동물생명공학

OC26001

Propiconazole impairs mitochondrial OXPHOS through disruption of electron transport chain activity in porcine endometrial luminal epithelial cells

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Propiconazole (PCZ) is a widely used triazole fungicide detected in environmental matrices and biological tissues and is also an endocrine disruptor with potential to impair mitochondrial function. We hypothesized that PCZ may influence implantation-related cells, a process strongly dependent on mitochondrial activity. To test this, porcine endometrial luminal epithelial (pLE) cells were analyzed to evaluate endometrial receptivity. PCZ reduced mitochondrial enzyme activity and content as demonstrated by live-cell imaging. Seahorse assays revealed decreased ATP production, oxygen consumption rate, and respiratory capacity, indicating impaired oxidative phosphorylation. Co-treatment with pyrroloquinoline quinone (PQQ) partially restored cell viability in a dose-dependent manner, as assessed by MTT assay. PCZ increased proton leak and reduced *VDAC1* expression, suggesting altered membrane permeability. qRT-PCR showed suppression of complex I-related genes, whereas transcripts associated with complex IV and ATP synthase were elevated. Genes involved in mtDNA replication, transcriptional stability, and antioxidant defense were upregulated, indicating compensatory biogenesis and oxidative stress responses. These findings demonstrate that PCZ disrupts mitochondrial respiration in pLE cells primarily through complex I inhibition. The induction of complex IV genes, mtDNA maintenance factors, and antioxidant pathways reflects a compensatory response. Overall, these results provide insights into the reproductive toxicity of PCZ.

Key words : Propiconazole, mitochondrial dysfunction, endometrial receptivity

OC26002

Nest-building intensity in prepartum period is associated with distinct gut microbial profiles in loose-housed sows

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Prepartum nest-building is an instinctive maternal behavior driven by endocrine changes. Given the bidirectional gut–brain axis, variations in this behavior may reflect distinct gut microbial compositions. Therefore, this study evaluated whether prepartum nest-building intensity is associated with gut microbiota profiles in loose-housed sows. Thirty-nine crossbred sows were categorized into two groups according to their nest-building duration in the 24 h preceding parturition: shorter nest-building (SN, n = 19, 144.04 ± 64.21 min) and longer nest-building (LN, n = 20, 310.99 ± 54.88 min). Faecal samples were collected on day 1 after farrowing. Beta diversity differed consistently ($p < 0.05$) between SN and LN groups. Taxonomic analysis at the genus level revealed *Lactobacillus* was more abundant in the LN groups, while *Romboutsia* and *Terrisporobacter* were reduced. At the species level, the LN group exhibited higher abundances of *Lactobacillus amylovorus* and *L. ruminis* and lower abundances of *Clostridium butyricum*. LEfSe analysis identified *Peptococcus*, *Treponema*, *Oribacterium*, *Megasphaera*, *Catenisphaera*, and *Lactobacillus* as key microbial biomarkers for the LN group ($p < 0.05$), while *Romboutsia* and *Turicibacter* were more abundant in the SN group ($p < 0.01$). Functional prediction analysis revealed enrichment of pathways related to core metabolism and biosynthesis processes in the LN group, whereas transport and signal transduction pathways were enriched in the SN group ($p < 0.05$). In conclusion, these findings suggest that variation in prepartum nest-building behavior is associated with early postpartum divergence in gut microbiota structure and predicted function in loose-housed sows.

Key words: farrowing environment, functional pathways, gut-microbial alteration, maternal behavior, nest-building categories

OC26003

Comparative analysis of SLA-DRB1 allelic diversity in Vietnamese and Jeju native pigs and their insights into antigen presentation potential

