

## Animal Health: Swine and Other Species

**587 Comparison of porcine cathelicidin expression between Jinhua and Landrace pigs.** Y. Gao\*, S. An, Y. Xie, Y. Liu, F. Han, C. Luan, and Y. Wang, *Institute of Feed Science, Zhejiang University, Hangzhou, Zhejiang Province, China.*

Cathelicidins are important antimicrobial peptides with both antimicrobial and immunomodulatory functions in porcine innate immunity. However whether the expression levels of cathelicidin genes contribute to the disease resistance capabilities between different pig breeds still remains unclear. Therefore the present study was aimed to investigate the tissue-specific and developmental expression of *Protegrin-1*, *Prophenin-2* and *PR-39* genes in Jinhua pigs, in addition compare the baseline differential expression of these 3 cathelicidin genes between Chinese local Jinhua pigs and foreign landrace pigs at 20-d of age (before weaning) and 40-d of age (after weaning) by using quantitative real-time PCR. The results showed that cathelicidin genes were expressed in bone marrow, spleen, liver, lung, mesenteric lymph node, kidney and duodenum in both Jinhua and landrace pigs at 20- and 40-d of age. Bone marrow is the major expression site for the above 3 cathelicidin genes and the expression levels in bone marrow were significantly higher than in other tissues ( $P < 0.05$ ). Spleen, liver, mesenteric lymph node and lung had moderate expression of cathelicidin genes. Jinhua pigs showed higher expression of *Protegrin-1*, *Prophenin-2* and *PR-39* in bone marrow, spleen, liver, mesenteric lymph node compared with landrace pigs at 20- and 40-d of age, and the difference were significant in bone marrow between breeds ( $P < 0.05$ ). Moreover, cathelicidin genes were developmentally regulated in Jinhua pigs from neonatal to 120-d of age. Taken together, those results indicated that bone marrow was the major expression site of cathelicidin genes in both Jinhua and Landrace pigs, and the higher baseline expression of cathelicidin in Jinhua pigs may contribute to the better disease resistance capabilities, suggesting the expression of cathelicidin genes could relate to the disease resistance capabilities between different pig breeds.

**Key words:** cathelicidin, gene expression, Jinhua pigs

**588 The effect of prenatal stress and dominance order on immune function in response to a DTH and LPS challenge in pigs.** B. L. Davis\*<sup>1</sup>, M. A. Sutherland<sup>1,2</sup>, and M. A. Ballou<sup>1</sup>, <sup>1</sup>*Texas Tech University, Lubbock*, <sup>2</sup>*Ruakura Research Centre, AgResearch, Hamilton, New Zealand.*

Prenatal stress (PNS) is caused by elevated maternal glucocorticoid concentrations crossing the placenta to the fetus, resulting in potentially long-term physiological, immunological and behavioral changes in the offspring. The objective of this research was to determine the effects of PNS and dominance order on the cell mediated and acute-phase immune responses. Sows were either injected with adrenocorticotropic hormone or sham handled 3 times a week, from d 76 to 115 of gestation. At weaning, barrows from the stressed (PNS;  $n = 15$ ) and sham (PNS;  $n = 15$ ) sows were housed in groups of 3 within the same treatment. At 28 d of age, a food deprivation test was used to determine the dominance order of pigs within each pen. Pigs were denoted as being dominant (DOM), intermediate (INT) or submissive (SUB). A delayed-type hyperresponsivity (DTH) test was performed to assess the cell mediated immune response. One week after the DTH test, each pig was injected intraperitoneally with (include the dose) lipopolysaccharide (LPS) to stimulate an acute phase response. Blood samples were collected from pigs before and 60, 120, 360 min and 24 h after the

LPS challenge to measure complete leukocyte counts and differentials; as well as plasma concentrations of cortisol and C-Reactive protein. Rectal temperatures were taken at the same time blood samples were collected. Data were analyzed using the MIXED procedures of SAS. Prenatally stressed pigs had a greater ( $P < 0.05$ ) DTH response than CON pigs and DOM pigs had a lower ( $P < 0.05$ ) DTH response than SUB pigs. Rectal temperature and total leukocyte counts changed ( $P < 0.05$ ) over time in response to the LPS challenge; however, neither prenatal stress nor dominance order influenced the response. Cortisol concentrations following the LPS challenge were greater ( $P < 0.05$ ) in DOM compared with INT pigs and tended ( $P = 0.084$ ) to be greater than SUB pigs. The neutrophil to lymphocyte ratio was greater ( $P = 0.05$ ) in SUB pigs than INT pigs. In conclusion, prenatal stress and dominance order affected both cell mediated immune function and the physiological response to an LPS challenge in barrows.

