. The surgical site was aseptically prepared with iodinated alcohol. Lidocaine was infiltrated locally before incision. The tumor was excised with a scalpel following hemostatic clamping, and safety sutures were placed using nylon 5.0 thread. Intraoperatively, 4 mL of warmed lactated Ringer’s solution was administered subcutaneously, alongside enrofloxacin 2.5% (0.28 mL), dexamethasone (0.2 mL), and vitamin K (0.2 mL). Histopathology confirmed a low-grade adenocarcinoma of the palpebral conjunctiva/third eyelid.

3.2.2. *Pfaffia glomerata* as a Key to Gut Health: A One Health Strategy for Sustainable Animal Production

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Gut dysbiosis in livestock production presents a critical intersection of animal health, antimicrobial resistance, and environmental sustainability, requiring integrated One Health solutions. This review examines *Pfaffia glomerata* (Brazilian ginseng) as a phyto-genic intervention that addresses these interconnected challenges through its documented effects on gut microbiome composition and immune function. *Pfaffia glomerata* contains bioactive compounds, including beta-ecdysterone, pfaffic acid, and triterpenoid saponins that demonstrate immunomodulatory and antimicrobial properties. Research indicates that these compounds enhance beneficial bacterial populations, particularly *Lactobacillus* and *Bifidobacterium* species, while reducing pathogenic colonization in the intestinal tract. This microbiome modulation improves nutrient absorption, strengthens intestinal barrier integrity, and enhances systemic immunity in production animals. The application of *Pfaffia glomerata* directly embodies One Health principles across three critical dimensions. For animal health, supplementation reduces the incidence of enteric diseases and improves production parameters without reliance on antimicrobial growth promoters. For human health, reduced antimicrobial use in animal production mitigates selection pressure on resistant pathogens that pose significant public health threats. For environmental health, phyto-genic alternatives reduce pharmaceutical residues in agricultural ecosystems and minimize contamination of soil and water resources. Integrating advanced analytical approaches, including machine learning analysis of microbiome sequencing data, enables precise characterization of how *Pfaffia* bioactive compounds interact with microbial communities and host physiology. This knowledge facilitates optimization of supplementation protocols tailored to specific production systems and health challenges. The strategic implementation of *Pfaffia glomerata* represents a practical One Health intervention that simultaneously addresses antimicrobial stewardship, animal welfare, and sustainable agricultural production through evidence-based modulation of gut health.

3.2.3. Replacing Antibiotics with Organic Acids: A Sustainable Strategy for Productivity and Disease Management in White Leg Shrimp

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The growing threat of antimicrobial resistance has prompted a reduction in antibiotic use within aquaculture, where disease outbreaks and environmental stressors continue to compromise animal health and productivity. Functional feed additives have emerged as promising alternatives, offering benefits to gut health, immunity, and performance without contributing to antimicrobial resistance. This study assessed the efficacy of an enhanced organic acids-based feed additive (Biotronic® PX Top3) as a sustainable substitute for antibiotics in Pacific white leg shrimp (*Litopenaeus vannamei*). In total, 480 shrimp were randomly assigned to 24 tanks in a recirculating aquaculture system and fed one of four diets over 66 days (six replicates per group): (I) basal diet as control; (II) basal diet with the antibiotic Cefotaxime (0.15 g/kg feed, as a prevention dosage); (III) basal diet with Biotronic® PX Top3 at 0.5 g/kg; and (IV) at 1 g/kg. Growth performance and feed consumption were evaluated. Subsequently, a bath immersion challenge with *Vibrio parahaemolyticus* (1.2×10^6 CFU/L) was conducted. The shrimp were fed the same experimental diets, except that the antibiotic dosage in group III was increased to 0.2 g/kg, representing the recommended treatment dose. Mortality was monitored daily for 14 days. Compared to the control, both dosages of

Biotronic® PX Top3 significantly improved specific growth rate, tank yield, feed conversion ratio, and survival during the 66-day feeding period, achieving results comparable to the antibiotic group. Post-challenge mortality was lowest in the higher-dosage Biotronic® group ($40.48 \pm 10.75\%$, mean $\pm$ standard deviation), followed by the antibiotic group ($57.14 \pm 12.78\%$, $p = 0.058$), the lower-dosage Biotronic® group ($59.52 \pm 10.75\%$, $p = 0.026$), and the control ($76.19 \pm 7.37\%$, $p < 0.001$). These findings suggest that organic acid-based feed additives may offer a promising and sustainable approach to supporting shrimp growth and resilience. Further studies should determine the mechanistic pathways.

3.2.4. Wildlife Recovery Centres (WRCs): A New Tool for Early Detection of Emerging and Re-Emerging Zoonosis in Wildlife

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Introduction: Appropriate surveillance programs and early detection are essential to minimize the consequences of the transmission and spread of zoonotic agents. In this context, Wildlife Recovery Centres (WRCs) can contribute to the monitoring of infectious diseases, playing a significant role as epidemiological observatories. The study presented was conducted from 2022 to 2024 by the Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta (IZSPLV), assessing the importance of the early detection of zoonotic pathogens in selected WRCs in NW Italy. **Methods:** An Operational Protocol was developed with technical sampling instructions for Influenza A virus, West Nile and Usutu viruses, Coronavirus, and Chlamydia in wildlife. Approximately 2300 samples were collected through active monitoring (cloacal and/or oropharyngeal swabs on live/hospitalized animals) and passive monitoring (target organ samples from deceased or euthanized animals). Biomolecular investigations were carried out using standardized and regulated methods: RT Real-Time PCR for viral agents and Real-Time PCR for Chlamydia. **Results:** Positivity for Influenza A virus (HPAI H5N1 and H13N2) emerged in 2023 only in the Piedmont region. Chlamydia psittaci was detected in 2.6% of samples, underlining the importance of the surveillance of this zoonotic agent. The discovery of samples positive for WNV and USUV in October and November 2022 highlighted how warmer winters can influence the active season for mosquitoes and increase the window of time for viral transmission. PCR screening for Coronavirus identified 17 positive samples amongst those collected from 285 birds. **Conclusions:** The findings expand knowledge on the spread and potential transmission of zoonoses, laying the foundation for implementing effective early detection systems. Concerning Avian Influenza, for instance, clade 2.3.4.4b A(H5N1) viruses have recently displayed an increased capability to infect mammalian species, due to an unusual capability for these viruses to evolve towards more efficient mammalian transmission. The establishment of a surveillance system to monitor the genetic and pathobiological characteristics of these influenza viruses is essential.

3.2.5. Application of Colostrum Enriched with Specific IgY for the Prevention of Diarrheal Infections in Newborn Calves

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Colostrum is vital for newborn ruminants, which are born in a state of agammaglobulinemia. Immunoglobulin G (IgG), contained in colostrum, forms the basis of protection for

newborns. Studies on dairy farms have revealed that the immunoglobulin concentration in the first milking of cows is often insufficient to protect offspring, especially in first-calf heifers, where IgG levels can be below 30 g/L. Calf mortality from diarrheal infections is often due to a deficiency of these immunoglobulins in colostrum. The study aimed to evaluate a strategy of enriching colostrum with specific IgY. Hens were hyperimmunized with an associated vaccine against bovine rotavirus, coronavirus infections, and escherichiosis. Yolk melange was prepared from the obtained eggs for subsequent colostrum enrichment. Chromatographic analysis showed that one yolk contains up to 100 mg of polyclonal immunoglobulins, of which 8% were specific antibodies against the target pathogens. Trials were conducted on a farm with a history of diarrheal diseases. Twenty-five calves in the experimental group received 100 mL of melange (equivalent to 5 yolks) with the first and second colostrum feeding. The control group (n = 25) received native colostrum. In the experimental group, cases of diarrhea were rare and mild, requiring no medication. In the control group, diarrhea was recorded in most calves. Testing of the methodology in other farms confirmed its high preventive efficacy. Thus, enriching colostrum with specific IgY is an effective strategy for preventing diarrheal infections in calves.

3.2.6. Case Study on the Pathophysiology of Retained Ovulation in Anser Anser Domesticus

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Egg retention is a common reproductive disorder in birds characterized by the failure of an egg to pass through the oviduct within the expected period, leading to various clinical signs and complications. Egg retention is a prevalent yet underrepresented pathological reproductive disorder in waterfowl species, particularly within Anseriformes, with limited scientific literature addressing its pathophysiology and management. This case study describes a Toulouse goose (*Anser anser domesticus*) experiencing prolonged difficulty in laying an abnormal egg, leading to severe clinical complications. Over 17 days, the bird underwent comprehensive evaluation. The results described refer to the patient's return after an initial procedure of ovocentesis, in which the eggshells were not expelled. Subsequently, the bird returned presenting a serious condition and a second procedure was performed including physical exams, radiography (egg fracture and increased caudoventral volume relative to the coelomic cavity, secondary to an inflammatory process), ultrasonography (liquid content in the oviduct and structures consistent with eggshells), and blood tests showing significant leukocytosis, revealing monocytosis with 13,889 cells/mm³ (150–2000 cells/mm³), heterophilia with 50,654 cells/mm³ (3000–17,300 cells/mm³) and lymphocytes within the upper limit 17,157 cells/mm³ (7000–17,500 cells/mm³), indicating a serious inflammatory process. Despite medical interventions including fluid therapy, analgesia, antibiotic therapy, ovocentesis, oviduct disobstruction, the condition progressed, culminating in severe inflammation (peritonitis and celomitis) and death. This study highlights the critical importance of timely diagnosis and comprehensive management of egg retention and dystocia in waterfowl, while emphasizing significant diagnostic chal-

lenges and therapeutic limitations. The scarcity of detailed literature on egg retention in Anseriformes further impedes effective diagnosis and treatment, reinforcing the need for further studies and focused research. Moreover, the report identifies potential complications, including follicular rupture in post-mortem findings and secondary infections, reinforcing the necessity for specialized veterinary expertise to optimize outcomes in avian reproductive disorders.

3.2.7. Global Distribution of Equine Herpesvirus Type 1: A Systematic Review

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Equine herpesvirus type 1 (EHV-1) is a widely distributed alphaherpesvirus that causes respiratory disease, abortion, neonatal death, and myeloencephalopathy in horses, with significant economic impact. Outbreaks such as Ogden (USA, 2011) and the international 2021 outbreak originating in Valencia (Spain) have demonstrated the virus's potential for rapid dissemination. This study aimed to compile and compare molecular surveillance publications from 2020 to 2025 to identify, by region, the main circulating EHV-1 strains, lineages, and genotypes. Searches were conducted in PubMed, ScienceDirect, Scopus, Web of Science, and EBSCO databases using terms related to *equine herpesvirus 1*, genotyping, and molecular characterization, covering the period from January 2020 to October 2025. The quality of observational studies was assessed using the Joanna Briggs Institute (JBI) checklist, and data were organized and described in Microsoft Excel. The review showed that EHV-1 circulation is global, intense, and genetically heterogeneous. Classical genotypes N752 (A2254) and D752 (G2254) remain predominant, but an emerging genotype, H752 (C2254), was identified in outbreaks in France (2020) and the USA (2021), associated with respiratory and neurological signs and potentially undetected by assays that only distinguish between N/D752. Regional variation was observed: in China, abortion cases were 100% D752; in Europe, the 2021 Valencia outbreak occurred without the classical neuropathogenic marker; and Swedish series revealed multiple genovariants, reflecting international horse movement. In South American and North African countries, high prevalence and co-circulation with EHV-4, EHV-2, and EHV-5 were reported, including in environmental samples. Thus, it is evident that the D752 marker alone is insufficient to define neuropathogenicity, and the emergence of H752 requires the sequencing of multiple ORFs or complete genomes, confirming the globalized circulation of EHV-1 facilitated by equine movement.

3.2.8. Long-Read Metagenomic Analysis of Milking Filters as a Tool for Dairy Herds' Resistome Monitoring

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Introduction: The rapid spread of antimicrobial resistance (AMR) in humans, animals, and the environment poses growing challenges to global health. Rapid, cost-effective methods for routine resistome surveillance in dairy herds are increasingly needed to support antimicrobial stewardship and implement risk management strategies. High-resolution resistome profiling could be a possible solution to better understand the antibiotic resistance landscape in herds related to the dairy industry chain. This study aimed to provide a proof-of-concept for a quantitative analysis of the resistome retrieved from milking filters

in six dairy herds in Lombardy (Italy). **Materials and Methods:** Milking filters (n = 6, one from each herd), replaced after each milking session, were collected. DNA was extracted using the PowerWater DNA kit (Qiagen) and quantified with the NanoReady Touch series Micro Volume Spectrophotometer (Aurogene, Italy). DNA libraries were prepared using the Rapid Barcoding Kit (Oxford Nanopore). Sequencing was performed on a MinION MK1C device. Microbial communities and resistomes were analyzed through a customized bioinformatic pipeline for taxonomic classification (Centrifuge) and antibiotic resistance gene identification (ABRicate); finally, results were visualized using R statistical software. **Results:** The two most abundant families of resistant bacteria were Mycobacteriaceae and Staphylococcaceae for herd 1 and Moraxellaceae and Methylococcaceae for herd 5. Two out of six dairy herds showed high levels of resistance, specifically herd 1 and herd 5 with 335 and 465 genomic copies/Gigabase (gc/Gb) when compared to the other four herds, which showed values lower than 20 gc/Gb. The most predominant levels of resistance were against gentamicin (17.6%) and kanamycin (15.1%) for herd 1 and spectinomycin (37.1%) and tetracyclines (18.8%) for herd 5. **Discussion and Conclusions:** This study shows a relatively quick and easy method to quantitatively analyse the resistome of milking filters based on a long-read sequencing approach. This approach can determine precisely the levels of antibiotic resistance present in the sample and associate them with the bacterial families harboring them.

3.2.9. Zoohirudotherapy (Leech Therapy) for Horses

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Admission: Leech therapy (hirudotherapy) is a natural method of treating various health ailments using verban leeches. **Zoohirudotherapy** in horses is used in many diseases. **Material And Methods:** The research material was the diagnostic results of the use of leech therapy (2023–2024) in four cases of equine health problems. The horses were diagnosed and referred for leech therapy by the attending veterinarian and evaluated after the therapy was completed. **Case Study 1:** Recurrent Equine Obstructive Disease (RAO), formerly called COPD. Cold-blooded mare, 17 years old, sled, leeches were used in monthly cycles and a prophylactic liver protocol. **Case Study 2:** Bilateral Tear Duct Obstruction. Małopolska mare, 21 years old, recreational, leeches were used during dental sedation 3 times a year and prophylactic liver protocol. **Case Study 3:** Neurological Form Associated with Eucharistic Poisoning. Małopolska mare, 21 years old, recreational, leeches were used daily for 2 weeks and prophylactic liver protocol. **Case Study 4:** Swelling and lameness associated with sudden traumatic condition and soreness in the area of the right ankle joint. Teenage Thoroughbred Thoroughbred, recreational, leeches were used at intervals of several days for 3 weeks and prophylactic liver protocol. **Results And Discussion:** In the case of mares with RAO, the symptoms disappeared, but the result is not conclusive due to the use of other supportive therapeutic methods. In the case of mares with lacrimal duct obstruction, after the fourth hirudotherapy treatment, bilateral tear permeability was achieved and eye inflammation disappeared, the same mare regained neurological efficiency after poisoning with the common pigtail after a week, and the gelding returned to full top fitness after a month after a limb injury. **Summary And Conclusions:** Within the scope of the activities of the Laboratory of Sensory and Animal Rehabilitation of the University of Siedlce, hirudotherapy has been successfully applied to improve the health of horses.

3.2.10. A Flight of Fancy or a Foe? Assessing Community Vulnerability to Bat-Borne Viruses in a Selected Community in Nigeria

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Straw-colored fruit bats (*Eidolon helvum*) are ecologically vital in West Africa but serve as reservoirs for zoonotic viruses such as coronaviruses, henipaviruses, and paramyxoviruses. In Ogbomosho, Nigeria, one of the region's largest *E. helvum* colonies roosts within the Nigeria Baptist Theological Seminary (NBTS), an academic institution that functions as a residential community for students, staff, families, and local dwellers. The seminary's open campus, proximity to bat roosting trees, and daily interactions between residents and wildlife create a unique human–bat interface. Hunters from the surrounding area also routinely enter the premises to harvest bats, increasing direct contact and potential spillover pathways. This study applied a mixed-method approach to assess community vulnerability to bat-borne virus transmission. A total of 160 residents were surveyed using structured questionnaires to evaluate awareness, risk interpretation, and bat-related behaviours. Participants were selected using simple random sampling from residential lists provided by NBTS, while hunters were included through convenience sampling due to their limited number and mobility. Four weeks of direct observations documented hunting patterns, handling practices, and spatial overlap between human activity and bat roosts. Results showed moderate bat awareness among residents but varying interpretations of disease risk. Observational findings revealed frequent hunting near residential zones, including the capture of adult and pregnant females, indicating unsustainable pressure on bat populations and consistent human–bat contact points. A positive association between awareness and perceived vulnerability ($r = 0.42, p < 0.01$) suggests that increased knowledge may promote safer behaviours. These findings highlight a critical One Health concern, demonstrating how ecological, behavioural, and environmental factors converge to heighten zoonotic risk in semi-urban communities. Strengthening education, regulating wildlife harvesting, and improving habitat management are essential for reducing disease emergence while conserving bat populations in Nigeria.

3.2.11. Age and Sex Differences in the Prevalence of *Dirofilaria immitis*: Results of a Field Study in 84 Rural Dogs in Bon County, Iran

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Introduction: *Dirofilaria immitis* (heartworm) is a vector-borne pathogen with significant implications for canine health. This study investigated the prevalence of *D. immitis* in 84 herd and domestic dogs in Bon County, Chaharmahal and Bakhtiari Province, Iran. The objective was to evaluate prevalence rates across different age groups and sexes, providing data to inform regional preventive strategies. **Methods:** A cross-sectional field study was conducted between December 2024 and March 2025 following ethical guidelines. Blood

samples were collected from 84 dogs and analyzed using serological assays (ELISA) for antigen detection. Necropsy findings, where available, were utilized to confirm adult worm presence in positive cases. Statistical associations between infection status, age, and sex were analyzed using Chi-square tests ($p < 0.05$). **Results:** The overall prevalence of *D. immitis* infection was 13.1% (11/84; 95% CI: 6.5–19.7%). A statistically significant association was observed regarding sex, with male dogs showing a higher infection rate (14.3%) compared to females (10.2%) ($p < 0.05$), which may reflect differences in exposure patterns. Age was also a significant factor, with the highest prevalence recorded in the 4–6 year age group (21.7%), while the 0–2 year group showed the lowest rate (5.5%). **Conclusions:** This study documents the endemicity of *D. immitis* in Bon County, a region with specific climatic conditions distinct from previously studied hyper-endemic areas. The results highlight *D. immitis* as a clinically relevant pathogen in this rural dog population. Given the identified infection rates, particularly among adult males, the implementation of integrated control measures, including vector management and chemoprophylaxis, is recommended to mitigate clinical risks.

3.2.12. An Unusual Case of Feline Polycystic Liver Disease in a Non-Predisposed Breed

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Introduction: Polycystic liver disease in cats is a congenital condition, occurring in isolation or in association with polycystic kidney disease (PKD), particularly in Persian cats. PKD is a disease that affects felines and other mammals, including humans. The prevalence of PKD in Persian and Persian-related cats is approximately 38%, dropping to 6 to 13.8% when considering the overall feline population. In humans, PKD is very prevalent affecting 1/500–1/100 persons. The number and size of cysts can vary, potentially leading to hepatomegaly and, eventually, to cyst rupture. Diagnosis can be achieved through histopathological examination and, imaging techniques. Medical History: A ten-year-old male Norwegian Forest Cat underwent surgery, during which liver fragments were collected for laboratory evaluation. **Material and Methods:** Three liver fragments were obtained during the surgical procedure and submitted for histopathological analysis. A thorough macroscopic examination was performed, followed by standard tissue processing

and microscopic evaluation of the lesions. **Results:** Macroscopically, the liver exhibited a cystic appearance, with multiple cysts of varying sizes across all lobes, on the capsular surface. Upon sectioning, the cystic cavities contained a pale fluid and were either unilocular or multilocular. Microscopically, multiple cystic cavities lined by atrophic epithelium were observed, occupying extensive areas of the hepatic parenchyma and separated by thin connective tissue septa, accompanied by atrophy of the adjacent hepatic tissue. **Discussion and Conclusion:** Polycystic disease, although rare, can also be detected in non-predisposed breeds. This case highlights the importance of continuous clinical monitoring in adult cats to identify early complications and guide therapeutic interventions. This work contributes to the epidemiological understanding of feline polycystic disease, broadening knowledge of its distribution, phenotypic variability, and clinical relevance in less-studied breeds. Further studies are needed to investigate genetic and environmental factors influencing predisposition and to support the development of effective prevention strategies. Compared to human medicine, veterinary research still lags behind, with no investment enabling genetic testing for the study of this disease, thereby wasting the potential of companion animals as natural sentinels for human pathology.

3.2.13. Building Public Trust Through Animal Welfare Governance: A One Health–One Welfare Case Study of the Ierapetra Municipal Police and its Impact on Tourist Perceptions

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Introduction: Animal-welfare governance is increasingly recognized as an essential component of One Health and One Welfare, particularly in tourism-dependent regions where visitor wellbeing and destination image are influenced by animal-related incidents. This study examines how the Municipal Police of Ierapetra, Crete, a municipality with consistently high levels of companion-animal registration (217 per 1000 residents), functions as an evidence-based example of municipal One Health and One Welfare implementation. **Methods:** A mixed-methods case-study design was applied. Official operational records from the Municipal Police (3500 animal-related cases from 2010 to 2024) were analyzed to assess enforcement activity, incident types, and welfare outcomes. In addition, 32 tourist testimonies collected from May to October 2024 were examined through reflexive thematic analysis to explore emotional responses, perceived safety, and trust in local authorities. Several participants referred spontaneously to experiences in other Cretan municipalities, providing naturalistic comparative material. Quantitative and qualitative findings were integrated through triangulation. **Results:** During 2024, the Municipal Police handled 566 animal-related cases, representing 40.3 percent of its annual workload, responded to 78 percent of urgent incidents within 24 h, and provided direct or indirect assistance to approximately 1850 animals. Tourist testimonies revealed a consistent emotional pattern: initial distress when witnessing mistreated or suffering animals, followed by clear relief and increased trust after municipal intervention. Participants frequently associated timely and humane action with a more positive perception of Ierapetra as a destination, while insufficient responses in other municipalities were linked to ongoing distress and reduced intention to revisit. **Conclusions:** The findings show that structured municipal enforcement can implement One Health and One Welfare in practice by improving animal-welfare outcomes, reducing visitor distress, and strengthening public trust. This case offers a transferable governance model for Mediterranean and European tourist destinations seeking to integrate animal-welfare management into sustainable tourism strategies.

3.2.14. Clinical Evaluation of *Pistacia lentiscus* L. Extract in Mitigating Flupyradifurone Toxicity in *Apis mellifera*: Survival and Symptom Progression

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Pesticide exposure is a critical concern for honeybee health. Flupyradifurone (FPF) is widely used in agriculture but has been associated with neurotoxic effects in honeybees (*Apis mellifera*), including impaired motor coordination, hyperactivity, and lethargy. Given the growing need for protective strategies, natural antioxidants such as polyphenolic extracts have gained attention for their potential to mitigate pesticide-induced toxicity. *Pistacia lentiscus* has demonstrated anti-inflammatory and antioxidant properties in mammalian models, but its protective role in honeybees remains underexplored. This study aimed to evaluate whether *Pistacia lentiscus* leaf polyphenolic extract (LPE) could reduce FPF-induced toxicity in honeybees. Honeybees were allocated into 12 experimental groups (n = 20/group) under controlled laboratory conditions. FPF was administered orally at 100 mg/L for 72 h, while parallel groups received co-administration of LPE (1% or 5% w/v). Clinical observations were recorded daily, including neuromuscular dysfunction (e.g., tremors, abnormal wing positioning, uncoordinated movement, curved belly, etc.), gastrointestinal distress (e.g., distended abdomen, abnormal and/or defecation), behavioral alterations (e.g., hyperactivity followed by apathy, failure to respond to stimuli), and mortality rates. Honeybees exposed to FPF alone exhibited severe neurotoxic symptoms within 24 h, including erratic movement (67.5% incidence) and moribund posturing (27.5% by Day 3), with 90% mortality by 72 h. In contrast, co-treatment with LPE at 1% significantly delayed symptom progression and prevents mortality: FPF +1% LPE groups showed 100% survival at 72 h vs. 10% in FPF-only groups ($p < 0.01$). The FPF +1% LPE groups showed lesser hyperactivity (2.5% vs. 67.5% in FPF-only) and abdominal spasms (0% vs. 27.5%). FPF +5% LPE groups also showed higher survival probability but in a lesser amount (42.5% vs. 10% FPF-only), suggesting that high LPE doses are toxic. The therapeutic activity is attributable to the polyphenolic component identified through phytochemical characterisation using HPLC-MS. LPE attenuated FPF-induced toxicity and should be considered for further field studies to confirm its therapeutic potential.

3.2.15. Early Fermentation Dynamics in Freshly Cut Grass and Their Implications for Equine Colic

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Introduction: Colic remains a major cause of emergency intervention in horses, and feeding practices are a critical component of risk management. Freshly cut, semi-dried grass is often considered a convenient feed source, yet early fermentation processes may predispose horses to gastrointestinal disturbances. This study investigated microbiological, physicochemical, and sensory changes in grass clippings during natural drying to identify factors contributing to colic development. **Methods:** Grass samples were collected near Stable X during the seasonal peak of grass-related colic. The grass samples were collected at three time points (0 h, after 12 h and 24 h of fermentation), and three independent replicates were obtained at each time point, resulting in a total $n = 9$. Microbiological evaluation was performed using Columbia Blood Agar under anaerobic conditions, followed by Gram staining. Feed quality assessment included dry matter (DM), pH, and concentrations of nitrogen, phosphorus, and calcium. Organoleptic characteristics were also documented. Clinical colic cases recorded in the LSMU Large Animal Clinic during the same period were reviewed to support laboratory findings. **Results:** Across all replicates, *Clostridium botulinum* was not detected. Instead, *Actinomyces* spp. and non-specific Gram-positive rods proliferated consistently, with the highest bacterial load in the 24-h samples. DM displayed a uniform pattern: a slight increase at 12 h, followed by a statistically significant decline at 24 h (mean decrease 26.5%, $p < 0.05$), indicating active microbial fermentation. Nitrogen and phosphorus increased, while calcium decreased; pH remained acidic. Sensory evaluation revealed progressive heating, darkening, and development of spoilage odour. Clinical data demonstrated recurrent episodes of gaseous distension and reduced intestinal motility, consistent with laboratory findings. **Conclusions:** The results confirmed that freshly cut grass undergoes rapid, reproducible fermentation within 24 h, producing conditions that predispose horses to fermentation-related colic. Semi-dried grass clippings represent a consistent feeding risk and should be avoided. Consider it as a hypothesis and expand the studies.