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The SLA-DRB1 gene encodes the beta chain of SLA-DR molecules, which play a vital role in recognizing and responding to pathogens by presenting antigens. Identification of DRB1 alleles in pig populations is an important consideration when developing a multi-epitope vaccine. This study aimed to investigate the SLA-DRB1 allele diversity in several Vietnamese native pig populations and compare it with that of the Jeju Korean native pig population. The Sequence-based typing (SBT), a high-resolution genotyping method combining Sanger sequencing and PCR-based sequence-specific primers (SSPs), was adapted for genotyping exon 2 of the SLA-DRB1 gene. In addition, the translated DRB1 amino acid sequences were combined with immunoinformatics to evaluate the potential of DR molecules to present pathogen peptides from circulating Foot and Mouth (FMD) immunological serotypes. As a result, a high number of novel DRB1 variants were discovered. Several peptide epitopes from FMDV VP1 serotype O were found to have strong and relatively stable binding potential to DR molecules. This work provides valuable insights into the SLA-DRB1 genetic diversity and suggests utilizing it in bioinformatics to rapidly assess their ability to resist specific pathogens, such as FMD virus.
This research was supported by the Regional Innovation System & Education (RISE) program through the Jeonbuk RISE Center, funded by the Ministry of Education (MOE) and the Jeonbuk State, Republic of Korea.(2025-RISE-13-JBU).

Key words : SLA-DRB1, foot and mouth disease, immunoinformatics, Korean native pig, Vietnamese native pigs

OC26004

Transcriptomic analysis of bovine peripheral blood mononuclear cells stimulated with antigens from bovine digital dermatitis associated treponemes

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Bovine digital dermatitis (BDD) is an infectious disease in cattle associated with multiple *Treponema* species yet information on the immune responses of circulating immune cells to these pathogens are limited. This study utilized RNA sequencing to characterize transcriptomic changes in bovine peripheral blood mononuclear cells (PBMCs) following stimulation with combined antigens derived from three BDD-associated *Treponema* species. A total of 3,898 differentially expressed genes were identified, including 2,013 upregulated and 1,885 downregulated genes (BH-FDR adjusted $p < 0.05$). Notable upregulated genes included *IFNγ, CXCL10, IL17F, IL1B, IL6, MMP9*, and *MMP14* suggesting activation of Th1 and Th17 associated inflammatory responses and extracellular matrix remodeling. In contrast, genes associated with antimicrobial activity, complement regulation, and immune modulation, such as *LYZ, KLF4, CCL23, CFD,* and *A2M,* were markedly downregulated, suggesting suppression of innate immune response and regulatory mechanisms potentially favoring a pro-inflammatory state in PBMCs. KEGG pathway analysis revealed enrichment of notable pathways like mitogen activated protein kinase signaling and tumor necrosis factor pathway which are associated in pro-inflammatory signaling and ECM processes. The enrichment of the NOD-like receptor pathway indicates that *Treponema* spp. antigens may influence inflammasome-associated and non-canonical inflammatory programs. Collectively, these findings suggest that PBMCs exhibit a pro-inflammatory transcriptional response following stimulation with *Treponema* spp. antigens, providing new insights into host–pathogen interactions in BDD.

Key words : bovine digital dermatitis; *Treponema* spp.; peripheral blood mononuclear cells; RNA sequencing; differentially expressed genes

OC26005

Effects of L-carnosine on oxidative stress and myogenic differentiation in bovine skeletal muscle-derived cells under heat stress

