980 Pre-hatch colonization of the chick gut with probiotic bacteria. J. E. de Oliveira*[1], J. M. B. M. van der Vossen[2], A. M. T. Ouwens[2], E. Hangoor[1], and T. A. Scott[1], [1]*Provimi*, *Velddriel, the Netherlands*, [2]*TNO*, *Zeist, the Netherlands*.

Poultry are believed to hatch with minimal amounts of intestinal bacteria present. Gut microflora is believed to be established during or after hatch. This is considered to be a key event with consequences that affect not only the bird's health and performance, but also relates to food safety risks with establishment of food borne pathogens, particularly into the ceca. The current view is that establishment and maintenance of "good" or probiotic microbiota can minimize or prevent overgrowth of pathogens. This study was designed to test the viability to colonize the chick intestine with probiotic bacteria before hatch. Embryonized (E17) chicken eggs were in ovo inoculated with medium containing *B. subtilis* (P1) or *E. faecium* (P2), and compared with embryos from non-inoculated eggs (control). Number of bacteria was calculated as cell equivalents (CE) based on amount of bacterial DNA determined by qPCR at 48 h after inoculation in the embryo's gizzard content, and at hatch (96 h after inoculation) in the ceca content. Ceca results were also confirmed by plate culturing. P1 inoculation greatly increased bacteria number compared with the controls in both, the gizzard (1×10^5 vs. 3×10^2 CE/mL, respectively, $P < 0.01$) and the ceca (4.5×10^4 vs. 3×10^3 CE/mL, $P < 0.05$). Similar increase was found for P2 in both, the gizzard (4×10^5 vs. 4×10^3 CE/mL, $P < 0.05$) and in the ceca (8×10^7 vs. 1×10^7 CE/mL, $P < 0.01$). Culturing of ceca contents showed that P1 inoculation significantly increased the number of total bacteria colonies compared with the control (respectively, 3×10^9 vs. 6×10^7 CFU/mL, $P < 0.05$), with P2 showing intermediate value (1×10^9 CFU/mL). Based on these findings we concluded that the chick intestine can be colonized with probiotic bacteria before hatching. Future research will focus in determining if chicks that hatch with gut probiotic bacteria are less susceptible to pathogenic bacteria such as *Salmonella*, *Campylobacter* and *Clostridia*.

Key Words: probiotic, gut colonization, chicken

981 Methionine hydroxy-analogue as antioxidant defence enhancer. Q. Swenen[1,3], J. Buyse[1], P.-A. Geraert[2], Y. Mercier*[2], N. Everaert[1], A. Stinckens[1], H. Willemsen[1], L. Yue[1], and E. Decuypere[1], [1]*K.U. Leuven*, *Laboratory for Livestock Physiology, Immunology and Genetics, Department of Biosystems. Kasteelpark Arenberg 30, 3001 Leuven, Belgium*, [2]*Adisseo France S.A.S*, *F-92160 Antony, France*, [3]*University of Hasselt*, *Center for Environmental Sciences, Agoralaan building C, 3590 Diepenbeek, Belgium.*

DL-Methionine (DLM) and the DL-methionine hydroxy-analog (HMTBA) are 2 bio-available methionine sources commonly used in the poultry feed industry. Previous studies on absorption and metabolism demonstrated that when compared with DLM, HMTBA metabolism produces more cysteine (Martin-Venegas et al., 2006) which is implicated in glutathione synthesis, one of the major intracellular antioxidant defense mechanisms. The present experiment involved 4 groups of broilers fed different diets: high (23%) or low (18%) dietary protein level and 2 methionine sources (DLM or HMTBA 0.25%) in a factorial design. As expected, the high protein level led to an improved zootechnical performance compared with that on the low protein diet. Whatever the protein level considered, higher slaughter weights were obtained with HMTBA than with DLM. Oxidative status was assessed in plasma by superoxide dismutase (SOD), uric acid and lipid peroxidation and in liver by total, as well as reduced and oxidised glutathione levels. Results showed that irrespective of the dietary protein level, HMTBA-fed birds were characterized by a higher plasma SOD activity compared with that of DLM-fed birds. Moreover, higher plasma levels of uric acid and lower lipid peroxidation were observed with the high protein-HMTBA combination compared with all other treatments. Hepatic concentrations of reduced glutathione as well as total glutathione were significantly higher in the low protein-HMTBA treatment compared with all other treatments. This work allows concluding that HMTBA, besides being a viable alternative dietary supplement for DLM, also improves both extra- and intracellular antioxidant status.

Key Words: methionine sources, antioxidant, glutathione

982 Comparative in vitro antimicrobial activity and mechanism of bovine lactoferricin-derived synthetic peptides. Y. Liu*, Y. Xie, F. Han, Y. Gao, C. Luan, and Y. Wang, *Zhejiang University*, *Hangzhou, Zhejiang, China*.