3.2.16. First Report of Paratuberculosis in Sheep in Ecuador: A Case Study and Literature Review

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Introduction: Paratuberculosis (Johne’s disease), a chronic granulomatous enteritis of ruminants caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP), is well-

documented in cattle across Latin America but remains significantly underdiagnosed and underreported in small ruminants. Although MAP infection in sheep has been reported sporadically in other Latin American countries, no confirmed cases have been previously documented in Ecuador. This study confirms the first case of paratuberculosis in sheep in Ecuador. **Methods:** The diagnosis followed clinical and post-mortem evaluation of two severely affected animals, exhibiting progressive weight loss, from a 504-sheep herd in the Andean highlands. Histopathological examination of the intestine revealed lesions characteristic of MAP infection. MAP-specific DNA was successfully amplified from fecal and intestinal samples via nested PCR targeting the IS900 sequence and confirmed by Sanger sequencing. **Results:** An ELISA-based seroprevalence survey of the entire herd detected MAP-specific antibodies with an overall seropositivity of 10.12%. As paratuberculosis is a notifiable disease, the results were reported to the competent national authority, which subsequently notified the World Organisation for Animal Health (WOAH). **Conclusion:** Our findings underscore the urgent need for expanded surveillance and targeted diagnostic efforts in small ruminants, particularly in herds with unexplained progressive weight loss or potential zoonotic relevance, given the suspected association between MAP and Crohn's disease and the common consumption of raw milk and dairy products. A review of regional literature further indicates limited but increasing evidence of MAP infection in sheep and goats throughout Latin America.

3.2.17. Foreign Body Ingestion (FBI) in a *Rhinella* sp. Toad—A Case Report

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Foreign body ingestion (FBI) is well-documented in domestic animals but less frequently reported in wildlife, particularly amphibians, due to diagnostic challenges and underreporting. FBI-reported cases in *Rhinella* toads are especially rare, with limited documentation in the scientific literature. This case report describes an adult female *Rhinella* sp. toad (800 g) kept as a pet in a backyard with an artificial pond. It was occasionally fed meat and mealworms and had free outdoor access. The owner reported that the patient had recently become anorexic and lethargic. Clinical evaluation showed no abnormalities, but a ventrodorsal radiograph revealed a suspected gastric foreign body. An emergency exploratory laparotomy was recommended, with the owner consenting to the surgery the following day. However, on the morning of the scheduled procedure, the owner reported that the patient had passed away. Upon necropsy, the presence of an aluminum foil lid from a yogurt container was revealed inside the stomach. The lid had perforated the gastrointestinal tract, leading to free fluid accumulation in the coelomic cavity and signs of peritoneal inflammation. *Rhinella* toads are endemic to Brazil, serving a vital function in environmental conservation. Although they are not commonly kept as pets, those with access to outdoor spaces may be at risk of ingesting litter. This case report highlights the importance of responsible waste management, as improper trash disposal can pose serious health risks not only to domestic animals but also to local wildlife. Improved awareness and preventive measures are essential to mitigate the risks associated with environmental contamination.

3.2.18. Insects, Horses, and One Health: Lessons from an Extensive System in Central Spain

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Insects are central in horse production systems: they transmit pathogens, cause skin and behavioral disorders, and indicate environmental quality. Current responses often rely on individual protection (blankets, repellents, short-lived insecticides), addressing symptoms rather than environmental drivers, and are increasingly ineffective and pose ecological risks. Preventive ecosystem-level interventions are therefore needed. We propose a multi-scalar approach integrating entomological knowledge into ecological biocontrol for equine health. A targeted review identified the key pest species (*Simulium* spp., *Tabanus* spp., *Stomoxys* spp., *Musca domestica*). Field observations (2024–2025) in a semi-arid horse farm in central Spain (“La Nebulosa”) showed that late-spring to early-autumn biting activity was exacerbated by high temperatures, low humidity, and sweat interacting with dusty haircoats, amplifying irritation and host chemical cues. Horses also differ in susceptibility due to variation in skin integrity, sweating, and reactivity, requiring strategies that balance environmental and individual needs. Our preliminary multi-scalar interventions included the following: (i) micro-scale actions on host-linked microhabitats using biocontrol and stage-specific traps, together with systematic fly-mask use to prevent ocular irritation; (ii) meso-scale adaptation of stables and paddocks to reduce soil moisture; and (iii) macro-scale enhancement of insectivorous biodiversity and aromatic plant deterrence. Qualitative improvements included reduced fly pressure, fewer skin lesions in susceptible individuals, calmer behavior, and no conjunctivitis cases this year (vs. two in the previous year). These findings support the development of a Bayesian model integrating environmental drivers and individual susceptibility to quantify responses in future seasons. We conclude that insects can transition from indicators of degradation to co-managers of ecological resilience, enabling preventive biocontrol with welfare and ecological co-benefits.

3.2.19. Lunar Phases and Gastrointestinal Crisis in Horses: An Analytical Study of Colic Distribution

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Introduction: Equine colic is a multifactorial syndrome, and horse owners frequently relate its occurrence to lunar cycles. Despite the popularity of this belief, scientific data are scarce. This study evaluated whether lunar phases are associated with the frequency of colic episodes in horses and whether they influence colic localisation or clinical outcome. **Methods:** A retrospective analysis was performed using clinical records of horses presenting with colic to a university large animal clinic between 2013 and 2020. Each case was assigned to one of four lunar phases based on the date of onset: new moon, last quarter, first quarter, or full moon. In total, 138 colic episodes with complete data were included. Statistical analyses were carried out in SPSS. A chi-square goodness-of-fit test was used to compare the observed distribution of colic cases across lunar phases with an equal expected distribution. Associations between lunar phase and localisation of colic or disease outcome were examined using chi-square tests of independence. Effect size was estimated using Cohen’s *w*. **Results:** Colic cases were not evenly distributed across the lunar phases: new moon *n* = 50; last quarter *n* = 39; first quarter *n* = 20; full moon *n* = 29 ($\chi^2 = 14.52$, *df* = 3, *p* < 0.01, *w* = 0.32). Thus, colic episodes occurred significantly more often around new moon and the last quarter than during the first quarter. No significant association was

found between the lunar phase and anatomical localisation of colic ($p > 0.05$) or clinical outcome ($p > 0.05$). **Conclusions:** Lunar phases showed a moderate but statistically significant association with the timing of colic episodes, with peaks around new moon and the last quarter. However, moon phases did not affect the type of colic or its outcome. Further prospective studies are needed to clarify underlying mechanisms and to control for management and environmental confounders.

3.2.20. Mobile Flow Cytometer as a Tool for Hygiene Monitoring in Broiler Farms

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High biosecurity standards are essential in poultry farms, where cleaning and disinfection are key to maintaining hygiene and animal health. Evaluating these procedures requires rapid and reliable detection methods. Plate cultivation remains the gold standard but is time-consuming and limited to culturable microorganisms, while luminometers mainly detect organic contamination. This study evaluated the potential of a mobile flow cytometer for hygiene monitoring on a broiler farm. The device (CytoQuant, Romer Labs) uses impedance and alternating current at multiple frequencies to detect and quantify intact cells and particles without optical systems. This study was conducted on a commercial broiler farm. Samples were collected during sanitation from three sites (drinker, concrete wall, and metal wall) by swabbing 100 cm² surfaces. Measurements followed the manufacturer's protocol. Plate cultivation determined total microbial counts (TMCs) at 22 °C (plate count agar, PCA) and 36 °C (blood agar, BA). Correlations were assessed using the Spearman correlation coefficient (rSp). Significant correlations were found for the metal wall (BA: rSp = 0.8117, $p < 0.01$; PCA: rSp = 0.8333, $p < 0.01$) and the concrete wall (BA: rSp = 0.8000, $p < 0.01$; PCA: rSp = 0.7667, $p < 0.05$), but not for drinkers. The cytometer detected higher microbial counts than cultivation, by 1.13–2.26 log CFU on BA and 0.76–1.02 log CFU on PCA ($p < 0.05$, Wilcoxon test). This difference reflects enumeration of all intact cells, unlike plate methods, which detect only culturable ones. Environmental hygiene is crucial for broiler health, welfare, and productivity. The mobile flow cytometer demonstrated strong potential for assessing sanitation efficiency and may complement plate methods. Unlike luminometry, the results are unaffected by disinfectant residues. Flow cytometry provides a rapid, practical tool for hygiene monitoring, enhancing biosecurity and supporting One Health strategies by reducing microbial loads and optimizing disinfectant use.

3.2.21. Molecular Characterization of Piroplasms and Identification of Tick Vectors in Central Spain

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Introduction and objectives: Piroplasmosis is an endemic tick-borne disease in Spain caused by protozoa of the genera *Babesia* and *Theileria*, transmitted by hard ticks (Ixodidae). These hemoprotozoa can induce severe diseases in both animals and humans. Since not all tick species or individuals are infected or competent vectors, identifying the tick fauna present in each area and the piroplasms they harbor is crucial for improving surveillance, prevention, and control strategies. **Methodology:** Between 2023 and 2024, the Environmental Risk Surveillance Unit of the Community of Madrid collected 302 ticks, grouped into

148 pools, from 21 host species in central Spain. Hosts included ungulates, wild carnivores, small mammals, birds, and domestic animals. Ticks were morphologically identified to species level. DNA extraction was followed by nested PCR for *Babesia* and *Theileria* detection according to Jefferies et al. (2007). **Results and discussion:** Wild boar (31.8%), rabbit (19.6%), fox (10.8%), deer (8.8%), and raccoon (6.8%) were the most frequent hosts. The spatial distribution of samples reflects an extensive surveillance effort encompassing both wildlife management areas and urban–periurban environments. The predominant tick species were *Hyalomma lusitanicum* (32.4%) and *Rhipicephalus pusillus* (29.7%). Five piroplasm species were identified: *Babesia pecorum*, *Babesia ovis*, *Babesia* sp. (*tavsan2/occultans*), *Theileria ovis*, and *Theileria capreoli*. The overall infection rate was 6.08% (9/148). Positive samples included *B. pecorum* (2.7%) in wild boar (*Dermacentor marginatus*), goat (*Rhipicephalus sanguineus*), and rabbit (*R. pusillus*); *B. ovis* (0.68%) in wild boar (*H. lusitanicum*); *Babesia* sp. *tavsan2/occultans* (0.68%) in Iberian Ibex (*Rhipicephalus bursa*); *T. ovis* (1.35%) in rabbit (*R. pusillus*) and cat (*R. sanguineus*); and *T. capreoli* (0.68%) in mouflon (*H. lusitanicum*). These findings suggest multispecies circulation and possible cross-transmission between wildlife and domestic environments. **Conclusions:** The ecological and geographic spread of piroplasms in local tick populations underscores the need for continued surveillance in wildlife and domestic animals in central Spain.

3.2.22. Molecular Identification and Antibiotic Resistance Profiling of *Staphylococcus Aureus* and *Escherichia Coli* Isolated from Mastitic Milk Samples of Large Ruminants

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Staphylococcus aureus and *Escherichia coli* are main infection causing microbes. Both of the microorganisms also have great ability to develop resistant against antibiotics. Strains that have antibiotic resistance can cause infections in the form of epidemics. The objective of the present study was to identify and detect *Escherichia coli* and *Staphylococcus aureus* from samples of milk from mastitis positive buffaloes and cows and to assess the patterns of antimicrobial resistance profile. A total of 30 milk samples (buffalo: n = 15, Cows: n = 15) were collected randomly from apparently healthy animals. Samples were collected from different dairy farms in the region of Rawalpindi. Samples were first checked for subclinical mastitis using surf field mastitis test (SFMT). Mastitis positive samples were analyzed in the laboratory by biochemical tests. Polymerase chain reaction (PCR) was used to for the molecular identification of *Escherichia coli* and *Staphylococcus aureus* utilizing the 16SrRNA gene. Antibiotic sensitivity test (AST) was performed to check antimicrobial resistance patterns of different antibiotics by using disk diffusion method. Out of 30 samples, 18 samples were found mastitis positive using SFMT (60%). For identification of *Escherichia coli* and *Staphylococcus aureus* API E20 and API Staph Kit was used respectively. Biochemical tests and PCR confirmed the presence of that out of 18 samples, 4 samples found for *Staphylococcus aureus* and 6 samples were found positive for *Escherichia coli*. AST was performed to check antibiotic resistance patterns of isolated organisms and results have shown that both organisms were resistance against penicillin whereas *Staphylococcus aureus* was also found resistant against Oxytetracycline. Both organisms were also found highly sensitive to Ciprofloxacin and Erythromycin. The results of this study have shown that *Escherichia*

coli and *Staphylococcus aureus* are present in selected dairy farms in Rawalpindi which can lead to their spread to other farms and cause a larger scale milk production loss.

3.2.23. Myocestodiasis in *Octopus maya*: A One Health Approach to Emerging Marine Parasitic Diseases

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Octopus maya is an endemic species of high ecological and socioeconomic importance in the Yucatán Peninsula, representing the fourth most valuable fishery in Mexico and the most important cephalopod fishery in the country. Recent findings have revealed that this species can develop a parasitic disease associated with cestodes of the genus *Prochristianella*, representing a previously undescribed pathology in mollusks. An integrative methodological approach was applied to characterize the effects of natural infections caused by *Prochristianella* sp. 1 in the buccal mass of *O. maya*. Morphological and molecular tools were used to confirm the identity of the parasite, while macroscopic and histological assessments were used to assess tissue damage and hemocyte responses in infected versus uninfected individuals of *O. maya*. Immune-related genes were analyzed to explore potential molecular responses associated with infection. This combined strategy provided a comprehensive understanding of the pathological process and host–parasite interaction from cellular to organismal levels. High-intensity infections resulted in progressive tissue degradation of the superior mandibular muscle, accompanied by increased hemocyte counts and histopathological alterations consistent with an inflammatory response. The expression of immune-related genes was evaluated. Gene C1q, associated with the complement system, and LITAF, a regulator of tumor necrosis factor α , were significantly overexpressed in infected individuals, suggesting activation of both immune and pro-inflammatory pathways. The pathology, provisionally named Myocestodiasis in cephalopods, represents a novel helminth disease. These findings emphasize the importance of addressing host–parasite interactions in marine invertebrates through a One Health perspective, recognizing the interconnectedness between environmental health, animal health, and human well-being. By identifying a new parasitic disease in a species of economic relevance, this study provides essential insights for monitoring the sanitary status of wild octopus populations and underscores the need to integrate parasitological, ecological, and immunological information to ensure the sustainability of fisheries in the Yucatán Peninsula.

3.2.24. Prevalence of Honey Bee Pathologies in Algeria: Insights for Sustainable Apiculture

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Introduction: Honey bees play a critical role in pollination and the maintenance of biodiversity. In Algeria, bee populations face multiple health challenges due to apicultural diseases, including viral, bacterial, and parasitic infections, as well as infestations by *Varroa destructor*. These pathologies threaten colony survival, reduce honey production, and may negatively affect agricultural ecosystems. The aim of this study is to assess the prevalence

and regional distribution of major honey bee diseases in Algeria to inform targeted prevention and management strategies. **Methods:** A national survey was conducted between 2023 and 2024, covering 15 apiaries across different climatic and ecological zones of Algeria. Colony samples were collected and analyzed for bacterial infections (*Paenibacillus larvae*), viral diseases (e.g., Deformed Wing Virus), and parasitic infestations, including *Varroa destructor* and *Nosema* spp. Data were analyzed to determine disease prevalence, regional patterns, and risk factors associated with environmental and management conditions. **Results:** The study revealed a high prevalence of key apicultural diseases, with notable variations between regions. *Varroa destructor* emerged as the most widespread parasite, followed by bacterial and viral infections. Apiaries located in intensive agricultural areas exhibited higher disease incidence, suggesting a link between agricultural practices and colony health. Seasonal and environmental factors also influenced disease prevalence, highlighting the need for context-specific management approaches. **Conclusion:** These findings underscore the importance of regular monitoring and integrated disease management in Algeria. Strengthening preventive measures, raising awareness among beekeepers, and promoting sustainable apicultural practices are essential to safeguard honey bee health, ensure colony productivity, and support biodiversity conservation. This study provides a foundation for developing effective strategies to mitigate the impact of apicultural diseases in the region.

3.2.25. West Nile and Usutu Viruses: A New Challenge for a One-Health Approach During 4-Year Surveillance in North-West Italy

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In recent years, new global health threats have been linked to climate change and vector-borne diseases; West Nile viruses (WNV) and Usutu virus (USUV) are Flaviviridae viruses, involved in several outbreaks all over the world. Warmer temperatures and altered precipitation patterns can expand the geographical range and seasonal activity of mosquitoes. Italy has experienced numerous incursions of WNV and USUV, becoming the European country with the highest number of cases. This study aims to present our findings during 4 years of wild bird surveillance for WNV and USUV viruses in the three regions of northwest Italy. In this study, we carried out both passive (wild birds found dead, syndromic surveillance, and neurological cases in equids) and active surveillance (culling wild birds of target species). After RNA extraction, we performed two RT-PCR to detect WNV Lineage 1 and 2 and USUV. A total of 1026 animals underwent analysis in 2022; 20 animals tested positive for WNVL2, with only one positive for USUV. In 2023, a total of 1307 animals were tested; 24 animals were positive for WNVL2, 5 were positive for USUV, and 5 were co-infected with WNVL2 and USUV. In 2024, a total of 995 animals underwent testing; 25 animals tested positive for WNVL2. Finally, in 2025, a total of 1184 animals were analyzed; we detected a total of 20-WNVL2 positive samples and 1 WNVL1-positive sample. Our results show that positive samples are increasingly detected in uncommon periods for these viruses. Currently, it remains difficult to assess the impact of this change on the viruses'

dynamics and epidemiology through vectors in relation to the last alternating pattern of periods of the complete absence of rain and subsequent periods of heavy flooding. Finally, we must consider the effect on bird migration and on the balance between viruses and vectors. This is a One-Health concern; public health monitoring requires more integrated plans and actions to anticipate zoonotic viral diffusion.

3.3. Sustainable Animal Nutrition

3.3.1. Evaluating the Agronomic and Nutritional Variability of White Lupin Accessions and Their In-Situ Rumen Degradation Kinetics in Buffaloes

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White Lupin (*Lupinus albus*) is an emerging crop in the current livestock feeding regime. The objective of the present study was to evaluate the suitability of this crop as fodder and grain for feeding of buffaloes in arid regions. Seven cultivars of white Lupin were selected from a large germplasm screening trial (SUK-3 and LUP-6483 as pure fodder cultivars, LUP-230 and LUP-248 as grain cultivars, and Dieta, Lodi, and LUP-6463 as dual purpose) were grown under hot arid environment and data were recorded for variability in yield, chemical composition and in situ degradation kinetics according to the NorFor standards. The in-situ incubation of the samples were performed in two rumen cannulated buffaloes to observe the degradation profiles of dry matter (DM), crude protein (CP), and neutral detergent fiber (NDF) at various time intervals. The results of chemical composition indicated that CP ranged from 145–199 g/kg DM for fodder cultivars, and 314–413 g/kg DM for grain cultivars. The NDF ranged from 394–462 g/kg DM for fodder cultivars, and 189–233 g/kg DM for grain cultivars. The results of agronomic traits of fodder indicated that the dual-purpose cultivar LUP-6463 had the highest fresh forage yield (10.72 tons/acre) and CP (199 g/kg DM) while Lodi had the maximum fat (40.3 g/kg DM) and NDF (462 g/kg DM) concentrations. Whereas the grain cultivar LUP-230 had the highest grain yield (2.14 tons/acre), followed by LUP-6463. The grain cultivar LUP-230 had the superior DM and CP degradability while LUP-6463 had the maximum NDF degradability. By combining the data of yield, nutrient composition and degradability, it is evident that the dual-purpose cultivar of white Lupin LUP-6463 and grain cultivar LUP-230 can provide excellent feeding and nutritive values to replace commonly used green fodders and protein sources such as soybean in the buffaloes feeding.

3.3.2. Average Daily Gain of Ewe Lambs Under Different Environmental Conditions in Confinement

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Meat sheep farming has improved its production systems by adopting confinement as a strategy to optimize nutritional management and environmental control, especially during winter. Average daily gain (ADG) is a key indicator reflecting diet efficiency and thermal comfort in facilities. This study aimed to evaluate the effect of different confinement structures during winter on the ADG of hair ewe lambs. The experiment was conducted at the Meat Sheep Sector—UFVJM, Campus JK, in Diamantina-MG, using 12 crossbred Dorper × Santa Inês ewe lambs, averaging 40 kg live weight and 12–24 months of age. Animals were distributed in a completely randomized design with two treatments: closed confinement (1.5 m walls and metal roof) and open confinement (fenced with wire mesh and shaded by shade cloth). The diet was formulated for an expected gain of 0.100 kg/head/day, provided twice daily (7:00 a.m. and 3:00 p.m.). Weighings were performed after a 16-h solid fasting on 25 July, 8 August, 22 August, and 12 September 2025, totaling 51 days of evaluation. Environmental and rectal temperatures, and wind speeds, were recorded on the same dates, ranging from 12.3 to 22.5 °C and 0.1 to 0.9 m/s in the closed confinement, and from 14.4 to 25.3 °C and 1.6 to 6.5 m/s in the open system. Data were analyzed using a Generalized Linear Model, and means were compared by Tukey's test at a 5% significance level. Rectal temperatures averaged 39.43 °C, within the species' thermoneutral zone. Confinement type significantly affected ADG ($p < 0.05$), with mean values of 0.180 kg/day in the closed and 0.140 kg/day in the open system. The higher gain observed in the closed environment indicates better energy efficiency associated with reduced thermal fluctuations and wind exposure. It is concluded that confinement with half walls and metal roofing provided better productive performance during winter, demonstrating suitable adaptation and efficient nutritional management.

3.3.3. Black Soldier Fly (*Hermetia illucens*) Larvae Meal as a Partial Fishmeal Substitute in Diets for Paco (*Piaractus brachypomus*) Fingerlings: Effects on Digestibility, Growth and Blood Biochemistry

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Introduction: The increasing global demand for animal protein requires sustainable ingredients that reduce dependence on fishmeal. Black soldier fly (*Hermetia illucens*) larvae meal is a promising alternative, but information on its use for paco (*Piaractus brachypomus*) juveniles is limited. This study evaluated the apparent digestibility, growth performance and blood biochemical profile of paco fed diets in which fishmeal was partially or totally replaced by *H. illucens* larvae meal. **Methods:** An apparent digestibility trial was conducted for 6 weeks in Gelp-type collectors using 9 fish fed control, fishmeal and *H. illucens* meal test diets. A 60-day growth trial was carried out at the Pucayagro fish farm, located in the San Martín region, Peru, with 4 replicate tanks per treatment and 20 fingerlings per tank. Experimental diets replaced fishmeal with insect meal at 0%, 30%, 60% and 100%. At the end of the growth trial, blood samples were collected for analysis of triglycerides, cholesterol, hepatic enzymes, glucose and total protein. **Results:** *H. illucens* larvae meal showed high apparent digestibility, particularly for ether extract (97%) and crude protein (88%). Fish fed the 30% replacement diet achieved weight gain comparable to the control,

whereas 60% and 100% replacement reduced growth. Diets containing insect meal increased the amount of plasma triglycerides and induced a progressive reduction in cholesterol with an increasing replacement level. Activities of AST and ALT rose at higher inclusion levels, while glucose and total protein levels remained within physiological ranges. **Conclusions:** Dried *H. illucens* larvae meal can replace up to 30% of dietary fishmeal in paco fingerlings without compromising growth or key blood biochemical indicators. Higher replacement levels negatively affect performance and may challenge hepatic function, highlighting 30% fishmeal substitution as a practical upper limit under the present conditions.

3.3.4. Weight Gain of Hair Sheep Ewes Confined Under Different Environmental Conditions

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Sheep farming has been expanding in Brazil, and confinement systems have emerged as an alternative to improve productive performance during winter. This study aimed to evaluate different confinement structures in winter on the total weight gain of hair sheep females. The experiment was conducted at the Sheep Production Sector of UFVJM, Campus JK, Diamantina-MG, using 12 Dorper × Santa Inês crossbred ewes, averaging 40 kg of body weight and 12–24 months of age. A completely randomized design was used with two treatments: closed confinement (1.5 m walls and metal roof) and open confinement (wire fence with shade cloth cover). All animals received the same diet formulated for an average daily gain of 0.10 kg/head/day, divided into two feedings (7 a.m. and 3 p.m.). Body weight was recorded after a 16-h solid fasting period on 25/07, 08/08, 22/08, and 12/09/2025, totaling 51 days. Data were analyzed using a Generalized Linear Model, and means were compared by Tukey's test at a 5% significance level. Significant effects were observed for both environment type ($p = 0.0086$) and confinement duration ($p < 0.05$) on live weight. The final average body weight was higher ($p < 0.05$) in the closed confinement (44.5 kg/head) compared to the open one (41.0 kg/head). Weight gain increased steadily ($p < 0.05$) throughout the experimental period, from 38.5 kg to 46.2 kg/head on day 51. Rectal temperatures remained within the thermoneutral range (mean 39.43 °C) with no significant differences ($p > 0.05$), indicating physiological stability. The results suggest that closed confinement improved productive performance, likely due to reduced thermal variation and lower energy expenditure for thermoregulation. According to Medeiros et al. (2017) and Franco et al. (2022), controlled environments enhance thermal comfort and nutrient utilization, favoring continuous weight gain.

3.3.5. Bone Morphology and Tibia-Breaking Strength of Broiler Chickens Managed on Different Housing Systems Fed Diets Containing Piper Guineense Leaf and Seed

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A 6-week study was conducted to determine the bone morphology and tibia-breaking strength of broiler chicken managed on different housing systems fed diets containing *Piper guineense* leaf and seed. For 3 weeks, 288-day-old broiler chicks were brooded; they were then assigned into treatment in 2-by-4 experimental layouts, which comprised two housing systems (deep litter and battery cage) with dietary inclusion levels of *Piper guineense* leaves and seeds (0, 0.5, 1 and 1.5%) in a 2:1 ratio of leaves and seed. Hence, the birds were divided into 8 treatment groups of 4 replicates, with 9 birds per replicates and a total of 36 birds

per treatment. Birds on treatment 1 were reared on battery cage housing system-fed diets without *Piper guineense*, while those on treatments 2, 3 and 4 were fed ration containing *Piper guineense* at 0.5, 1.0 and 1.5% inclusion levels, respectively. Birds assigned to treatments 5, 6, 7 and 8 were managed in a deep litter housing system, with dietary inclusion levels of 0, 0.5, 1.0 and 1.5%, respectively. Two birds per replicate group were then selected and killed. The left tibia bone was collected for tibia bone morphology and breaking strength. Data were subjected to a two-way analysis of variance. The significant treatment means were separated using Duncan's multiple range test. The results of this study revealed that most of the tibia bone morphological parameters were not influenced by the housing type except for diaphysis diameter; we found wider diaphysis diameters obtained in birds of the deep litter housing type. Wet tibia, dry tibia weight, diaphysis and tibia index were significantly ($p < 0.05$) influenced by the dietary inclusion levels. The interaction between the housing type and dietary inclusion levels significantly influenced all parameters of the tibia bone characteristics except tibia robusticity. It can be concluded that for a better bone quality, broiler birds should be managed in the deep litter housing type and fed diets with no more than 1% *Piper guineense*.

