동물생명공학

PC26001

Economic evaluation of ultrasonographic fetal sex prediction: implications for profitability and market dynamics in Hanwoo cattle

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This study aimed to evaluate the diagnostic accuracy and optimal timing of ultrasonographic fetal sex determination in Hanwoo cattle and to examine its economic implications for breeding farm profitability and market dynamics. Specifically, we investigated whether early fetal sex identification could affect the transaction price of pregnant cows and shift profit distribution between breeding and purchasing farms. A total of 107 pregnant Hanwoo cows were examined by transrectal ultrasonography between 55 and 100 days of gestation; after excluding indeterminate cases, 104 were included in the final analysis. Fetal sex was determined by identifying the position of the genital tubercle and external genital structures. Diagnostic performance was assessed across three gestational periods (55–70, 71–85, and 86–100 days). An economic scenario analysis was conducted to estimate changes in asset value, net profit, cost–benefit ratio, and return on investment under different pricing strategies reflecting male–female calf price differentials. Sensitivity analyses further evaluated the effects of ultrasound costs and market fluctuations. The economic analysis indicated that farms adopting this technology could increase profitability by differentiating market prices according to fetal sex, with male calves contributing substantially to herd asset value. However, the magnitude of economic benefit depended on market conditions, implementation costs, and sex ratio variation. Ultrasonographic fetal sex determination has strong potential as a management tool to improve profitability and long-term sustainability in Hanwoo production systems, although its economic effectiveness requires strategic adoption that accounts for associated costs and market variability.

Key words : particulate matter, metal, testis, sperm, reproductive toxicity

PC26002

Isolation and characterization of a novel Stx1-converting temperate bacteriophage St1-gc

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Shiga toxin-producing *Escherichia coli* (STEC) is a major foodborne pathogen responsible for hemorrhagic colitis and hemolytic uremic syndrome in humans. Shiga toxins are encoded by Stx-converting bacteriophages (phages), which facilitate horizontal gene transfer and drive the emergence of new pathogenic strains. As ruminants are recognized as major reservoirs of STEC, understanding the biological and genomic characteristics of Stx-converting phages is essential for food safety and sustainable livestock management. In this study, we report a novel temperate phage, St1-gc, induced from a STEC isolate by mitomycin C treatment. The phage exhibited siphovirus-like morphology and showed strong lytic activity against its indicator *E. coli* strain in both planktonic and biofilm states, along with high tolerance to environmental stresses. Notably, phage St1-gc successfully mediated transduction of the *stx1* gene into a non-STEC strain. Genomic analysis revealed that phage St1-gc possessed a double-stranded DNA genome (50,192 bp) encoding 85 predicted ORFs, and the stx1 subunits A and B were identified within the genome. These findings highlight the potential role of phage St1-gc in the dissemination of STEC virulence factors and provide insights into strategies for controlling STEC to support sustainable livestock production.

Key words : STEC (Shiga toxin producing *Escherichia coli*), Stx-converting bacteriophage, transduction

PC26003

Isolation and characterization of a novel bacteriophage KW1 infecting *Lactococcus lactis*

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Lactococcus lactis is a Gram-positive lactic acid bacterium widely used as a starter culture in dairy fermentation because of its roles in rapid acidification, curd formation, flavor development. However, bacteriophage (phage) infection can disrupt these processes, leading to fermentation failure and economic losses. Despite ongoing control efforts, phage infection remains a persistent challenge due to the diversity and adaptability of lactococcal phages, many of which remain poorly characterized. In this study, a *L. lactis*-infecting phage, KW1, was isolated and characterized. The phage formed clear plaques with distinctive halos on its host strain, consistent with a lytic lifestyle, and exhibited a high degree of host specificity. Morphological analysis identified KW1 as a siphovirus-like phage, and stability tests showed high tolerance to pH and thermal stress. Genome sequencing revealed the phage possessed a 29,600-bp genome with 35.8% GC content, encoding 44 ORFs with no tRNAs, with no identifiable virulence-associated or antibiotic resistant genes. Interestingly, Gp38-like protein associated with Abi (abortive infection) resistance and multiple recombination-related proteins were detected, suggesting potential anti-host defense mechanisms and genomic adaptability. These results provide insights into the biology of lactococcal phages and support the development of control strategies against phage contamination in dairy fermentation.

Key words : *Lactococcus lactis*, bacteriophage, lytic phage, characterization, genome analysis, dairy fermentation

PC26004

Breaking host barriers: a novel bacteriophage infecting hybrid strains of Enterotoxigenic/Shiga toxin-producing *Escherichia coli*

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Escherichia coli is a Gram-negative bacterium commonly found in the intestinal tract of humans and animals. Certain pathotypes possess distinct virulence factors and can cause enteric and extraintestinal disease in both humans and livestock. Among these, enterotoxigenic *E. coli* (ETEC) is a major cause of post-weaning diarrhea in piglets, while Shiga toxin-producing *E. coli* (STEC) is an important foodborne pathogen. Recently, hybrid ETEC/STEC strains carrying virulence factors from both pathotypes have been increasingly reported, complicating disease control in the livestock industry. In the context of ongoing efforts to reduce antibiotic use, bacteriophages (phages) have emerged as a promising biocontrol agent. Here, we isolated and characterized a novel phage, SJ25, capable of infecting a broad range of ETEC and ETEC/STEC hybrid strains. Transmission electron microscopy revealed that SJ25 exhibits myovirus morphotype with an icosahedral head and contractile tail. The phage also demonstrated strong bacteriolytic activity and typical lytic characteristics against several ETEC *E. coli* isolates. In addition, SJ25 remained stable across various pH and temperature conditions. Genomic analysis showed that SJ25 possessed a 166,101 bp double-stranded DNA genome with a G+C content of 35.5%, encoding 261 predicted ORFs and 8 tRNAs without virulence or antibiotic resistance genes. Phylogenetic analysis classified SJ25 within the genus *Tequatrovirus* of the class Caudoviricetes. Overall, SJ25 represents a promising biocontrol candidate for the control of both ETEC and ETEC/STEC hybrid strains, supporting its potential application as a sustainable alternative to antibiotics in livestock production under a One Health framework.

Key words : bacteriophage, enterotoxigenic *Escherichia coli* (ETEC), ETEC/STEC hybrid, post-weaning diarrhea, biocontrol

PC26005

Spirobenzofuran isolated from *Acremonium* mitigates the negative effects of Ochratoxin A in porcine intestinal epithelial cells

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In the pig industry, the consumption of feed exposed to Ochratoxin A (OTA) causes various adverse effects, and absorbed OTA particularly targets the small intestine, resulting in hindered intestinal function. Antimicrobial peptides (AMPs) are small peptides present in the immune system that are revealed various functions such as toxin neutralization, wound healing, and cell growth. We confirmed through RNA-seq that Beta-defensin 1 (DEFB1), one of the AMPs, is significantly reduced by OTA, and hypothesized that the recovery of OTA-induced reduction in beta-defensin 1 could have a mitigating effect. The present study evaluated the mitigating effect in porcine intestinal epithelial cells (IPEC-J2 cells) exposed to OTA through the construction of DEFB1 overexpressing cell line and discovery of DEFB1-induced natural product. The study results showed that OTA induced cytotoxicity in IPEC-J2 cells, reduced cell migration, and increased the expression of inflammatory marker mRNA. Importantly, the overexpression of DEFB1 and the administration of Spirobenzofuran, a DEFB1-inducing natural compound selected through high-throughput screening, significantly mitigated the cytotoxicity, reduced cell migration, and increased inflammatory marker expression induced by OTA. These findings provide insights into the molecular mechanisms by which mycotoxins affect the intestinal epithelium and can aid in the selection of feed additives in the pig industry.