**Key words:** pigs, prenatal stress, immune

**589 Effects of *Lactobacillus fermentum* 15007 on the redox state of healthy and oxidative-stressed piglets.** C. J. Cai\*, A. N. Wang, L. C. Chu, S. Y. Qiao, and D. F. Li, *China Agricultural University, Beijing, China.*

The study was conducted to investigate the effects of *Lactobacillus fermentum* 15007 (LF) on the redox state of healthy and oxidative-stressed piglets. A total of 24 weaned barrows ( $7.19 \pm 0.22$  kg) were randomly assigned to 1 of 4 treatments: control group (T1), stress group (T2), control group orally administrated LF (T3), and stressed group orally administrated LF (T4). The trial period lasted 21 d. Pigs in T3 and T4 were orally administrated with 20 mL/d ( $10^8$  cfu/ml) LF. On d 8, pigs in T2 and T3 were injected intraperitoneally with diquat at 10 mg/kg body weight, while pigs in T1 and T4 were injected the same volume of isotonic saline. Following the injection, blood was collected at 0.5 h, 1.5 h, 3.5 h, 7.5 h and 14.5 h. At the end of the experiment, all pigs were killed and the liver was sampled. Data were analyzed using the GLM procedure of SAS. The effects of diquat, LF and their interaction were included in the statistical model. The results showed that compared with saline-injected pigs, the diquat-injected pigs had decreased growth performance ( $P < 0.05$ ) during 2 wks after injection, and the increased levels of cortisol (26.90 vs 144.72 pg/ml,  $P < 0.01$ ), adrenaline (3.68 vs 27.90 ng/ml,  $P < 0.01$ ), the free fatty acid (67.02 vs 186.75  $\mu\text{mol/L}$ ,  $P < 0.01$ ), glucose (26.90 vs 144.72 mg/dl,  $P < 0.01$ ), malondialdehyde (MDA, 3.67 vs 4.03 nmol/ml,  $P < 0.01$ ), and carbonyl (0.93 vs 1.06 nmol/mg protein,  $P < 0.05$ ) in the plasma from 1.5 h to 14.5 h. Regardless of diquat, supplementation of LF improved the ADG (416 vs 446 g,  $P < 0.05$ ) and ADFI (630 vs 667 g,  $P < 0.05$ ); increased superoxide dismutase (105.23 vs 120.89 U/mg protein,  $P < 0.05$ ), glutathione peroxidase (93.94 vs 103.54 U/mg protein,  $P = 0.05$ ) and the ability to inhibit superoxide anion production (AISP, 316.90 vs 351.34 U/g protein,  $P = 0.05$ ), and reduced the levels of MDA (3.66 vs 3.45 nmol/mg protein,  $P < 0.05$ ) in pig liver. In conclusion, this study indicated that LF increased the growth performance of pigs, improved the antioxidative defense system, and alleviated the oxidative damage caused by oxidative stress.

**Key words:** *Lactobacillus fermentum*, piglet, oxidative stress

**590 In vitro antibacterial activity, cytotoxicity and mechanisms of cathelicidin peptides against enteric pathogens in weaning pig-**

lets. Y. Liu\*, S. An, C. Luan, and Y. Wang, *Institute of Feed Science, Zhejiang University, Hangzhou, Zhejiang Province, China.*