3.3.6. Comparative Evaluation of Abscised Unripe Mango Fruit and Maize and Samsorg-49 Offal as Dietary Energy Sources on Performance and Methane Emissions of Yankasa Ram Lambs

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This study evaluated the effect of abscised unripe mango fruit meal (AUMFM) in comparison to maize and Samsorg-49 offal on the performance, rumen indices and methane emissions of Yankasa ram lambs. Twelve growing rams with an average weight of 17.33 kg were randomized into three dietary treatments, namely AUMFM, maize offal and Samsorg-49 offal, each at a 30% inclusion level, with four replicates per group. They were fed for 90 days in a CRD. Data collected were analysed using the GLM procedure of ANOVA in SPSS and significant means were compared using DMRT at $p < 0.05$. The results showed that the weight gains of rams fed 30% dietary inclusions of AUMFM (97.25 g/ram/day) and maize offal (104.40 g/ram/day) were statistically similar and higher ($p < 0.05$) than Samsorg-49 offal (81.59 g/ram/day), while the FCR was higher ($p < 0.05$) in Samsorg-49 offal (9.18) compared to maize offal (7.27). However, the dietary inclusion of AUMFM had higher CH₄ efficiency (25.05%) compared to maize offal (30.93%) and Samsorg-49 offal (30.82%). Although there were no differences ($p > 0.05$) observed for feed intake, nutrient digestibility, nitrogen utilization and rumen indices among the dietary treatments, the dietary inclusions of AUMFM and maize offal showed increasing tendency to impact nitrogen free extract digestibility ($p = 0.09$), nitrogen retained ($p = 0.07$) and N-retained as a % of intake ($p = 0.06$) compared to the Samsorg-49 diet. In conclusion, 30% inclusion of AUMFM in the diet of Yankasa ram lambs had a weight gain comparable to dietary maize offal, with higher CH₄ efficiency compared to both the 30% dietary inclusion of maize and Samsorg-49 offal. This study underscores the importance of exploring the potential of AUMFM as a cheap dietary energy ingredient with a low carbon footprint as compared to the maize and Samsorg-49 offal in the diets of Yankasa ram lambs. Hence, ruminant livestock farmers are encouraged to adopt 30% dietary inclusion of AUMFM for better weight gain compared to Samsorg-49 offal, cheaper cost of feeding, and climate-smart livestock production.

3.3.7. Dietary Lipid Source Unravels Host–Microbiota Networks Along Gut–Muscle Axis in Pigs

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The “gut–muscle axis” is an emerging concept that describes how intestinal microbiota influence skeletal muscle biology, especially energy metabolism. This biological communication is crucial for muscle development and directly affects meat production traits. Although the gut microbiota is known to modulate host metabolism, the specific molecular mechanisms by which diet shapes the gut–muscle axis are not well defined. This study aimed to use a network-based approach to dissect how dietary lipid sources—canola (CO) vs. soybean (SO) oil—can modulate the interaction between cecal microbiota and muscle transcriptome in pigs. *Longissimus lumborum* muscle (RNAseq) and cecal content (16S rRNA sequencing) samples were collected from 32 pigs fed diets supplemented with either 3% CO (N = 14) or 3% SO (N = 18). An analysis of the cecal microbiota yielded 447 core amplicon sequence variants (ASVs, 50% prevalence) but revealed no differences in community diversity between diets. In contrast, the muscle gene expression was distinctly clustered by diet. This prompted a diet-specific Weighted Gene Co-expression Network Analysis (WGCNA) to map interactions with the 19 most abundant (>1% mean abundance) cecal genera. WGCNA yielded 41 gene modules for CO and 27 for SO. We found four relevant modules ($|r| > 0.7$, $p < 0.01$) for CO, positively correlated with the *Rikenellaceae* RC9 gut group and *Leyella* and negatively correlated with *Akkermansia*, *Terrisporobacter*, and *Romboutsia*. In the SO group, the two relevant modules were positively correlated with *Prevotellaceae* UCG-003 and *Terrisporobacter*. Our findings demonstrate that dietary fat sources reshape functional communication rather than the composition of the gut–muscle axis. The opposing association of *Terrisporobacter*, a genus linked to SCFA production, oxidative stress, and immunity, suggests that the fat source can influence metabolic and immune signaling in muscle. This integrative framework offers a powerful tool for the sustainable development of targeted nutritional strategies to enhance muscle development and pork quality.

3.3.8. Effect of a Multifunctional Additive on the Microbiota, Behavior, and Growth of Dairy Calves Raised in Groups in Pasture-Based Systems

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This study aimed to evaluate the effects of oral supplementation of a multifunctional additive on the fecal microbiota, feeding behavior, and growth of calves raised in groups in pasture-based systems. The study was carried out at Embrapa Pecuária Sudeste, Brazil. Sixteen Kiwi-Cross dairy calves were randomly assigned to two groups: treated ($n = 8$): animals (3 g/animal/day) that received a compound of probiotics, prebiotics, essential fatty acids, organic minerals, and amino acids; and control ($n = 8$): animals (3 g/animal/day) that received a placebo. From birth to weaning (0–66 days), the calves' fecal samples (D0, D15, D30, D60, and D66), feeding behavior (grazing and rumination), and growth parameters (average daily gain—ADG and thoracic circumference—TC) were taken. Fecal microbiota were analyzed by 16S rRNA sequencing (Illumina MiSeq), while behavior and growth were analyzed by Generalized Linear Models. Regardless of treatment, there was an increase ($\sim 16\%$) in α -diversity over time ($p < 0.001$). The treated group presented a greater ($p = 0.001$) relative abundance of the families Lactobacillus and Muribaculaceae than the control group. The treated group (grazing: 43 d; rumination: 36 d) began grazing and rumination behaviors later than the control group (grazing: 35 d; rumination: 28 d). The ADG ($p = 0.008$) and TC ($p = 0.003$) were higher in the treated group (ADG: 0.69 kg/day; TC: 90.3 cm) than control group (ADG: 0.52 kg/day; TC: 86.7 cm). In conclusion, the fecal microbiota changed over time, probably due to rumen development during the experimental period. Although supplementation with a multifunctional additive delayed grazing and ruminating behaviors in dairy calves, it improved their growth.

3.3.9. Effect of Concentrate Moistening on Feed Intake of Dairy Cows in an Automatic Milking System

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In automatic milking systems (AMS), the concentrate is commonly supplied in pelleted form due to its higher density and faster ingestion rate. However, this type of feed has a higher cost and lower storage convenience. As an alternative, the use of mash feed is economically advantageous but may result in a low intake rate during milking. Thus, adding water to mash feed can be a potential strategy to shorten feeding time during milking. This study aimed to evaluate the effect of adding water to mash feed on the intake of dairy cows managed in AMS. The study was conducted at *Embrapa Pecuária Sudeste*, São Carlos, SP, Brazil, during 60 consecutive days. Data from 69 dairy cows (3.99 ± 2.54 years; 149.3 ± 88.3 days in milk) were used. The animals had free access to an AMS (DeLaval VMSTTM V300), located (~ 160 m) around the pasture area. During milking, the system automatically supplied mash feed in the trough, individually adjusted based on milk production data. The experiment was divided into two periods; in the first period (30 d),

dry mash feed was supplied, while in the second period (30 d), the mash feed was offered with the addition of water. Individual intake was automatically recorded by the AMS system as the difference between the amount of feed offered and the amount consumed. As the data did not present normality, comparisons were performed using the Wilcoxon test ($p < 0.05$). Cows that consumed mash feed with water showed a higher percentage of intake proportion ($91.2 \pm 26.6\%$; $p < 0.001$) compared to the cows that consumed mash feed without water addition ($84.4 \pm 24.9\%$). In conclusion, adding water to the mash feed increased feed intake during milking in the automatic system.

3.3.10. Effects of Dietary Inclusion of Piper guineense Leaf and Seed on Gut Development and Organ (Liver and Kidney) Histology in Broiler Chickens

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Gut development and organ integrity are central to nutrient utilization, immune competence, and overall performance in broiler chickens. With restrictions on antibiotic growth promoters, phytogenic feed additives have emerged as natural strategies to enhance gut function while ensuring food safety. Piper guineense, a West African spice rich in alkaloids and essential oils, has demonstrated antimicrobial and growth-promoting potential, yet its effects on intestinal morphometry and organ histology in poultry remain poorly studied. This study was therefore conducted to evaluate the influence of dietary *P. guineense* leaf and seed (2:1 ratio) on intestinal morphometry and organ (liver and kidney) histology of broiler chickens. A total of 288-day-old broiler chicks were randomly assigned to four dietary treatments containing 0%, 0.5%, 1.0%, or 1.5% *P. guineense* and reared on deep litter for 6 weeks. At the end of the trial, two birds per replicate were humanely sacrificed. Intestinal segments (duodenum, jejunum, ileum, pancreas, colon, and caecum) were excised and their length weight were measured, while liver and kidney tissues were fixed in 10% buffered formalin, processed using standard paraffin embedding, sectioned at 5 μ m, stained with hematoxylin and eosin, and examined under light microscopy. Results revealed that birds fed 1.0% inclusion exhibited significantly ($p < 0.05$) longer colons and heavier colons and caecums, suggesting enhanced hindgut development and fermentation capacity, with no detectable alterations in liver and kidney tissues. Conversely, birds fed 1.5% inclusion showed reduced jejunal and ileal weights, increased duodenal length, and histopathological lesions in the liver and kidney, including hepatocellular degeneration and mild renal tubular damage. In conclusion, moderate inclusion (1.0%) of *P. guineense* supports beneficial gut development without compromising organ health, whereas excessive inclusion (1.5%) may impair small intestinal growth and induce metabolic organ damage. These findings highlight the dual efficacy and safety considerations of *P. guineense* as a phytogenic additive and underscore the importance of dose optimization in broiler nutrition.

3.3.11. Effects of Prepartum Dietary Protein-to-Energy Ratio on Postpartum Lactation Performance of Dairy Cows: A Meta-Analysis

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Introduction: Dietary protein and energy supplies are critical during the transition period of dairy cows. Although prepartum restricted energy has several benefits, supplies of protein and energy vary concurrently, and it remains unclear whether it is the effect of prepartum protein and energy supplies independently or it is the protein-to-energy ratio that matters. Therefore, the objective of this meta-analysis was to observe the effects of the prepartum dietary protein-to-energy ratio on the postpartum performance of dairy cows fed for prepartum restricted or ad libitum intake. **Methods:** Studies conducted on the manipulation of prepartum dietary protein or energy were included in the analysis. A total of 16 experiments were included with 47 dietary treatments. To distinguish linear trends from non-linear responses, a linear GLM model was used to assess the overall direction of the response, whereas a quadratic MIXED model was applied to test curvilinear responses while accounting for between-study heterogeneity. **Results:** The prepartum protein-to-energy ratio of the experiments ranged from 40.7 to 84.3 g/Mcal per kg of dry matter. Postpartum milk fat yield increased quadratically with an increase in the protein-to-energy ratio only in those studies where restricted energy intake was compared with ad libitum/high-energy intake ($p = 0.03$). Milk yield and postpartum feed intake remained unaffected ($p > 0.05$). However, milk yield tended to increase ($p = 0.09$), while the milk yield/kg of dry matter linearly increased with increasing prepartum metabolizable protein intake ($p = 0.02$). In prepartum ad libitum intake experiments, milk fat content and postpartum body condition score decreased with increasing prepartum metabolizable protein intake ($p < 0.05$). Milk yield increased linearly in those studies where prepartum feed was offered for ad libitum/high-energy intake but at the cost of increased body condition loss ($p < 0.01$). **Conclusions:** Overall, beyond their individual effects, the ratio of prepartum dietary protein to energy influences the postpartum performance of dairy cows. Nevertheless, feeding strategies (restricted vs. ad libitum) should be considered while balancing prepartum dietary protein and energy contents.

3.3.12. Effects of Steam-Flaked Corn on Rumen Microbial Composition and Metabolism in Yak

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This study aimed to investigate the effects of steam-flaked corn on growth performance, serum biochemical parameters, rumen parameters, microbial community composition, species function, and metabolism in yaks using multi-omics technologies. Fourteen healthy Zhongdian yaks aged 3–4 years with similar body weights were selected and randomly divided into two groups. They were fed total mixed rations containing either ground corn (GC) or steam-flaked corn (SFC), respectively. The trial lasted for 65 days, including a 10-day adaptation period. At the end of the trial, rumen fluid samples were collected for metagenomic and metabolomic analyses. The results show that the dominant bacterial phyla in the yak rumen were Bacteroidetes and Firmicutes, and the dominant genus was Prevotella. The abundance of Fibrobacter was significantly higher in the SFC group than in the GC group. Firmicutes bacterium CAG:103 was significantly positively correlated with acetate, propionate, and butyrate, Ruminobacter sp. RM87 and Ruminobacter amylophilus

were significantly positively correlated with propionate, and *Butyrivibrio hungatei* was significantly positively correlated with isobutyrate and isovalerate. CAZy database annotation revealed that the abundance of GH was significantly higher in the SFC group than in the GC group, while the abundances of CBM and CE were extremely significantly higher in the SFC group. *Prevotella* sp. CAG:1124 and *Ruminococcaceae* bacterium YAD3003 were significantly positively correlated with ko00500. *Succinimonas amylolytica* and *Tolomonas lignilytica* were significantly positively correlated with ko00540 ($p < 0.05$). The rumen of yaks in the SFC group was characterized by the accumulation of purine degradation products (e.g., 3-methyladenine) and neuroactive substances (e.g., tryptamine), whereas the GC group was enriched with various bacterial fermentation products and signaling molecules, including indole-3-carboxylic acid, and showed significant enrichment in the sphingolipid metabolism pathway. *Succinivibrionaceae* bacterium WG-1 was significantly positively correlated with indole-3-carboxylic acid. Steam-flaked corn altered the structure and metabolism of the rumen microbial community in yaks.

3.3.13. Evaluation of Body Condition Score in Ewe Lambs Under Different Environmental Confinement Conditions

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The growing demand for sheep meat has encouraged the adoption of confinement systems, which allow greater environmental and nutritional control, improving animal productive performance. This study aimed to evaluate different confinement structures during the winter on the body condition score (BCS) of hair ewe lambs. The experiment was carried out at the Sheep Production Sector, UFVJM, Diamantina-MG, using 12 Dorper × Santa Inês crossbred ewe lambs, with an average body weight of 40 kg and aged 12 to 24 months, distributed in a completely randomized design with two treatments: closed confinement (1.5 m walls and metal roof) and open confinement (wire fence and shade cloth cover). The animals received a diet formulated for an average daily gain of 0.100 kg/day, offered twice daily at 7 a.m. and 3 p.m. The BCS assessments and weighings were performed after 16 h of fasting on 25 July, 8 August, 22 August, and 12 September 2025 totaling 51 days of evaluation. On the same dates, environmental temperature and wind speed were recorded at 7 a.m., 10 a.m., 1 p.m., and 4 p.m. using a thermometer and anemometer. Temperature and wind speed averaged 12.3–22.5 °C and 0.1–0.9 m/s in the closed confinement and 14.4–25.3 °C and 1.6–6.5 m/s in the open one. Rectal temperatures, measured on weighing days, remained within the thermoneutral range, averaging 39.43 °C, with no difference between confinements ($p < 0.05$). The type of confinement did not influence BCS ($p < 0.05$), but there was a significant effect of time ($p < 0.001$), increasing from 3.29 to 3.93 throughout the experimental period. BCS values ≥ 3.0 before mating are recommended for better reproductive performance. It is concluded that both systems were effective in maintaining welfare and improving ewe body condition during winter.

3.3.14. Growth Performance and Visceral Histopathological Assessment of Broiler Chickens Fed Processed Senna Seed (*Senna occidentalis* (L.)) Meal-Based Diets

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Senna occidentalis (L.) seed is a nutrient dense plant protein ingredient for poultry diets. However, its utilisation is constrained by the presence of anti-nutritional factors which may

elicit metabolic disorder and poor performance in birds. Processing techniques have been shown to alleviate these limitations. This study therefore evaluated the effects of *Senna occidentalis* seed meal-based diets on growth performance and liver-jejunal histopathology of broiler chickens. One hundred and sixty 17-day old Ross 308 broiler chicks were randomly allotted into four dietary treatments with five replicates of eight birds each in a completely randomised design. Diet 1 was a highly digestible protein (HDP); a control and diets 2, 3 and 4 contained Raw Senna Seed Meal (RSSM), Fermented Senna Seed Meal (FSSM), and Boiled Senna Seed Meal (BSSM) supplemented diets. Performance indices were measured. On day 21, liver and jejunal samples were harvested and examined for histopathological evaluations following standard procedures. Data were analysed using ANOVA at $p = \alpha 0.05$. Birds fed BSSM diet recorded the highest ($p < 0.05$) average daily weight gain (34.80 g/b) and average daily feed intake (58.19 g/b) compared with the other diets. Liver of birds fed BSSM diet showed normal architecture with intact hepatocytes, with no observable necrosis while birds fed RSSM diet exhibited periportal fibrosis, sinusoidal dilation, and inflammatory infiltration, indicating hepatic stress. Jejunal sections of the birds fed the raw and processed SSM displayed intact villi and mild crypt hyperplasia without vascular changes, fibrosis or necrosis. In conclusion, boiling improved the nutritional quality of *Senna occidentalis* seed meal which resulted in enhanced growth performance, and preserved hepatic and intestinal integrity. Therefore, the inclusion of 10% processed *Senna occidentalis* seed meal is considered as a viable and promising alternative protein source for broiler chickens.

3.3.15. Growth Response and Carcass Quality of Broiler Chickens Fed Graded Levels of Sesame (*Sesamum indicum*) Meal Supplemented with a Multi-Enzyme

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The poultry industry seeks innovative strategies to mitigate rising feed costs and competition for conventional feedstuffs towards achieving sustainable livestock. This study evaluated the impact of graded levels of sesame meal (SM), supplemented with a multi-enzyme complex, as a sustainable partial replacement for highly priced conventional feed ingredients on the growth response and carcass characteristics of broiler chickens. A total of 210 Arbor Acre broiler chicks were randomly allotted to seven dietary treatments in a completely randomized design, comprising 0–25% SM inclusion with enzyme supplementation, which lasted for 8 weeks. The growth response showed that higher SM inclusion levels ($>10\%$) significantly ($p < 0.05$) depressed the body weight gain, average daily gain, and feed intake, while FCR increased. However, birds fed up to 10% SM inclusion exhibited comparable growth performance to the control, with an FCR of 1.96–1.97. Carcass evaluation indicated that increased SM inclusion significantly reduced abdominal fat (1.00–0.31%), thereby suggesting SM's potential anti-lipogenic effect. Additionally, breast yield declined with higher inclusions (e.g., 31.50% at 20% SM vs. 35.37% at 15% SM). The study concluded that SM supplemented with a multi-enzyme can successfully replace up to 10% of conventional feed ingredients in broiler diets without compromising growth or carcass quality. However, higher inclusion levels require an increased enzyme dosage to reduce anti-nutritional effects and support sustainable and cost-effective poultry feeding.

3.3.16. Growth Response and Haematological Indices of Noiler Birds Fed Varying Dietary Levels of Sodium Benzoate

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There have been consequential side effects from the use of antibiotics growth promoters in poultry, such as drug resistance and residual effects on consumers. This has led to international restriction on the use of antibiotics, putting pressure on the poultry industry to search for suitable natural alternatives that have similar beneficial effects (Braconi et al., 2021). This trial investigated the growth response and haematology of Noiler birds fed dietary additive levels of sodium benzoate (SB). One hundred and twenty (120)-day-old Noiler chicks were used for the 8-week trial. Randomized into four treatments with three replicates of ten (10) birds each, commercial diet (HYBRID™) was used with the inclusion levels of SB at 0 g, 200 g, 400 g, and 600 g per 100 kg of feed, representing T1, T2, T3, and T4, respectively. Data collected on final body weight (FBW), total weight gain (TWG), average daily weight gain (ADWG), dressed carcass weight (DCW), total feed intake (TFI), feed cost/kg gain (FC/kg gain), packed cell volume (PCV), red blood cell (RBC), and haemoglobin (HB) were subjected to a One-Way Analysis of Variance (ANOVA) and significantly different means were separated using Duncan's New Multiple Range Test at a 5% level of probability. FBW, TWG, ADWG, DCW, TFI, and FC/kg gain were significantly ($p < 0.05$) better in T4 (1356.87 ± 0.57 g, 1321.82 ± 0.41 g, 23.60 ± 0.39 , 1061.75 ± 1.50 , 7292.48 ± 1.21 and 1366.48) than in T1 (1198.87 ± 0.80 , 1162.63 ± 0.91 , 20.76 ± 0.21 , 1011.11 ± 1.17 , 7103.28 ± 1.22 and 1512.8), respectively. Treatment 4 significantly ($p < 0.05$) improved the haematological parameters, PCV, RBC, and HB ($35.67 \pm 0.21\%$, $11.27 \pm 0.33 \times 10^6$ and 9.46 ± 0.05 g/dL) compared to T1 ($33.33 \pm 0.42\%$, $10.42 \pm 0.19 \times 10^6$, and 8.40 ± 0.07 g/dL, respectively). The study revealed that at all the levels of inclusion of SB in the experimental diets, there was no recorded mortality, indicating zero toxicity for the birds. It was concluded that incorporating 600 g SB/100 kg feed in Noiler diets was a more economical approach to feeding the birds, produced healthier birds, and more effectively improved growth performance.

3.3.17. In Ovo Administration of Oyster Mushroom (*Pleurotus ostreatus*) Extract: A Sustainable Nutritional Strategy for Improved Humoral Immunity in Broiler Chickens

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Broiler chickens, because of the intensive production systems in which they are bred and their high metabolic demands, are generally vulnerable to infectious diseases and thus, immune support is an essential element of sustainable poultry production. Traditional prophylactic methods using antibiotics and synthetic immune boosters raise concerns regarding antimicrobial resistance and environmental impact. Consequently, plant-based natural immunomodulators represent a considerably safer and more environmentally sustainable option. Thus, the oyster mushroom (*Pleurotus ostreatus*)—a mushroom widely attested for its bioactive compounds with immunostimulatory properties—was thus evaluated for its ability to enhance the humoral immunity of broilers through in ovo administration. A total of 300 hatching eggs were incubated, out of which 165 were fertile (55% fertility rate). On the 18th day of incubation, the fertile eggs were divided into three groups for injection (55 eggs each): a control (without injection), 0.1 mL/egg extract, and 0.2 mL/egg extract. The hatched chicks were reared for 8 weeks under a deep litter system and were given feed and water ad libitum. The birds' immune status was evaluated by immunization with sheep red blood cells (SRBC) on day 29 post-hatch, and the antibody titres were measured by the haemagglutination assay. Results showed that broilers from eggs injected with 0.2 mL extract were found to have a significantly higher ($p < 0.05$) humoral immune response

(mean titre of 5.96) than those of the control and 0.1 mL groups. In summary, in ovo injection of not more than 0.2 mL of water extract of *Pleurotus ostreatus* can be a method to activate the humoral immune system of broiler chickens and, therefore, can be regarded as a safe and effective nutritional approach for the contemporary poultry sector.

3.3.18. In Vitro Evaluation of Digestibility and Methane Gas Production of *Urochloa brizantha* and *Inga edulis* as Forage Alternatives for Tropical Livestock Systems

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This study evaluated the potential of tropical forage species to improve nutrient use efficiency and reduce enteric methane emissions using the in vitro gas production technique. The objective was to assess digestibility and methane (CH₄) production of *Brachiaria brizantha* (Hochst. ex A. Rich.) Stapf and *Inga edulis* Mart., evaluated individually and in mixtures, as a strategy to enhance the sustainability of tropical livestock systems. Forages were incubated in vitro for 48 h, either alone or combined, and total gas production, dry matter degradation (DMD), and methane production were measured. Data were analyzed using a completely randomized block design. *I. edulis* showed a substantially higher crude protein content than *U. brizantha* (18.16 vs. 6.31%), while neutral detergent fiber (NDF) was also higher in *I. edulis* (78.88 vs. 67.28%). After 48 h of incubation, *U. brizantha* and the mixture containing 15% *I. edulis* produced the highest total gas volumes (179.33 and 175.04 mL/g DM, respectively), followed by the mixture with 30% inclusion (153.83 mL/g DM). In contrast, pure *I. edulis* showed the lowest gas production (31.46 mL/g DM). Significant differences in DMD were observed among treatments. At 24 h, *U. brizantha* showed the highest degradation (50.39%), followed by mixtures with 15% (49.10%) and 30% (44.88%) *I. edulis*, while the legume alone had the lowest value (14.03%). A similar pattern was observed at 48 h. Methane production at 24 h was highest in *U. brizantha* (5.45 mL/g DM), followed by mixtures with 15% (5.21 mL/g DM) and 30% (4.26 mL/g DM) *I. edulis*. Pure *I. edulis* produced negligible methane (0.03 mL/g DM). These results indicate that moderate inclusion (15%) of *I. edulis* maintains fermentative activity similar to that of the grass alone, while higher inclusion levels reduce gas production, dry matter degradation, and methane emissions.

3.3.19. Metabolic Biomarkers in Heifers Fed with r-Zeta® Additive in Their Diets

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The aim of this work was to evaluate different rearing diet strategies with r-zeta® additive in heifers (192.6 ± 19.0 kg body weight, (BW)). The additive formulation includes flavorings that regulate intake, enzymes, microorganisms that modulate the gut microbiota, liver protectants, and anti-inflammatories. The treatments were HR + CS (hay roll (HR) and control supplementation at 1% BW—80% whole corn grain and 20% pelleted protein concentrate); HR + Sr-zeta® (HR and balanced pellet r-zeta® supplementation at 1% BW); HR + SFr-zeta® (HR and self-feeding balanced pellet r-zeta®) and SFr-zeta® (self-feeding balanced pellet r-zeta®). In treatments with the additive and self-feeding, food was offered ad libitum from the first day. Whole-blood samples were collected on days 0, 28, 56 and 83 to evaluate metabolic biomarkers (lactate dehydrogenase (LDH), uremia and glycemia).

Continuous variables were analyzed using repeated measures ($\alpha = 0.05$). All treatments showed similar values of LDH (1200 to 1400 mU/mL; $p = 0.0713$). The different feeding alternatives affected uremia ($p = 0.0143$). The animals fed SFr-zeta[®] had higher values on day 28 compared to supplemented groups. Furthermore, both self-feeding groups showed higher values on day 56 in comparison to the supplemented groups. In relation to the reference values (20 to 40 mg/dL), the supplemented animals showed even lower values, which could be considered an insufficient supply of nitrogen compounds in the diet. The self-feeding groups showed normal urea levels throughout the study (30 to 40 mg/dL). A lower value was observed in the blood glucose levels in HR + CS treatment on day 28 compared to the HR + SFr-zeta[®] group on day 56 ($p = 0.0174$). For the remaining sampling times, all groups showed similar means. Only the HR + CS group had the range established by other authors (60 to 80 mg/dL), while all groups fed with r-zeta[®] were above the reference values. The results demonstrate that there was no negative effect on the metabolic variables studied when the additive was included in self-feeding diets without adaptation protocols.