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This study investigated the effects of L-carnosine on oxidative stress responses and myogenic differentiation in bovine skeletal muscle-derived cells (BSMCs) under heat stress conditions. BSMCs were isolated from the *longissimus thoracis* muscle of slaughtered Korean native cattle, cultured for 2 days of growth, and then induced to differentiate for 6 days under either normothermic or heat-stress conditions. Cells were treated with L-carnosine at concentrations of 0, 0.01, 0.1, 1, and 10 mM during differentiation. In the heat stress experiment, cells were exposed to 41°C from days 2 to 4 of differentiation with the same L-carnosine treatments, followed by recovery at 37°C until day 6. Cell viability, malondialdehyde (MDA) levels, myogenic and stress-related gene expression, and myotube morphology (fusion index and myotube width) were evaluated. Under normal conditions, 10 mM L-carnosine significantly increased cell proliferation, while 1 mM L-carnosine increased MyoG and Myf6 expression and enhanced myotube width, suggesting promotion of myotube maturation. Heat stress significantly decreased cell viability and impaired myogenic differentiation compared with the 37°C control group. However, 1 and 10 mM L-carnosine significantly restored cell viability, fusion index, and myotube width under heat stress conditions. In addition, 1 mM L-carnosine significantly decreased MDA levels compared with the 0 mM heat-stressed group. Heat stress decreased Myf5 and Myf6 expression compared with the 37°C control group, whereas 1 and 10 mM L-carnosine increased MyoD, MyoG, and Myf6 expression compared with the 0 mM heat-stressed group ($p < 0.05$). L-carnosine treatment also increased HSP70, GPX1, SOD2, and NRF2 expression compared with the 0 mM heat-stressed group ($p < 0.01$). During the recovery period after heat stress, 1 and 10 mM L-carnosine increased myotube width compared with the 0 mM heat-stressed group ($p < 0.01$), suggesting enhanced myotube maturation during recovery. In conclusion, L-carnosine improved cell viability and myogenic differentiation and reduced oxidative damage in BSMCs under heat stress conditions. These findings suggest that L-carnosine may be a potential functional nutrient for supporting skeletal muscle development in cattle under heat stress.

Key words : bovine skeletal muscle-derived cells, heat stress, L-carnosine, myogenic differentiation, oxidative stress

OC26006

Protein engineering of a novel recombined lytic enzymes against rumen methanogens in livestock

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Agricultural activities account for one-third of anthropogenic methane, with 70% of these emissions generated by rumen methanogens such as *Methanobrevibacter ruminantium* and *Methanobrevibacter smithii*. Various strategies, including endolysin therapy, have been investigated to inhibit methanogen growth. In this study, we evaluated MetRE, a novel lytic enzyme constructed by combining binding and catalytic domains from two distinct endolysins. The structure of the recombined enzyme was predicted via AlphaFold3, and domain conservation was analyzed against natural counterparts using TM-align. Furthermore, both the wild-type and the novel recombined enzymes were expressed in a *Lactiplantibacillus plantarum*. Structural alignment of MetRE yielded a TM-score of 0.9800 with the natural binding domain and 0.9954 with the catalytic domain. Western blot analysis confirmed the successful expression of both wild-type and recombined enzymes in *L. plantarum*. Consequently, the recombined enzyme maintains the 3D structure of its natural counterparts with high precision while exhibiting stable expression. These findings suggest that the newly designed lytic enzyme can be produced in practice as a promising tool for inhibiting rumen methanogens, supported by its high structural precision and stable microbial expression. Supported by NRF/MSIT (RS-2024-00344849).

Key words: ruminant, rumen, methanogen, protein engineering, endolysin

OC26007

Comparative analysis of 2D and 3D culture systems using Hanwoo skeletal muscle-derived cells

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This study aimed to establish a three-dimensional (3D) culture using bovine skeletal muscle-derived cells (BSMCs) from Hanwoo cattle and to compare it with a conventional two-dimensional (2D) culture. BSMCs were isolated from the longissimus thoracis muscle of Hanwoo cattle, and 3D constructs were bio-printed into scaffold using a gelatin methacryloyl(GelMA)-based bioink. Cell viability and proliferation were evaluated, and mRNA expression of myogenic regulatory factors and myosin heavy chain (*MYH*) isoforms were analyzed by RT-qPCR. Myotube formation and alignment were evaluated by immunocytochemistry (ICC) based on MYH-positive area, myotube length, and directionality. Cell viability remained above 90% throughout the culture period in the 3D. Proliferation in 2D increased up to day 5 and decreased at day 7, whereas proliferation in 3D was maintained after day 5 ($p < 0.05$). During differentiation, *MyoG* and *Myf6* expression increased in both systems, and at day 8, 3D showed higher expression of *MyoG, Myf6, MYH1*, and *MYH2* than 2D ($p < 0.05$). ICC showed that myotubes were longer in 2D than in 3D at day 4 ($p = 0.0002$), but longer in 3D at days 6 and 8 ($p = 0.0019$ and $p < 0.0001$). MYH-positive area was higher in 3D than in 2D at days 6 and 8 ($p = 0.0343$ and $p = 0.0013$). Directionality analysis showed less organized myotube alignment in 2D but directional alignment in 3D. In conclusion, the 3D culture promoted sustained proliferation, enhanced late-stage myotube formation, and improved directional alignment compared with the 2D culture.

