

## Swine Species

**759 Casein glycomacropeptide (CGMP) in the diet of early weaned piglets reduces the *Escherichia coli* attachment to the intestinal mucosa and increases lactobacillar numbers in digesta.** R. G. Hermes\*, F. Molist, J. F. Pérez, A. G. de Segura, M. Ywazaki, and S. M. Martín-Orúe, *Universitat Autònoma de Barcelona, Barcelona, Spain.*

Adherence of bacteria to the intestinal mucosa is considered a prerequisite step for the colonization of pathogens. In this study we evaluated if CGMP in the diet can reduce the attachment of *E. coli* to the intestinal mucosa of oral challenged animals by enterotoxigenic *E. coli* (ETEC) K88. The experiment included 72 piglets (24 ± 3 d old) with an average BW of 6.9 ± 0.46 kg, divided into 4 treatments according to a 2 × 2 factorial design (2 diets including or not 1.5% of CGMP; ETEC challenged or not). Four days after a single 2-mL oral dose (10 CFU/ml) of the ETEC, 24 animals were killed and ileal (digesta and tissue) and colonic samples (digesta) were collected. Enterobacteria and lactobacilli were quantified in digesta by real time PCR (using SYBR green dye). Attachment of *E. coli* to ileal mucosa was monitored by FISH (fluorescent in situ hybridization) staining technique using EC1531 oligonucleotide probe, specific for *E. coli* and labeled with cyanine 3). The data were analyzed using the GLM procedure of SAS (1999), with factorial ANOVA design ( $P = 0.05$ ). The ETEC challenge produced a mild diarrhea with increases in enterobacteria in ileal (9.2 vs. 8.4 log of 16S rDNA copies/g,  $P = 0.033$ ) and colonic digesta (11.2 vs. 10.2 log of 16S rDNA copies/g,  $P = 0.004$ ), and *E. coli* attachment to the ileum mucosa (34.6 vs. 8.6% of villi with bacteria adhered,  $P = 0.031$ ). The inclusion of CGMP did not produced significant changes in the animal performance. However, it increased the lactobacilli in the colonic digesta (11.7 vs. 10.9 log of 16S rDNA copies/g,  $P = 0.007$ ) and reduced the ileal enterobacteria, especially in the challenged animals (8.3 vs. 10.1 log of 16S rDNA copies/g,  $P$  interaction = 0.006). The CGMP most relevant result was observed on the reduction of *E. coli* adhered to the ileal mucosa (10.9 vs. 32.4% of villi with bacteria adhered,  $P = 0.034$ ), although it was not observed significant interaction. Our results suggest that the inclusion of 1.5% of CGMP in piglet diets modify the intestinal microbial populations and impair the *E. coli* attachment to the intestinal mucosa after an ETEC oral challenge.

**Key Words:** casein glycomacropeptide, *Escherichia coli*, piglet

**760 Early- vs. late-gestation dietary lysine requirement of young sows.** R. S. Samuel\*<sup>1</sup>, S. Moehn<sup>1</sup>, P. B. Pencharz<sup>1,2</sup>, and R. O. Ball<sup>1,2</sup>, <sup>1</sup>Department of AFNS, University of Alberta, Edmonton, Alberta, Canada, <sup>2</sup>Research Institute, Hospital for Sick Children, Toronto, Ontario, Canada.

Pregnant sows are traditionally fed a single dietary formulation during the entirety of gestation. The optimal feeding strategy should account for changing dietary requirements due to the linearly increasing growth rate of fetuses during the last third of gestation and the development of the mammary gland close to parturition. Young, rapidly growing sows might be expected to have greater requirements than older, slower growing sows. The objective of this study was to determine the lysine requirement of a population of 2nd and 3rd parity sows in early- (d 24 – 45) and late- (d 86 – 110) gestation. Pregnant Hypor Hybrid (Hypor Inc.) sows ( $n = 7$ ; 185.7 ± 9.6 kg BW) were adapted to individual intakes of a semi-synthetic diet containing 14.0 MJ ME/kg. Each sow received 6 different test diets, in random order. These ranged from 60 – 150% and from 60 – 185% of the requirement suggested by NRC (1998) in early- and late-gestation, respectively. These were equivalent to dietary lysine