3.3.20. Phytochemical Properties of Processed Senna Seed (*Senna occidentalis* L.) and Its Influence on Serum Biochemical Indices in Broiler Chickens

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Senna occidentalis (L.) is a tropical legume with promising nutritional value for poultry feeding. However, its utilisation in broiler diets is limited by the presence of anti-nutritional factors that impair overall performance. Processing techniques have been reported to mitigate these constraints and enhance its nutritional quality. This study evaluates the phytochemical properties of processed *Senna occidentalis* seed and its effect on the serum biochemical profile of broiler chickens. One hundred and sixty (160) 17-day old Ross 308 broiler chicks were randomly assigned to four dietary treatments with five replicates of eight birds each in a completely randomised design. Diet 1 was a highly digestible protein (HDP), diets 2, 3 and 4 contained 10% Raw Senna Seed Meal (RSSM), 10% Fermented (FSSM), and 10% Boiled (BSSM) supplemented diets. Phytochemical screening of the processed seed meals was determined using standard procedures. On day 21, 2 mL of blood samples were collected from three birds per treatment for serum biochemical assay. Data were analysed using ANOVA at $p = \alpha 0.05$. Flavonoids and alkaloids were abundantly present (++ve) across treatments. Saponins and tannins were more pronounced (++ve) in FSSM and RSSM while anthraquinones were abundantly present (++ve) in BSSM. Alkaloids content was highest in the RSSM (2.70%) and BSSM (2.40%), while FSSM had increased flavonoids (2.23%) and terpenoids (2.25%) contents. BSSM showed moderate levels of phytochemicals across compounds. Serum biochemical indices revealed elevated total protein (3.77 g/dL) and albumin (1.03 g/dL) in birds fed BSSM diet, while birds fed RSSM (3.83 mg/dL) and FSSM (3.43 mg/dL) diets had higher urea levels. Other parameters showed no significant differences. In conclusion, fermentation and boiling enhanced the nutritional value of *Senna occidentalis* seed meal and improved the serum biochemical profile of broiler chickens, suggesting its potential as a safe and effective alternative protein ingredient in broiler diets.

3.3.21. Postbiotic Supplementation Enhances Growth and Feed Efficiency in Sahiwal Heifers: A Sustainable Alternative to Antibiotics

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The latest generation of functional feed additives, known as postbiotics, provides stable and bioactive metabolites that improve cattle gut health and nutrient absorption. This study investigated the effects of the postbiotic-based feed supplement extra pure metabolites (XPM), which is generated from the fermentation of *Saccharomyces cerevisiae*, on digestibility and growth performance in native Sahiwal heifers grown in tropical environments. Twelve heifers of the same age and weight were split into two groups; one group was given a conventional basal diet (control), which consisted of green fodder (sorghum + wheat straw), while the other group received XPM supplements for ninety days. Feed intake, body condition score, average daily gain (ADG), and nutritional digestibility (DM, CP, and NDF) were among the parameters assessed. The results showed that XPM supplementation increased the digestibility of dry matter and crude protein and considerably ($p < 0.05$) improved ADG and feed conversion efficiency. In comparison to controls, treated heifers also showed improved rumen stability and greater physical health. Yeast-derived postbiotics optimize rumen microbial activity, reduce gut pathogens, and promote positive fermentation pathways, all of which contribute to improved nutrition utilization and metabolic efficiency. The study shows that XPM supplementation can enhance animal health and production efficiency without the need for antibiotics, providing a sustainable and safe method of feeding dairy cattle. The use of postbiotics like XPM promotes increased productivity in tropical dairy production and is consistent with the global movement toward livestock systems free of antibiotics.

3.3.22. Sawdust Utilization in WAD Goat Diets: A Comparative Analysis of Wood Ash, Hot Water, Caustic Soda, and Molasses Pre-Treatments

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This study investigated the potential of using differently treated sawdust-based diets to sustainably enhance livestock production, specifically focusing on West African Dwarf (WAD) goats in Nigeria. Thirty-six (36) intact male West African Dwarf (WAD) goats, with an initial mean body weight range of 9–10 kg, were utilized in an 8-week feeding trial. The experimental design was a Completely Randomized Design (CRD). The animals were randomly allocated to the four dietary treatment groups such that nine (9) goats served as replicates per treatment group. Sawdust of fine-milled *Gmelina arborea* was harvested from the nearby sawmill, picked to remove dirt, and treated using wood ash (T1)—10% w/v , hot water (T2)—100 °C for 2 h, caustic soda (T3)—4% w/v , and molasses (T4)—1:9 w/w of molasses/sawdust before being mixed with other feed ingredients. The feed was offered at 4% of the body weight of the animals. Data were collected on crude protein content, dry matter intake (DMI) (g/day), volatile fatty acids (VFAs), packed cell volume (PCV), red blood cells (RBCs), CO₂, and total volatile organic compounds. The results showed significant differences in the chemical composition of the diets. Molasses-treated sawdust diet (T4) had the highest crude protein content (5.69%), highest concentrations of VFAs, and lowest TVOC emissions (0.40 mg/m³), showing its potential as a high-quality feed option with lower environmental impact. In contrast, caustic soda-treated sawdust (T3) had the highest TVOC levels (0.52 mg/m³). Carbon dioxide emissions were highest in T4 (4996.67 ppm) and lowest in T1 (3911.50 ppm). Results demonstrated that pre-treatment significantly altered the sawdust composition and animal performance. All treated diets

had a positive effect on haematological parameters. This study shows the feasibility of incorporating treated sawdust-based diets into livestock management practices to ensure the suitability of the diets and the high productivity of West African Dwarf goats.

3.3.23. The Effect of Using Garlic (*Allium sativum* L.) on Intestinal and Liver Morphology in Broilers

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In recent years, feed additives have been widely used for their positive effects of improving health status, productive performance, and carcass quality in both livestock and poultry. Numerous studies have highlighted the fact that a series of medicinal plants, in the form of powder, extracts, or essential oils, both as single compounds and mixed preparations, can be introduced into the feed of broiler chickens. They exhibit beneficial effects on both the intestinal microflora and digestibility of nutrients, aspects that are reflected in the quality of meat and eggs. Among them, garlic occupies a special place due to its broad spectrum of action and its availability for poultry farmers. The present study aims to evaluate the effects of supplementing the diet of broilers with garlic powder (*Allium sativum*) on their growth performance and the morphology of their intestine and liver. Twenty-four one-day-old ROS 308 male hybrids were randomly assigned to two groups, each group with two replications, of six individuals: the control group (LC), which was fed with the basic diet, and the experimental group (LGa), in whose diet 1% garlic powder was introduced. Food and water were provided ad libitum until the end of the experiment (42 days). The obtained results show that supplementing the basic diet with 1% garlic powder in broilers had a significant effect on body weight, feed intake, and FCR ($p < 0.05$), as well as on the studied histomorphometric parameters: the height, width, area, perimeter of the intestinal villi ($p < 0.01$), and depth of the crypts ($p < 0.05$). In conclusion, supplementing the basic diet of broiler chickens with 1% garlic powder, from 1 to 42 days, improves growth performance and increases the absorptive surface of the intestinal villi, as well as implicitly increasing the absorption capacity of nutrients; these aspects prove that garlic can be considered a growth promoter.

3.4. Animal Genetics and Genomics

3.4.1. Adaptive Genomics and Conservation Objectives: Balancing Hybrid Vigor and Climate Resilience in Pakistan's Yak Population

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The yak (*Bos grunniens*) is not only an animal; it is the biological foundation sustaining life for thousands of pastoral families across the high-altitude Karakoram–Himalayan region of Northern Pakistan (Gilgit-Baltistan and Chitral). At altitudes of up to 4500 m, this species and its vital first-generation cross with cattle known locally as Zo or Zomo provide the only reliable sources of milk, meat, fiber, and transport. This system supports

a significant population of approximately 25,000 pure yak and over 100,000 crossbreds, representing immense socioeconomic value. However, this critical livestock system is facing a profound genetic crisis. Local herders rely heavily on the Zo/Zomo hybrid for its superior strength and productivity at mid-altitudes (known as hybrid vigor). This practice, while beneficial for immediate production, unintentionally introduces cattle genes into the pure yak population (genetic dilution). This threatens to erase the yak's unique, millennia-old genetic adaptations to extreme cold and oxygen deprivation, jeopardizing its role as a climate-resilient resource for future generations. To secure this irreplaceable heritage, we propose a strategic overhaul of conservation breeding, driven by population genetics. Our approach is two-fold. (a) Genomic Conservation: We will use high-density SNP genotyping to measure the pure yak population's genetic worth with unprecedented precision. The approach permits us to monitor genetic diversity, estimate the effective breeding population size (N_e), and establish secure germplasm repositories to protect its irreplaceable adaptive genes. (b) Hybrid Optimization: We will integrate genomic selection into the crossbred population for a systematic improvement in economic performance while strictly controlling and minimizing the harmful influx of cattle DNA into the pure yak genome. By embracing this modern, genomics-based strategy, we aim to maximize the economic benefits of productive hybrids while guaranteeing the long-term genetic integrity and survival of the pure indigenous yak, ensuring the resilience of high-altitude livestock farming against climate change.

3.4.2. An Integrated SNP Genotyping Assay for Genomic Monitoring of the Koala (*Phascolarctos cinereus*)

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The koala (*Phascolarctos cinereus*), an iconic Australian marsupial, continues to experience severe population declines due to habitat loss, disease, and climate change, and is now listed as *Endangered* across eastern Australia. Effective conservation of koalas requires a standardised genomic tool to ensure data is directly comparable over time and space, strengthening conservation efforts. We therefore developed an integrated single nucleotide polymorphism (SNP) genotyping assay, incorporating ~5000 informative loci derived from published genomic datasets, and novel SNPs identified from candidate fitness-related genes and major koala pathogen genomes/genes. This unified assay incorporates both host and pathogen markers, enabling comprehensive genomic monitoring through a single, streamlined workflow. The assay, which was validated using over 1000 koalas collected across the species' distribution, demonstrated high genotyping success and reproducibility across sample types including blood, tissue, urogenital swabs, hair, scat, and archived DNA. It supports multiple applications such as assessing population structure and connectivity, provenance, relatedness estimation, sex determination, and screening for pathogens including koala retrovirus and papillomavirus. The assay provides a robust, cost-effective framework for both fine- and broad-scale management by standardising data generation and comparison across studies and regions. By integrating host and pathogen genomics, this multi-purpose approach improves understanding of koala biology and strengthens

evidence-based decision-making in conservation programs. The platform establishes a scalable model for wildlife genomics, enabling efficient, evidence-based management of threatened species.

3.4.3. Association of the Inbreeding Coefficient on Milk, Protein, and Fat Production in the First Lactation of Holstein Cows

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Inbreeding increases homozygosity, favoring the expression of deleterious recessive alleles and resulting in the phenomenon of inbreeding depression, which can compromise the productive and reproductive performance of animals. The objective of this study was to estimate the inbreeding coefficients of Holstein cows from genealogical records and evaluate their association with the 305 d milk yield (MY305), 305 d fat yield (FY305), and 305 d protein yield (PY305) of the first lactation. The data analyzed belong to an agricultural company in the southeastern region of Brazil. The pedigree file contained information on 7561 animals born between 1911 and 2023, of which 763 founders were identified, in addition to 790 bulls and 4953 cows with progeny, covering 16 generations. Within this relationship matrix, 5307 animals are inbred, with an average inbreeding coefficient (F) of 2.26% and a maximum of 28%. The F value of each animal was used as a linear covariate in the statistical model, including the contemporary group effect (year and month of birth), age class at calving, and additive genetic and residual random effects. In this analysis, the first lactations of 2228 cows were used, with averages of $10,383 \pm 1291$ kg, 369 ± 87 kg, and 312 ± 63 kg for MY305, FY305, and PY305, respectively. The estimated heritabilities for MY305, FY305, and PY305 were 0.17 ± 0.05 , 0.19 ± 0.04 , and 0.12 ± 0.01 , respectively. The F effect prediction solution described a decrease in MY305, FY305, and PY305 of 33.96, 95.81, and 21.6 kg for each 1% increase in F. These results show the importance of monitoring inbreeding and directing animal mating, given that inbreeding depression leads to economic losses.

3.4.4. Carbonic Anhydrase Polymorphism Reveals Striking Genetic Diversity and Strong Selection Signatures in Nigerian Indigenous Cattle Breeds

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Introduction: Cattle production underpins Nigeria's agricultural economy and rural livelihoods, with over 20 million indigenous animals adapted to harsh environments. However, genetic erosion from cross-breeding threatens these resources. Blood protein polymorphisms like carbonic anhydrase (CA) offer a cost-effective marker for diversity assessment, revealing adaptation and selection patterns. This study characterised CA polymorphism in four key Nigerian breeds—White Fulani, Red Bororo, Sokoto Gudali, and Kuri—to inform conservation and breeding strategies. **Methods:** Blood samples (5 mL) were collected from 351 healthy cattle (121 White Fulani, 97 Red Bororo, 100 Sokoto Gudali, 33 Kuri) across southwestern Nigeria. Erythrocytes were lysed and analysed via cellulose acetate electrophoresis (pH 5.6, 200 V, 45 min). Allele/genotype frequencies were calculated

by direct counting, and the Hardy–Weinberg equilibrium (HWE) was tested via χ^2 and G^2 . Diversity metrics (Shannon's I and Nei's heterozygosity), F-statistics, genetic distances (Nei's test), and neutrality (Ewens–Watterson test) were computed using POPGENE 1.32. **Results:** Two co-dominant alleles (A, B) and three genotypes (AA, AB, BB) were detected. Allele B predominated in White Fulani (0.926) and overall (0.667), while A was highest in Kuri (0.697). Only White Fulani met HWE ($P = 0.057$); others showed severe heterozygote deficits (e.g., Sokoto Gudali observed AB = 1 vs. expected = 50; $p < 0.001$). Diversity was highest in Sokoto Gudali ($n_e = 1.990$, $I = 0.691$, $Nei = 0.498$) and lowest in White Fulani ($n_e = 1.220$, $I = 0.325$, $Nei = 0.180$). $F_{ST} = 0.191$ indicated moderate differentiation; Red Bororo and Sokoto Gudali clustered closely ($I = 0.982$). The Ewens–Watterson test suggested selection occurred in all three breeds considered in this study. **Conclusions:** CA polymorphism highlights breed-specific diversity and selection pressures in Nigerian cattle, underscoring the need for targeted conservation of unique lineages like Kuri. These findings support sustainable genomics-informed breeding for climate-resilient livestock.

3.4.5. Deep Learning-Enabled Detection of Regulatory Variants from Single-Cell Chromatin

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Although genomic prediction has improved markedly in cattle, buffalo, sheep, and poultry, a substantial proportion of additive genetic variance remains unexplained, prompting continued debate on where “hidden heritability” resides. Since many livestock traits appear to be shaped by myriad regulatory variants with context-dependent effects, one might reasonably consider whether bulk-tissue datasets are masking cell-type-specific regulatory architecture. As recent breakthroughs in single-cell chromatin profiling illustrate, regulatory activity varies sharply across epithelial, immune, and stromal compartments, yet livestock breeding programs have scarcely incorporated this information.

This study employed single-cell ATAC-seq datasets newly available for bovine and ovine tissues to map cell-resolved open-chromatin peaks. Variants overlapping these peaks were then interpreted using a hybrid convolutional transformer deep learning model inspired by Enformer, enabling prediction of enhancer disruption and directionality of regulatory effects. Because deep models can integrate long-range chromatin interactions, they provide an opportunity to observe regulatory dependencies that conventional annotations often overlook. In comparison to baseline SNP models, cell-type-specific regulatory scores increased prediction accuracy across growth, mastitis resistance, and fertility traits by 10–18%. Immune-cell-specific regulatory polymorphisms expressed disproportionately significant effects on parasite-resilience traits and mastitis, supporting previous multi-tissue eQTL studies. Despite the limited tissue diversity and sample number, these advancements may represent the early promise of single-cell functional genomics.

The combination of deep learning and single-cell chromatin landscapes does, however, seem a rational and physiologically sound way to deal with latent heredity in cattle. This approach prioritizes variants with actual regulatory relevance, strengthening genomic selection processes and mechanistic interpretation.

3.4.6. Gene-Content Multiple-Trait REML Improves the Reliability of Genetic Evaluation for Milk Production Traits in Spanish Palmera Goats

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Genetic evaluations of milk production traits in dairy goats typically do not explicitly account for variability in major genes such as the polymorphisms at *CSN1S1* (α S1-casein), despite its substantial impact on milk protein synthesis. This study assessed the effect of incorporating *CSN1S1* genotypes on the reliability of genetic evaluations in the Palmera goat breed. The dataset comprised 37,875 test-day records from 3168 goats, 4342 pedigree animals, and the *CSN1S1* locus genotypes for 156 bucks, being sires of 1069 phenotyped animals. Traits analyzed included milk yield (MY), and protein (PC) and fat contents (FC). A gene-content multi-trait REML model (GCMT-REML), treating gene content at the *CSN1S1* locus as a correlated additional trait, was implemented and compared against a baseline univariate animal model. Heritability estimates were similar between models, ranging from 0.19 (FC) to 0.31 (PC). The incorporation of gene content information increased reliability across traits, with overall gains from 0.07% (PC) to 1.82% (MY). Improvements were markedly higher in males than females, and in genotyped (5.52% to 9.54%) compared to non-genotyped animals ($\approx 0\%$). Individuals with low baseline reliability benefited the most, showing gains from 7.2% to 14.27%. These results demonstrate that including *CSN1S1* genotypes in routine genetic evaluations can enhance reliability in the Palmera breed. Future work should focus on expanding the genotyped population, including females, so that dominance effects in the locus could also be accounted for.

3.4.7. Genetic Diversity and Population Structure Analysis of Goat Breeds of Pakistan

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Goats are among the earliest domesticated livestock species, known for their adaptability to diverse climates. Pakistan, with thirty-five indigenous goat breeds and the world's third-largest goat population, requires genomic characterization to support effective conservation and selection programs. In this study, the data for 827 individuals representing seven Punjab breeds (Beetal, Barbari, Daira Din Panah, Nachi, Pahari, Pothwari, and Teddi) genotyped using the GoatSNP50 Illumina chip were retrieved. After stringent quality control (call rate $\geq 90\%$, MAF $\geq 5\%$, HWE $P < 0.0001$), 38,890 autosomal SNPs were retained for further analysis. Genetic diversity and relationships among breeds were investigated using principal component analysis (PCA) and linkage disequilibrium (LD) decay profiles with PLINK v1.9.0 and PopLDdecay, respectively. Population structure was evaluated with ADMIXTURE v1.3.0 ($K = 2-7$), selecting the model with the lowest cross-validation error. Allele-based breed-specific markers were identified using a pipeline already established by our group to support breed conservation programs. PCA revealed discrete clustering of breeds based on genetic structure, with Teddi being most distinct breed. Other breeds, including Barbari, Beetal, Daira Din Panah, Nachi, Pahari, and Pothwari, showed partial overlap but remained generally distinguishable. LD decay analysis (using SNP correlation, r^2 , over 100 kb) indicated that Barbari, Daira Din Panah, and Nachi were more uniform as they maintained higher LD at longer distances; however, Teddi and Beetal exhibited faster

LD decay, reflecting greater genetic diversity. ADMIXTURE analysis revealed the higher allelic sharing and extensive crossbreeding among breeds, precluding the identification of any breed-specific markers. In conclusion, this study demonstrates genetic stratification and diversity among goat breeds from the Punjab region of Pakistan, but exhibits considerable allelic sharing and gene flow, likely due to crossbreeding. Future studies should target pure-bred animals with higher-density SNP panels or whole-genome resequencing for better understanding of population structure and to validate breed-specific markers.

3.4.8. Genetic Polymorphism in the Exon 5 of the Prolactin Gene on Two Duck Breeds Adapted to Nigeria

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Purpose: This study aimed at determining genetic variants of prolactin gene in the Muscovy and Mallard ducks in Nigeria, and to evaluate the genetic relatedness or diversity of the duck species at the exon 5 of Prolactin (PRL) gene loci in the two selected Duck breeds, by determining the genetic polymorphisms, genetic parameters and genetic variation in the duck breeds. 80 adult ducks sourced from Lagos, Ogun and Oyo states of Nigeria, were used for this study. **Method:** Blood samples (2 mL) collected by jugular venipuncture from the ducks, using sterile needles and syringes, were stored in ethylene diamine tetra acetic acid (EDTA) bottles, to prevent coagulation and preserve the integrity of the samples, were transported in ice packs, to the laboratory for analysis. Genomic DNA was extracted from whole blood samples, amplified using PCR were digested using *HinfI* Restriction Enzyme. The data were analyzed using POPGENE 1.32 software. **Results:** Two alleles with frequencies A (0.1034, 0.2188), B (0.8966, 0.7812), and three genotypes with frequencies AA (0.0000, 1.0000), AB (6.0000, 5.0000) and BB (23.000, 10.0000) were identified at the Exon 5 of PRL gene in Mallard and Muscovy ducks respectively. Indicating that prolactin gene was polymorphic in both populations. The Chi-square test (0.367, 0.2381) suggests that the populations conforms to Hardy-Weinberg equilibrium. Also, noted were observed heterozygosity (0.2069, 0.3125) and expected heterozygosity (0.1887, 0.3528). **Conclusion:** The sampled populations were polymorphic for PRL gene at exon 5, and are also in Hardy-Weinberg equilibrium, based on the observed parameters and statistical analysis. The values of observed heterozygosity and expected heterozygosity, indicates higher genetic variation within the Mallard breed population, compared to the Muscovy population, which can be attributed to inbreeding. This indicates the need for Genetic intervention to prevent this indigenous breed of ducks from extinction.

3.4.9. Genomic Approaches for Enhancing Disease Resistance and Climate Adaptation in Livestock

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Climate change poses increasing challenges to livestock productivity and health, particularly in tropical and subtropical regions. Genomic technologies have emerged as powerful tools to enhance both disease resistance and environmental adaptability in animals. Several studies have demonstrated that integrating genomic selection, genome-wide association studies (GWAS), and functional genomics can accelerate breeding for resilience traits (Boettcher et al., 2015; Knap & Doeschl-Wilson, 2020). This review synthesizes evidence

from seven recent studies addressing genomic adaptation and disease resistance in livestock species, including cattle, sheep, goats, and pigs. The selected works applied methodologies such as GWAS, SNP mapping, transcriptomic profiling, and selection signature analysis (Boettcher et al., 2015; Genomic Responses to Climatic Challenges in Beef Cattle, 2024; BMC Genomics, 2024; Genome Biol. Evol., 2020). These approaches were evaluated in the context of climate-related stressors such as heat tolerance, pathogen pressure, and environmental variability. Across species, genomic regions associated with heat stress tolerance and immune response have been consistently identified. For instance, heat-adapted cattle populations show selection signatures in genes such as TRPM8, HSP70, and SOD2 (Genomic Responses to Climatic Challenges in Beef Cattle, 2024). In goats, climate adaptation was linked to immune-related loci detected through landscape genomics (BMC Genomics, 2024). Likewise, studies in pigs and sheep revealed that resilience to polymicrobial diseases and weather variation is heritable and can be enhanced through genomic selection (Knap & Doeschl-Wilson, 2020; BMC Genom. Data, 2020). Collectively, these findings emphasize that genomic tools can identify adaptive variants and accelerate breeding for combined resilience to disease and climate stress. Genomic-based breeding therefore represents a sustainable strategy to improve livestock productivity and welfare under global climate change. Future directions should include the integration of multi-omics datasets and large-scale genomic databases to strengthen precision livestock adaptation strategies.

3.4.10. Genomic Evaluation of Productive, Health, and Economic Traits in Ecuadorian Jersey Dairy Cattle

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Genomic analysis in dairy cattle is considered a practical technology in the genetic management of livestock. The objective of this study was to analyse the results derived from genomic testing of a commercial Ecuadorian Jersey herd between 2021 and 2025. A total of 409 genomic analyses using the Illumina Bovine SNP50 BeadChip (Clarifide–Zoetis, San Diego, CA, USA) were performed from hair root samples to evaluate the traits of interest related to milk production (quantity and quality), health, and economic indices, all of which are considered in the Council on Dairy Cattle Breeding (CDCB) database. In order to determine the genetic progress of the population, the year of birth of each animal was considered as a fixed factor. The data were processed and analysed using RStudio v. 1.4.1564 software. It was observed that between 2021 and 2025, production parameters such as milk (37.75 lb to 496.86 lbs), fat (−13.58 to 8.3 lbs), and protein (−6.8 to 14.52 lbs) increased dramatically in value, while the average percentage of fat (−0.078–0.081%) and protein (−0.042–0.020%), as well as somatic cells ($2.94\text{--}2.97 \times 100,000$ cells/mL), improved slightly, albeit with high variability. On the other hand, no individuals were found to be carriers of the recessive BLAD and DUMPS genes (0%), and only one animal was a carrier of the CMV gene (0.24%). In addition, the Beta Casein A1/A2 ($p = 0.001$), Alpha S-1 Casein ($p < 0.0001$), and Kappa Casein I ($p < 0.0001$) genes were found to be in Hardy–Weinberg disequilibrium, while β -lactoglobulin was the only gene found to be in equilibrium ($p = 0.0872$). Economic indices such as net merit (\$−211.89–38), cheese merit (\$−221.93–37.95), and fluid merit (\$−186.89–38.69) increased over time. In conclusion, genomic evaluation improves the selection process for the best Jersey cows by increasing the efficiency of traditional genetic analyses, which only took into account the genetic characteristics of the sire (imported bulls), thus efficiently improving genetic progress in this breed.

3.4.11. Host Genomic Regulation of Gut Microbiota Resilience Under Environmental Stress in Pigs

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Climate change and intensive genetic selection for greater productivity have increased the need for breeding livestock with better adaptive capacity, supported by microbiome–host interactions that promote resilience. The gut microbiota plays a key role in maintaining host homeostasis under thermal and oxidative stress, yet the genomic regulation underlying microbiota resilience remains poorly understood. In this context, the main objective of this study was to investigate host genetic variants associated with bacterial genera linked to adaptive and metabolic resilience in pigs. A genome-wide association study (GWAS) was performed using genotypes from a commercial 50K SNP chip and microbial profiles obtained by 16S rRNA sequencing of cecal and fecal samples (ASV level, 10% prevalence). These data were integrated with expression quantitative trait loci (eQTL) derived from RNA-seq (TPM-normalized) across brain, liver, and muscle tissues of 72 immunocastrated Large White males fed diets containing soybean, fish, or canola oil. Both cis (≤ 1 Mb) and trans (> 1 Mb) eQTL were considered (FDR < 0.05). Thirty-seven bacterial genera were associated with eQTL, among which *Monoglobus*, *Intestinimonas*, *Fournierella*, *Helicobacter*, *Coprococcus*, *Anaerostipes*, *Faecalibacterium*, *Roseburia*, and *Oscillospira* were linked to metabolic and physiological pathways that support host resilience. These include short-chain fatty acid metabolism, mucin utilization, immune modulation, and neuroendocrine regulation. Most associations involved fecal microorganisms linked to trans-eQTL in the brain. Thirty-eight host genes were identified across cis- and trans-regulatory regions. Associated loci covered genes involved in epithelial barrier integrity and mucosal protection, including tight junction claudins (*CLDN17* locus) and *TFF2*, as well as stress-response candidates such as *CASP2*, indicating a genomic contribution to microbiota-mediated resilience under environmental challenges. The identified microbial groups play a crucial role in maintaining metabolic stability and intestinal homeostasis during physiological challenges, suggesting that host regulation of microbial diversity may help sustain good welfare and adaptive capacity in intensive production environments.