In the last few decades, long-term and high-dose usage of antibiotics in livestock diets induces the emergence of antibiotic resistance bacteria, antibiotics residues in animal products and environmental pollution, which have adverse effects on animal health and well-being. This study investigated 5 cathelicidin peptides from different animal origins (i.e., protegrin-1[PG-1], PMAP-23, LL-37, indolicidin and cathelicidin-BF[C-BF]) as antibiotic replacements with higher antimicrobial activity and lower cytotoxicity and to study their mechanisms of action toward enteric pathogens in vitro. The antibacterial activity was evaluated via minimum inhibitory concentration (MIC) determinations, killing kinetics and synergy assays. The morphology of peptide-treated bacteria was observed by transmission electron microscopy and intracellular function was determined by DNA binding and cell-free protein synthesis assays. Finally, cytotoxicity was assessed by hemoglobin release, cell viability and lactate dehydrogenase release assays. PG-1 from porcine and C-BF from snake had the most effective bacteriocidal properties and widest spectra of activity, with the MIC values equal to or lower than commonly used antibiotics toward several *Escherichia coli* isolates and *Salmonella* strains, and showed a synergistic effect with aureomycin. Mechanism studies for PG-1 and C-BF suggested the C-BF killing mechanism was based on membrane permeability, while multiple targets existed for PG-1, including membrane and intracellular biomacromolecules. Cytotoxicity tests showed PMAP-23 and C-BF exhibited the lowest cytotoxic effects, while PG-1, LL-37 and indolicidin displayed cytotoxicity by dose. Although PG-1 showed strong cytotoxic activity, there was less than 20% lysis at 8  $\mu$ g/mL at which there was remarkable antimicrobial activity. This study demonstrated that C-BF has the capacity to inactivate enteric pathogens with lower cytotoxicity and is potentially a novel anti-bacterial agent. The activity of PG-1 is highly efficient, with the potential to reduce cytotoxicity using molecular design.

**Key words:** cathelicidin peptides, antibacterial activity, mechanism of action

**591 Microbial transmission and assembly of the gut microbiota in neonatal pigs on day 7 and 14 postfarrowing.** E. E. Hinkle\*, I. Martinez, J. Walters, P. S. Miller, and T. E. Burkey, *University of Nebraska-Lincoln, Lincoln.*

The gastrointestinal microbiota (GM) effects gut maturation, nutrient metabolism, host immunity, and protection from pathogens. We employed 454-pyrosequencing of 16S rRNA tags to evaluate 1) effect of dam parity (P) on microbial ecology, and 2) microbial transmission from sows to their progeny. Fecal samples were collected from P1 and P3 sows (n = 6/P; d 7 postfarrowing) and 1 piglet/litter on d 7 and 14 postfarrowing. Microbial DNA was extracted and pyrosequenced (Roche Genome Sequencer GS-FLX Titanium). Quality controlled, chimera checked sequences were taxonomically assigned (Classifier, Ribosomal Database Project). An  $\alpha$  diversity (Shannon) measure was determined using Qiime. Phylogenetically blasted  $\beta$  diversity was obtained (UniFrac). There were no P effects on GM composition or diversity. The phyla Firmicutes and Bacteroidetes dominated in P1 (83.8% and 5.6%, respectively), and P3 sows (91.0% and 3.1%, respectively). A parity  $\times$  d ( $P < 0.05$ ) interaction was observed within the phylum Firmicutes. Specifically, P1 sows (83.8%) and d 7 progeny (81.48%) had increased Firmicutes compared with their d 14 progeny (61.7%), and P3 sows (91.0%) had increased Firmicutes compared with their d 7 (60.8%) and 14 (63.0%) progeny. Time effects ( $P <$

0.05) were observed for Bacteroidetes, Fusobacteria, and Proteobacteria among progeny from both parities. Bacteroidetes were increased ( $P < 0.001$ ) in piglets compared with sows (11.0 and 25.4% vs. 5.6% for P1, 22.8 and 27.4% vs. 3.1% for P3; respectively, for d 7 and 14 vs. sows on d 7). Fusobacteria and Actinobacteria in progeny were greater on d 7 compared with d 14 (Fusobacteria, 4.13 and 0.17%, Actinobacteria, 4.49 and 0.45%; respectively). Proteobacteria in progeny was increased on d 14 (5.8 and 7.4% for P1 and P3 piglets, respectively) compared with d 7 (0.76 and 3.70% for P1 and P3 piglets, respectively). Sows had increased ( $P < 0.001$ ) Shannon's index compared with progeny. The GM of progeny had greater  $\beta$  diversity than sows ( $P < 0.001$ ). These results represent an initial investigation into transmission and establishment of microbial populations from sows to their progeny.