Key words : Ochratoxin A, porcine intestinal epithelial cells, beta defensin 1, high-throughput screening, spirobenzofuran

PC26006

Standardized safety evaluation protocols for *Enterococcus* spp. as probiotic candidates

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Enterococcus spp., such as *Enterococcus faecium* (*E. faecium*) and *Enterococcus faecalis* (*E. faecalis*), are recognized for their ability to inhibit the growth of pathogens and exhibit strong adhesion to the intestinal mucosa, underscoring their potential as probiotics. However, their safety is highly strain-dependent, as certain strains are associated with opportunistic pathogenicity and specific virulence determinants. Nevertheless, no globally harmonized safety standards currently exist for these bacteria. Therefore, this study aimed to establish a scientific foundation for the standardization of safety criteria for *Enterococcus* spp. The proposed evaluation framework encompassed seven key safety aspects: antibiotic resistance, hemolytic activity, lactate dehydrogenase activity, antimicrobial substance production, and biogenic amine production, along with genotoxicity assessments, specifically the bacterial reverse mutation test and the micronucleus assay. Validation was conducted using type strains of *E. faecium* and *E. faecalis*. Our results demonstrated that these standardized protocols were highly accurate and suitable for the safety evaluation of *Enterococcus* spp., providing a reliable framework for future industrial applications.

Key words : *Enterococcus*, probiotic safety, safety assessment

PC26007

Stress-associated cortisol elevation is associated with impaired nursing behavior and gut microbial alterations in periparturient sows

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Parturition is accompanied by substantial physiological stress, which may influence maternal behavior and the composition of the gut microbiota in sows. This study investigated whether stress-associated variation in cortisol levels is associated with gut microbial composition and maternal behavior in periparturient sows. A total of 22 primiparous sows were classified into low-cortisol (LC, n = 12) and high-cortisol (HC, n = 10) groups based on postpartum cortisol concentrations. Fecal samples were collected on day 1 after farrowing and analyzed using full-length 16S rRNA sequencing (PacBio). The HC group showed higher total nursing termination and lower carefulness scores than the LC group ($p < 0.05$ for all). The relative abundance of *Campylobacter coli* was significantly higher in the HC group than in the LC group. Correlation analysis indicated that *C. coli* tended to be negatively correlated with total nursing duration (r = –0.40, $p = 0.06$), total nursing bout duration (r = –0.40, $p = 0.06$), and unsuccessful nursing bout duration (r = –0.39, $p = 0.07$). The results indicate that elevated cortisol levels around parturition are associated with altered maternal behavioral patterns and distinct gut microbial composition in sows. The correlation between *C. coli* abundance and impaired nursing behavior further suggests a potential involvement of gut microbiota in maternal performance during the periparturient period.

Key words : *Campylobacter coli*, carefulness score, maternal behavior, microbial changes, parturition

PC26008

Comparison of electroporation conditions in mouse zygotes for improving CRISPR/Cas9-mediated ssODN knock-in efficiency

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Successful gene editing is essential for the generation of disease model animals, functional validation of genes, and gene therapy applications. The CRISPR/Cas9 system induces artificial double-strand breaks (DSBs) in the genome, which are repaired through either homology-directed repair (HDR) or non-homologous end joining (NHEJ). Although NHEJ-mediated knock-out can be achieved relatively easily, HDR-mediated knock-in remains technically challenging. To generate genetically engineered mice using the CRISPR/Cas9 system, genome-editing components are delivered into zygotes. Single-stranded oligodeoxynucleotides (ssODNs) shorter than 200 nt have been reported to exhibit relatively high knock-in efficiency. Compared with microinjection, electroporation is easier to perform and enables efficient delivery of genome-editing components into embryos. Therefore, this study evaluated the electroporation efficiency of ssODNs in mouse zygotes using the NEPA21 electroporator. A 200-nt ssODN containing a 34-bp loxP sequence was used to assess knock-in efficiency. Overall, embryo developmental rates decreased following electroporation regardless of electrode type compared with the control group. However, knock-in efficiency at the right-arm target locus was significantly increased in the group treated with high concentrations of ribonucleoprotein (RNP) complex and ssODN using the CUY505P5 electrode.

Key words : CRISPR/Cas9, ssODNs, electroporation, knock-in efficiency

PC26009

C. maltaromaticum-derived factors enhance white adipose beiging and lipolysis, ameliorating metabolic dysfunction in an HFD-induced obese mouse model

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Obesity drives metabolic syndromes like type 2 diabetes and non-alcoholic fatty liver disease, making probiotics a compelling alternative to traditional pharmaceuticals for restoring metabolic homeostasis. This study investigated the therapeutic potential and molecular mechanisms of *Carnobacteria maltaromaticum* and its culture supernatant in a high-fat diet (HFD)-induced obese mouse model. C57BL/6L mice were fed an HFD for six weeks while receiving *C. maltaromaticum* or its medium, compared against *Lactobacillus gasseri* BNR17 and Semaglutide controls. Metabolic health was evaluated via glucose and insulin tolerance tests and serum lipid profiling, while molecular changes in adipose tissues were analyzed through histology and qPCR. *C. maltaromaticum*-derived factors significantly suppressed weight gain without affecting food intake, suggesting direct metabolic modulation rather than appetite suppression. Intervention reduced total cholesterol and triglycerides while improving insulin sensitivity. At the tissue level, treatment decreased adipocyte hypertrophy and ectopic lipid accumulation. Molecular analysis showed the downregulation of adipogenic factors (SREBP1C, FAS, ACC) and the upregulation of lipolytic enzymes (ATGL, HSL). Importantly, increased expression of UCP1 and PGC1α in both white and brown adipose tissues indicates promoted energy expenditure through WAT beiging and BAT activation. In conclusion, *C. maltaromaticum* comprehensively ameliorates obesity by inhibiting adipogenesis, promoting lipolysis, and activating thermogenic programs, establishing it as a promising probiotic candidate for metabolic syndrome management.

Key words : *C. maltaromaticum*, obesity, probiotics, adipogenesis, lipolysis, browning, beige adipocytes, thermogenesis, UCP1

PC26010

Protective effects of Stanniocalcin 2 against oxidative stress in muscle cells

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Excessive accumulation of reactive oxygen species (ROS) disrupts cellular homeostasis and contributes to muscle damage and atrophy. In poultry, rapid growth and high metabolic rates increase susceptibility to oxidative stress in skeletal muscle, leading to impaired muscle development and breast muscle abnormalities. Therefore, clarifying the cellular mechanisms related to oxidative stress is essential. *Stanniocalcin 2* (*STC2*) is known to be involved in stress-related cellular functions; however, its specific role in skeletal muscle under oxidative conditions remains unclear. This study examined the function of *STC2* in H_2O_2-induced oxidative stress in muscle cells. Upon H_2O_2 exposure, *STC2* expression was upregulated in the quail muscle cell line. To evaluate its functional role, *STC2*-overexpressing muscle cells were established, allowed to differentiate for 4 days, and subsequently exposed to oxidative stress. *STC2* overexpression significantly improved cell survival, suppressed ROS accumulation, reduced cell death, and preserved myotube structure compared to control cells. These findings suggest that *STC2* plays a protective role in skeletal muscle cells under oxidative stress and may serve as a potential target for preventing oxidative stress-related muscle damage.