3.4.12. In Silico Prediction of DNA-RNA Triplex Formation Sites Reveals Novel Regulatory Links Between Key Fertility Genes and lncRNAs in Retinta Beef Cattle

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Long non-coding RNAs (lncRNAs) are increasingly being recognized as key regulators of gene expression in several organisms. In this study, we aimed to identify lncRNAs that may regulate protein-coding genes that had been previously associated with fertility in Retinta cattle via the formation of DNA:RNA triplexes. Sixteen lncRNAs located within ± 50 kb genomic windows, mainly found on BTA5, around the transcription start sites of target genes linked with female fertility or male scrotal circumference were further

selected. We observed a range of distances, from 0.61 to 49.8 kb, which is compatible with *cis*-regulation. A total of 341 triplex-forming oligonucleotides (TFOs) of high quality were identified by LongTarget, with TFO lengths falling between 20 and more than 70 nucleotides. *AMHR2* was the target gene with the higher proportion of lncRNA-binding sites, as well as the one with the most stable interactions. Furthermore, the accessibility of the lncRNAs in the context of their secondary structure was predicted using RNAplfold, revealing the presence of predicted TFOs in exposed regions for two lncRNAs, ENSBTAT00000117549 and ENSBTAT00000088580, located in the proximity of genes *AMHR2* and *KRT8*, respectively. Our results also showed that the presence of lncRNA-binding sites in DNA are frequent in the upstream regions of the target genes, where promoters and other regulatory regions lie. This observation is consistent with the putative regulatory role of lncRNAs at the gene expression level. Our findings provide a first step towards improving our understanding of the molecular basis behind the lncRNA-mediated regulation of the reproductive performance in cattle.

3.4.13. Mathematical Modeling of Genetic Relationships Among Dog Breeds Using Matrix-Based DNA Analysis and Predictive Trait Simulation

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Genetic variation among domestic dog breeds is a determining factor in physiological traits, behavioral characteristics, and susceptibility to hereditary diseases. While conventional genomic approaches provide biological insights, mathematical modeling offers an innovative perspective to systematically analyze DNA variability and predict cross-breed genetic outcomes. This study proposes an interdisciplinary framework combining genetics, matrix theory, and mathematical optimization to investigate DNA connections among multiple dog breeds. DNA sequence datasets were numerically encoded into binary matrix structures to represent nucleotide patterns. Linear algebraic techniques, including eigenvalue decomposition and vector space mapping, were applied to identify dominant genetic markers and quantify inter-breed genetic similarity. A predictive mathematical model was formulated to simulate genetic inheritance patterns, enabling estimation of potential outcomes in cross-breeding scenarios. Model validation was performed using comparative genomic references and statistical error minimization methods. Results revealed that matrix-based classification accurately differentiated dog breeds with characteristic phenotypic traits. Eigenvalue distribution patterns indicated significant clustering around genes associated with immunity and neurological behavior. The predictive model demonstrated high reliability, with simulation accuracy exceeding 93% in identifying probable trait inheritance. Experimental simulations suggested a potential enhancement of 15–18% in genetically inherited health-related markers under controlled breeding strategies. This research confirms that mathematical modeling, specifically through the application of linear algebra to genomic data, provides a powerful and novel analytical pathway in animal genetics. The proposed model supports sustainable and health-oriented selective breeding in dogs and demonstrates potential application to broader animal genetic studies. Future work will explore integration with nonlinear modeling and AI-based machine learning techniques for advanced predictive analytics.

3.4.14. Multi-Omics Integration to Understand Pathogen Impacts in Farmed and Aquaculture Animals

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The outbreak of infectious diseases is one of the major challenges in the farming and aquaculture industries, causing significant economic losses. Recent advances in high-throughput sequencing technologies have provided detailed analyses of how these diseases affect host animals at multiple molecular levels. Multi-omics approaches allow for a comprehensive investigation of complex interactions, providing a holistic view of the interrelationships between the biomolecules and their functions. The integration and analysis of these heterogeneous datasets are essential steps towards generating knowledge in the framework of precision livestock and aquaculture. This research focused on studies that use multi-omics data integration rather than those that apply multiple omics approaches separately without integration. Moreover, it summarises recent publications addressing the application of integrative multi-omics techniques in economically relevant livestock species such as cattle, pigs, goats, sheep, and aquatic organisms affected by diverse pathogens (virus, bacteria, and fungi, among others). This study provides an up-to-date overview of the integration methods used in this field, the omic tools available for different species, and the molecular mechanisms underlying host responses to infection. Our findings highlight the fact that multi-omics integration improves our understanding of disease mechanisms. However, this approach presents challenges due to inconsistent methodology and a lack of rigour and standardization in statistical analysis, particularly in farming and aquaculture studies.

3.4.15. Omega-6-Associated Gene Modules Reveal Novel Regulatory Mechanisms in Porcine Liver

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Identifying diets with desirable ratios of omega-6 (n-6) and omega-3 (n-3) fatty acids (FAs) plays a key role in regulating inflammation, maintaining membrane fluidity, facilitating β -oxidation, and promoting overall metabolic health. Previously, we showed that pigs fed 1.5% soybean oil (SOY1.5) had greater hepatic linoleic acid (an n-6 FA) deposition than pigs fed 3.0% soybean oil (SOY3.0). A balanced dietary ratio of n-6:n-3 FA is crucial to support optimal health and reduce the risk of chronic diseases. Thus, this study aimed to identify gene co-expression modules correlated with n-6 deposited in hepatic tissue of pigs fed diets containing either SOY 1.5 or SOY3.0. mRNA-seq data from 35 immunocastrated Large White pigs (CEUA2018-28) were analyzed using standard bioinformatics pipelines. Co-expression modules were identified with WGCNA, followed by functional enrichment using DAVID. Strong co-expression patterns related to n-6 were observed in both diets, but they involved distinct modules and pathways. In pigs fed SOY1.5, one gene cluster was negatively correlated with linoleic acid and total n-6. In pigs fed SOY3.0, however, another module was negatively correlated with the n-6:n-3 ratio. Functional enrichment analysis

(FDR < 0.05) highlighted the fatty acid degradation pathway (ssc00071) in SOY1.5-fed pigs and Bile secretion pathway (ssc04976) in the SOY3.0 group. These findings suggest that dietary oil inclusion modulates distinct co-expression networks and metabolic pathways in porcine liver. These molecular insights can optimize nutritional strategies, thereby enhancing animal health and aligning with the FAO's One Health initiative.

3.4.16. Post-GWAS Mapping of Genomic Regions in Quiescent Chromatin Linked to Average Feed Intake per Visit in Landrace Pigs

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In this study, we conducted a GWAS analysis to further investigate the genomic regions associated with average feed intake per visit (AFIV) in Landrace pigs. Three QTL regions, located on chromosomes 12, 13, and 14, were identified based on visualizations in the Integrative Genomics Viewer (IGV). To assess the functionality of these regions, significant SNPs were compared with publicly available ATAC-Seq data from relevant tissues, including muscle, liver, cerebral cortex, and adipose tissue. All regions associated with AFIV were found to be within the “E15” chromatin state, characterized as a quiescent region with low transcriptional activity. This association suggests a potential regulatory role for these regions, which may act during specific developmental stages or in particular tissues, despite their epigenetically silent state. Our findings underscore the complexity of genetic and epigenetic regulation of feeding behavior in pigs and provide valuable insights for genetic improvement strategies targeting feed efficiency in Landrace pigs. Future functional studies are needed to clarify the role of these quiescent regions in regulating feed intake, thereby contributing to advances in genetic selection. Moreover, this work aligns with the United Nations Sustainable Development Goals (SDGs), particularly SDG 2 (Zero Hunger and Sustainable Agriculture), by promoting progress in efficient and sustainable animal production, and SDG 12 (Responsible Consumption and Production), by contributing to the optimization of feed resource use in pig farming.

3.4.17. Seed Dispersal Coevolution with the Extinct Proboscidean *Stegodon trigonocephalus* Restricts Current *Platymitra macrocarpa* Distribution in Indonesia

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The coevolution between angiosperms and megafaunal frugivores remains poorly understood despite its importance in shaping modern plant community dynamics and dispersal systems. *Platymitra macrocarpa* (Annonaceae) is a Southeast Asian tree species predicted to have coevolved with extinct proboscideans. This study investigates whether the extinct Javan proboscidean *Stegodon trigonocephalus* served as its primary seed disperser, influencing the plant's current restricted distribution. Data were collected from literature reviews and secondary sources, including *Herbarium Bogoriense* for *P. macrocarpa* distribution and fruit-seed dimensions, and the Sangiran Museum for *Stegodon* fossil localities. Statistical correlation between *P. macrocarpa* distribution and *S. trigonocephalus* fossil records across Java was analyzed using SPSS, followed by spatial mapping with ArcGIS. The logistic

regression analysis revealed a strong positive correlation (Nagelkerke $R^2 = 0.854$, $p < 0.05$) between *S. trigonocephalus* fossil distribution and *P. macrocarpa* occurrence, with a model accuracy of 94.4%. These results support a historical ecological association between the two species. Phylogenetic evidence indicates *P. macrocarpa* descended from *Polyalthioxylon platymitroides*, a Miocene taxon that migrated from India to Southeast Asia, reinforcing its long evolutionary history with megafaunal browsers. The extinction of *Stegodon* likely caused dispersal limitation and population fragmentation in *P. macrocarpa*, a phenomenon consistent with megafaunal dispersal evolutionary anachronism. This study concludes that *P. macrocarpa* distribution in Indonesia is a relict of its coevolution with *S. trigonocephalus*, and emphasizes the ecological legacy of extinct megafauna on tropical plant biogeography.

3.4.18. Whispers of the Wild: Genetic Diversity of Carnivores Across Southern Saudi Arabia Through Multilocus DNA Barcoding

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Carnivores represent important ecological components as apex predators, but their diversity is still poorly described in Saudi Arabia due to a deficiency of field data. This study provides the first molecular assessment of carnivores across southern Saudi Arabia using a multilocus DNA barcoding approach (COI, Cytb, and 16S). Out of 155 biological samples (scats and tissues), we successfully generated and analyzed 152 sequences, identifying 11 species (*Panthera pardus*, *Caracal caracal*, *Acinonyx jubatus*, *Felis lybica*, *Vulpes vulpes*, *Canis lupus*, *Canis lupus familiaris*, *Hyaena hyaena*, *Genetta genetta*, *Ichneumia albicauda*, and *Herpetes javanicus*). Phylogenetic and haplotype network analyses uncovered clear resolution at the species level with substantial intraspecific variation, especially within *Vulpes vulpes*, *Canis lupus*, and *Panthera pardus*. We have also confirmed, for the first time since the sighting in 2014, the presence of the critically endangered Arabian leopard, *Panthera p. nimr*. The observed high genetic diversity among the studied leopard samples likely reflects the temporal divergence of the sampled specimens rather than the true current population diversity. The cheetah (*Acinonyx jubatus*) samples in our study likely represent introduced individuals from Africa via illegal trade, rather than native Arabian populations. Genetic divergence among haplotypes reveals historical isolation and recent population expansions favored by habitat fragmentation. The proposed methodology of integrating molecular barcoding with traditional field approaches may hold great potential for the protection of the elusive and threatened carnivore fauna of Saudi Arabia.

3.4.19. Whole-Genome Comparative Study of Greek Black Pigs and Wild Boars: Insights into Adaptation and Genomic Divergence

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Background: The Greek black pig is an autochthonous breed of notable historical, cultural, and agricultural importance. Renowned for its adaptability to extensive farming systems and high-quality meat, it represents a valuable genetic resource within European pig populations. Despite its significance, its genomic landscape remains largely unexplored. To address this, we performed whole-genome sequencing (WGS) of Greek black pigs and wild boars from Greece, aiming to uncover genomic variation, identify functional

divergence, and highlight genes and pathways that may be shaped by domestication and environmental adaptation. **Materials and Methods:** Blood-derived DNA from both populations underwent WGS. Following bioinformatic analysis, missense single-nucleotide polymorphisms (SNPs) and indels were extracted due to their potential functional impact. Per-gene SNP and indel density was calculated. A comparative analysis was conducted to identify genes with unique or shared variants between populations. Gene Ontology (GO) and KEGG pathway enrichment analyses were performed to explore the biological significance of the findings. **Results:** Distinct sets of missense variants and indels were identified across the two groups. In wild boars, genes such as *KRTAP22-1*, *LCE7A*, and *TAS2R1* showed high variant density, with functions related to keratin structure and sensory perception. GO and KEGG analyses revealed enrichment in muscle contraction and immune-related pathways. In contrast, Greek black pigs exhibited increased SNP density in genes like *TAS2R39*, *OR5L1Q1*, and *OR4K2*, reflecting alterations in olfactory and taste receptor families. Enriched pathways included calcium signaling and various metabolic processes, potentially linked to domestication and diet. **Conclusions:** This comparative genomic analysis highlights divergent selection pressures in the studied populations of wild boars and black pigs, offering insights into adaptation, domestication, and the unique genetic profile of the Greek black pig. The results support conservation and sustainable use of local genetic resources.

3.5. Environmental Challenges to Animals and Precision Livestock Farming

3.5.1. Climate-Smart Sustained-Release Formulation of Mustard Oil Bioactives for Methane Mitigation in Ruminants

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Methane (CH₄) is a potent greenhouse gas with a global warming potential approximately 84–86 times higher than carbon dioxide (CO₂) over a 20-year period. Reducing CH₄ emissions is essential to achieving the EU's climate neutrality goals under the European Green Deal and the EU Methane Strategy. Ruminant livestock are responsible for up to 90% of agricultural CH₄ through enteric fermentation. With the global population expected to reach 9.7 billion by 2050, growing food demand will further intensify emissions, underscoring the urgent need for scalable, sustainable mitigation technologies. Current CH₄ reduction approaches include feed additives, dietary manipulation, vaccination, and microbial inhibitors. However, these methods often suffer from limited efficacy, short duration of action, high cost, or the need for frequent supplementation, making them impractical for large-scale adoption. To overcome these limitations, this project proposes the development of a polymer-based sustained-release formulation that continuously delivers a natural, long-acting, CH₄-reducing compound in the rumen. Mustard oil, produced from mustard cake, a common high-protein feed for animals, contains natural compounds like allyl isothiocyanate (AITC), known for their antimicrobial and anti-inflammatory effects. This project aims to develop a sustained-release formulation using mustard oil and biodegradable polymers to help reduce CH₄ emissions from ruminant animals. After assessing AITC's properties and antibacterial activity, it was blended with five biodegradable polymers of both synthetic (PCL, PEO) and natural (PHB, PLA, Chitosan) origin by solvent casting. FTIR and DSC analyses showed clear miscibility of AITC with PCL and PHB, without new or shifted peaks, and compatible thermal behaviour, further supported by SEM images confirming uniform dispersion within these polymer matrices. Antimicrobial assays using

both Gram-positive and Gram-negative rumen bacteria also produced promising results, highlighting that PCL and PHB are the most suitable biodegradable polymers to act as matrix materials for incorporating AITC to formulate a sustained-release formulation for CH₄ mitigation.

3.5.2. Comparison of a Laser Methane Detector with the GreenFeed for On-Farm Measurements of Enteric Methane Emissions from Goats

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The current study aimed to evaluate the diagnostic accuracy of the Laser Methane Detector (LMD) compared to the GreenFeed (GF) system for measuring methane emissions in goats. It employed two analytical frameworks to analyze agreement: continuous agreement of paired readings and diagnostic accuracy at a designated clinical limit of 13 g/day, using a significance level of $\alpha = 0.05$. The study involved 45 Spanish Boer goats aged 4–6 months, with methane levels measured over 14 days. Goats had free access to the GreenFeed unit, and readings were taken using the LMD, directed at the nostrils for one minute at a fixed distance. The results showed that the accuracy was 47.6% (95% CI 39.7–55.5%), the no-information rate was 59.1%, and $p(\text{Acc} > \text{NIR}) = 0.9989$, meaning it was not better than guessing the majority class. The sensitivity was 34.0% (missed $\sim 2/3$ of GF positives) and the specificity was 67.2% (moderate ability to correctly call negatives). Similarly, the PPV/NPV was 60.0%/41.3%, respectively, with balanced accuracy of 50.6%. In terms of the agreement, Kappa was 0.011—essentially indicating no agreement beyond chance. McNemar's test indicated that $p = 9.82 \times 10^{-6}$, with many more false negatives (64) than false positives (22); this means that the LMD systematically under-calls positives at this threshold. The experiment concluded that the LMD's diagnostic performance against GF is insufficient for stand-alone use at the current threshold (AUC ≈ 0.54 ; sensitivity ≈ 0.34 ; $\kappa \approx 0.01$). Finally, there was no clear correspondence between GF and LMD device measurements. Given the under-calling of positives and near-chance discrimination, the LMD should not replace GF without recalibration and threshold optimization.

3.5.3. Effect of a Heat Wave on Milk Yield of Cows Raised on Pasture with Access to an Automatic Milking System

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This study aimed to evaluate the effect of a heat wave on the milk yield of cows raised in a pasture-based system with access to an automatic milking system. The study was conducted at *Embrapa Pecuária Sudeste*, Brazil, using data from 32 days of milk from 68 dairy cows (Holstein $\times$ Jersey). The animals had free access to an AMS (DeLaval VMS300),

located ~160 m around the pasture area. The microclimate data (air temperature, AT °C, and relative humidity, RH%) were recorded by automatic weather stations (15 min interval). So, we calculated the Black Globe-Humidity Index (BGHI) and determined the heat wave (HW) period. The HW was defined according to the TX90P index (maximum air temperature above the 90th percentile = 31.6 °C, for at least five consecutive days). For the HW, three different periods were defined: pre-heat wave (10 days), heat wave (12 days), and post-heat wave (10 days). Milk yield data were tested for normality using the Shapiro–Wilk test and analysed using a mixed linear model, considering the period as a fixed effect and the cow as a random effect. The highest average values of microclimate variables were found in the heat wave period (AT: 27.1 °C; RH: 50.1%; and BGHI: 77) followed by the pre-heat wave period (AT: 22.1 °C; RH: 77.9%; and BGHI: 73.5) and the post-heat wave period (AT: 22.2 °C; RH: 67.8%; and BGHI: 72.7). A progressive reduction ($p < 0.001$) in milk yield was observed across the periods (pre-heat wave: 17.6 ± 0.8 ; heat wave: 17.1 ± 0.8 ; post-heat wave: 16.1 ± 0.8 kg/day). The continued decline in milk yield during the post-heat wave period indicates that the physiological effects of hyperthermia extended beyond the end of the event, reflecting a slower productive recovery. Our results indicate that periods of heat waves compromise milk yield and that this effect persists even after normal thermal conditions are restored.

3.5.4. Effects of Heat Stress on Testicular Physiology in Cattle: A Review

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Introduction: Global warming has intensified the occurrence of heat waves, directly impacting animal reproduction, especially in cattle. Heat stress affects male reproductive efficiency. Increased body temperature compromises essential reproductive processes, particularly spermatogenesis. Understanding how heat interferes with testicular physiology and sperm quality in cattle is fundamental for the development of management strategies that minimize its negative effects on reproduction. This study aimed to evaluate, based on the scientific literature, how heat stress compromises testicular function in cattle. **Methodology:** The review was conducted in the PubMed and ScienceDirect databases, including original scientific articles published in English in the last 10 years, with full-text access. The descriptors used were heat-shock response, testis, cattle, thermoregulation, and spermatozoa. Initially, 21 studies were identified, of which 13 met the inclusion criteria. **Results:** Evidence has shown that environmental heat stress promotes increased testicular temperature and alterations in blood flow, compromising scrotal thermoregulation. These alterations result in oxidative stress, sperm DNA damage, and epigenetic modifications. Significant reductions in sperm quality have been observed, including decreased motility, impaired sperm structural integrity, and Sertoli and Leydig cell dysfunction, negatively affecting spermatogenesis. Studies have also reported early diagnostic methods for heat-induced testicular changes. Furthermore, mitigation strategies, including ventilation, cooling systems, and chemical approaches, have proven effective in reducing thermal damage and preserving fertility. **Conclusion:** The studies demonstrate that heat stress reduces sperm quality parameters and alters the morphology and physiology of seminiferous tubules, ultimately leading to decreased fertility. Even moderate increases in testicular temperature are sufficient to significantly compromise sperm quality.

3.5.5. Evaluation of Dairy Heifers' Drinking Behavior Dynamics in Agrivoltaic Systems in a Tropical Region

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This study aimed to evaluate the effect of an agrivoltaic system on the water intake behavior of dairy heifers raised in a tropical climate. The experiment was conducted between July and August 2025, during 12 consecutive days at FMVZ/Unesp–Botucatu, São Paulo State, Brazil. Visits to the water trough of 21 dairy heifers (550 ± 55 days old; 247 ± 37 kg, mean $\pm$ SD) were registered continuously from 9 h to 16 h. In a crossover design, the heifers were divided into three groups (7 heifers/group) and located in three pasture systems: an open pasture system (OPS), an agrivoltaic system with horizontally arranged photovoltaic panels (AGRIhor), and an agrivoltaic system with vertically positioned panels (AGRIver). The agrivoltaic systems comprised 72 panels divided into 6 paddocks (12 panels/paddock). The data were analyzed using a generalized linear mixed model (Poisson family), considering treatment and hours as fixed effects and date as a random effect. In total, 334 water intake events were recorded in the OPS, 343 in the AGRIhor, and 305 in the AGRIver. There was no effect ($p > 0.05$) of agrivoltaic systems in relation to OPS. However, a difference was found between agrivoltaic systems ($p = 0.02$). The AGRIhor had, on average, 23% more events of water intake than AGRIver. Additionally, there was an interaction ($p < 0.05$) between treatment and hours. The number of water intake events decreased progressively over the observation hours, with a decrease of 22% to 42% compared to the beginning of the observation. The highest number of water intake events (196) was recorded in the first hour of observation (9 h–9:59 h), while the lowest number of events (94) occurred between 12 h and 12:59 h. In conclusion, the arrangement of the agrivoltaic system influenced the heifers' water intake behavior, indicating that the shading generated by agrivoltaic systems, especially in the horizontal configuration, can modify the behavioral dynamics of heifers in tropical conditions.

3.5.6. Sensor-Derived Heat-Tolerance Traits in Sheep: Heritability, Prediction, and On-Farm Decision Triggers

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Sheep reproduction, survival, and welfare are increasingly challenged by heat, humidity, and unstable forage. Modeling in *Nature Food* estimates heat stress already causes ~ 2.1 million lamb losses annually in Australia, rising to ~ 3.3 million with $+3^\circ\text{C}$ warming, with mating on hot days ($\geq 32^\circ\text{C}$) especially harmful. The Temperature–Humidity Index (THI) for small ruminants provides actionable thresholds—moderate 82–84, severe 84–86, extreme ≥ 86 useful for both management and analysis. Precision Livestock

Farming (PLF) offers a data driven response. Sensor streams accelerometers, location data, and computer vision can generate selection-worthy phenotypes and enable early stress detection. In a Merino cohort ($n = 160$), accelerometer-derived grazing time showed heritability $h^2 = 0.44 \pm 0.23$ and repeatability 0.70 ± 0.03 , indicating sensor traits are improvable through genetic selection. Genomic work in dairy sheep reports heat-resilience heritability around 0.26, suggesting thermotolerance can be selected without sacrificing production. Modern CV pipelines (e.g., YOLO/DeepSORT families) reliably classify eating, lying, and rumination, enabling automated welfare/intake monitoring at scale. Management strategies triggered by PLF signals—timely shade/ventilation and ration adjustments improve comfort and performance, reducing heat-load behaviors. We propose an integrated five-stage system for climate-resilient sheep breeding and operations: (1) quantify heat load with on-farm loggers and THI; (2) extract PLF features (activity bouts, rumination, shade-seeking, grazing time); (3) link features to fertility and survival via mixed-effects models and gradient boosting; (4) estimate genetic parameters and breeding values for sensor-derived heat-tolerance indicators; and (5) deploy real-time triggers (shade/soakers) when locally validated THI thresholds are exceeded. This pipeline converts environmental pressures into measurable, heritable, and decision-useful phenotypes. By coupling continuous sensing with predictive analytics and genomic selection, producers can improve reproductive success and lamb survival while safeguarding welfare—offering a practical path toward climate-resilient sheep systems.

3.5.7. Thermal Environment and Daily Patterns Shaping the Water Intake of Caracu Young Bulls in a Tropical Region

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The aim of this study was to evaluate the effects of the thermal environment, body weight, and hours on the water intake of Caracu young bulls raised in a tropical region. Forty-six animals (mean $\pm$ SD: 247 ± 230 days old; 226 ± 35 kg body weight) participated in a Feed Efficiency Test over 68 days (June–September 2024) in Sertãozinho, Brazil. Hourly individual water intake (L) and daily body weight (BW, kg) were recorded using electronic water troughs (Intergado®). Hourly environmental data were obtained from NASA-POWER, and the 3-h moving average of the Black Globe Humidity Index (BGHI) was calculated. Data were analyzed using a Generalized Additive Mixed Model (GAMM), including smooth terms for BGHI and BW, hour (24 h) as a fixed effect, and a random intercept for animal, using REML. BGHI had a non-linear effect ($p < 0.05$), with two inflection points (71 and 76.7). Up to BGHI 71, water intake increased ($+0.105$ L/BGHI unit); between 71.1 and 76.7, there was a reduction (-0.041 L/BGHI unit); and above 76.7, intake increased again ($+0.0128$ L/BGHI unit). The model also showed a diurnal pattern in water intake, with low consumption during the night and early morning (hours 0–4; $p > 0.05$). Intake rose around 5 h ($+0.43$ L; $p = 0.018$) and reached its highest levels between 8 h and 11 h ($+1.6$ to $+2.1$ L; $p < 0.001$). In the afternoon, consumption gradually declined and returned to baseline by late afternoon (12 h–17 h; $p < 0.01$). A secondary increase occurred at 22 h ($+0.27$ L; $p = 0.045$). BW influenced ($p < 0.001$) water intake; for each additional kilogram, it increased by 0.018 L. Overall, these findings show that thermal environment drives water intake. Although individual increases may seem small, when multiplied across many

animals over several days under heat stress, they can reduce water-use efficiency in the system. This reinforces the need for management practices that reduce heat load and ensure adequate water availability in tropical production systems.

3.5.8. Utilising the Enviro-Detect System to Comprehensively Monitor the Environmental Conditions in European Pig, Poultry and Cattle Production Facilities

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10. Carnex d.o.o., Vrbas, Serbia
11. ILVO, Merelbeke-Melle, Belgium
12. SLU Skara, Skara, Sweden

Effective environmental monitoring within livestock facilities is fundamental for ensuring optimal animal welfare, promoting farm worker safety, and demonstrating environmental stewardship. Airborne pollutants, such as ammonia, particulate matter, and carbon dioxide can negatively impact animal health, welfare, productivity, the working conditions of staff, and the environmental sustainability of the farming operation. In addition, the precise and continuous evaluation of thermal conditions in livestock buildings is becoming an essential management duty, as climate change-related impacts can severely compromise whole-farm efficiency. Traditionally, the comprehensive assessment of in-barn environmental conditions has required the deployment of multiple, often non-integrated devices, complicating data collection and analysis. As part of three EU-funded projects, such as LivestockSense (<https://livestocksense.eu/>), aWISH (<https://www.awish-project.eu/>), and ET4D (<https://www.et4d.eu/>), a number of integrated environmental monitoring systems or Enviro-Detect units (ED; PLF Agritech, Brisbane, Australia) were deployed in commercial pig, poultry, and cattle buildings in Estonia, Israel, Germany, Hungary, Poland, Serbia, Spain, and Sweden to evaluate (1) the capacity of a single platform to continuously track a suite of critical environmental parameters under commercial conditions, (2) the practical feasibility of such technology in real-world farm settings, and (3) the utility of these measurements for assessing animal health, welfare, and production efficiency. The ED systems were installed in the above-mentioned countries between 2021 and 2026, with weekly environmental reports delivered to farm management. Comparative analysis with data from these pig, poultry, and dairy buildings were undertaken and the results will be presented during this conference. The findings showcased the ED system's ability to deliver actionable, real-time data on barn climate and pollutant levels, empowering farm managers to rapidly respond to suboptimal conditions and optimize production efficiency.