Key words : 2D culture, 3D culture, bovine skeletal muscle-derived cells (BSMCs), myogenic differentiation, Hanwoo cattle

OC26008

Sensor-based detection of alterations in social relationships in laying hens and dairy calves with diseases

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These studies focused on the changes in social interaction between animals (dairy calves and laying hens) in relation to sickness signs. Two studies were conducted to compare social interaction data obtained from human observations with data collected using wearable sensors.

In the hen study, 20 previously unvaccinated hens (Hy-Line Brown, 25-35 weeks old) were used in 5 replicated experiments (4 hens/replicate). Hens were placed in groups of 4 to 180 m^2 room. On day 1, TrackLab sensor (Tracksys Ltd.) was mounted on each hen's back to detect changes in social interaction (m/day) from day 1–12. On day 3, saline (control) was administered to all birds by nasal and ocular drops. On day 6, birds were given ILT (Infectious Laryngotracheitis) vaccine using the same method. In the calf study, 69 dairy calves (Holstein, 3–25 days old) were born at the farm. Calves were distributed into 3 Pens (22-25 calves per pen) according to their birth date. At 3–7 days of age, each calf was fitted with a GoChip sensor (GoChip Pet tech.) to detect social distance for 16 days. Behavioural changes were analysed from 3 days before to 3 days after the onset of clinical signs (day 0). All data was analysed in RStudio using linear mixed models.

In the hen study, there was a significant difference between days in the mean proximity frequency from TrackLab sensors (p = 0.003). In the calf study, the estimated social distance detected by GoChip devices also showed a significant day effect (p = 0.001). There were no significant differences between days in social data via human observations.

These findings suggest that wearable sensor technologies may provide a more sensitive tool for detecting disease-associated changes in social behaviours than human observations.

Key words : social behaviour, animal disease, disease detection, wearable sensor, social distance

OC26009

Effects of TLR signaling on immune activation and metabolic regulation in chicken macrophages under distinct modes of *Salmonella* uptake

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Salmonella survives and replicates within macrophages by evading host immune defenses and adapting to the intracellular environment. Although Toll-like receptor (TLR) signaling is implicated in host defense against *Salmonella*, its role in avian macrophages remains unclear. Here, we investigated how TLRs regulate bacterial handling, immune activation, and metabolic adaptation in gene edited chicken macrophages under phagocytosis and opsonophagocytosis conditions. Among them, TLR4$^{-/-}$ macrophages showed the most distinct phenotype. Under phagocytosis conditions, TLR4$^{-/-}$ macrophages exhibited reduced bacterial uptake, attenuated activation associated responses, impaired bacterial clearance, and transcriptional reprogramming away from immune related pathways toward cell cycle associated programs. In contrast, under opsonophagocytosis conditions, TLR4$^{-/-}$ macrophages showed enhanced bacterial clearance together with enrichment of innate immune pathways. Metabolic analyses further revealed that TLR4 deficiency promoted enhanced glycolytic dependence under both uptake conditions, while metabolomic changes differed according to the mode of uptake. Histidine metabolism was enriched in WT macrophages under phagocytosis, whereas tyrosine metabolism and tryptophan metabolism were preferentially associated with WT and TLR4$^{-/-}$ macrophages, respectively, under opsonophagocytosis. Integration of transcriptomic and metabolomic pathway analyses revealed that directional coupling according to the mode of uptake. These findings show that the effects of TLR4 on macrophage immune and metabolic responses to *Salmonella* in chickens depend on the mode of bacterial uptake.