intakes of 7.5 – 19.3 g/d in early- and 8.1 – 23.7 g/d in late-gestation. Oxidation of the indicator amino acid L-[1-<sup>13</sup>C]-phenylalanine (Phe) during a primed-constant oral infusion was measured. The average number of piglets born alive was 13.7 ± 1.9, but ranged from 4 to 20. The average piglet birth weight was 1.5 ± 0.1 kg. Sows gained 600 g/d from breeding and weighed 258.8 ± 8.3 kg before farrowing. Break-point analysis of Phe oxidation indicated that the lysine requirement of 2nd parity sows was 13.1 g/d and 18.7 g/d in early- and late-gestation, respectively. For 3rd parity sows, the dietary lysine requirement was 8.2 g/d and 13.0 g/d for early- and late-gestation, respectively. The dietary requirements for lysine in early- and late-gestation were greater than previously reported by NRC (1998). Growing evidence indicates that phase feeding of sows would be economically advantageous by reducing feed costs and maximizing lifetime productivity.

**Key Words:** sow, gestation, lysine

**761 A wheat bran extract shows a high attachment to K88 *Escherichia coli* in vitro.** F. Molist, R. G. Hermes\*, J. F. Pérez, and S. M. Martín-Orúe, *Universitat Autònoma de Barcelona, Barcelona, Spain.*

The aim of this study was to elucidate in vitro the ability of wheat bran (WB) and other fiber sources to bind K88 *E. coli* as a likely blocking mechanism of its attachment to the intestinal mucosa. The in vitro adhesion test was done in polystyrene 96-well plates using WB, rice hulls, soybean hulls, cereal straw, sugar beet pulp, pea hulls and oat hulls as possible blocking agents and 2 *E. coli* strains (K88 and non-fimbriated), isolated from the feces of post-weaning piglets. The fiber sources were diluted in phosphate buffer (PBS, 4%, w/v), sonicated 3 times and centrifuged. The supernatant was introduced in the plate and incubated at 4°C overnight. After that, the plate was washed with sterile PBS and the non-specific adhesion sites blocked with 1% BSA. The test of adhesion started when *E. coli* strains were inoculated at a concentration of 10<sup>8</sup> CFU/mL and allowed to adhere during 30-min at room temperature. Finally the plate was washed with sterile PBS to remove the non-attached bacteria and Luria Broth media was added to promote the growth of the attached bacteria. Plates were incubated at 37°C/10 h in a spectrophotometer where the optical density (OD, 650 nm) was recorded every 10 min. All OD data were processed using the PROC NLIN of SAS. The parameters thus obtained were used to calculate  $t_{OD=0.05}$  (delay time (h) for the cultures to reach an OD of 0.05 at 650nm). Analysis of variance of the  $t_{OD=0.05}$  values was done using the PROC GLM of SAS. Results showed that the non-fimbriated *E. coli* adhered less to the fiber substrates compared with the K88 (3.14 vs. 2.72h,  $P = 0.001$ ) which indicates that the presence of fimbria (F4) play an important role in the interaction bacteria-fiber substrate. Regarding the fiber sources, more bacteria bind to the WB (0.94 and 2.73 h for K88 and non-fimbriated, respectively,  $P = 0.001$ ) compared with the other fiber sources (average 2.97 and 3.20 h). Our results suggest that WB could act as an anti-adhesive ingredient against pathogenic *E. coli* and improve the animal health in the post-weaning period.

**Key Words:** wheat bran, *E. coli*, adhesion

**762 Effect of a softer surface in the farrowing crate on feed intake of lactating sows.** A. Da Silva\*, S. S. Anil, J. Deen, and S. K. Baidoo, *University of Minnesota, Saint Paul.*