Key words : Stanniocalcin 2, oxidative stress, QM7 cell line, muscle differentiation, myotube

PC26011

Artepillin C triggers mitochondria-dependent apoptosis in canine osteosarcoma cell lines

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Artepillin C (ARC) is a prenylated derivative of p-coumaric acid and a major phenolic constituent of *Brazilian green propolis*. This compound has been reported to exhibit diverse pharmacological activities, such as antioxidant, anti-inflammatory, immunomodulatory, and anticancer properties. As osteosarcoma (OS) is the most prevalent skeletal malignancy in dogs, we investigated the anticancer effects of ARC on the canine OS cell lines DSN and D17.

In this study, we demonstrated that ARC significantly inhibited cell proliferation and induced apoptosis by triggering excessive ROS generation. Mechanistically, ARC treatment decreased cytosolic Ca^{2+} levels while increasing mitochondrial Ca^{2+} accumulation, leading to a significant loss of mitochondrial membrane potential. These findings suggest that Artepillin C is a promising therapeutic agent for canine OS that acts by inducing mitochondria-mediated apoptosis.

Key words : Artepillin C, osteosarcoma, apoptosis, mitochondria

PC26012

Effect of calving season on postpartum ovarian activity in Holstein cows

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This study investigated the effect of calving season on postpartum resumption of ovarian activity in Holstein cows under Korean climatic conditions, hypothesizing that summer heat stress delays reproductive recovery in spring-calving cows. A total of 20 multiparous cows (spring: n = 9; summer: n = 7; autumn: n = 4) were evaluated. Blood samples were collected weekly from 10 to 100 days postpartum. Resumption of luteal activity was defined as progesterone (P4) ≥ 1 ng/mL, with ovulation estimated 5 days prior to this detection. Fisher's exact test revealed that the proportion of cows with delayed ovarian activity resumption (61–80 days postpartum) was significantly higher in spring-calving cows (55.5%) than in summer (14.3%) and autumn (0%) groups ($p < 0.05$). Conversely, spring-calving cows tended to show lower early resumption rates (within 40 days) compared to other seasons (22.2% vs. 63.6%; $p < 0.1$). In conclusion, calving season may influence postpartum reproductive recovery in Holstein dairy cows under Korean climatic conditions. These results suggest that heat stress during the summer breeding period could negatively affect reproductive performance in spring-calving cows.

Key words : dairy cattle, heat stress, Holstein, postpartum ovarian activity, progesterone

PC26013

Effects of nonylphenol on oxidative stress and implantation-related functions in porcine luminal epithelial cells

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Nonylphenol (NP) is a widely distributed environmental endocrine-disrupting chemical that may be associated with reproductive dysfunction. In this study, we investigated the effects of NP exposure on endometrial function and implantation-related cellular responses using porcine luminal epithelial (pLE) cells. NP treatment decreased pLE cell viability in a concentration-dependent manner, indicating NP-induced cytotoxicity. qPCR analysis showed altered expression of inflammation-related genes, including IL18, IL21, NFKBIA, TNFα, and TGFβ1. NP also changed the expression of implantation-, adhesion-, and cell function-related genes, including VCAM1, ICAM1, IGF1, FGF2, LOX, and CCND1. Changes in antioxidant-related genes, including GCLC, NQO1, GPX1, SOD1, and GPX4, suggest that NP may disrupt cellular redox homeostasis. Annexin V assay demonstrated that NP induced apoptosis. Spheroid culture showed that NP altered three-dimensional structure formation in pLE cells. In addition, pTr transwell assay and pTr/pLE co-culture transwell assay indicated that NP may affect trophoblast cell migration and endometrial–trophoblast cell interactions. Taken together, these results demonstrate that NP can induce cytotoxicity, inflammatory responses, apoptosis, and implantation-related functional impairment in pLE cells. These findings suggest that NP may negatively affect porcine endometrial function and early implantation processes. This study provides evidence that pLE- and pTr-based *in vitro* models can be useful for evaluating the reproductive toxicity of environmental endocrine-disrupting chemicals.

Key words : nonylphenol, pLE cell, pTr cell, endometrium, oxidative stress, implantation, reproductive toxicity

PC26014

Adenovirus-mediated CRISPR/Cas9 system for efficient knockout of the ovalbumin gene in chicken cells

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Ovalbumin (OVAL) is the major protein component of egg white and an important target in food allergy and biopharmaceutical research. In this study, we aimed to establish a CRISPR/Cas9-based knockout system targeting the chicken OVAL gene and evaluate its in vitro editing efficiency. To induce deletion of exon 2 in the OVAL gene, two guide RNAs (gRNAs) targeting intron 1 and two gRNAs targeting intron 2 were designed. A total of four gRNAs (cOVAL_int1_gR1, cOVAL_int1_gR2, cOVAL_int2_gR1, and cOVAL_int2_gR2) were generated, and a dual-gRNA strategy was applied to induce exon 2 deletion. Adenovirus-based CRISPR/Cas9 vectors containing paired gRNAs were constructed and transduced into chicken embryonic fibroblasts (CEFs). Initial in vitro validation confirmed cleavage activity for all four gRNAs using PCR-based analysis. Subsequently, genome editing efficiency was evaluated for each dual-gRNA combination. Expected deletion bands ranging from approximately 258 to 315 bp were detected, along with additional deletion products of various sizes in some combinations, suggesting diverse genomic rearrangements at the target locus. The present study demonstrated the feasibility of establishing an adenovirus-based OVA knockout system in chicken cells. Further analyses, including gel extraction and Sanger sequencing, will be performed to characterize the editing patterns.

Key words : CRISPR/Cas9, genome editing, adenovirus, ovalbumin, chicken

PC26015

Development of bta-miR-375 biomarker associated with intramuscular fat deposition in Korean Native cattle

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Although SNP markers have been widely used for genetic improvement, they have limitations in explaining complex economic traits. This study aimed to elucidate the important role of bta-miR-375 in bovine adipogenesis and to evaluate its potential as a miRNA biomarker. Preadipocytes were isolated from Hanwoo adipose tissue and transfected with bta-miR-375, followed by adipogenic differentiation. Total RNA extraction, cDNA synthesis and qRT-PCR were performed to analyze the expression of adipogenesis and lipid metabolism-related genes. For biomarker developemnt, Plasma bta-miR-375 was also quantified in Hanwoo blood samples using miRNA-specific qRT-PCR. These results demonstrated that bta-miR-375 significantly upregulated the expression of adipogenesis- and lipid metabolism-related genes, including PPARγ, CEBPα, ADIPOR2, and GPAM, in bta-miR-375-treated cells compared with the negative control at day 14 of differentiation. Furthermore, bta-miR-375 was stably detected in all plasma samples within a Ct range of 20–27, demonstrating its technical feasibility as a circulating miRNA biomarker. These findings indicate that bta-miR-375 promotes lipid accumulation during early adipogenic differentiation in Korean native cattle. Taken togather, these study suggests the potential application of bta-miR-375 as a biomarker for improving meat quality traits.