3.6. Animal Physiology, Reproduction, and Sustainable Animal Production

3.6.1. Characterizations of the Production System and Breeding Practices of Surma Cattle Population in Bench Maji Zone, Southwest Ethiopia

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The study was carried out in Bench Maji Zone, Southwest Ethiopia. The objective of the study was to identify the production systems of the Surma cattle population under farmer management conditions and to identify Surma cattle breeding practices of pastoralists and agro-pastoralists in the study area. A total of 184 households were considered for questionnaire administration. A purposive multistage stratified random sampling technique was used to determine the number of districts, Kebeles, and households. The data were gathered through field observations, focus group discussions, and semi-structured questionnaires. Livestock rearing was the main (73.4%) means of livelihood for the community in the study area. Natural pasture was the only feed resource for Surma cattle, and cattle were herded on communal grazing land. Traditionally named “Fora”, this nonconventional mineral source is used for supplementing Surma cattle. Most (70.7%) of the respondents practiced castration of males for the purpose of controlling inbreeding, followed by fattening (25.5%). The majority (70.1%) of the farmers in the study area depended on uncontrolled natural mating. The majority (92.9%) of the respondents practiced selecting replacement animals to achieve their breeding objectives. Sixty-seven-point four percent (67.4%) of the pastoralists also mostly depended on coat color (red, 44.6%, Fawn (spotted), 28.3%, white, 23.9%, and black, 3.3%) to replace their animals. The average lactation length was approximately 5.69 months, and the average daily milk per cow was 1.45 L for Surma cattle. This study provided basic information on traditional knowledge associated with their cattle rearing systems, breeding concepts, selection strategies, breeding objectives, and utilization in the agro-pastoral and pastoral communities in the Surma area. Therefore, substantial interventions must be made to make the sector sustainable, improved, and productive, especially in areas such as herd selection and breeding practices.

3.6.2. Effect of Mating Rate on Offspring Sex Ratio in Thoroughbred Stallions

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Changes in factors like an increase in mating frequency, age, and subfertility have been associated with a female-biased sperm sex ratio and may result in a female-biased offspring sex ratio. In the New Zealand Thoroughbred industry, there is a preference for male foals due to a greater potential economic return from successful racing and breeding careers. If female-biased sperm sex ratios are linked to higher mating rates, this has economic implications and practical implications for stallion breeding management. Within the industry, there are multiple commercial and economic drivers to mate large number of mares (31–114 mares within a season) within a heavily condensed breeding season (approximately 110 days). Therefore, stallions have a high daily reproductive rate (mating up to four mares per day), with mating concentrated within the narrow mare reproductive season and breeding window. This study investigated the effect of average daily mating rate from 7 to 0 weeks before conception on the likelihood of conceiving a male foal using logistic regression and data derived from official breeding records (2005–2015) of New Zealand

Thoroughbred stallions ($n = 220$ stallions, 33704 observations). The 7-week window was included to capture the effect of oxidative stress from mating on stages of spermatogenesis. Other relevant variables such as sire, season, stud fee category, age, book size, and fertility were also investigated. Variables were tested using univariable analysis (significance obtained using Chi-square test) followed by a forward stepwise approach. Only the increased mating rate (≥ 3 mares/day) at 4 weeks (28–35 days) prior to conception significantly reduced the likelihood of conceiving a male foal (Odds ratio = 0.93, CI: 0.89–0.98, $p < 0.01$). This coincides with the meiosis stage of spermatogenesis, where greater Y-chromosome susceptibility to stress-induced damage during early development has been reported in the literature. These findings are consistent with observations in previous studies and highlight the relationship between economic optimisation and biological pressures associated with intensive production practices.

3.6.3. Seroprevalence and Risk Factors of Equine Piroplasmosis (EP) in Competition Horses in Spain

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Introduction and objectives: Equine piroplasmosis (EP) is a parasitic disease caused by *Theileria equi*, *Babesia caballi*, and, to a lesser extent, *Theileria haneyi*. It is transmitted by hard ticks, mainly belonging to the genera *Dermacentor*, *Haemaphysalis*, *Rhipicephalus*, and *Hyalomma*. The aim of the present study was to determine the seroprevalence of EP in sport horses in Spain and to identify its associated risk factors. **Methodology:** A total of 1022 serum samples collected in 2023 ($n = 356$), 2024 ($n = 353$), and 2025 ($n = 313$) from healthy horses of various breeds residing in 16 autonomous communities across Spain were analyzed. The samples were tested using two commercial cELISA kits (VMRD[®] Inc., Pullman, WA, USA). Apparent and true seroprevalence were calculated, and a risk factor analysis was performed using binary logistic regression to evaluate different variables, including year, sex, age group, breed group, geographical area (north or south), use/purpose, and paddock access. **Results and discussion:** Results showed an overall true seroprevalence of EP (*T. equi* and/or *B. caballi*) of 30.3% (95% CI: 27.5–33.2%), with a decreasing trend over the three years for both *T. equi* and *B. caballi*. Older age ($p < 0.001$) and breed (crossbred and Iberian horses, $p < 0.001$) were identified as significant risk factors. The southern region of Spain showed a significantly higher EP seropositivity compared to the northern region ($p = 0.018$). Neither the horse's use/purpose ($p = 0.66$) nor paddock access ($p = 0.19$) was identified as a risk factor. **Conclusions:** Breed, age, and geographical location were identified as risk factors associated with the presence of EP in sport horses. Increasing the number of leisure horses included in the study, as well as collecting more detailed information on paddock access and other management-related variables for all horses, could improve the assessment of potential risk factors associated with EP.

3.6.4. Anethole Preserves the Viability of Epididymal Spermatozoa in Wistar Rats

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Reproductive biotechnologies, such as *in vitro* fertilization, are efficient alternatives in both human and animal contexts, enabling couples to achieve pregnancy and contributing to genetic improvements in animals, respectively. However, the excessive production of Reactive Oxygen Species (ROS) in the *in vitro* environment compromises sperm integrity and reduces fertilizing potential. In light of this challenge, anethole, a phenolic compound with recognized antioxidant activity, has been investigated as a supplement capable of attenuating oxidative stress. Therefore, the aim of the present study was to evaluate the effect of anethole supplementation on sperm viability. The experimental protocol was approved by the Ethics Committee for Animal Use (CEUA/UECE). Epididymal spermatozoa from Wistar rats (N = 6) were collected by diffusion and incubated for 1 h in α -MEM medium supplemented with 1.25 mg of BSA in the presence of ascorbic acid (50 μ g/mL; positive control) and anethole at concentrations of 30 μ g/mL and 300 μ g/mL. Both antioxidants were used individually or combined with H₂O₂ (negative control). Sperm viability was assessed using eosin–nigrosin staining and was expressed as the percentage of live cells. The results were analyzed using one-way ANOVA in the GraphPad Prism software using $p < 0.05\%$. After 1 h of incubation, both anethole concentrations maintained sperm viability similar to the fresh control and the other treatments, including ascorbic acid ($p > 0.05$). No differences were observed among the other groups tested ($p > 0.05$). Anethole supplementation proved to be safe for the *in vitro* culture of Wistar rat spermatozoa, maintaining viability and showing no cellular toxicity. These findings reinforce the potential of anethole as an antioxidant substitute to ascorbic acid in commercial formulations of media in reproductive protocols. However, its potential beneficial effects on embryo production still need to be investigated.

3.6.5. Body Development of Lambs Fed Extruded Supplements with Different Protein Levels

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Maintaining an appropriate balance between energy and protein in feedlot diets is crucial to ensure productive performance while preserving animal health. This study aimed to evaluate the body development of lambs fed whole-corn diets supplemented with extruded protein concentrates containing different protein levels. The project was approved by the Animal Ethics Committee of the Federal University of Uberlândia (protocol no. 092/16). A total of 22 crossbred (Dorper $\times$ Santa Inês) lambs, with an initial body weight of 21.46 ± 6.36 kg and aged 3 ± 1 months, were used. The animals were housed in collective pens for 70 days in a randomized block design and assigned to four experimental groups differing in the crude protein content of the extruded supplements (20%, 24%, 28%, and 32%). Feeding was carried out three times daily at 08:00, 12:00, and 16:00. Biometric measurements and body condition scores (BCSs) of the animals were assessed every two weeks. For body growth evaluation, *in vivo* biometric measurements were taken using a measuring tape by the same evaluator to minimize subjectivity. BCS was assessed by three trained evaluators through palpation of the 12th and 13th lumbar vertebrae using a 1–5 scale. The mean of the three evaluators was used to improve data accuracy. The data

were tested for variance assumptions using SAS software and analyzed by contrast tests to evaluate the linear and quadratic effects of protein levels (PLs), considering significance at $p \leq 0.05$. There was no effect of PL on biometric measurements ($p > 0.05$). However, a negative quadratic effect was observed for BCS ($p = 0.05$), with the quadratic response indicating a minimum value of 3.42 at a 28% PL. It was concluded that the protein level of the extruded supplement in high-grain diets for lambs affects body condition score without influencing body development measurements.

3.6.6. Characterization of the Seminal Microbiome and Its Association with Testosterone Levels and Testicular Morphometry Across Different Ram Fertility Groups

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The reproduction efficiency of rams has a strong impact on flock productivity, and the fertility of a flock is very diverse even when it is exposed to similar environmental and management conditions. There is no clear information on how these differences are caused by biological factors, especially in the heat stress seasons such as summer. There is also emerging evidence that the seminal microbiome can have an effect on the reproductive performance of the male, although the interactions between the seminal microbiome and hormonal profiles and testicular characteristics in rams has not been well established. It is important to clarify this critical gap in order to identify reliable fertility biomarkers and enhance the choice of superior breeding males. Of note, this experiment investigated the relationships among seminal microbiome composition, testicular morphometry, and reproductive parameters in rams during summer. A total of 386 ewes and 9 rams were used in an entirely randomized study, and rams were categorized and grouped based on the level of superior, good, and acceptable fertility according to the rate of pregnancy. The microbiome was characterised through 16S rRNA gene sequencing. Clostridiaceae was positively associated with fertility status, and the relative abundance of Clostridiaceae was 98.38%, 87.03%, and 59.57% in superior, good, and acceptable fertility categories, respectively. Superior-fertility rams had a higher concentration of testosterone (3343.75 pg/mL) than acceptable-fertility rams did (1242.72 pg/mL, $p < 0.0001$). Specific bacterial signatures were found to be fertility-specific, with some families specific to certain fertility groups. The results form a basis for establishing microbiome-based strategies for enhancing ram reproductive performance.

3.6.7. Comparative Efficacy of Single-Dose Versus Multi-Dose FSH Regimens in Dromedary Camels: Assessment of Ovarian Response, Embryo Yield, Endocrine Profiles, and Molecular Signatures

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This study aimed to compare two donor superovulation protocols (SOP) and embryo transfer (MOET) technology in dromedary camels by analyzing the mRNA gene expression of *osteopontin* (OPN) and *vascular endothelial growth factor* (VEGFA), in conjunction with follicular activity, embryonic development, and hormonal profiles. Ten healthy female camels were assigned to two treatment groups to evaluate different superovulation regimens: a conventional 5-day protocol (5dSOP) and a simplified single-dose protocol combined with hyaluronic acid (1dSOP), both utilizing a mixture of 500 µg porcine follitropin and 100 µg porcine lutetropin. The 1dSOP treatment consistently yielded significantly higher mean fol-

licular counts (27.2 ± 0.74 vs. 22.6 ± 0.76 ; $p < 0.05$), larger follicular diameters (1.82 ± 0.04 vs. 1.68 ± 0.05 cm), and a superior embryo recovery rate (19.29 ± 0.39 vs. 13.21 ± 0.43) compared to the 5dSOP. Furthermore, the 1dSOP resulted in significantly shorter response times (7.75 ± 0.23 days) than the 5dSOP (8.88 ± 0.27 days). A highly significant interaction between protocol and time was observed, with the 1dSOP group reaching peak follicular values at 144 and 192 h. While no significant differences were found in *OPN* expression, *VEGFA* expression was notably elevated in the 5dSOP group, suggesting potential benefits for endometrial receptivity. In conclusion, the 1dSOP is a highly successful, simplified method that demonstrates superior follicular growth and embryo yield while enhancing animal welfare through decreased intervention and improved follicular harmonization.

3.6.8. Development of a Big Additive Model to Predict Boar Semen Quality

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The quality of semen in pig farms is a key factor for the efficiency of artificial insemination programs. Semen quality is influenced by genetic, environmental, and management factors that affect parameters such as sperm motility, viability, or morphology. However, boar selection for semen collection is usually based on the technician's experience. Therefore, this study aimed to develop an artificial intelligence-based model to predict ejaculate quality and support data-driven decision-making. A database comprising 6899 semen samples collected from 78 Duroc boars was used, including information on animal (age), handling during collection and semen preparation, sperm quality parameters, season, and environmental conditions (temperature and humidity). A Big Additive Model (BAM) was applied to capture nonlinear relationships and temporal trends. The model was used to predict the "number of good sperm" in an ejaculate, calculated from sperm concentration (spz/mL), ejaculate volume (mL), and the percentage of motile and morphologically normal sperm (divided by 100). Additionally, semen samples were categorized into four quality groups (low, medium-low, medium-high, and high) to evaluate the model's ability to predict boar semen quality. The model achieved a coefficient of determination (R^2) of 0.485, with a root mean square error (RMSE) of 17730.56 and a mean absolute error (MAE) of 13832.95, indicating moderate predictive performance. In the categorical classification, the BAM showed an overall accuracy of 48.79%, with greater consistency in the extreme categories (low—58.38% and high—63.59%). Interestingly, the model reached an accuracy of 88.87% when classifying medium-high/high semen samples and 86.03% for low/medium-low samples. In conclusion, the BAM achieved moderate predictive accuracy but high reliability in distinguishing two categories of semen quality. This study forms part of the Agroalnext programme and was supported by MCIU with funding from European Union NextGenerationEU (PRTR-C17.I1) and by Comunidad Autónoma de la Región de Murcia—Fundación Séneca.

3.6.9. Dietary Noodle Waste-Induced Alterations in Ovarian Biometry, Histological Integrity and Oxidative Status in Wistar Rats

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Nutrition plays a vital role in regulating reproductive performance in humans and animals. Recently, the inclusion of agro-industrial by-products such as noodle waste (NW) in animal diets has increased, often without the adequate evaluation of their potential reproductive consequences. This study investigated the effects of NW consumption on ovarian function in twenty (20) healthy adult female Wistar rats. The rats were randomly assigned to four groups (n = 5). Group A (control) received standard rat feed, while Groups B, C, and D were fed diets containing NW only, 50% NW supplemented with 50% fish meal (FM), and 30% NW supplemented with 70% FM, respectively, for 35 days. Serum samples were analyzed for reproductive hormones (progesterone, oestradiol, and follicle-stimulating hormone (FSH)) using an enzyme-linked immunosorbent assay (ELISA). Ovaries were excised to evaluate ovarian biometry, gonadosomatic index (GSI), oxidative stress markers, antioxidant enzymes, and histopathology. There were no significant differences ($p > 0.05$) in the mean serum levels of FSH, progesterone, and oestradiol among the treatment groups. Similarly, mean malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GPx) levels showed no significant variation ($p > 0.05$), although Group B (NW only) exhibited higher MDA and antioxidant enzyme levels. Significant differences ($p < 0.05$) were observed in right ovarian weight (Group B: 0.03 ± 0.01 g and Group C: 0.06 ± 0.02 g), left ovarian weight (Group B: 0.02 ± 0.01 g and Group C: 0.06 ± 0.01 g), and GSI (Group B: 0.03 ± 0.02 and Group C: 0.07 ± 0.00). Histological examination revealed extensive follicular atresia, reduced ovarian vascularization, and smaller ovarian size in Group B compared to other groups. These findings suggest that exclusive consumption of noodle waste may impair ovarian function through oxidative stress and structural degeneration, emphasizing the need for appropriate dietary supplementation to mitigate its adverse effects.

3.6.10. Effect of Factors Related to Reproductive Protocols on Conception Rate in Girolando Cows

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This study evaluated the effects of reproductive management factors on the conception rate of Girolando cows. Data were obtained from a commercial dairy farm located in Patrocínio, Minas Gerais, Brazil, using the Ideagri software. Reproductive records from 145 Girolando cows of different breed compositions, collected between January 2020 and

August 2025, were analyzed. The variables evaluated included insemination condition (natural estrus or reproductive protocol), type of reproductive protocol, insemination center, and year of bull birth. Three reproductive protocols were assessed: protocol 1 consisted of two doses of gonadotropin-releasing hormone (GnRH) administered on days 0 and 10, with insemination using conventional semen on day 12; protocol 2 involved natural estrus paired with prostaglandin (PG) administration on day 0, followed by insemination after 48 h using conventional semen; protocol 3 included pre-synchronization with a progesterone implant, followed by GnRH and estradiol administration on day 0, PG on day 7, estradiol plus PG on day 9, and insemination with sexed semen on day 10. Semen from 46 Holstein bulls, born between 1989 and 2024 and originating from two artificial insemination centers, was used. Data were analyzed using Pearson's chi-square test at a 5% significance level, and Fisher's exact test was applied when expected frequencies were below five. Significant associations were observed between conception rate and insemination condition, reproductive protocol, insemination center, and year of bull birth ($p < 0.0001$). Cows subjected to reproductive protocols showed higher conception rates than those inseminated during natural estrus (40.59% vs. 31.88%). Protocols 1 and 3 resulted in higher conception rates compared to protocol 2 (40.63% and 40.00% vs. 31.88%, respectively). Insemination center 1 showed superior performance compared to center 2 (36.40% vs. 28.29%). The highest and lowest conception rates were observed with semen from bulls born in 2010 and 2019, respectively (52.27% vs. 26.62%). It is concluded that reproductive management strategies and bull-related factors significantly influence the conception rate of Girolando cows.

3.6.11. Effect of Genetic and Management Factors on Milk Production of Primiparous Girolando Cows

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This study aimed to evaluate the effects of factors related to reproductive management on the conception rate of Girolando cows. Data were collected from a commercial dairy farm located in Patrocínio, Minas Gerais, Brazil, using the Ideagri software. The dataset extracted from farm reports included reproductive information from 145 Girolando cows of different breed compositions recorded between January 2020 and August 2025. The number of females subjected to reproduction, number of inseminations performed, and conception rates were obtained. Inseminations were carried out under two conditions (natural estrus or reproductive protocol) and three reproductive protocols: 1—two doses of gonadotropin-releasing hormone (GnRH); 2—natural estrus + prostaglandin (PG); and 3—pre-synchronization with a progesterone implant followed by a protocol consisting of d0: implant + GnRH + estradiol, d7:PG; d9: estradiol + PG, and d10: insemination with sexed semen. Semen from 46 Holstein bulls born between 1989 and 2024 was used, originating from two artificial insemination centers. Data were analyzed using Pearson's chi-square test (χ^2) at a 5% significance level in SAS software. When expected frequencies were below 5, Fisher's exact test was applied. Significant associations were found between the conception rate and the variable insemination condition, type of protocol, insemination center and year of bull birth ($p < 0.0001$). Cows subjected to reproductive protocols showed higher conception rates than those inseminated during natural estrus (40.59% vs. 31.88%). Similarly, animals in protocols 1 and 3 presented higher conception rates compared with protocol 2 (40.63% and 40.00% vs. 31.88%, respectively). Insemination

center 1 outperformed center 2 (36.40% vs. 28.29%). Conversely, the highest and lowest conception rates were observed when semen from bulls born in 2010 and 2019 was used, respectively (52.27% vs. 26.62%). It can be concluded that the insemination condition, type of protocol, insemination center, and bull year of birth affect the conception rate of Girolando cows.

3.6.12. Effect of Hydroponic Green Fodder Feeding on Carcass and Meat Quality Characteristics of Gerze Native Chickens

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In recent years, the increasing cost of feed ingredients and the need for environmental sustainability in the poultry sector have necessitated the exploration of alternative feed sources. In this context, the production of hydroponic green fodder (HGF) is attracting attention, as it is an environmentally friendly method that minimizes water and land use. It has been reported that hydroponic fodder can positively impact production performance, welfare, and digestive health, particularly in poultry. This study was conducted to investigate the effects of HGF supplementation in the last quarter of the growing season on growth performance, carcass, and meat quality traits in the slow-growing Gerze Turkish native chicken breed. In the study, chicks were fed starter feed until 8 weeks of age, grower feed between 8 and 14 weeks, and either finisher feed alone (control group, CD) or finisher feed plus HGF (treatment group) ad libitum between 14 and 18 weeks of age. At the end of the trial, the CD group was found to have significantly higher abdominal fat weight, dry matter percentage, and protein levels than the HGF group ($p < 0.05$). In contrast, ash content was lower in both the pectoralis major (PM) and gastrocnemius (GN) muscles in the control group ($p < 0.05$). In chickens who were fed HGF, drip loss and intramuscular fat content in the GN muscle on the second storage day increased significantly ($p < 0.05$), while cooking loss in the GN muscle and freezing loss in the PM muscle decreased ($p < 0.05$). Furthermore, the hardness and adhesiveness values of the PM muscle in the CD group were higher than in the HGF group ($p < 0.05$).

In conclusion, it was determined that the use of hydroponic green feed can improve meat quality traits in Gerze chickens without negatively affecting growth performance. These findings suggest that HGF is a potential alternative feed source in sustainable and efficient poultry farming practices.

3.6.13. Effects of in Ovo Injection of Aqueous Extracts of Phytobiotics on Growth Performance, Blood Profile and Sex Ratio of Egg-Type Chickens

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This study investigated the effects of in ovo injection of aqueous extracts of phytobiotics (oyster mushroom, garlic, and ginger) and their combinations on growth performance, blood profile, and sex ratio of egg-type chickens. A total of 500 hatching eggs of egg-type chickens were examined. On the 14th day of incubation, 432 (86.40%) eggs of the egg-type chickens were fertile. The egg-type chicken eggs were divided into eight in ovo injection

(0.1 mL/egg) groups of phytobiotics: T1 (control); T2 (oyster mushroom); T3 (ginger); T4 (garlic); T5 (ginger + oyster mushroom); T6 (garlic + oyster mushroom); T7 (garlic + ginger); and T8 (garlic + ginger + oyster mushroom). Upon hatching, 284 (65.74% hatchability) hatched chicks were obtained. Data were subjected to One-Way Analysis of Variance in a completely randomized design. Feed intake and feed conversion ratio were significantly ($p < 0.05$) influenced by in ovo injection of aqueous extracts of the phytobiotics in pullets at 126 days of age. In the combinations of the phytobiotic extracts, there was a significant ($p < 0.05$) increase in the percentage of male (77.77%) to female (22.23%) chicks. Pullets from eggs that underwent in ovo injection of a combination of phytobiotics had the best ($p < 0.05$) feed conversion ratio (8.66). Packed cell volume, haemoglobin, red blood cell, and eosinophil counts were significantly ($p < 0.05$) influenced by the in ovo injection of aqueous extracts of phytobiotics and their combinations, with the highest values (76.10 and 76.20 g/dL) being from birds from eggs that received in ovo injection of a combination of phytobiotics. The study concluded that in ovo injection of phytobiotics at 0.1 mL/egg could be adopted to improve growth performance and manipulate the sex ratio without any deleterious effects on the blood profile of egg-type chickens.

3.6.14. Effects of Vitamin C and E Supplementation on Hen Day Production, Egg Quality Characteristics and Ovarian Morphometry of Improved Fulani Ecotype Hens

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This 12 weeks study examined effects of supplemental vitamins C and E on the hen day production (HDP), egg quality characteristics (EQCs) and ovarian morphometry (OM) of improved Fulani ecotype (IFE) hens. A total of 400 hens (17 weeks old) were divided into four groups, each had 100 birds and five replications of 20 birds. Hens in group 1 were fed a basal diet (control). Hens in groups 2, 3 and 4 were fed a diet with supplemental vitamin C (VC—500 mg/kg), vitamin E (VE—400 mg/kg) and VC + VE (500 + 400 mg/kg), respectively. The study applied a completely randomized design and HDP was calculated on the basis of the treatment. The EQCs determined included egg weight (EWT), shell weight (SWT), yolk weight (YWT), yolk height (YH), albumen height (AH) and albumen weight (AWT), while Haugh unit (HU) was estimated using a standard formula. Four hens from each replicate were sacrificed and their reproductive organs harvested to determine oviduct length, oviduct weight, ovary weight and stroma weight. The number of follicles and diameter of large yellow (>10 mm), small yellow (5 mm) and white follicles were determined, respectively. Data were subjected to an analysis of variance, and Tukey's HSD test was used to compare the difference between means. HDP (%) and OM were not influenced ($p > 0.05$) by the antioxidants. A lower EWT ($p < 0.05$) was observed in the control group (49.58 g) compared to the VC (57.14 g) and VE groups (58.03 g). A pattern similar to that of EWT was observed in SWT, while a heavier YWT was observed in the VE group (19.54 g). The AH of the VC (7.63 mm), VE (7.55 mm) and VC + VE (7.70 mm) groups were better than the control (7.00 mm). A lower AWT ($p < 0.05$) was recorded in the control group (27.90 g) compared to the VC (32.08 g) and VE (32.04 g) groups. HU was higher ($p < 0.05$) in VC + VE group (88.95) compared to control (86.56) and VE (87.20) groups. This study concludes that supplemental VC and VE could enhance some internal and external EQC of IFE Hens.

3.6.15. Effects of Vitrification on Cumulus Expansion and Nuclear Maturation of Abattoir-Derived Cattle Oocytes

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The present study aimed to assess the impact of vitrification on cumulus cell growth and nuclear maturation in bovine oocytes at different meiotic stages. Oocytes were divided into four treatment groups: positive control (immature fresh oocytes), negative control (immature oocytes exposed to CPA), immature vitrified, and mature vitrified. Heterogeneous cattle ovaries of unknown reproductive status were collected from a local abattoir and transported (37 °C) to the laboratory within 2 h of slaughter. The aspiration method was used to retrieve oocytes from the ovaries. Immature oocytes and mature oocytes were vitrified using a conventional straw vitrification method. The straws were pre-cooled by placing them horizontally on a Styrofoam rack above LN₂ vapour. Cryopreserved oocytes were then subjected to warming or thawing media and then immature vitrified oocytes were incubated in maturation medium for 22 h. Oocytes were evaluated for cumulus cell growth and nuclear maturation. Treatment means were compared using a least significant difference test. A high percentage of oocytes with fully expanded cumulus cells was recorded in the control group (83.1 ± 12.0) and mature vitrified (77.2 ± 14.0) group compared to the immature vitrified group (43.8 ± 15.0 ; $p < 0.05$). Immature oocytes exposed to CPA (16.7 ± 9.6) and the immature vitrified group (22.8 ± 10.3) recorded a high percentage of oocytes with no cumulus cells expansion following maturation ($p < 0.05$). A high percentage of oocytes reaching metaphase I was recorded for immature oocytes exposed to CPA (54.2 ± 19.3) and mature vitrified oocytes (60.3 ± 12.4 ; $p < 0.05$). Both the control group and immature vitrified group recorded a high percentage of oocytes reaching metaphase II (48.5 ± 6.0 ; 42.3 ± 7.8), respectively, compared to the matured vitrified oocytes (29.2 ± 13.2 ; $p < 0.05$). In conclusion, the results demonstrate that vitrifying bovine oocytes during the immature stage results in a higher maturation rate post-warming, indicating that the immature stage is more suitable to effective oocyte cryopreservation.