**Key words:** parity, gut microbiota, swine

**592 Viability of *Parascaris equorum* eggs intermittently exposed to the interior of a windrow composting system.** J. C. Gould\*, E. T. Lyons, L. M. Lawrence, and M. G. Rossano, *University of Kentucky, Lexington.*

*Parascaris equorum* generally infects horses less than 18 mo; its pathological effects can be severe. The purpose of this study was to examine the effects of windrow composting on the viability of *P. equorum* eggs intermittently exposed to the interior of a windrow. ANKOM F57 filter bags were used as sentinel chambers and spiked with manure confirmed positive for *P. equorum* eggs. Starting on d 0, chambers were placed within the center of the windrow at 5 different locations, then alternated between resting on top of, or inside, the windrow whenever it was turned. Chambers from each location and control chambers were removed at d 2, 4, 6, 8, 10, 12, 14, and 18; chambers incubated for 21 d at room temperature (24°C). After incubation, chamber material was diluted with a 10% bleach solution, subsampled, and eggs were recovered using double centrifugation with Sheather's solution. Eggs were evaluated using a microscope and classified as viable or nonviable. Efficacy was assessed by 2-tailed *t*-test; a *P*-value of  $< 0.05$  was deemed to be significant. The average % viability of *P. equorum* eggs exposed to composting were 10.77, 0.31, 0.00, 0.00, 0.00, 0.00, and 0.00 on sampling d 2, 4, 6, 8, 10, 12, 14, and 18 respectively. The average % viability of control *P. equorum* eggs were 91.34, 79.97, 90.37, 89.93, 81.30, 86.74, 83.29, and 86.94 on sampling d 2, 4, 6, 8, 10, 12, 14, and 18 respectively. Intermittent windrow composting treatment reduced the percent viable eggs compared with the control on d 2 and 4 ( $P < 0.000002$ ). By d 6, percent viable eggs for sentinel containers under the intermittent windrow composting treatment dropped to 0.00 and remained this way for d 8, 10, 12, 14, and 18. The results of this study demonstrate that a well maintained windrow composting system is capable of rendering *P. equorum* eggs nonviable even under intermittent exposure within the windrow.

**Key words:** parasite, *Parascaris equorum*, compost

**593 Effect of a yeast nucleotide product on performance and health status of broilers.** A. Ganner\*, S. Schaumberger, J. Uhlik, and G. Schatzmayr, *BIOMIN Research Center, Tulln, Lower Austria, Austria.*

Nucleotides are involved in various essential biochemical processes; in animal studies dietary nucleotide supplementation has been shown to exert positive effects on performance, growth, gut health and the immune system. Yeasts as feed additives are an economical and practi-

cal way to provide concentrated nucleotides to the animal. The present study was conducted to evaluate the efficacy of a product, consisting of purified nucleotides, on performance and health status of broilers. The nucleotide product contained 27% RNA-monomers (AMP 9%, GMP 6%, CMP 5%, UMP 4%, nucleosides 3%). In a 35-day study, 675 1-d-old mixed sexed broilers were distributed into 3 experimental groups with 8 replicates: control group A, group B and C with 0 kg, 0.2 kg and 2 kg of nucleotide product per ton feed. Directly after housing the chicks were supplied with the experimental diets. Feed and water were provided ad libitum, feeding was done manually several times a day. Chicks were group weighed at day 1, day 14 and single weighed at day 35. On day 14, statistical significant differences ( $P < 0.05$ ) for weight and average daily weight gain could be observed in group C (2 kg per ton feed) compared to group B (0.2 kg per ton feed) and the control group. At the end of the trial no more statistical differences could be observed in group C ( $P > 0.05$ ), but group B showed positive trends over all parameters (weight day 35,  $P = 0.5$ ; average daily weight gain (ADWG) day 15-35,  $P = 0.1$ ; ADWG d 1-35,  $P = 0.5$ ; FCR d 15-35,  $P = 0.09$ ; FCR d 1-35,  $P = 0.2$ ) compared to group C and the control group. Mortality was slightly reduced in both trial groups with 1.3%, compared to the control (1.8%). Our results indicate that proper nucleotide dosage and supplementation period may have beneficial effects on broilers growth and performance; however over-dosage of nucleotide additives which could cause loss in performance through potential over-reaction of the immune system should be avoided.