Key words : bta-miR-375, circulating biomarker, adipogenesis genes, intramuscular fat deposition, Korean native cattle

PC26016

Preliminary deep learning-based image analysis for segmentation-assisted measurement of jejunal mucosal morphology of weaning pigs under different nutritional conditions

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Nutritional imbalance can affect jejunal mucosal morphology and intestinal function in piglets. Accordingly, villus height (VH), crypt depth (CD), and the VH/CD ratio are widely used indicators of intestinal morphology, but these parameters are commonly measured manually from hematoxylin and eosin-stained tissue sections, which is time-consuming and may be affected by observer-dependent variability. This study aimed to develop a preliminary deep learning-based image analysis approach for segmentation-assisted measurement of VH and CD in porcine jejunal tissue under different nutritional conditions. A total of 223 jejunal tissue images were collected from weaning pigs at day 21 and day 42 after feeding either a basal diet (CON) or a restricted nutrition diet (TRT). Images were annotated using four mutually exclusive classes: background, villus, crypt, and lumen, with labeling focused on villus-crypt structures suitable for morphometric evaluation. A deep learning-based image analysis model was trained using the annotated dataset. Conventional morphology data showed that the TRT group had greater VH, lower CD, and a higher VH/CD ratio than the CON group. Mask-derived morphometric indices showed a similar biological pattern, particularly for the age-related increase in VH and the increased VH/CD ratio in the TRT group. The VH/CD ratio, which was the primary morphometric indicator in this study, was accurately showing a relative agreement of 89.0% compared to conventional morphology results. These preliminary findings suggest that deep learning-based image analysis has potential as an objective tool for intestinal morphometry in piglets.

Key words : piglet, jejunum, villus height, crypt depth, deep learning, image analysis, intestinal morphometry

PC26017

Effects of commercial feed additive on methane reduction and microbial communities in Korean Native cattle

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Recent implementation of national carbon-neutrality policies has increased interest in commercial feed additives for reducing enteric methane emissions from ruminants. Therefore, this study evaluated the methane mitigation effect of an allicin-containing commercial feed additive through *in vitro* rumen fermentation and field validation trials. *In vitro* fermentation was conducted using rumen fluid in the AnkomRF Gas Production System with a TMR substrate control, a monensin-supplemented control, and commercial methane-reducing feed additive treatments. Among the treatments, T4 showed the lowest methane production, corresponding to 92.35% of the control, and was selected for field validation. The selected additive was administered to 12 Korean Native cattle at 1% of dietary DM, and daily CH_4 emissions were significantly reduced from 140.4 g/d in the control group to 124.5 g/d in the treatment group, representing an 11.3% reduction ($p = 0.049$). Full-length 16S rRNA sequencing was performed using rumen fluid samples on the PacBio Sequel II platform, and microbial community analysis will be conducted based on non-chimeric ASVs. Taken together, these findings suggest that the allicin-containing commercial feed additive effectively mitigated methane emissions without compromising dry matter intake, indicating its potential as a practical methane mitigation strategy for Korean Native cattle.

Key words : Korean native cattle, ANKOM system, methane emission reduction, commercial feed additive, 16S rRNA full-length sequencing

PC26018

Development of a prediction model for methane emission in Korean Native cattle

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Methane (CH_4), a potent greenhouse gas 28 times more warming than CO_2, mainly originates from ruminant enteric fermentation. For its measurement, direct tools like respiration chambers and the Greenfeed system exist, yet limited sampling capacity hinders their use in diverse conditions. Therefore, multiple linear regression (MLR) equations have been widely utilized for indirect methane prediction due to their simplicity, interpretability, and applicability across datasets. The aim of this study was to develop a predictive equation capable of estimating CH_4 emissions (g/day) from Korean native cattle under field conditions. A MLR equation was constructed using body weight (BW), dry matter intake (DMI), and the CH_4/CO_2 ratio as input variables. Equation performance was estimated using adjusted R^2, root mean square error (RMSE), and the concordance correlation coefficient (CCC). The equation incorporating the three predictors achieved the highest accuracy, yielding an adjusted conditional R^2 of 0.860, an RMSE of 18.382 and a CCC of 0.951. The developed MLR equation demonstrates strong predictive performance and may serve as a practical tool for estimating CH_4 emissions from Korean native cattle. Furthermore, it is expected to contribute benchmark data and technical foundations for national methane mitigation strategies and the improvement of livestock GHG inventory systems in Korea.

Key words : methane emission, Korean native cattle, multiple linear regression, respiration chamber, ruminant greenhouse gas

PC26019

Effects of vitamin E and glutamine on stress-responsive and myogenic gene expression in heat-stressed bovine skeletal muscle-derived cells

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Heat stress (HS) impairs skeletal muscle development by inducing oxidative stress and disrupting myogenic programs. This study evaluated the individual and combined effects of vitamin E (Vit E; 0.1 or 1 μM) and L-glutamine (Gln; 1 or 10 mM) on antioxidant, apoptotic, and myogenic gene expression in bovine skeletal muscle-derived cells (BSMCs) isolated from three Korean native cattle. BSMCs were cultured at 37°C (control) or 41°C (HS) for 24 h at differentiation day 6 across six treatment groups. One-way ANOVA was performed using PROC GLM of SAS 9.4. HS elevated ($p < 0.05$) HSP70, SOD1, and MYOD while reducing FBXO32, an atrophy marker. Gln supplementation (1 and 10 mM) upregulated MYOG expression in a dose-dependent manner, suggesting enhanced terminal myogenic differentiation. Vit E (0.1 μM) and low Vit E + low Gln upregulated ($p < 0.05$) MYF5, an early myogenic regulator. The combination of high Vit E and high Gln uniquely upregulated ($p < 0.05$) NFE2L2 and GPX1, activating Nrf2-mediated antioxidant defense, and downregulated ($p < 0.05$) the apoptotic marker CASP3. These findings suggest that combined Vit E and Gln supplementation enhances antioxidant capacity and suppresses apoptosis in heat-stressed BSMCs.