Key words : avian immunology, macrophages, Toll-like receptor, *Salmonella*, cell metabolism

OC26010

Comparative genomic analysis of *Akkermansia muciniphila* and *Akkermansia massiliensis* and their potential impact on growth performance in livestock

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Akkermansia muciniphila is an intestinal symbiont recognized as a biomarker for host health and weight regulation. While it has recently been identified in cattle and swine, functional research on its related species in livestock remains insufficient. This study conducted a comparative genomic analysis between *A. muciniphila* and *A. massiliensis* to verify the identification of weight-loss-related genes and their potential impact on animal growth. and their distribution in Korean and Chinese broilers by sequencing. Genomic data from 24 complete genomes (12 per species) were analyzed via RAST Subsystem, revealing that the two species share 156 out of 191 functional subsystems (about 81%). P9-like weight-loss gene was identified in *A. massiliensis*, with blastn (100% coverage, 91.4% identity) and blastp (99% coverage, 98.24% identity) confirming high homology. Microbiota analysis of the ileum showed a high prevalence *A. muciniphila* in both groups, 75% of Korean (67/89) and 100% of Chinese (41/41) broilers. In contrast, *A. massiliensis* was absent in Korean samples and only rarely detected in 4.9% of Chinese samples (2/41). These findings indicate that *A. massiliensis* possesses subsystems similar to those of *A. muciniphila*, which can modulate energy metabolism and nutrient utilization. Thus, microbiome strategies like targeted vaccines to regulate specific *Akkermansia* species, offer a promising approach to enhancing livestock productivity by promoting weight gain. Supported by NRF/MSIT (RS-2024-00344849).

Key words: gut microbiome, comparative genomics, *Akkermansia muciniphila, Akkermansia massiliensis*, livestock productivity

OC26011

Computational investigation of PD-L1 dimer interfaces in human and canine systems for cross-species analysis of small-molecule inhibitors

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Small-molecule inhibitors targeting the PD-1/PD-L1 immune checkpoint have emerged as promising alternatives to antibodies for cancer therapy, offering improved tissue penetration and oral bioavailability. Studies show that compounds such as BMS-202 stabilize PD-L1 dimerization, forming a protein–protein interface that blocks PD-1 binding. However, cross-species variation in PD-L1 highlights the need to evaluate inhibitor binding in canine systems. In this study, computational approaches were used to investigate species-specific structural differences. Sequence alignment was performed, followed by canine structure prediction and dimer construction based on human crystal templates. The dimer interface was refined by protein–protein docking, yielding a modified binding-pocket architecture in the canine complex. Comparative docking of PD-L1 inhibitors revealed ligand-dependent binding, including retained or lost binding, variations in affinity, and differences in binding modes relative to the human structure. These results indicate that, although the canine pocket shares features with the human interface, subtle but functionally significant structural differences influence ligand recognition and binding efficiency. These findings provide structural insights for designing species-specific inhibitors and a foundation for developing targeted cancer therapeutics in canine systems.
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Key words: cancer immunotherapy, computational modeling, PD-L1, small-molecule inhibitors, cross-species analysis

OC26012

Predicting solute-solvent interactions via paired molecular graph attention networks

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Solute-solvent interactions are a fundamental determinant of thermodynamic properties in chemical and biological systems, governing solvation free energies, partition coefficients, and aqueous solubility across diverse molecular environments. In this study, we developed and evaluated SolvGAT, a dual-graph attention network architecture that jointly encodes solute and solvent molecular graphs and learns pairwise interaction representations through a cross-graph attention pooling mechanism. The model was trained and validated on solute-solvent pairs sourced from the BigSolDB 2.0 and MixtureSolDB databases encompassing aqueous, organic, and mixed-solvent systems, using ten-fold cross-validation data partitioning strategy. Under a random split, SolvGAT achieved an R^2 of 0.91 ± 0.01 and RMSE of 0.14 ± 0.02 logS.

Key words : drug discovery, ADMET, aqueous solubility, molecular SMILES, feature extraction