**Key words:** yeast nucleotides, broiler performance, growth

**594 The effect of *Vernonia amygdalina* leaf extract on Alloxan-induced diabetic rats.** A. H. Ekeocha\*, P. C. Ekeocha, and T. Fashola, *University of Ibadan, Ibadan, Oyo, Nigeria.*

The hypoglycaemic or sugar reducing effect of the bitter leaf extract (BLE) was determined using Alloxan-induced diabetic rats. Thirty male Albino rats were divided into 6 groups of 5 rats. Four groups with basal blood sugar levels of  $38 \pm 0.16$ ,  $39.2 \pm 0.23$ ,  $35.2 \pm 0.27$ , and  $35.8 \pm 0.25$  mg/dl were injected with 10% alloxan in saline to make them diabetic ( $277.6 \pm 6.55$ ,  $284.8 \pm 3.80$ ,  $256.4 \pm 1.39$  and  $265.6 \pm 4.41$  mg/dl fasting blood sugar (FBS) respectively). The 4 diabetic groups were then treated with different doses (g/kg body weight,

BW) of an aqueous extract of dried bitter leaf herein referred to as BLE. A fifth group (non diabetic) was treated with 400mg BLE /kg BW. BLE was administered twice daily for 2weeks using an oral cannula. The sixth group (non-diabetic) received no BLE as a positive control. Blood was collected from the tail to determine blood on a glucometer. The FBS levels of the 6 albino rat groups were recorded every 2 days for 2 weeks. At the end of wk 2, the rats were slaughtered and their liver, kidney and pancreas examined. Data were analyzed using ANOVA (SAS, 1999). All the rats injected with alloxan became diabetic as their fasting blood sugar (FBS) levels exceeded the normal range of between 80 and 100 mg/dl. The FBS of the diabetic albino rats significantly ( $P < 0.05$ ) decreased as BLE levels increased from 50 to 400 mg/kg BW on days 2, 4, 6, 8, 10, 12 and 14 (Table 1). The plant extract was observed to have a hypoglycemic effect on each group of diabetic rats as it reduced FBS levels (mg/dl) from  $277.6 \pm 6.55$  to  $92.0 \pm 1.68$  (Group 1),  $284.8 \pm 3.80$  to  $68.8 \pm 0.41$  (Group 2),  $256.4 \pm 1.39$  to  $55.8 \pm 0.49$  (Group 3) and  $265.6 \pm 4.41$  to  $38.4 \pm 0.21$  (Group 4) over a period of two weeks. The extract reduced the level of damage to the kidney, liver and pancreas when administered on diabetic rats. The rats were considered treated when their FBS returned to almost their basal blood sugar (BBS) levels. *Vernonia amygdalina* has anti-diabetic properties as it reduced the blood sugar level of albino rats.

**Table 1.** Fasting blood sugar (mg/dl) of normal and induced-diabetic albino rats administered with varying doses of *Vernonia amygdalina* leaf extracts

No of days	Gp1	Gp2	Gp3	Gp4	Gp5	Gp6	SEM
2	237.4 <sup>a</sup>	226.6 <sup>b</sup>	203.2 <sup>c</sup>	201.8 <sup>c</sup>	65.6 <sup>d</sup>	54.4 <sup>e</sup>	2.1
4	231.2 <sup>a</sup>	200.0 <sup>b</sup>	151.6 <sup>c</sup>	94.0 <sup>d</sup>	60.0 <sup>e</sup>	46.0 <sup>f</sup>	2.1
6	196.0 <sup>a</sup>	176.0 <sup>b</sup>	94.0 <sup>c</sup>	72.0 <sup>d</sup>	55.0 <sup>e</sup>	44.0 <sup>f</sup>	1.3
8	163.4 <sup>a</sup>	132.0 <sup>b</sup>	89.0 <sup>c</sup>	62.0 <sup>d</sup>	47.6 <sup>e</sup>	41.8 <sup>f</sup>	1.6
10	142.2 <sup>a</sup>	102.0 <sup>b</sup>	75.0 <sup>c</sup>	50.8 <sup>d</sup>	42.2 <sup>e</sup>	41.2 <sup>e</sup>	1.5
12	110.4 <sup>a</sup>	80.0 <sup>b</sup>	61.0 <sup>c</sup>	44.0 <sup>d</sup>	42.2 <sup>de</sup>	40.0 <sup>e</sup>	1.7
14	92.0 <sup>a</sup>	68.8 <sup>b</sup>	55.8 <sup>c</sup>	38.4 <sup>d</sup>	38.0 <sup>d</sup>	40.0 <sup>d</sup>	1.6

Means on the same row with different superscripts differ significantly.

**Key words:** *Vernonia amygdalina* leaf extract, Alloxan-induced diabetic rats