Lactoferricins are positively charged, highly basic peptides exhibiting multifunctional immunoregulation of antibacterial, antifungal, antiendotoxin, and antiviral activities. Lactoferricin B (LFcinB), a 25 residue peptide derived from N-terminal part of bovine lactoferrin, causes depolarization of the cytoplasmic membrane in susceptible bacteria, but the exact mode of action of LFcinB is not fully understood. In the present study, synthetic bovine lactoferricin and 2 derivatives with 15–residue and 11–residue peptides were prepared to investigate their antimicrobial nature and mechanism. The antimicrobial properties of the peptides were measured against *Escherichia coli* ATCC25922, *Escherichia coli* K88, *Staphylococcus aureus*, *Salmonella typhimurium*, *Salmonella choleraesuis* and *Pseudomonas aeruginosa* and hemolytic activity of these peptides were examined using the erythrocytes of pig. We found that 15-residue and 11-residue bovine lactoferricin almost maintained the same level of growth inhibition as LFcinB against tested bacteria in the minimal inhibitory concentrations (MIC) range of 16–128 μg/mL and the minimal bactericidal concentrations (MBC) range of 64–256 μg/mL. Also, the 11-residue lactoferricin was found to have the lowest hemolytic activity compared with the intact peptide. The mechanism of lactoferricin on *E. coli* and S. aureus was investigated by imaging the cells with scanning electron microscopy (SEM). After 1 h of exposure to MIC of LFcinB, a profound effect on the cell morphology of *E. coli* was observed. Compared with the control, the LFcinB-exposed cells became filamentous and elongated. The cells increased over 4-folds in length, and did not appear to be dividing in a regular manner. S. aureus cells exposed to MIC of LFcinB for 1 h appeared to be significantly smaller and somewhat paler than the cells not exposed to LFcinB. These results showed that LFcinB has a minor permeabilizing effect on the cytoplasmic membrane of both gram-positive and gram-negative bacteria, indicating a possible intracellular target.

Key Words: bovine lactoferricin, antimicrobial activity, mode of action

983 Microbial programming in the gut of neonatal pigs. D. Petri* and A. G. Van Kessel, *University of Saskatchewan*, *Saskatoon, Canada*.

To investigate possible long-term effects of first colonizing bacteria on post weaning gut commensal microbiota composition, a gnotobiotic study was conducted using 27 germ-free piglets derived by caesarian

section. Pigs were assigned to one of 4 isolators and were inoculated with either *L. delbrueckii* (L), *S. infantarius* (S), *C. perfringens* (C) or *E. coli* (E). Piglets were conventionalized on d 7 with sow feces, merged and transferred to group pens. On d 20 of age, piglets were weaned and on d 28 samples of digesta were collected from the stomach, jejunum and colon. Using 16S rRNA gene-based molecular methods, analysis of anal swabs taken on d 4 confirmed monoassociation of S, C and E pigs and di-association with *E.coli* for L pigs. Data was analyzed as a 2x2x4 factorial ANOVA (gender, litter, treatment; SPSS17 GLM function with Tukey's HSD). A significant gender effect ($P = 0.03$) was observed for ADG between d 7 and d 28. DNA was extracted from contents and qPCR used to quantify bacteria as a percentage of log10 total bacteria 16S rRNA gene copies per g of contents. In the stomach, Bifidobacterium spp. were significantly lower ($P = 0.05$) in E vs. L (60.4% vs. 70.8%) and showed a trend ($P = 0.07$) toward a gender effect. Enterobacteria tended ($P = 0.08$) to be lower in L (90.6%) vs. C (96.0%). In the jejunum, enterobacteria were not different between E (85.0%) and L (86.7%) but these were lower ($P < 0.01$ and $P = 0.02$, respectively) than in C (92.7%). Clostridium cluster 1 spp. in E (97.0%) were significantly lower ($P = 0.03$) than in L (109.0%). Bifidobacterium spp. tended ($P = 0.07$) to be lower in C (54.4%) than L (66.1%). Lactobacillus spp. also showed a significant gender effect ($P < 0.01$) and a trend ($P = 0.06$) toward Lactobacillus spp. levels in treatment S (93.4%) being lower than in treatment L (98.5%). Enterobacteria showed a trend ($P = 0.07$) toward a litter effect. In colon a gender by treatment interaction ($P = 0.02$) was observed for Bifidobacterium spp. Early postnatal microbial colonization pattern and gender affect postweaning gut microbial profile.

Key Words: intestinal microbiota, qPCR, gnotobiotic

984 Efficacy of water-soluble antioxidants on chicken embryos challenged by hypoxia. J. E. de Oliveira*[1], Y. Li[2], H. Willemsen[2], E. Decuypere[2], and T. A. Scott[1], [1]*Provimi*, *Velddriel, the Netherlands*, [2]*Department of Biosystems*, *K.U. Leuven, Belgium*.

