188 Development of a *Bacillus subtilis* product for a large commercial swine farm to reduce *Clostridium perfringens* and *Clostridium difficile* in neonatal pigs. A. Baker*[1], E. Davis[1], D. Rosener[1], K. Novak[1], R. White[2], A. Veldkamp[1], and T. Rehberger[1], [1]*Agtech Products, Inc.*, *Waukesha, WI*, [2]*Iowa Select Farms*, *Iowa Falls, IA*.

Disease caused by *Clostridium perfringens* Type A and *Clostridium difficile* is common in neonatal pigs and can lead to scouring, low weaning weights, and pre-weaning mortality. This study evaluated the diversity of *C. perfringens* Type A and *C. difficile* in neonatal pigs in a large commercial swine farm to develop a *Bacillus*-based direct-fed microbial to control the incidence of disease. Neonatal pigs with clinical signs of scours and a small number of healthy pigs were swabbed rectally during nine months at 11 sites throughout the commercial swine farm. Samples were plated on selective media for *C. perfringens* and *C. difficile*, isolated colonies were picked, and DNA was extracted. Multiplex PCR confirmed the identification of 794 isolates as *C. perfringens* Type A and 476 isolates as *C. difficile* from the 333 pigs swabbed. *C. perfringens* Type A was isolated from 89.8% of the pigs and *C. difficile* from 57.6% of the pigs. Dendrograms were constructed using RAPD PCR to cluster the toxigenic isolates according to banding patterns. The *C. perfringens* Type A dendrogram consisted of 138 clusters representative of 794 isolates at a similarity coefficient of 80%. The largest cluster in the *C. perfringens* Type A dendrogram contained 131 isolates, 47 of which had identical RAPD types. The *C. difficile* dendrogram was comprised of 476 isolates grouped into 126 clusters. The largest cluster contained 30 isolates — 13 with identical RAPD types. At least one isolate per cluster was tested against six unique strains of *Bacillus subtilis* and inhibition of clostridial growth was measured by light absorbance. Of the six *Bacillus* strains tested, four provided 90% or greater inhibition of *C. perfringens* and *C. difficile*, whereas inhibition by two strains varied depending upon type of clostridia (type × strain interaction, $P \leq 0.001$). The results of this evaluation indicate the presence of a high degree of diversity in *C. perfringens* and *C. difficile* populations within this commercial swine farm and suggest that these populations can be controlled by the specific selection of *Bacillus* strains.

Key Words: swine, clostridia, microbial flora

189 Effects of water-soluble and in-feed organic acids on the growth performance of weanling pigs. R. O. Gottlob, J. M. Benz*, S. S. Dritz, M. D. Tokach, R. D. Goodband, J. M. DeRouchey, and J. L. Nelssen, *Kansas State University*, *Manhattan*.

A total of 360 weanling pigs (initially 5.2 kg and 18 ± 3 d of age, PIC) were used in a 42-d growth assay to determine the effects of water-soluble antimicrobials and organic acids in feed or water on nursery pig growth performance. Pigs were blocked by BW and allotted to one of nine experimental treatments. There were five pigs per pen and eight pens per treatment. The nine treatments included: 1) control (no feed or water antimicrobials or acids); 2) water containing 38 mg/L neomycin sulfate; 3) water containing 0.06% Activate WD; 4) water containing 0.12% Activate WD; 5) feed containing neomycin and oxytetracycline (154 ppm neomycin sulfate, 154 ppm oxytetracycline HCl; neooxy); 6) feed containing 0.50% Activate DA; 7) feed containing 0.45% Starter L; 8) feed containing 0.45% Multimax L; and 9) feed containing 0.50% Activate DA and 0.10 % Mintrex P. Activate™ WD (water dispersible), DA (dry acid), Starter L (liquid), and Multimax L (liquid) organic acid blends are combinations of Methionine hydroxyl analog (MHA) and butyric, propionic, and/or lactic acids with methionine activity ranging from 29% to 31%. Mintrex P is a mixture of organic trace minerals including zinc, copper, and manganese with some residual methionine activity as a result of having MHA as the carrier. From d 0 to 14, pigs provided antimicrobials, Multimax L, or the combination of Activate DA and Mintrex had greater ($P < 0.05$) ADG than pigs fed the control. Overall (d 0 to 42), pigs provided neo/oxy in the feed had greater ($P<0.05$) ADG, compared with pigs in all other treatments, except pigs provided the combination of Activate DA and Mintrex P which were intermediate. Pigs provided neo/oxy in the feed had greater ADFI ($P<0.02$) than did pigs provided the control treatment. There were no differences in feed efficiency between any of the treatments. These data demonstrate that pigs provided in-feed antimicrobials had improved growth, whereas those provided organic acids in feed and water did not.

Key Words: nursery pig, antimicrobials, organic acids

190 Effect of probiotics supplementation on growth performance and immune response in weaning pigs. Y. D. Jang*, H. K. Oh, H. B. Choi, and Y. Y. Kim, *Seoul National University*, *Seoul, Korea*.

Probiotics are defined as a live microbial feed supplement which beneficially affects the host animals by improving its intestinal microbial balance. It is known that probiotic effects were mediated through immune regulation, particularly through balance control of cytokines. There have been several efforts to search for alternatives to antibiotics. The objective of this study was to investigate the effect of probiotics supplementation on the growth performance and immune response of weaning pigs. Treatments were 1) NCON (basal diet), 2) PCON(basal + 0.12% avilamycin) and 3) A(basal + 0.2% Aspergillus oryzae), 4) B(basal + 0.2% Lactobacillus casei), 5) C(basal + 0.2% Bacillus subtilis), 6) D(basal + 0.2% Lactobacillus cripticus). A total of 120 pigs ([L x Y] x D, 7.16 ± 0.01 kg average body weight, weaned at 23 ± 3 days of age) were assigned to 6 treatments of 5 replicates of 4 pigs per pen in a randomized complete block design. During the whole experimental period, ADG (217, 294, 223, 236, 226, 225 g, $P<0.01$) and ADFI (474, 548, 451, 489, 476, 467 g, $P<0.05$ for NCON, PCON, A, B, C, D, respectively) of group PCON were greater than other treatments but G:F ratio in treatment A(Aspergillus oryzae) was similar to group PCON (0.538, 0.495, $P<0.05$ for PCON, A, respectively). Lipopolysaccharide was injected to induce immune response in weaning pigs. At 3h post-injection, blood was collected and analyzed for CD4+ and CD8+ T-cells. The CD4+/CD8+ ratio was determined using the fluorescence cytometry method. The number of CD4+ T cells was significantly lower in group C than in other treatments ($P<0.10$). Pigs in the NCON and PCON group had more CD4+ T cells than the pigs in the probiotics treatments (1026.7, 1075.7, 906, 758, 421, 758, $P<0.10$ for NCON, PCON, A, B, C, D, respectively). The number of CD8+ T cells was not significantly different among treatments. There were no differences in the ratios of CD4+/CD8+ among treatments. These results demonstrated that supplementation of 4 different probiotics had potential effects on growth performance and immune response in weaning pigs.

Key Words: probiotics, weaning pigs, CD4