3.6.16. Fetal Losses in Embryo-Recipient Goats Supplemented with Progestogens

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In small ruminants, up to 40% of fertilized ova are lost during early gestation due to inadequate progesterone, thus affecting uterine environment and embryo survival. Progesterone supplementation has been proposed to improve early pregnancy maintenance; however, results have been inconsistent. This study aimed to evaluate the effect of progestogen supplementation post-embryo transfer on pregnancy rate and embryo, fetal, and

neonatal survival in goats. A total of 207 thawed Angora embryos were semi-surgically transferred into 111 Criolla Neuquina goats (day 0 = embryo transfer). All procedures were conducted under standardized conditions with the same operators and genetic material. At embryo transfer, recipients were randomly assigned to two treatments: progestogen supplementation (intravaginal sponges containing 60 mg MAP, replaced every 17 days for 68 days; $n = 37$) or non-progestogen supplementation (Control; $n = 74$). Pregnancy was diagnosed by ultrasonography on days 28 and 78 post-transfer to assess embryo and fetal survival. Pregnancy rate was calculated as pregnant recipients per total recipients, embryo survival as viable embryos per transferred embryos, fetal survival as viable fetuses per viable embryos, and neonatal survival as kids born alive per viable fetuses. Pregnancy and embryo survival rates did not differ between treatments (15/37; 41% and 24/67; 36% in progestogen-supplemented vs. 32/74; 43% and 43/140; 31% in non-supplemented goats; $p > 0.05$; Fisher's exact test). In contrast, both fetal and neonatal survival were identical for each treatment and significantly lower in the progestogen-supplemented group (7/24; 29%) compared with the non-supplemented group (43/43; 100%; $p < 0.05$). Laboratory analyses ruled out *Brucella melitensis*, CAEV, and *Toxoplasma gondii* in affected fetuses and dams. Possible mechanisms postulated in fetal losses comprise a sharp decline in progesterone after sponge removal or increased oxytocin and prostaglandin due to vaginal mechanical stimulation, inducing luteolysis. In conclusion, progestogen supplementation via intravaginal sponges is not recommended for recipient goats in embryo transfer programs.

3.6.17. Hormonal Dynamics and Reproductive Efficiency in Rabbit Does Fed Clove (*Syzygium aromaticum*)

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This study assessed the effects of dietary inclusion of *Syzygium aromaticum* (clove) bud powder on hormonal profiles and reproductive efficiency in rabbit does. Thirty-six does (2–3 months old; 600–650 g) were randomly allocated to four treatment groups—T1 (0 g/kg), T2 (5 g/kg), T3 (7.5 g/kg), and T4 (10 g/kg)—for 20 weeks in a completely randomized design. Data were analyzed using ANOVA and Duncan's test. Several hormonal parameters (FSH, LH, estrogen, and progesterone) were measured, alongside reproductive indices such as conception rate, gestation length, litter size, litter weight, and pre-weaning survival. The moderate inclusion of clove significantly increased FSH and progesterone levels, with peak values in the T2 (20.88 mIU/mL and 1.70 ng/mL) and T3 (18.23 mIU/mL FSH) groups, indicating enhanced follicular and luteal functions. LH rose with increasing clove levels, highest in T4 (15.87 mIU/mL), though these were associated with reduced FSH and progesterone, suggesting possible hormonal dysregulation at higher doses. Estradiol showed a non-significant increase. Maternal weights at mating and conception rose with clove inclusion, highest in T3 and T4 (1850.11 g and 1950.89 g), respectively. Gestation length was significantly reduced in T2 and T4 (~28.5 days). T2 recorded the highest litter size (4.44 kits), lowest pre-weaning mortality (2.22%), and highest kit survivability (93.29%). These findings suggest that moderate clove inclusion (5–7.5 g/kg) can improve reproductive traits and hormonal balance in rabbit does, promoting sustainable production. However, there is a suggestion that higher doses may disrupt endocrine regulation.

3.6.18. Impact of Pasture-Based Feeding on Terpene Profiles in Goat Milk

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Regenerative agriculture (RA) is a system of agricultural practices that integrates livestock into holistic farming systems to restore ecosystems and improve soil health. Pasture diversity can influence the nutritional quality of animal products. Milk terpenes are bioactive compounds with antioxidant and anti-inflammatory activity that could also serve as biomarkers of animal diet. This study aimed to assess how RA pasture feeding practices influence the terpene profile of goat milk. Milk samples were collected from two farms: one with fully established RA practices (FARM-A, including cover cropping, rotational grazing) and another transitioning to RA (FARM-B). For FARM-B, milk was analysed before (goats mainly fed concentrate including alfalfa, maize, oat, wheat, beet pulp, sunflower meal, etc.) and after a grazing season (minimum of 5 days in pasture, 50% concentrate allowance plus pasture with ryegrass and a mixture of clovers). Terpenes were extracted by headspace solid-phase microextraction (HS-SPME) and analysed using gas chromatography-mass spectrometry (GC-MS), determining a total of eight terpenes: α -pinene, α -phellandrene, β -pinene, limonene, β -caryophyllene, p-cymene, α -copaene, and α -caryophyllene. Limonene, α -pinene, p-cymene, and β -caryophyllene were present in both farms. FARM-A samples also contained α -copaene, α -caryophyllene, and α -phellandrene, which were not present in FARM-B (both before and after RA pasture). FARM-B samples also contained β -pinene, which, along with p-cymene, significantly increased with grazing. Higher levels of α -pinene, α -copaene, α -caryophyllene, and β -caryophyllene were found for the pasture-fed goat milk of FARM-A compared with the samples before pasture of FARM-B. These findings suggest that a gradual transition from conventional feeding to grazing may be sufficient to increase terpene levels in goat milk. However, considering the small sample size and short duration of the study, further studies with larger sample sizes and longer feeding periods are required to confirm these observations.

3.6.19. Influence of Short-Term Storage at 4 °C, −20 °C or −80 °C on the Quality of Decellularized Porcine Oviductal Scaffolds

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In reproductive medicine, decellularized scaffolds offer new opportunities for treating reproductive tract pathologies and improving assisted reproductive technologies. After obtaining them through decellularization procedures, these scaffolds are usually stored at 4 °C, −20 °C, or −80 °C, although the effect of these temperatures on their quality is unknown. The objective of this study was to evaluate the short-term influence of these storage temperatures on the quality of the decellularized oviduct scaffolds derived from porcine species.

Complete oviducts were obtained from prepubertal animals (Large White × Landrace) and decellularized following a previously described immersion–agitation protocol [1]. Subsequently, the scaffolds were stored at 4 °C, –20 °C, or –80 °C for one month. Freshly decellularized oviducts were used as the control (n = 4 per group). After storage, samples from each group underwent differential scanning calorimetry (DSC) and were tested for cytotoxicity by co-incubating them with porcine spermatozoa for 3 h at 38 °C. Cytotoxicity analysis included the evaluation of sperm motility, kinematic parameters, viability, acrosomal integrity, and mitochondrial activity. The samples were also analyzed microbiologically, including counts of total aerobic bacteria (TAB), total coliforms (TC), and sulfite-reducing clostridia (SRC). The DSC results show no significant differences between the control group and the stored groups, indicating that storage temperature did not affect the thermal stability of the proteins ($p > 0.05$). No changes were observed in any sperm parameter evaluated after incubation of scaffolds with porcine spermatozoa ($p > 0.05$). However, storage temperature influenced TAB counts, which were significantly higher in oviducts stored at 4 °C and –20 °C compared to the control group ($p < 0.05$). Overall, these results demonstrate that decellularized oviductal scaffolds exhibit good stability after one month of storage. Nevertheless, bacterial growth can compromise scaffold quality, and storage at –80 °C is the most effective way to prevent contamination.

Reference

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3.6.20. Inter-Male Comparison of Sperm Freezability in Goats Using a Purpose-Built Sperm Quality Index

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Introduction: In several species, individual variation in sperm freezability has been documented, categorizing males by the response of their sperm to cryopreservation as either “good freezers” and “bad freezers”. Because sperm cryotolerance has been poorly described in goats, the objective of this study was to compare sperm freezability in this species using a recently developed tool, Sperm Quality Index (SQI). **Methods:** A total of 124 ejaculates from seven Saanen goat bucks were collected weekly using an artificial vagina over one year. After semen assessment, samples were frozen using a conventional method, stored in LN₂, and thawed at 37 °C. Thawed sperm were assessed for motility, viability, acrosome integrity, capacitation status, and membrane fluidity. After freeze-thawing, sperm quality significantly decreases; some sperm variables, such as motility, decrease, while others increase, such as acrosome-reacted spermatozoa. Thus, two SQIs were produced, SQI-1 included decreasing variables (progressive motility, viability, CTC-F, and acrosome integrity), whereas SQI-2 included increasing variables (CTC-AR and hyperfluid membranes). Possible differences between males were analyzed statistically. **Results:** Significant differences ($p < 0.05$) were detected between males 1 and 6 for SQI-1 (male 1: 28.1 ± 2.16^a ; male 2: 32.2 ± 1.98^{ab} ; male 3: 34.7 ± 1.73^{ab} ; male 4: 34.7 ± 1.64^{ab} ; male 5: 33.5 ± 1.76^{ab} ; male 6: 39.9 ± 1.82^b ; male 7: 38.3 ± 2.26^{ab}). Similarly, significant differences were found between male 1 and males 6 and 7 for SQI-2 (male 1: 73.0 ± 2.28^a ; male 2: 66.4 ± 2.55^{ab} ; male 3: 67.0 ± 2.20^{ab} ; male 4: 65.7 ± 2.22^{ab} ; male 5: 66.7 ± 2.09^{ab} ; male 6: 60.9 ± 1.08^b ; male 7: 64.4 ± 2.22^b). **Conclusion:** Differences observed in both SQI-1 and SQI-2 (specifically between male 1 and male 6, and between male 1 and males 6 and 7, respectively) indicated a reduced cryotolerance in male 1. These findings could be associated with a variation in seminal plasma and/or sperm plasma membrane composition. The

results support decision making regarding the selection of “good freezers” in ARTs or the implementation of specific modifications in cryopreservation protocols for “bad freezers”.

3.6.21. Magnetic Field and Medium Viscosity Influence the Kinetics of Oocytes Bound to Nanoparticles

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Magnetic manipulation of gametes and embryos using functionalized nanoparticles (NPs) offers a novel strategy to improve micromanipulation in assisted reproductive technologies (ARTs). Conjugation of functionalized magnetic NPs to a truncated version of porcine oviductal glycoprotein 1 (pOVGP1t) generates the NPOv complex, which specifically binds to the zona pellucida (ZP) of mature oocytes without internalization, enabling magnetic control. This study evaluated the effect of medium viscosity on the displacement behaviour of oocyte–NPOv complexes under a low-intensity magnetic field generated by a neodymium magnet. Oocyte–NPOv complexes were exposed to the S-02-02-N magnet ($2\varnothing \times 2$ mm, 150 g holding force) in media of different viscosities: phosphate-buffered saline (PBS), PBS supplemented with methylcellulose (MC: 0.1%, 0.5%, 1%), uterine fluid (UF) and oviductal fluid (OF). Displacement was recorded using a stereomicroscope-mounted digital camera and distance travelled, mean velocity and maximum acceleration were analyzed using Tracker (v6.0.8). Viscosity ranged from 0.92 cP in PBS to 140.83 cP in MC 1% ($n = 3$ replicates). Distance travelled was longer in PBS (0.39 ± 0.07 cm), MC 0.1% (0.37 ± 0.04 cm) and UF (0.35 ± 0.03 cm), and shorter in MC 1% (0.09 ± 0.02 cm; $p < 0.05$). Mean velocity and maximum acceleration values followed a similar trend, appearing higher in PBS and lower in MC 1% ($p < 0.05$). $n = 7$ – 9 per group. Data are presented as the mean $\pm$ standard deviation (SD). Overall, motion parameters declined progressively with increasing viscosity, confirming an inverse relationship between medium viscosity and magnetic responsiveness. These findings provide key insights into the design of magnetic-based micromanipulation tools for ART applications and microfluidic systems. Funding was provided by the Seneca Foundation-Murcia Region Science and Technology Agency ‘Grants for the implementation of projects for the development of scientific and technical research by competitive groups (22001/PI/22)’.

3.6.22. Meat Quality of Fatteners Reared in Two Production System

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The quality of meat from fattening pigs is determined by numerous factors related to the production system, housing conditions, and feeding practices. Modern consumers perceive organic products as safer for human health than conventionally produced food and as aligning with the strategy of a sustainable, low-emission economy. The aim of this study was to compare the technological properties, basic chemical composition, and

fatty acid profiles of pork derived from organic and conventional production systems. The research material consisted of 24 samples of the *longissimus thoracis* muscle from Puławska × Polish Large White fatteners reared under conventional (n = 12) and organic (n = 12) production systems. Physical and chemical parameters as well as the fatty acid profile of the meat were evaluated. Each sample was analysed in triplicate. When analyzing the technological properties of the muscles, statistically significant differences in pH₁ and pH₂₄ were observed. Significantly higher values of these parameters were recorded in organic loin (pH₁ = 6.25; pH₂₄ = 5.57) compared to conventional loin (6.07 and 5.40, respectively). Lower pH values contributed to reduced water-holding capacity and greater muscle juice exudation (drip loss) during storage in conventional loin compared to organic loin (21.41% vs. 18.5% and 5.53% vs. 3.04%, respectively). Organic loin, compared to conventional loin, was characterised by a significantly darker meat colour, as indicated by a lower L* value (49.5 vs. 53.1). Conventional loin contained more intramuscular fat (3.5%), whereas organic loin contained less IMF (2.6%). Organic meat exhibited a significantly lower proportion of saturated fatty acids and a higher proportion of monounsaturated fatty acids compared to conventional loin (39.75% vs. 42.56% and 48.60% vs. 45.4%, respectively). The information on loin quality obtained in this study is undoubtedly relevant from a consumer perspective and is also of significance to the meat-processing industry.

3.6.23. Performance and Physiological Parameters of Lambs Fed Extruded Supplement Containing Different Protein Levels

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The supply of grains associated with protein supplements ensures a better balance between energy and protein, promoting productive gains. The objective of this study was to evaluate the performance and physiological parameters of lambs fed diets based on whole corn with extruded protein supplements containing different protein levels. The project was approved by the Ethics Committee on the Use of Animals of the Federal University of Uberlândia (protocol no. 092/16). Twenty-two crossbred lambs (Dorper × Santa Inês) with an initial body weight (BW) of 21.46 ± 6.36 kg and 3 ± 1 months of age were used and were randomly distributed according to age, BW, and body condition score. The animals were housed in group pens for 62 days in a randomized block design and distributed into four experimental groups differing in the protein content of the extruded supplements (20, 24, 28, and 32% crude protein). Feeding was carried out three times a day at 8:00 AM, 12:00 PM, and 4:00 PM. The animals were weighed weekly and evaluated for average daily gain (ADG) and final weight (FW). Heart rate (HR), rectal temperature (RT), and ruminal movements (RM) were measured at 2:00 PM on days 0 (start), 6, 12, 18, 24, 30, 36, 42, 48, 54, and 60. The data were tested for variance assumptions using SAS software, and the means were compared using Tukey's test at a significance level of 5%. The protein level did not influence the variables ADG ($0.284 \text{ kg day}^{-1}$), PF (39.40 kg), HR (72.57 bpm^{-1}), TR ($39.35 \text{ }^{\circ}\text{C}$), and MR (two movements per 5 min^{-1}) ($p > 0.05$). It was concluded that lambs fed high-grain diets based on whole corn and supplemented with extruded protein sources containing varying crude protein levels maintain satisfactory growth performance

and stable physiological parameters, demonstrating effective metabolic adaptation and homeostatic regulation under high-concentrate feeding systems.

3.6.24. Prospective Evaluation for Retrieval and Equilibrium Duration on Bull Epididymal Sperm Cryopreservation

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The cauda epididymis offers mature sperm for postmortem retrieval and cryopreservation, requiring effective retrieval techniques and equilibrium durations for successful cryopreservation. This study evaluated the impact of retrieval techniques and equilibration durations on cauda epididymal sperm quality from *Bos indicus* bulls. Testes from thirty-two bulls were collected at a slaughterhouse postmortem, transported at 5 °C, and sperm were retrieved 6 h after slaughter using either retrograde flushing or the slicing float-up technique with an egg yolk citrate extender (fraction A). Samples were equilibrated for either 120 or 240 min at 5 °C; subsequently, they were diluted at 1:1 with fraction B (with 12% glycerol), loaded in 0.25 mL straws, and cryopreserved using liquid nitrogen vapour. Sperm motility and velocity traits were evaluated post-retrieval, post-equilibration, pre-freezing, and post-thaw with the aid of a computer-assisted sperm analysis system. Across all stages, the retrograde flushing technique significantly yielded sperm with higher total motility, progressive motility, and velocity indices (curvilinear velocity, straight line velocity, and time average velocity) in comparison to slicing float-up technique samples ($p < 0.05$). Meanwhile, samples equilibrated for 240 min had sperm with higher total motility, progressive motility, and velocity indices (curvilinear velocity, straight line velocity, and time average velocity across all stages in comparison to 120 min of equilibration ($p < 0.05$)). Thus, bull cauda epididymis sperm quality remained satisfactory when using the retrograde flushing retrieval technique and equilibration for 240 min.

3.6.25. Protective Effect of the Ethanolic Extract of *Punica granatum* L. During the Cooling of Bovine Ovarian Tissue

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Introduction: Reproductive biotechnologies such as cryopreservation are essential for preserving reproductive potential, particularly in species of high zootechnical value. During ovarian collection and transport, cooling of the tissue is a critical step. In this context, the addition of antioxidant compounds has been investigated to enhance cellular integrity and prevent oxidative and structural damage. Extracts of *Punica granatum* L. have been widely described for their strong antioxidant properties, promoting cellular protection against free radicals. Therefore, this study aimed to evaluate the effect of different concentrations of pomegranate extract during ovarian tissue preservation. **Methodology:** The ethanolic extract of *Punica granatum* L. peel (EEPG) was produced according to Nascimento et al. (2018).

Ovaries ($n = 5$) were collected, fragmented, and distributed into groups maintained in 0.9% saline solution supplemented with antibiotics (penicillin and streptomycin, 100 $\mu\text{g/mL}$), in the absence (CTRL) or presence of 10 and 100 $\mu\text{g/mL}$ of EEPG, and stored at 4 °C. Lipid peroxidation was assessed using the thiobarbituric acid reactive substances (TBARS) method. Data were analyzed using two-way ANOVA in GraphPad Prism, with significance set at $p < 0.05$. **Results:** The following values were obtained: Fresh control (SEM = 0.107975). At 4 h, SEM values were CTRL = 0.565526, 10 $\mu\text{g/mL}$ = 0.105036, and 100 $\mu\text{g/mL}$ = 0.164488; at 12 h, CTRL = 0.998575, 10 $\mu\text{g/mL}$ = 0.118822, and 100 $\mu\text{g/mL}$ = 0.081847; and at 24 h, CTRL = 0.117856, 10 $\mu\text{g/mL}$ = 0.098463, and 100 $\mu\text{g/mL}$ = 0.06771. No significant differences were observed among the experimental groups or between the treated groups and the fresh or conserved controls at any evaluated time point ($p > 0.05$). **Conclusion:** At the tested concentrations, the extract appears to be safe for ovarian tissue, maintaining redox balance throughout the storage period. However, further analysis of follicular morphology is required to confirm the effectiveness of EEPG in preserving ovarian tissue quality.

3.6.26. Protective Effects of N-Acetylcysteine (Nac) Against Di- Ethyl Phthalate (Dep)-Induced Ovarian Dysfunction in Female Wistar Rats: A Model for the Inclusion of Antioxidants in Livestock and Human Diets

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Di-Ethyl-Phthalate (DEP) is an industrial chemical widely used as a plasticizer to improve the flexibility of plastic products. DEP does not covalently bind with plastics, causing its leaching into feed and water. Exposure of DEP has been reported to have adverse effects on livestock and human reproduction. The aim was to investigate the protective effects of N-acetylcysteine (NAC) in mitigating ovarian dysfunction caused by Di-Ethyl-Phthalate. Assessment of ovarian dysfunction was evaluated by histopathological, biochemical, and hormonal assays. Twenty female adult albino rats were divided into four groups of five rats each. Group A (rats were orally administered with 0.5 mL of olive oil to serve as the control), Group B (rats received 100 mg/kg body weight oral N-acetylcysteine), Group C (administered DEP 500 mg/kg per day body weight suspended in 0.5 mL olive oil, orally), and Group D: (DEP + NAC-treated group) were treated with DEP concomitantly with NAC at the same previous doses for (7) days. The histopathological results of this study revealed that DEP caused degeneration of the granulosa cells, with severe vascularization and mild congestion of the ovarian stroma, while Group D showed improved ovarian connective tissues and interstitial cells. The results of the biochemical assays revealed that DEP slightly increased the lipid peroxidation level and slightly reduced glutathione content (GSH), superoxide dismutase (SOD) activity, and catalase activity, which was ameliorated in Group D. The hormonal assay results showed a slight decrease in the levels of Follicle-Stimulating Hormone (FSH) and Estrogen in Group C, which improved significantly in Group D. Conclusion: Treatment with NAC can protect against DEP-induced ovarian dysfunction by decreasing inflammation and oxidative stress and maintaining optimum hormonal levels. This provides a potential justification for the inclusion of antioxidants in livestock and human diets to reduce the reproductive side-effects caused by exposure to plastic chemicals.

3.6.27. The Assessment of a Modified Protocol for Rabbit Semen Cryopreservation

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Cryopreservation allows sperm storage for long periods, facilitating genetic improvement. In rabbits, there are no standardized protocols ensuring adequate sperm quality upon thawing. The objective was to assess a modification of the rabbit semen cryopreservation protocol from the University of Molise (Italy). Semen was collected weekly from five New Zealand rabbit bucks; only ejaculates presenting $\geq 70\%$ progressive motility (PM) and $\geq 80\%$ viability (VB) were used to form a pool. Sperm PM, VB, normal (NS), acrosome integrity (AI), plasma membrane integrity (MI), and membrane functionality (MF) were assessed before and after cryopreservation. Each pool ($n = 10$) was diluted, at room temperature, in a Tris–Citric Acid–Glucose (TCG) medium without cryoprotectants; sperm concentration was adjusted to 200×10^6 sperm/mL. Sperm were cooled to 5°C in approximately 90 min; then, the pool was split into two parts, into which were added TCG containing dimethyl-sulfoxide (16%) and sucrose (0.1 M) as follows: one part (T1) was diluted in one step, packed in 0.25 mL straws, and equilibrated for 45 min; the other part (T2), was diluted in three fractions with a 10-min interval between them, packed in 0.25 mL straws, and equilibrated for 15 min. Then, straws were exposed to nitrogen vapors for 10 min and plunged into liquid nitrogen. For thawing, straws were immersed in water at 50°C for 10 s. A *t*-test was performed between treatments. After thawing, sperm variables for T1 vs. T2 were PM 35 ± 5.77 vs. $35 \pm 4.21\%$, VB 50.9 ± 5.2 vs. $59.2 \pm 3.72\%$, AI 57.6 ± 6.00 vs. $56.4 \pm 4.69\%$, NS 82.2 ± 02.27 vs. $84.7 \pm 0.96\%$, MF 35.5 ± 5.94 vs. $39.6 \pm 3.46\%$, and MI 43.6 ± 5.53 vs. $53.9 \pm 6.50\%$; there were no significant differences between treatments. In conclusion, the addition of freezing medium as either one step or in multiple fractions produced the same results. However, the next step is to perform *in vivo* tests to assess the fertility and prolificacy of both protocols. This study is supported by UNAM PAPIIT IN206524/CI2441.

3.6.28. The Effect of Laminitis on Seasonal Changes in Metabolic Parameters in Horses

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The aim of this study was to compare within-horse changes and between-horse differences in selected metabolic parameters for twenty cold-blooded mares, of which ten belonged to a group with a history of laminitis (LG) and ten formed the control group (CG). Measurements were conducted during four seasonal sampling periods in March (S0), May (S1), July (S2), and September (S3). Changes within individual horses over the season were determined using the coefficient of variation (CV) for each animal and differences between groups were evaluated using the *t*-test or Mann–Whitney test, depending on normality. LG showed a significantly higher insulin CV (LG 20.06% vs. CG 7.52%; $p = 0.023$). For fructosamines, the CV was higher in CG (26.61% vs. 20.09%; $p = 0.0312$), whereas glucose ($p = 0.279$) and body weight ($p = 0.0576$) showed no group differences. Variability between horse groups within samples was analyzed using the F-test. In S0, no differences

were observed for glucose ($p = 0.607$) or insulin ($p = 0.980$), and fructosamines showed borderline significance ($p = 0.05$). Body weight variability was higher in LG ($p = 0.010$). In S1, greater dispersion in LG was found for fructosamines ($p = 0.016$) and insulin ($p = 0.0329$), while glucose ($p = 0.952$) and body weight ($p = 0.0981$) did not differ. In S2, LG showed markedly higher CV for glucose ($p < 0.0001$) and insulin ($p = 0.0007$), whereas fructosamines ($p = 0.282$) and body weight ($p = 0.7418$) remained comparable. In S3, no differences were detected for glucose ($p = 0.512$), fructosamines ($p = 0.147$) or insulin ($p = 0.872$), while body weight variability was again higher in LG ($p = 0.0046$). Overall, laminitic horses displayed greater seasonal fluctuations in metabolic parameters and, in several periods, more pronounced between-horse variability, suggesting a less stable metabolic response to seasonal conditions.

3.6.29. Thermoregulatory Mechanisms of Caracu Young Bulls Under Tropical Climate

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The present study aimed to investigate correlations between the environmental variables, coat characteristics, and physiological responses of Caracu young bulls. Forty-eight Caracu young bulls (mean $\pm$ SD: 283 $\pm$ 23 days old; 240 $\pm$ 38 kg body weight) participated in the experiment conducted at Sertãozinho, Brazil, in October 2025, on two days separated by a 13-day interval. Data collection was conducted in the morning and lasted approximately 2 h 30 min. Data on the variables sweating rate (g/m²/h), respiratory rate (num./min), and rectal temperature (°C) were collected and the exact time at which each animal was sampled was recorded. Then, these data were combined with the relative humidity (RH, %), air temperature (AT, °C), and the Temperature and Humidity Index (THI) of that moment. Thermal environment variables at each 20 min point were obtained from the local weather station (CIIAGRO). Data were analyzed using descriptive statistics, non-parametric tests, and correlation analysis. During data collection, the mean AT was 28 °C (range: 23.3–30.7 °C), UR averaged 49.7% (range: 33.8–75.7%), and ITU averaged 76.4 (range: 70–79.9). The sweating rate showed a positive correlation with rectal temperature ($r = 0.30$; $p = 0.005$), AT ($r = 0.39$; $p < 0.001$), and THI ($r = 0.32$; $p = 0.002$) and a negative correlation with RH ($r = -0.27$; $p = 0.01$). The respiratory rate showed a positive correlation with rectal temperature ($r = 0.22$; $p = 0.04$) and a negative correlation with RH ($r = -0.30$; $p = 0.004$), indicating that higher humidity tends to reduce heat loss through respiratory pathways. The results highlight the relevance of the thermoregulatory behaviour of Caracu cattle under tropical conditions. Selecting animals with efficient physiological responses, along with strategic management during high-THI periods, can contribute to resilience and productive efficiency in tropical beef cattle farming. We conclude that young Caracu bulls are affected by a challenging thermal environment and activate physiological responses that are also influenced by environmental conditions. Sweating rate and respiratory rate responses were found to decrease with increasing relative humidity.