Lameness in swine herds is both an economic and welfare concern. The pain associated with lameness may decrease lactation feed intake. An

important cause for lameness has been suggested to be claw lesions. Claw lesions in pigs are the result of the interaction between claw and the floor surface. However, the effect of floor type on lameness and its effects on feed intake of sows are poorly understood. The present study aimed to explore the effect of providing rubber mats in farrowing crates as a measure to minimize the adverse effects of lameness on feed intake of lactating sows. This study involved 70 lame and 70 non-lame gestating sows (gestation stall  $n = 63$  and group pens with ESF  $n = 77$ ) identified based on their ability to bear weight on all limbs without favoring any particular limb, on d 109 of gestation when they were moved from the gestation housing systems to farrowing rooms. Equal numbers of sows were randomly allocated to farrowing crates with cast iron total slatted floor or to crates with rubber mats on the cast iron total slatted floor in the posterior half. Sows in farrowing crates were hand-fed twice daily weighed amount of feed which was recorded. Feed consumed was assumed to be equal to that fed if the feeder was empty. Orts were weighed on weaning day to estimate feed intake during the lactation period. The frequency of the number of lactation days was calculated for all sows using daily feed intake (d 2 to 14 of lactation) category of 0 to 5 lbs (2.27 kg). The feed intake of lame and non-lame sows with and without rubber mats were compared using separate models for each category, controlling for the effect of housing system (Proc Genmod, SAS v 9.2). The number of days sows consumed less than 5 lbs (2.27 kg) was 42% higher in lame sows and 30% lower in stall housed sows ( $P < 0.05$  for both). Rubber mat was not found to be associated with feed intake in this study.

**Key Words:** lameness, sow, feed intake

**763 Effect of P.G. 600 on estrous cycles in gilts.** M. J. Estienne\* and R. J. Crawford, *Virginia Polytechnic Institute and State University, Blacksburg.*

A combination of eCG (400 IU) and hCG (200 IU) (P.G. 600, Intervet/Schering-Plough Animal Health, De Soto, KS) is labeled for stimulating first estrus in prepubertal gilts. Variation exists among farms, however, in the estrual response. Perhaps at least some of the variation is due to inadvertent treatment of gilts that are already cycling. The objective was to determine the effect of i.m. P.G. 600 on estrous cycle length. Prepubertal gilts (110 kg BW and 175 d of age) were checked for estrus in the presence of a boar daily throughout the experiment. Gilts in Treatment (TRT) 1 ( $n = 16$ ) received P.G. 600 at the onset of boar exposure. Gilts in TRT 2 – 5 ( $n = 16$ /TRT) were allowed to express a natural first estrus and were then treated with P.G. 600 as follows: TRT 2, at d 6; TRT 3, at d 12; and TRT 4, at d 18 of the cycle. Gilts in TRT 5 received no P.G. 600. More ( $P < 0.05$ ) gilts in TRT 1 (43.8%) were in estrus by d 7 of the experiment compared with gilts in TRT 2 – 5 (20.3%), and for gilts in estrus by d 7, the interval from the start of the study was lesser ( $P < 0.01$ ) for TRT 1 compared with TRT 2 – 5 ( $4.7 \pm 0.3$  vs.  $6.1 \pm 0.3$  d). Gilts displaying estrus during the entire experiment (97.5%) was not affected by treatment ( $P = 0.28$ ). Gilts that displayed a normal estrous cycle (17 – 24 d) was greater ( $P < 0.05$ ) for TRT 4 (100%) and 5 (100%) compared with TRT 1 (73.3%) and 3 (60%), with TRT 2 having a value (87.5%) that was not different from the other groups. Abnormal cycle lengths were  $37.0 \pm 9.4$  d for TRT 1,  $23.7 \pm 13.2$  d for TRT 2, and  $32.5 \pm 7.6$  for TRT 3 ( $P < 0.75$ ). The percentages of gilts that expressed a first estrus but then were anestrous for the remainder of the experiment was 6.7 for each of TRT 1 and 3, and 0 for TRT 2, 4 and 5 ( $P < 0.23$ ). Although mechanisms responsible for these effects must be further scrutinized, the results demonstrate to swine producers the need to correctly classify replacement gilts as prepubertal or cycling before administering P.G. 600.