Key words : heat stress, bovine skeletal muscle-derived cells, glutamine, vitamin E, myogenesis, antioxidants

PC26020

Heat stress-induced temporal shifts in the fecal microbiome and predicted functional pathways of laying hens using 16S rRNA gene sequencing

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Heat stress impairs productivity, immune function, and nutrient utilization in laying hens; however, its temporal effects on the fecal microbiome and associated functional pathways remain unclear. This study investigated time-dependent changes in the fecal microbiome and 16S rRNA-based predicted functional pathways during prolonged heat stress. A total of 48 Lohmann Brown laying hens (26 weeks old) were exposed to heat stress for 14 days at THI 82 (33°C, 66% relative humidity), and fecal samples were collected on day 0, day 7, and day 14. Fecal bacterial 16S rRNA V3–V4 sequencing data were processed with QIIME2, followed by alpha and beta diversity analyses, LEfSe-based discriminative taxa identification, and PICRUSt2-based functional prediction. Alpha diversity remained unchanged; however, PCoA based on Bray-Curtis, unweighted UniFrac, and weighted UniFrac distances revealed time-dependent shifts in microbial community structure during prolonged heat stress. LEfSe identified 50 discriminative taxa (LDA > 3.5); Lactobacillus, Enterobacteriaceae, Klebsiella, and Veillonella were enriched at day 14. PICRUSt2 predicted 39 functional pathways (FDR < 0.05), mainly involving carbohydrate utilization and fermentation at day 14. In summary, prolonged heat stress alters the fecal microbiome of laying hens by shifting microbial community composition and predicted metabolic functions, particularly pathways related to carbohydrate utilization and fermentation, which may be associated with microbial responses to prolonged heat stress.

Key words: laying hens, heat stress, fecal microbiome, gut microbiota, 16S rRNA sequencing, LEfSe, PICRUSt2

PC26021

Comparative analysis of physiological and transcriptomic responses to heat stress in laying hens in different time period using RNA-seq

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Increasing global temperatures and humidity associated with climate change have intensified heat stress as a major challenge in poultry production. This study evaluated the effects of different THI conditions on laying performance, physiological responses, and transcriptomic changes in laying hens over a 14-day period. Forty-eight 26-wk-old Lohmann Brown laying hens were assigned to THI 68 or THI 85 (24 birds / group) and blood samples for RNA-seq were collected on days 0, 7, and 14. RNA-seq analysis was performed to identify DEGs (FDR < 0.05, $|\log2FC| \geq 1$), followed by functional enrichment via DAVID. In the THI 85 group, feed intake, body weight gain, egg production, and egg quality traits decreased, while water intake, rectal temperature, and serum potassium levels increased. DEGs identified were 40 in Day 0 vs. 7 (12 up, 28 down), 292 in Day 0 vs. 14 (152 up, 140 down), and 22 in Day 7 vs. 14 (15 up, 7 down). At Day 7, upregulated *Hmox1* and *Jchain* linked to heme binding and detoxification-related pathways indicated acute oxidative stress. By Day 14, upregulation of *Oma1*, *Bnip3*, and *Acsl1* was associated with mitochondrial homeostasis and metabolic reprogramming (fatty acid biosynthesis and pentose phosphate pathway) for chronic stress adaptation. In summary, high THI impairs laying hen productivity and egg quality. Transcriptomic analysis suggests a time-dependent shift from acute oxidative stress toward long-term cellular adaptation. These findings provide insights into the importance of environmental management and future selection strategies for enhancing heat tolerance in laying hens.

Key words : laying hen, heat stress, RNA-seq, transcriptome, physiological response

PC26022

Immunoinformatics-based design and *in silico* evaluation of a multi-epitope vaccine candidate against porcine reproductive and respiratory syndrome virus

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Porcine reproductive and respiratory syndrome virus (PRRSV) causes significant economic losses in the global swine industry. As the high genetic variability of circulating strains limits the cross-protective efficacy of current modified-live and inactivated vaccines, we employed an immunoinformatics pipeline to design a multi-epitope subunit vaccine (MESV) targeting conserved and immunologically relevant regions of the PRRSV proteome. We prioritized three proteins (GP3, GP4, and RPP1TF) based on their antigenicity and immune accessibility. The MESV was then assembled by systematically screening and combining predicted B- and T-cell epitopes. Linkers (EAAAK, KK, and AAY) were incorporated to maintain epitope integrity and ensure optimal antigen processing. To enhance the immunostimulatory properties of the vaccine construct, the 50S ribosomal protein L7/L12, a well-characterized TLR4 agonist, was utilized as an adjuvant. Physicochemical characterization indicated favorable stability and antigenicity. The docking analyses revealed stable interactions with porcine Toll-like receptors (TLR3 and TLR4), which were further confirmed by molecular dynamics simulations and MM-GBSA binding free energy calculations. Immune simulations predicted robust activation of both humoral and cellular immunity. These in silico findings suggest that the proposed MESV is a promising candidate for further *in vitro* and in vivo validation to improve PRRSV control strategies.

Key words : porcine reproductive and respiratory syndrome virus (PRRSV), multi-epitope subunit vaccine, immunoinformatics, molecular docking, molecular dynamics simulation

PC26023

Engineering the delta-9 pathway for *de novo* synthesis of eicosapentaenoic acid (EPA) in *Yarrowia lipolytica*

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Eicosapentaenoic acid (EPA) is an omega-3 polyunsaturated fatty acid involved in inflammatory regulation and cardiovascular health, with broad applications in food, feed, and nutraceutical products. In particular, because mammals have a limited ability to biosynthesize omega-3 fatty acids, dietary intake is essential. However, current EPA production largely relies on fish oil, which raises concerns over marine resource depletion and heavy metal contamination. Thus, sustainable microbial production systems are needed as alternative sources of EPA. In this study, an EPA-producing strain (EPA strain) was constructed using the oleaginous yeast *Yarrowia lipolytica*, which naturally produces fatty acids only up to linoleic acid. To establish the EPA biosynthetic pathway, four heterologous pathway genes (*Δ9E*, *Δ8D*, *Δ5D*, and *Δ17D*), encoding one elongase and three desaturases, were sequentially integrated into the *A08* pseudogene locus at intervals of less than 500 nt using the CRISPR-Cas9 system. After establishing the EPA strain, *DGA1*, encoding diacylglycerol acyltransferase involved in neutral lipid synthesis, was inserted into the *PEX10* locus, which is involved in peroxisome biogenesis, to further improve EPA productivity. This one-step strategy was designed to increase fatty acid accumulation by enhancing neutral lipid synthesis while reducing peroxisomal fatty acid degradation. The engineered strains were analyzed by qRT-PCR, GC-FID, HPLC, and lipid body staining to evaluate gene expression, fatty acid composition and EPA content, carbon source consumption and organic acid production, and intracellular lipid accumulation. As a result, the *DGA1*-integrated EPA strain (EPA-DGA1 strain) showed approximately 3-fold higher total fatty acid content and 2-fold higher EPA content than the EPA strain. These findings support the use of engineered *Y. lipolytica* as a sustainable microbial EPA source, with potential food and feed applications to improve omega-3/omega-6 fatty acid balance in humans and livestock.

Key words : yeast, EPA, omega-3, CRISPR Cas-9, *Yarrowia lipolytica*

PC26024

Evaluation of antioxidant probiotic potential of lactic acid bacteria isolated from swine feces and fermented foods

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Lactic acid bacteria with antioxidant potential are considered useful candidates for improving gut health and maintaining redox balance. This study aimed to screen antioxidant probiotics candidates isolated from 3-week-old piglet feces and kimchi. Approximately 40 bacterial isolates were obtained by selective anaerobic culture, and 9 lactic acid bacterial strains were selected for evaluation. Their probiotic properties were assessed by acid tolerance, bile tolerance, intestinal adhesion ability, auto-aggregation, and cell surface hydrophobicity. Antioxidant activity was determined using DPPH and ABTS radical scavenging assays. Based on overall probiotic and antioxidant properties, three strains were selected as final candidates, and their safety was confirmed by hemolytic activity and bile salt hydrolase associated assessments. Among the candidates, kimchi-derived *Limosilactobacillus reuteri* showed excellent probiotic potential, including 94.95% acid tolerance, 93.05% bile tolerance, and 61.65% intestinal adhesion ability. It also showed the highest antioxidant activity, with 51.06% DPPH and 41.02% ABTS radical scavenging activity. In LPS-stimulated RAW 264.7 cells, this strain increased *Nqo1* expression at 3 h and strongly induced *Hmox1* expression at 6 h, indicating a possible association with NRF2/KEAP1-related antioxidant responses. These findings suggest that kimchi-derived *L. reuteri* is a promising antioxidant probiotic candidate.