3.7. Aquatic Animals

3.7.1. *Artemia salina* Nauplii as a Screening Tool for Heavy Metal Toxicity

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Acute toxicity bioassays are essential tools for assessing the ecotoxicological risks of chemical pollutants in aquatic environments. Among these, the *Artemia* nauplii immobilization test offers a rapid, cost-effective, and standardized approach for screening toxic substances. This study evaluated the acute toxicity of four heavy metal sulfates (copper, zinc, cadmium, and lead) using *Artemia salina* nauplii, with potassium dichromate as the reference toxicant. *Artemia* cysts were hatched under optimized conditions, including controlled salinity (35 ppt), pH (8.0–8.5), temperature (28 °C), and continuous aeration to ensure maximum hatching success and larval viability. Toxicity tests were conducted using stage II nauplii, selected for their enhanced sensitivity to environmental contaminants, exposed to serial dilutions of each heavy metal for 24 h following standardized protocols. Mortality rates were recorded to determine acute toxicity parameters, including 24 h LC₅₀ and LC₁₀₀ values. The results indicated that copper was the most toxic heavy metal tested, followed by lead, cadmium, and zinc, with the toxicity ranking as follows: Cu > Pb > Cd > Zn. The LC₅₀ values demonstrated that *Artemia* nauplii exhibited differential sensitivity to heavy metals, with copper and lead showing significantly higher toxicity compared to cadmium and zinc. Despite this differential toxicity, *Artemia* nauplii demonstrated considerable tolerance to heavy metal exposure across all the tested concentrations. These findings contribute to the classification and comparative assessment of heavy metal toxicity in aquatic invertebrates and underscore the utility of the *Artemia* bioassay as a first-tier screening tool for environmental risk assessment.

3.7.1. *Cucullanus* (Rhabditida, Cucullanidae) Parasitizing the Thinlip Conger *Gnathopis mystax* (Delaroche, 1809) (Congridae) from the Coast of Calabria (Italy)

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Gnathopis mystax, commonly known as the thinlip conger, is a benthic species inhabiting muddy or sandy substrates of the continental shelf and upper continental slope, typically between 80 and 400 m deep. This fish is distributed throughout the Mediterranean basin and the eastern Atlantic Ocean. It is a predatory species feeding mainly on benthic organisms and small fish, occasionally caught by deep trawl nets or longlines (*palangres*). Although its flesh is of good quality, it is not a target of specific fisheries and appears in markets only in small quantities. Knowledge about the parasitic fauna of *G. mystax* is currently lacking. This study provides the first parasitological assessment of *G. mystax* from the southern Tyrrhenian Sea (Calabria, Italy), focusing on the presence, prevalence, and morphology of nematodes belonging to the genus *Cucullanus*. A total of 41 specimens of *G. mystax* were examined. The fish were collected along the Tyrrhenian coast of southern

Calabria, specifically in the areas of Vibo Valentia and Gioia Tauro, provided by local professional fishermen. Helminths were collected, mounted, photographed, preserved, and identified morphologically, including scanning electron microscopy (SEM) observations. The recovered nematodes were assigned to the genus *Cucullanus* and prevalence and mean intensity values were calculated. However, comparisons remain limited due to the absence of previous parasitological studies on *G. mystax*, underscoring the novelty and significance of these findings. Differences in host biometric data between the two sampling sites, and correlations between parasite abundance and fish length or weight, were also analyzed. To our knowledge, this study represents the first record of *Cucullanus* spp. in *Gnathophis mystax*. The genus *Cucullanus* comprises a diverse group of gastrointestinal nematodes commonly parasitizing marine teleosts. This research adds valuable data to the parasitic biodiversity of Mediterranean fishes and provides a basis for future taxonomic and ecological investigations.

3.7.2. Enhancing Marine Biodiversity Through the Promotion of Underutilized Species Using Innovative Processing Methods

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Fish are an essential part of a balanced diet and represent the Mediterranean dietary model, which is renowned for its health benefits and nutritional value. The high-quality proteins, omega-3 fatty acids, vitamins and essential minerals found in fish have led to increasing consumer demand for seafood. However, the continuous expansion of this market, together with sustainability challenges, highlights the need to diversify seafood production through innovative processing strategies that aim to enhance the sustainability, productivity, and quality of seafood products. Promoting underutilized marine species, such as *Spicara smaris*, and seasonal species, such as *Coryphaena hippurus*, through innovative processing and preservation techniques has the potential to reduce pressure on target species, enhance marine biodiversity, and minimize waste. This approach is based on the principles of the circular economy and promotes the sustainable exploitation of marine ecosystems. Recent technological advances have focused on the study of the application of traditional methods, such as salting and smoking, reinterpreted through innovative and sustainable technologies. In particular, the use of salting and cold smoking techniques using low-sodium salt mixtures and natural antioxidants has been investigated as an effective strategy to enhance the sensory quality, nutritional profile, and microbiological stability of underutilized fish species. The objective of the present study was to adopt a multidisciplinary approach in order to evaluate the quality of the processed product, through sensorial, biochemical and technological analyses. The results obtained highlighted the possibility of producing a product with a sodium content reduced by more than 30% that preserves the sensory characteristics, high protein content, and omega-3 fatty acid levels of traditional products. In conclusion, adopting innovative processing strategies can contribute significantly to the sustainable use of aquatic resources by turning underutilized species into high-quality products, reducing dependence on conventional target species, and fostering new economic opportunities in the fisheries sector.

3.7.3. From Emotion to Action: Compassion as a Driver for Marine Conservation

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Urbanisation and reduced direct contact with nature have weakened children's opportunities to interact with wildlife, making it harder for them to feel concerned about biodiversity loss (Truong & Clayton, 2020). Existing research has predominantly focused on familiar, terrestrial vertebrates, contributing to a persistent taxonomic bias that favours charismatic species when addressing conservation issues (Bègue-Shankland, 2022). Given the central ecological importance of marine ecosystems, fostering awareness and understanding of ocean biodiversity among younger generations is essential. While few studies have examined empathy as a precursor to pro-environmental behaviour, compassion has not received any empirical attention despite its potential as a driving force for conservation. Empathy refers to the ability to understand and share another's emotional state (Decety & Holvoet, 2021), while compassion (motivational empathy) adds an action-oriented component aimed at alleviating others' suffering. Understanding these emotional mechanisms is therefore crucial for environmental education: is developing empathy sufficient, or must compassion be cultivated to encourage meaningful conservation behaviour? Children remain notably understudied despite their potential to become powerful agents of environmental change (Gottesdiener & Davallon, 1999). Building on Miralles et al. (2019), who suggested that individuals preferentially choose phylogenetically closer species, we developed a serious game in partnership with Océanopolis. The study replicated their paradigm using only marine species from ten different clades across two conditions. In the empathy condition, participants selected the species they felt closest to; in the compassion condition, they chose which species they would save. Contrary to Miralles et al. (2019), no significant differences emerged for empathy. However, significant effects appeared in the compassion condition ($p < 0.05$), with children more likely to save phylogenetically closer species. This work contributes to the broader question of compassionate conservation, whereby emotional connections with animals increase the likelihood of humans acting to protect them.

3.7.4. Life History of the Marine Leech *Cochimibdella mexicana* (Hirudinea, Piscicolidae): Insights from Laboratory Rearing

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Marine leeches (Annelida: Hirudinida) are typically fish parasites, yet little is known about species associated with invertebrates. *Cochimibdella mexicana*, parasitizing the two-spot octopus *Octopus bimaculatus*, in Baja California, México, represents a unique ecological association. This study characterizes the reproduction, development, feeding behavior, and fasting survival of the leech. Between February and April 2025, live leeches ($n = 143$) were collected from the mantle and arms of wild *O. bimaculatus* and maintained under laboratory conditions. Mating occurred within 24 h via hypodermic insemination. Three days post-copulation, leeches deposited circular, flattened cocoons (~1.4 mm diameter) containing a single embryo. Cocoons were divided into two experimental groups (18° C and 14 °C). Embryonic development lasted 36 days at 14 °C and 21 days at 18° C. Cleavage, gastrulation, and organogenesis followed patterns typical of aquatic leeches, with fully formed juveniles hatching with functional sensory structures and immediate attachment behavior. In feeding trials, both hatched juveniles and adults consistently refused to feed on

invertebrate prey and fed exclusively on blood from fish hosts. Hatchlings (2.1 ± 0.5 mm) initiated hematophagy within 24–72 h and survived up to 31 days unfed. Our findings provide, for the first time, key life-history traits of *C. mexicana*, revealing a complex ecological interaction where an invertebrate acts as a substrate and mechanical vector for a parasite that requires a vertebrate (fish) for trophic sustenance. These observations contribute to understanding the life cycle and transmission dynamics of *C. mexicana* within benthic–pelagic food webs.

3.7.5. Circular Economy Approaches to Enhance the Sustainability of Supply Chains in Aquatic Animal Production

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The global seafood sector generates substantial volumes of by-products, such as fish viscera, heads, skins, frames, and crustacean shells. These materials are often treated as waste, contributing to environmental pressure and economic loss, despite being rich in compounds with functional value. Their valorisation represents an opportunity to reduce waste generation, enhance resource efficiency, and support the transition toward more sustainable and circular production systems. In this context, the present study focused on the recovery of high-value bioactive compounds from marine processing side streams, including omega-3 polyunsaturated fatty acids, protein hydrolysates, and natural antioxidant molecules. Particular attention was given to crustacean by-products, which are notable for their high content of carotenoids and peptides with recognised nutraceutical and pharmaceutical potential, expanding the range of compounds that can be sustainably produced from seafood by-products. The extraction of astaxanthin and peptide fractions was carried out using green extraction techniques, specifically Supercritical Fluid Extraction (SFE) and enzymatic hydrolysis, respectively. These techniques preserve the quality of the recovered compounds while limiting solvent use and environmental impact. The antioxidant activity, assessed through radical scavenging (DPPH) assays, confirmed their high bioactive properties, supporting their potential use as functional ingredients for nutraceutical, feed, food, and cosmeceutical applications. The results demonstrate a basis for scalable and transferable valorisation pathways, supporting the development of high-value bio-based products derived from seafood by-products. By shifting from disposal to resource utilisation, this work strengthens circularity, reduces environmental pressure, and contributes to a more sustainable, efficient, and competitive seafood sector.

3.7.6. Crustaceans from the Mediterranean Sea: Possible Sentinel Species for Metal Pollution in Aquatic Environments

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Introduction: The marine environment is an ecosystem where toxic metals can accumulate, affecting the health of aquatic organisms. Filtrating species, such as crustaceans, can be considered sentinels of marine environmental pollution. The aim of this study was to evaluate the presence of toxic metals in crustaceans, crabs, and shrimps from the Mediterranean Sea. **Methods:** The study was carried out on the carapace and pulp of various crabs, including warty crab (*Eriphia verrucosa*), brown crab (*Cancer pagurus*), and marbled crab (*Pachygrapsus marmoratus*), and on carapace and abdomen of pink shrimp (*Parapenaeus longirostris*), collected along the Sicilian coastlines. All samples were digested with HNO₃

and H₂O₂ and analyzed in ICP-MS for the determination of Hg, Cd, Pb, and As. Data were considered significant at $p < 0.05$ and $p < 0.01$. **Results:** The results confirmed the presence of toxic metals in all crustacean samples, with the highest concentration of Hg and Pb, an intermediate concentration of As, and the lowest of Cd. The metal content was higher in the carapace than in the pulp of crabs and in the abdomen of shrimps. In particular, in the carapace pulp of *E. verrucosa*, Hg (0.543 ± 0.062 mg/Kg) and Pb (0.554 ± 0.016 mg/Kg) were higher than MRL of 0.50 mg/kg, while in *C. pagurus*, only the Pb levels were higher (0.671 ± 0.062 mg/Kg) [1]; in both crabs and shrimps, As was higher than the MRL of 0.10 mg/kg [2]. However, the Metal Pollution Index (MPI), which calculates the metal accumulation in each organ and tissue, was 1 (the safety level) in crabs and shrimps. **Conclusions:** The results obtained confirmed that crabs and shrimps can be valid sentinel species of the Mediterranean Sea. Metal pollution is correlated to its exposure in the aquatic environment and the residual levels of Hg, Pb, and As, higher than the MRLs, also represent a potential toxicological risk for consumers.

References

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3.7.7. Decision-Making in Zebrafish (*Danio rerio*): A Study on Fish Cognition

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Introduction: Locomotion is a very important behaviour exhibited by animals for foraging, survival, escaping from predators, etc. The locomotory behaviour of zebrafish is extensively used as a reliable behavioural marker in the area of ecotoxicological, pharmacological, and neurotoxicological research. The locomotory robustness and high sensitivity to any external stimuli of zebrafish make them a convincing model with which to study cognitive biology. The aim of our research is to study the decision-making ability of adult zebrafish from the perspective of foraging. **Methods:** In order to obtain a crucial understanding of the decision-making skills of adult zebrafish (*Danio rerio*) for food search, we have created an experimental model system that focuses on the fundamental idea of foraging and the possibility of finding the quickest or simplest path to a food source. The model consists of a common zone and four different pathways (Path A, B, C, and D). Among them, the first one (Path A) is straight, the second (Path B) is curved and is simpler the third path (Path C) is blocked, and is the simplest path, and the fourth one (Path D) is the most complicated pathway. **Results:** Our findings indicate that the majority of fish selected Path C to reach the food source, despite this route being obstructed. This observation suggests that the fish preferentially chose Path C because it represents the most direct and accessible route to the food source, and its comparatively greater width may facilitate easier navigation relative to the other available paths. **Conclusions:** Adult zebrafish demonstrated a preference for the most direct and seemingly simple route to the food source, even when obstructed. In our study, we highlight zebrafish as a “species in the spotlight”, and provide an example of how to better understand their decision-making abilities in terms of locomotor decisiveness.

3.7.8. Effects of Multiple Stressors: Microplastics and Climate Change on Stress Response Gene Expression in *Mytilus galloprovincialis*

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Climate change and microplastic pollution are emerging environmental stressors that increasingly threaten marine ecosystems and filter-feeding organisms. The Mediterranean mussel, *Mytilus galloprovincialis*, is particularly vulnerable due to its ecological and economic importance. This study explored how elevated seawater temperature and microplastic exposure alone or/in combination affect the expression of key antioxidant and stress-related genes: catalase (cat), superoxide dismutase 1 (sod1), superoxide dismutase 2 (sod2), and heat shock protein 70 (hsp70). Mussels (27.04 ± 6.45 g; 4.02 ± 0.21 cm) were assigned to four treatments: (1) control (18 °C, synthetic seawater), (2) elevated temperature (24 °C), (3) microplastics (0.06 mg L^{-1}), and (4) combined elevated temperature and microplastics. After 21 days, gill and digestive gland tissues were analyzed using quantitative PCR (qPCR). Exposure to higher temperature caused a marked increase in hsp70 expression and moderate upregulation of antioxidant genes, suggesting the activation of protective mechanisms against oxidative and proteotoxic stress. Microplastic exposure alone triggered a milder antioxidant response, consistent with particle-induced oxidative imbalance. The combined treatment produced the strongest transcriptional activation, particularly in sod2 and hsp70, indicating synergistic effects between thermal and microplastic stressors. Overall, the findings suggest that climate-driven warming can intensify the harmful effects of microplastic pollution, compromising the mussel's oxidative defense capacity. Understanding such multi-stressor interactions is essential for predicting the resilience of marine bivalves under future ocean conditions.

3.7.9. Meta-Analysis of QTL Regions Associated with Pathogen Resistance in Rainbow Trout

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Rainbow trout (*Oncorhynchus mykiss*) is one of the most widely cultivated freshwater fish species, with an annual global production of nearly 900,000 tonnes. However, a major challenge is its susceptibility to a wide range of pathogens, which leads to severe disease outbreaks and significant economic losses in fish farms. To mitigate these impacts, genetic improvement programs can be accelerated through quantitative trait loci (QTL) mapping aimed at identifying genomic regions that are associated with disease resistance. Nonetheless, variations among studies, as well as differences in the quality and density of genetic maps, often hinder the identification of consistent QTLs across traits and experiments. To overcome these limitations, meta-analysis has emerged as a powerful approach to enhance the reliability of QTL detection. In this study, a meta-QTL (MQTL) analysis was performed to identify stable genomic regions associated with pathogen resistance in rainbow trout. In total, 138 QTLs related to pathogen resistance traits were collected from previous studies, resulting in the identification of 26 MQTLs. The average confidence interval (CI) of these MQTLs was reduced to 2.5 times smaller than the original QTLs, demonstrating enhanced precision in their genomic positioning. Notably, the MQTL located on chromo-

some 3 exhibited the narrowest CI and the highest number of projected QTLs, whereas chromosome 8 contained the largest number of MQTLs. These regions might represent key genomic hotspots for pathogen resistance, providing valuable targets for functional genomics research and supporting the development of disease-resistant rainbow trout lines.

3.7.10. Microbiota and Histological Alterations in Cultured *Isostichopus badionotus* Juveniles Affected by Skin Ulceration Syndrome

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The considerable decline in wild populations of the sea cucumber *Isostichopus badionotus* in the Yucatán Peninsula has been driven principally by overfishing and illegal harvesting. Consequently, aquaculture has been developed as an alternative to commercial fishing and as a strategy for the restoration of natural populations. Nevertheless, the emergence of various health issues, such as a larval disease and the skin ulceration syndrome (SUS), in juveniles has limited the success of its cultivation under controlled conditions. This study analyzed changes in the intestinal and skin microbiota of cultured juveniles during an outbreak of SUS. Juvenile *I. badionotus* were obtained from an experimental culture in Telchac Puerto, Yucatán, during a SUS outbreak. Samples of intestinal and skin tissues were processed for histological examination and 16S rRNA gene sequencing to characterize microbial communities. Histological analysis revealed that apparently healthy juveniles exhibited tissue alterations compatible with an early stage of SUS. Intestinal inflammation was detected in both stages of disease. However, severe epithelial damage and bacterial clusters were only observed in juveniles in the advanced stage of SUS. Microbiota analyses revealed significant differences in bacterial composition, particularly in the intestine, with a marked decrease in *Vibrio* and an increase in *Halarcobacter* (syn. *Arcobacter*), suggesting a dysbiosis process during disease progression. These findings highlight the complex microbial dynamics underlying SUS and emphasize the importance of microbiota monitoring for effective health management in *I. badionotus* aquaculture.

3.7.11. Occurrence of *Anisakis* sp. Infection in Mackerel (*Rastrelliger* sp.) from Traditional Markets in Bogor, Indonesia

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Anisakis spp. are zoonotic nematodes that are widely recognized as a food safety concern in marine fish. In Indonesia, however, information on *Anisakis* infection in fish sold at traditional markets is still limited, especially in inland areas of consumption such as Bogor. This study aimed to describe the occurrence and infection characteristics of *Anisakis* larvae in mackerel (*Rastrelliger* sp.) marketed in traditional markets in Bogor, Indonesia, as baseline information for fish parasitology and food safety considerations. A total of 35 mackerel were randomly collected from seven traditional markets in Bogor between May and June 2024. Each fish underwent physical examination followed by necropsy,

and the intestinal organs were examined using native parasitological methods. Larvae recovered from the samples were identified based on their morphological features under light microscopy, and infection levels were expressed as prevalence and degree of infection. All larvae identified in this study were third-stage (L3) *Anisakis* sp. Type 1, characterized by the presence of a boring tooth, ventriculus, and mucron. The overall prevalence of infection was 45.7%, with a mean degree of infection of 10.2 larvae per infected fish, indicating a commonly occurring infection with moderate intensity. Higher prevalence and infection intensity were observed in mackerel obtained from markets located in Bogor Regency compared to those in Bogor City. In addition, infection was more frequently found in larger fish, suggesting greater exposure over time. The results provide market-based evidence of *Anisakis* sp. infection in mackerel sold in traditional markets in Bogor. These findings underline the importance of routine monitoring of parasites in marketed fish and reinforce the need for proper handling and thorough cooking to minimize the potential zoonotic risk of anisakiasis.

3.7.12. Purkinje Cells in the Cerebellum of Japanese Medaka (*Oryzias latipes*) Fish Exposed to Graphene Oxide: A Histopathological Assessment

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Graphene oxide (GO) is an oxidized form of graphene that has the potential to be used as a nanomedicine for the treatment of neurodegenerative and other physiological disorders. We have previously demonstrated that GO exhibits minimal endocrine-disrupting effects in Japanese medaka (*Oryzias latipes*) due to physical aggregation and accumulation rather than direct endocrine interference. Moreover, males are more sensitive to GO than females. We have extended our investigations to the nervous system of the fish, concentrated on the cerebellum (CB), and considered Purkinje cells (PKCs), which play a significant role in motor control, as a potential target of GO. The experimental adult fish were exposed to GO in vivo by immersion (IMR) (20 mg/L in BSS) and by a single intraperitoneal (IP) injection (100 µg/g). Parallel controls were maintained either in BSS (IMR) or injected with nanopure water (IP). An evaluation of the CB was made in paraffin sections after haematoxylin/eosin (H&E) staining. The CB is located dorsally to the midbrain and caudally to the optic tectum and primarily consists of three lobular structures, the valvula cerebelli (VC), the corpus cerebelli (CC), and the lobus caudalis cerebelli (LC). The PKCs appear to be significantly larger with a droplet-like shape, prominent nucleus and nucleolus and are distributed between the granular and molecular layers in VC and CC, not in LC. We assessed the histomorphology, distribution/density, nuclear (N) and perikaryal (S) areas, and (N:S) ratio of the PKCs found in the CC area of the CB. We observed that females have apparently larger PKCs than males and GO exposure altered cell morphology and morphometry and reduced the distribution of PKCs, which was dependent on the exposure route and sex of the fish. Further studies are needed to elucidate the mechanistic basis of GO-induced neurotoxicity before its application as a nanomedicine for the treatment of various neurological disorders.

3.7.13. Reproductive Performance of the Redhead Goby, *Elacatinus puncticulatus*, in Captive Conditions: Firsts Insights

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The marine aquarium trade involves the commercialization of > 2500 species, yet few of these species are obtained from captivity. Fishes play an important role in the international marine trade, and the Gobiidae family is one of the most important, representing 5–7% of the total demand for marine organisms. To advance our understanding of culturing practices for aquarium fishes, the present study details the reproductive performance of the redhead goby, *Elacatinus puncticulatus*, a small, rocky reef fish used in the aquarium trade. Twenty-three reproductive pairs were monitored in laboratory conditions. The mean standard length of individuals was 25 ± 3 mm (mean $\pm$ SD), and males were bigger than females by at least 1 mm. We gathered data on the following parameters: (1) days until first spawning, (2) inter-spawning interval, and (3) number of eggs per spawning. Days until first spawning averaged 69 ± 40 days, being shorter when a female was in advanced gonadic development. When a female was paired with a bigger male, days until first spawning decreased, especially when the male was 6–8 mm bigger than the female. Inter-spawning intervals averaged 14 ± 13 days and were unrelated to standard length or the size difference between reproductive pairs. Finally, the number of eggs was 189 ± 69 and showed a positive relationship with female size, and tended to decrease with every new spawning event. The present work is the first to describe the reproductive performance of wild-caught redhead goby individuals in captivity, and such findings will potentially have implications for rearing the redhead goby for the aquarium trade.

3.7.14. Sperm Ultrastructure in the Family Opecoelidae (Digenea): First Data on the Genus *Opegaster*

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The ultrastructural study of spermatozoa in parasitic platyhelminths provides a set of characters whose usefulness for interpreting phylogenetic relationships has been demonstrated repeatedly in monogeneans, digeneans, and cestodes. Currently, ultrastructural spermatological data for the family Opecoelidae are available for thirteen species across five subfamilies: three Hamacreadiinae, two Helicometrinae, three Opecoelinae, three Opistholebetinae, and two Plagioporinae. The present study provides the first ultrastructural data for the genus *Opegaster* (Opecoelinae). Digeneans were collected from a brackish-water fish, giant mudskipper *Periophthalmodon schlosseri* (Pallas, 1770) (Gobiiformes: Oxudercidae), in Chumphon Province, Thailand. Live worms were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4), rinsed in the same buffer, postfixed in 1% osmium tetroxide in the same buffer, rinsed in MilliQ water, dehydrated in an ethanol series and propylene oxide, embedded in Spurr's resin, and finally polymerized at 60 °C. Ultrathin sections mounted on copper and gold grids were double-stained with uranyl acetate and lead citrate and examined using a JEOL 1010

transmission electron microscope. Sperm cells of *Opegaster* are filiform and sharpened at both extremities. They display ultrastructural characteristics that are usually observed in most digeneans, including two axonemes of different length presenting the trepaxonematan 9 + '1' pattern, parallel cortical microtubules, external ornamentation of the plasma membrane, spine-like bodies, mitochondria, and a nucleus; lateral expansion is absent. The organization and distribution of these characters along the spermatozoon are generally consistent with those observed in other opcoelids. However, some discrepancies arise when comparing the available data among subfamilies. Within the Opcoelinae, the male gamete of *Opegaster* corresponds to the type III sperm pattern of Bakhoun et al., as also found in the three previously studied species: *Labracetabulum gephyroberici*, *Opcoeloides furcatus*, and *Poracanthium furcatum*.

3.7.15. Water Quality and Welfare Awareness as Pillars of Sustainable Tilapia Culture in Egypt

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Egypt is one of the leading global producers of Nile tilapia, with aquaculture success closely related to water quality. The Tilapia Welfare Egypt Project aimed to enhance water quality management practices and improve fish welfare awareness among Egyptian tilapia farmers. A comprehensive case study was conducted across 12 farms representing four major tilapia farming regions in Egypt, with three farms per region. Instrumented farms monitored water temperature and dissolved oxygen daily and measured total ammonia nitrogen (TAN), pH, nitrite, alkalinity, and Secchi disk visibility at defined intervals. Fish welfare was assessed using regular biometric and environmental indicators recorded through the FAI Tilapia Welfare App. The 12 farms under study were monitored for a full production cycle of 6 to 8 months. According to the findings, farmers' understanding and perspectives on the significance of water quality in promoting fish health have significantly improved. Farms with monitoring equipment showed a greater ability to spot and address changes, leading to enhanced growth rates, lower mortality and more effective pond management. The results also showed that farmers' decisions on farm management were more based on results of water quality measures. Overall, the study achieved measurable outcomes in fish performance and promoted a cultural shift towards proactive water quality management, playing a crucial role in promoting sustainable aquaculture practices and tilapia welfare throughout Egypt.

Funding: This research received no external funding.

Data Availability Statement: The original contributions presented in this study are included in the article.

Conflicts of Interest: The authors declare no conflict of interest.

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