**Key Words:** P.G. 600, estrous cycle, gilts

**764 Analysis of the association between lameness and claw lesions in stall-housed gestating sows.** A. Da Silva\*, S. S. Anil, J. Deen, and S. K. Baidoo, *University of Minnesota, Saint Paul.*

Lameness in sows has both economic and welfare implications. Severe claw lesions can cause lameness in pigs. Not all claw lesions may lead to lameness in pigs. Housing conditions and management practices may be associated with the development of claw lesions. An evaluation of the association between claw lesions and lameness would provide strategies to minimize the incidence of such lesions and reduce removal of sows for lameness. The objective of this study was to analyze the association of lameness with different types of claw lesions in sows. Claws of 63 stall-housed sows (parities 1 to 8) in a breeding herd at the University of Minnesota, Southern Research and Outreach Center, Waseca, MN were individually examined for lesions on d 110 of gestation when sows were in the farrowing crates, before farrowing. Lesions included erosions, cracks, and overgrowths. Areas in the claw were categorized as side wall, heel including overgrown heel, sole, heel-sole junction, white line and overgrown dew claw and toe. Lesions were scored on a scale of 0 (no lesion) to 3 (severe lesion). The total score for each claw area was obtained by adding the scores for that area in the lateral and medial claws of front and hind limbs. The level of lameness in these sows was assessed when they were moved to the farrowing crates, based on their ability to bear weight on all 4 limbs without favoring any particular limb. A multivariate logistic regression analysis was performed (Proc Logistic, SAS v 9.2) to assess the association of lesion scores of different claw areas with lameness (lame vs. non-lame). Lesions on the heel and the white line were associated with lameness. Total heel lesion score was negatively associated with lameness (odds ratio 0.65,  $P < 0.05$ ). For every unit increase in total white line lesion score, the likelihood of lameness increased by 31% ( $P < 0.05$ ). The likelihood of lameness increased with an increase in the severity of lesions in other claw areas as well, though the associations were not statistically significant.

**Key Words:** claw lesions, lameness, gestation stall

**765 Design of porcine lactoferricin-based antimicrobial peptides with improved activity.** F. F. Han\*, Y. F. Liu, Y. G. Xie, Y. H. Gao, and Y. Z. Wang, *Feed Science Institute of Zhejiang University, Hangzhou, Zhejiang, China.*

Structure-function relationships in antimicrobial peptides have been extensively investigated to obtain improved analogs. In the present study, a series of synthetic derivatives of porcine lactoferricins were prepared with an aim to understand the structural basis of activity as well as improve its activity. We found that the 20-residue porcine lactoferricin (LP20) and its derivatives LF2A, LF-1, LF-3 displayed antimicrobial activity against *Escherichia coli*, *Staphylococcus epidermidis*, *Salmonella choleraesuis*, and *Salmonella typhimurium*. The minimal inhibitory concentrations of LP20 ranged from 64 to 128  $\mu\text{g}/\text{mL}$ ; LF2A, LF-1 and LF-3 were 2–8 times more effective than LP20. The studies demonstrate that replacing the Cys with Ala not only increased the activities against gram-negative bacteria but also decreased hemolytic activity. Replacing the Ile with Trp both increased the antimicrobial and hemolytic activity at 4, 32, 64, 128, and 256  $\mu\text{g}/\text{mL}$  ( $P < 0.05$ ). The cytotoxic potential of LP20 analogs were quantified by colorimetric WST-1 and LDH assays in PBMC. LF2A, LF-1 and LF-3 increased cell proliferation and viability in a dose dependent fashion. Compared with LP20, 25–200  $\mu\text{g}/\text{mL}$  LF-1 improved the cell proliferation significantly ( $P < 0.05$ ), while 400  $\mu\text{g}/\text{mL}$  LF-1 decreased the cell proliferation ( $P < 0.05$ ). Both 200  $\mu\text{g}/\text{mL}$  and 400  $\mu\text{g}/\text{mL}$  LF-1 induced an increase ( $P < 0.05$ ) in LDH release from PBMC whereas 25–50  $\mu\text{g}/\text{mL}$  decreased the LDH release ( $P < 0.05$ ). Moreover, LF-1, LF-3 able to disrupt the

cytoplasmic membranes at relatively low concentrations. In contrast, LP20 and LF2A had more-modest antibacterial activities, a lesser ability to depolarize the cytoplasmic membrane.

**Key Words:** porcine lactoferricin, antimicrobial peptide, cytotoxic activity