Key words : *Limosilactobacillus reuteri*, antioxidant activity, NRF2/KEAP1 pathway

PC26025

Identifying time-dependent molecular adaptations to heat stress in finishing pigs through longitudinal blood transcriptome profiling

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Heat stress poses a major challenge to swine production by reducing feed intake, metabolic efficiency, and immune function. However, the molecular basis of metabolic adaptation to chronic heat exposure remains poorly understood. This study investigated time-dependent metabolic changes in finishing pigs exposed to heat stress for 14 days. Nine pigs [(Landrace × Yorkshire) × Duroc] were assigned to thermoneutral (TN, 23–24°C, n = 5) and heat-stressed (HS, 32–33°C, n = 4) groups, and whole-blood samples were collected at weeks 0, 1, and 2. RNA sequencing, differential expression, and functional enrichment analyses were performed to characterize longitudinal responses to heat stress. Using a linear mixed model with group × time interaction, differentially expressed genes (DEGs) were identified based on FDR < 0.05 and $|\log_2 FC| > 1$. A total of 257 DEGs were identified at week 1, 131 DEGs at week 2, and 608 DEGs from the interaction term, indicating a temporal shift in transcriptional responses. Early responses were enriched for cell cycle, heme biosynthesis, and porphyrin metabolism pathways, whereas later responses were associated with erythrocyte differentiation, AMPK signaling, and ferroptosis pathways. These findings suggest that chronic heat stress induces dynamic transcriptional changes associated with metabolic and homeostatic adaptation in finishing pigs, providing molecular insight into the biological processes underlying heat tolerance.

Key words : heat stress, finishing pigs, transcriptome, RNA-seq, longitudinal design, repeated measures

PC26026

전사체 분석(RNA-seq)을 통한 돼지 고환 탈장 관련 차등발현유전자 및 조절 네트워크 발굴

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돼지 고환탈장은 장기 탈출에 따른 성장 지연 및 폐사 등으로 농가에 경제적 손실을 초래하는 복합 유전 질환이다. 동일 사양 조건에서도 개체별 발생 차이가 뚜렷하나, 기존 특정 유전자 중심 연구로는 전반적인 기전 설명에 한계가 있다. 따라서 본 연구는 전사체 분석(RNA-seq)을 통해 정상 개체와 환축 간의 mRNA 발현 차이를 비교하여 분자 유전학적 발병 기전을 구명하고자 하였다. 제주 난지축산연구센터에서 사육한 랜드레이스와 제주재래돼지의 교배로 생산된 자돈을 대상으로 이유시점인 3주령에 표현형 조사를 실시하여 고환탈장 환축 6두와 정상축 10두를 사용하였다. 선별된 개체의 등심 조직에서 RNA를 추출하여 RNA-seq을 수행하였다. 추출된 발현 데이터는 edgeR 패키지 이용해 차등발현 유전자 분석하였고, Gene Ontology 및 Kyoto Encyclopedia of Genes and Genomes 네트워크 분석을 실시하였다. 정상축과 환축 간 총 3,218개의 유의한(FDR < 0.05, $|\log_2 FC| \geq 1$) 차등발현 유전자를 확인하였다. 기능 분석(GO, KEGG, 네트워크) 결과, 세포 유착(cell adhesion), 세포외기질(ECM) 항상성, 국소 부착(focal adhesion) 및 조직 형태 형성(tissue morphogenesis) 등 결합조직 구조 조절 경로들의 복합적인 상호작용이 두드러지게 나타났다. 결론적으로, 이러한 구조적 네트워크 및 유전자 발현 패턴의 변화는 고환탈장의 해부학적 배경과 연관될 가능성을 시사한다. 발굴된 주요 차등 발현 유전자 신호전달 경로는 고환탈장 발생 기전 이해 및 추가연구를 위한 기초 자료를 넘어, 향후 양돈 산업에서 고환탈장 발생률을 낮추고 저항성 개체를 선별하기 위한 핵심 지표로 적극 활용될 수 있을 것이다.

Key words : 고환탈장, 제주재래돼지, 전사체 분석, 차등발현유전자, 세포외기질

PC26027

Effect of *Cordyceps militaris* powder on dextran sulfate sodium-induced colitis in mice

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This study evaluated the effects of *Cordyceps militaris* (CM) powder on intestinal inflammatory response, enteroendocrine cell hormone secretion, intestinal receptor expression, and intestinal barrier function using an *ex vivo* mouse model. The ileum was collected from 12-week-old male ICR mice (n = 6) and cultured in Krebs-Ringer Bicarbonate/HEPES (KRB/HEPES) buffer under two conditions: control (CON) and CM treatment (100 mg/kg) at 37°C with 5% v/v CO_2. Following incubation, glucagon-like peptide-1 (GLP-1) concentration in the culture medium was measured by ELISA, and the relative mRNA expression of GCG, Tas1r2, NfkB1, TNF-α, IL-6, ZO-1, OCLN and CLDN4 was analyzed by RT-qPCR. GLP-1 secretion did not differ significantly between the CON and CM groups ($p > 0.05$), as GLP-1 secretion in vivo involves complex regulatory mechanisms encompassing intestinal L cells, gut microbiota, short-chain fatty acids, and various luminal nutrients. CM treatment significantly improved the relative mRNA expression of inflammation-related genes compared to the control group ($p < 0.05$). These results suggest that the diverse bioactive compounds present in CM may attenuate intestinal inflammatory responses. Further in vivo studies are warranted to elucidate the mechanisms by which CM modulates intestinal endocrine function and inflammatory regulation.

Key words : *Cordeyceps militaris*, *ex vivo*, enteroendocrine cells, GLP-1, intestinal inflammation

PC26028

Modeling interferon-associated transcriptomic dynamics following kidney xenograft transplantation through gene regulatory network inference and time-lagged dynamical modeling

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Kidney xenotransplantation has emerged as a promising strategy to overcome donor organ shortage, yet its clinical application remains constrained by systemic immune and inflammatory dysregulation. This study investigated survival-associated temporal immune dynamics using longitudinal whole-blood transcriptomic profiles obtained from one recipient in each of three survival categories after pig-to-nonhuman-primate kidney xenotransplantation. Trajectory-based gene selection, functional enrichment analysis, Dynamic bayesian network inference, and delay differential equation modeling were integrated to characterize shared and group-specific expression dynamics. The transcriptomic response may reflect host recognition of the xenograft as a persistent foreign antigenic stimulus, leading to activation of interferon-stimulated antiviral defense programs. Representative interferon-stimulated genes showed earlier and sustained induction in short-term survival, whereas long-term survival exhibited delayed and attenuated activation. Network inference identified survival group-dependent regulatory edge patterns, and dynamic modeling achieved coefficients of determination greater than 0.8 for 18 of the 35 shared genes, with *USP18*, *DDX60*, and *OAS1* of exceeding 0.9. Permutation validation confirmed that temporal structure was essential for model performance. These findings suggest that longitudinal transcriptomic modeling can reveal dynamic risk signals and provide a framework for post-transplant host responses.

Key words : kidney xenotransplantation, longitudinal modeling, transcriptomics, gene regulatory network, interferon-stimulated genes

PC26029

Transcriptomic and microbiome profiling of heat stress responses in the broiler jejunum

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Heat stress (HS) disrupts intestinal physiology, host metabolism, and gut microbial balance in broiler chickens. Although transcriptomic and microbiome changes under HS have been widely studied, the regulatory interactions among long non-coding RNAs (lncRNAs), mRNAs, and gut microbiota remain unclear. In this study, RNA-seq-based jejunal transcriptome and microbiome data from four HS-exposed and four thermoneutral broilers were analyzed using an integrated multi-omics framework. Differential expression analysis (FDR < 0.05, $|\log_2 FC| > 1$) identified 139 mRNAs and 140 lncRNAs associated with HS, together with microbial features linked to stress conditions. Functional enrichment analysis indicated that HS-responsive genes were mainly involved in metabolic, membrane-associated, and immune-related pathways, including retinol metabolism and cholesterol-related processes. Several bacterial taxa associated with inflammatory responses, mucosal immune activity, and nutrient metabolism were enriched under HS conditions. In addition, opposite expression tendencies between mRNAs and lncRNAs may suggest potential lncRNA-mediated regulation of stress-responsive genes. Overall, these findings provide insight into host transcriptional and microbial alterations associated with HS in the broiler jejunum and improve understanding of host–microbiome interactions under heat stress conditions.

Key words : chicken, heat stress, jejunum, microbiome, transcriptome, lncRNA

PC26030

Multi-tissue longitudinal transcriptomics identifies adaptive immunity and lymphoepithelial homeostasis as drivers of interindividual PRRSV susceptibility in weaning piglets

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Porcine reproductive and respiratory syndrome virus (PRRSV) remains a major economic burden to the global swine industry due to its high genetic diversity and different virulence levels. Elucidating the biological basis of individual variation in clinical outcomes is crucial for disease control. To identify host factors underlying differential susceptibility, we performed longitudinal, multi-tissue RNA-seq based transcriptomic profiling of the lungs, bronchial lymph nodes (BLNs), and tonsils of PRRSV-infected 34 piglets. Animals were classified into non-susceptible and susceptible phenotypes based on average daily gain (ADG). While early innate antiviral responses were similar, non-susceptible pigs exhibited stronger, metabolically supported adaptive immunity in lymphoid tissues during later infection stages. Conversely, susceptible pigs showed upregulation of genes associated with epithelial damage and remodeling, particularly in the tonsils. Weighted gene co-expression network analysis (WGCNA) revealed that genes governing epithelial barrier integrity and keratinization—specifically *KRT78*, *IVL*, and *KLK10*—acted as central regulatory hubs distinguishing the susceptible phenotype. These findings demonstrate that PRRSV susceptibility depends on both robust adaptive immunity and the maintenance of lymphoepithelial homeostasis. This approach provides novel insights into the biological basis of disease resilience, offering potential host-directed strategies for PRRSV control.

Key words : PRRSV, susceptibility, RNA-seq, lymphoid tissues, epithelial homeostasis

PC26031

Application of exosomes as a functional culture supplement for bovine muscle satellite cells in cultured meat production

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Bovine muscle satellite cells (BMSCs) are a major cell source for cultured meat production because of their proliferation and myogenic differentiation capacity. However, continuous passaging can reduce their proliferative capacity and accelerate cellular aging, limiting their long-term use. This study evaluated the effects of exosome supplementation on the proliferation and long-term culture stability of BMSCs. Exosomes were analyzed using nanoparticle tracking analysis and transmission electron microscopy, which confirmed their particle characteristics and typical round vesicle-like morphology. Cell experiments were conducted at passages 5 and 10 using cell proliferation assay, live/dead staining, and immunofluorescence analysis. The comparison between P5 and P10 showed that 100 μg/mL exosome-treated BMSCs maintained significantly higher cell growth even at P10 compared with the control group ($p < 0.0001$). Live/dead staining showed continued cell growth in the 100 μg/mL exosome-treated group, and differentiated cells formed comparable myotube-like structures at both P5 and P10. These results suggest that exosome supplementation helps maintain the viability, proliferation, and myogenic differentiation potential of BMSCs during continuous passaging. Therefore, exosomes could be used as a functional culture supplement for cultured meat production.

Key words : exosome, cell culture, bovine muscle satellite cells, proliferation, long-term culture

PC26032

Development of gelatin-based edible scaffolds for muscle satellite cell culture in cultured meat production

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In cultured meat production, edible three-dimensional scaffolds with extracellular matrix (ECM)-like properties are needed to support cell adhesion, proliferation, and differentiation under in vitro culture conditions. In this study, wet-spinning-based edible composite scaffolds were fabricated using gelatin and plant-derived materials and applied to muscle satellite cell culture. The gelatin based composite solutions showed a gradual increase in viscosity as the content of plant-derived materials increased. Cell proliferation was compared on days 1, 3, 5, and 7, and cells cultured on the T2 scaffold showed a significantly higher growth rate than those cultured on the other scaffold groups (p <0.001). Live/dead staining confirmed that cells were stably attached to the scaffold surface on day 1 and gradually proliferated by day 7. These results suggest that gelatin-based composite scaffolds can effectively support muscle satellite cell attachment and proliferation and may be used as edible three-dimensional scaffolds for cultured meat production.

Key words : scaffold, cell culture, bovine muscle satellite cells, proliferation, wet-spinning

PC26033

CRISPR/Cas9-mediated knockout of ISG15 and IDO1 in porcine alveolar macrophages for PRRSV resistance studies

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Porcine reproductive and respiratory syndrome virus (PRRSV) remains one of the most economically important viral pathogens in swine production, highlighting the need for host-directed strategies to improve resistance. In this study, we generated CRISPR/Cas9-mediated ISG15 and IDO1 knockout porcine alveolar macrophage (PAM) cell lines as candidate models for PRRSV resistance research. Four guide RNAs were designed for each target gene, and genome editing was followed by single-cell dilution cloning. Because both genes are inducible immune-related factors, protein expression was assessed after interferon stimulation. ISG15 protein was detected in parental cells after interferon treatment but was absent in ISG15-edited cells. Genotyping PCR and Sanger sequencing confirmed ISG15 clone 27 as a successfully edited clone harboring an approximately 254-bp deletion near the target region. Similarly, IDO1 protein expression was induced by IFN-γ in parental cells but was not detectable in IDO1-edited cells. Genotyping PCR and sequencing identified IDO1 clone 9 as a candidate edited clone with an approximately 105-bp deletion at the target site. These results confirm the successful establishment of ISG15- and IDO1-deficient PAM cell lines. Because both genes are closely linked to interferon-mediated antiviral and immunoregulatory pathways, these edited cells provide a useful platform for investigating host-gene-dependent modulation of PRRSV infection. Ongoing challenge studies will determine whether loss of ISG15 or IDO1 alters viral replication and macrophage antiviral responses.

Key words : PRRSV, CRISPR/Cas9, ISG15, IDO1, porcine alveolar macrophages, genome editing, antiviral immunity

PC26034

Comparative molecular docking and dynamics analysis of milk β-casomorphin binding across opioid receptor family

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β-Casomorphins (BCMs) are opioid-like peptides generated from β-casein during gastrointestinal digestion. Although BCM7 has been well studied for its interaction with the μ-opioid receptor (MOR), the binding properties of other BCM variants remain unclear. This study investigated the receptor-specific binding behavior of BCM3–BCM11 against MOR, δ-opioid receptor (DOR), κ-opioid receptor (KOR), and nociceptin/orphanin FQ peptide (NOP) receptors. Three-dimensional BCM structures were generated and docked to each opioid receptor subtype. Docking scores and binding poses were compared to identify receptor-preferred BCM variants by using Glide SP-Peptide docking. Selected high-affinity complexes were further analyzed using molecular dynamics simulations by 500ns. Docking analysis showed that BCM7 had the lowest docking score for MOR (−10.503 kcal/mol), whereas non-MOR receptors preferentially interacted with other BCM variants, including BCM11 for DOR (−9.039 kcal/mol) and KOR (−11.959 kcal/mol), and BCM9 for NOP (−10.798 kcal/mol). Molecular dynamics and protein–ligand contact analyses showed that representative BCM–receptor complexes reached equilibrated conformational states and maintained recurring receptor–peptide interaction networks supported by hydrogen bonding, hydrophobic contacts, ionic interactions. Overall, these findings suggest that BCM7 dominance is mainly MOR-specific, while other BCMs may preferentially bind non-MOR opioid receptors.

Key words : opioid receptor, β-casomorphins, peptide docking, molecular dynamics simulation

PC26035

Lipopolysaccharide-induced alterations in barrier integrity and inflammatory signaling in porcine small intestinal organoids

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Inflammation-associated diseases in pigs damage the intestinal tract and impair its function, posing a major threat to animal health and productivity. However, current *in vitro* models do not adequately replicate porcine physiological conditions. This study aimed to establish a novel inflammation model using porcine small intestinal organoids (pSIOs) derived from adult jejunum and stimulated with lipopolysaccharide (LPS). Inflammatory responses were evaluated through cytokine mRNA expression, intestinal barrier integrity, and changes in the expression and localization of nutrient transporters, receptors, and an enteroendocrine hormone associated with absorptive function using quantitative real-time PCR and immunofluorescence staining. Transcriptome analysis was also performed to identify LPS-responsive genes and enriched pathways. LPS successfully induced inflammation in pSIOs, as shown by increased cytokine expression, impaired barrier function, and altered nutrient transporter expression. Transcriptomic profiling further revealed broad transcriptional changes, including enrichment of immune- and inflammation-related pathways, as well as pathways associated with barrier maintenance and cellular adaptation to inflammatory stress. Collectively, these findings demonstrate that pSIOs represent a physiologically relevant *in vitro* model for studying porcine intestinal inflammation. This platform may be useful for investigating disease mechanisms and screening therapeutic candidates while supporting the 3Rs principles by reducing animal use.

Key words : lipopolysaccharide, inflammation, porcine, small intestinal organoid, disease model

PC26036

Sustained lactogenic differentiation of bovine mammary epithelial cells through RAC1 overexpression

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In the lactating mammary gland, mammary epithelial cells (MECs) are embedded within the extracellular matrix (ECM). Interactions between MECs and ECM activate RAC1, a Rho family small GTPase, which regulates signaling mechanisms involved in lactogenic differentiation. MEC–ECM interactions also regulate physiological processes, including cell polarization, differentiation, and reduced cell death, thereby facilitating long-term cell culture. This study aimed to investigate the effects of RAC1 activation in MAC-T cells on sustained lactogenic differentiation. MAC-T cells were transduced with lentiviral vectors to overexpress either GFP (GFPO) or RAC1 (RAC1O). To mimic an ECM-surrounding condition, GFPO cells were cultured on Matrigel-coated plates (M_GFPO). All cells were differentiated using lactation hormones. Increased active RAC1 levels, indicated by RAC1-GTP, were observed in both RAC1O and M_GFPO cells. In GFPO cells, cell viability and lactogenic differentiation persisted for only 10 days, after which cytotoxicity increased and differentiation capacity decreased. By comparison, RAC1O cells sustained cell viability and lactogenic differentiation for up to 20 days, whereas M_GFPO cells maintained these characteristics for up to 15 days. Collectively, these results demonstrate that RAC1 contributes to the prolonged maintenance of lactogenic differentiation and suggest that RAC1 activation may underlie ECM-mediated regulation of mammary gland function.

Key words : RAC1, matrigel, bovine mammary epithelial cell, lactogenic differentiation

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Dynamic molecular transitions of sperm storage tubules are associated with long-term sperm retention after artificial insemination in chickens

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Artificial insemination (AI) is an important reproductive technology in poultry breeding and production facilitating efficient genetic dissemination and reproductive management. In birds, AI enables prolonged fertility after a single insemination, largely due to sperm storage tubules (SST) located in the uterovaginal junction (UVJ). However, the molecular mechanisms by which SST support long-term sperm retention remain incompletely understood. In this study, we investigated time-dependent histological and transcriptomic changes in SST-containing UVJ tissues containing SST after AI. Histological analysis showed that sperm were absent before AI, became clearly detectable in SST at 3 days after AI, remained abundant at 7 days, and gradually declined thereafter, although sperm were still present in some SST at 21 days. No obvious structural damage to the SST epithelium was observed throughout the observation period. To characterize molecular responses during this process, RNA sequencing was performed on UVJ tissues containing SST collected at multiple post-AI time points. Among 8,522 expressed genes, distinct time-dependent expression patterns were identified, with the largest transcriptomic shift occurring at 3 days after AI (2,553 upregulated and 2,867 downregulated genes versus control), followed by persistent but gradually reduced transcriptional changes at later time points. Functional enrichment analyses revealed that genes showing gradual increases over time were associated with calcium, MAPK, and Wnt signaling, extracellular matrix interaction, focal adhesion, actin cytoskeleton regulation, and lipid-related metabolic pathways, whereas genes showing gradual decreases were enriched in immune-, inflammatory-, and defense-related pathways, including phagosome, necroptosis, and infection-associated terms. GO analysis further supported a transition from an early reactive state toward a more regulated functional state. Real-time RT-PCR validation of selected representative genes generally confirmed the major temporal trends identified by RNA-seq. Collectively, these findings indicate that long-term sperm storage after AI is accompanied by staged histological and transcriptomic reprogramming in UVJ tissues containing SST and suggest that prolonged sperm retention in birds is associated with dynamic molecular adaptation of the SST microenvironment.

Key words : uterovaginal junction, artificial insemination, sperm storage tubules, chicken