Searching for natural antioxidants (AO) is challenging because many sources, doses and conditions fail to demonstrate consistent results in vivo. Chick embryo models can be used as tools to indicate bioavailability and bioactivity of feed additives. This trial was designed to investigate the effect of in ovo administration of increasing doses of vitamin E analog Trolox (VEA) or grape extract (GRP) on development and antioxidant capacity (AOC) of chick embryos. Chicken eggs were challenged at 14 d of incubation (E14) by partially sealing egg shell pores to induce mild hypoxia. Embryos were challenged by hypoxia as a way to induce oxidative stress. At E17, a group of 120 eggs was assigned to one of the following treatments: control (CTR, no AO); injection with VEA at 3 concentrations (10, 30 or 100 ppm); injection with GRP at 2.5, 12.5 or 25 ppm, or injection with the combination of VEA 10 ppm and GRP 2.5 ppm (COMB). Sixteen embryos from each treatment were sampled at E18 and E19 to measure embryo yolk-free body mass (YFBM), yolk-sac mass (YKM), and liver mass (LM). Liver samples were analyzed for levels of superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), and glutathione disulphide (GSSG) to indicate AOC. VEA 10 and 30 ppm significantly increased YFBM in both ages compared with CTR ($P < 0.01$), with all other treatments showing intermediate values. There was no treatment effects on YKM or LM. Liver AOC showed VEA 30 ppm and GRP 12.5 ppm with higher CAT then CTR ($P < 0.01$), with other treatments showing intermediate values. SOD results at E18 showed GRP 25 ppm, COMB, and VEA 100ppm having significantly higher values then CTR ($P < 0.05$), with other treatments showing intermediate values. For GSH at E18 there were differences only among VEA levels, with VEA 10 ppm being significantly lower than CTR ($P < 0.01$). No differences were found for SOD or GSH at E19, or for GSSG at both ages. We concluded that hypoxia-challenged chick embryos are good models to test AO, and that VEA 30 ppm, GRP 12.5 ppm and COMB improved growth and liver AOC of challenged chicken embryos.

Key Words: antioxidants, chick embryo, hypoxia

985 Growth response, carcass evaluation and hematology of broilers fed graded levels of enzyme treated cocoa bean shell based diets. M. D. Olumide, O. A. Ogunwole*, and O. A. Adebiyi, *Department of Animal Science, University of Ibadan, Ibadan, Nigeria*.

Cocoa bean shell (CBS) is a waste from cocoa processing industries in Nigeria and it constitutes a serious disposal problem. Previous trials revealed that addition of enzyme reduces the theobromine (anti-nutrient in CBS). Hence, this study focused on evaluating the growth response, carcass characteristics and hematological parameters of broilers fed graded levels of enzyme treated CBS based diets. A total of 150 birds at 1d of age were allotted to 5 dietary treatments (3 replicates/treatment, 10 birds/replicate) in a completely randomized design. The treatments were: A (0% CBS-control diet); B (5% CBS with enzyme); C (10% CBS with enzyme); D (15% CBS with enzyme) and E (20% CBS with enzyme). Each of the diet was fed ad libitum to the experimental birds. The trial lasted 8 weeks. The feed intake, weight gained, carcass characteristics and hematological indices were then evaluated. There were significant differences ($P < 0.05$) in feed intake, weight gain and carcass characteristics of broilers fed the experimental diets. The results revealed that enzyme treated CBS can effectively replace up to 15% maize in the diets of broilers without any adverse effects.

Key Words: broiler, cocoa bean shell, hematology

986 Evaluation of the efficacy of Myco-Ad in preventing aflatoxin toxicity in broiler chicks.. C. A. Mallmann[1], P. Dilkin[1], L. Giacomini[1], R. H. Rauber[1], and D. Zaviezo*[2], [1]*Universidade Federal de Santa Maria, Laboratorio de Analises Micotoxicologicas (LAMIC)*, *Santa Maria, RS, Brasil*, [2]*Special Nutrients*, *Miami, FL*.

The dietary use of 0.25% Myco-Ad has been proven to effectively prevent the toxic effects of aflatoxin B1 (AFB), ochratoxin and T-2 toxin in broilers. Studies were conducted to evaluate the AFB adsorption capacity of Myco-Ad and its efficacy in preventing the deleterious effects of high levels of AFB in broiler chicks; as part of the regulatory anti-mycotoxin additives (AMA) approval process in Brazil. Three hundred day-old Cobb male chicks were placed in battery cages randomly distributed into 5 treatments with 6 replications each and fed a basal corn-soy diet containing or exceeding NRC recommendations. All ingredients used were tested free of mycotoxins contamination. Treatments were: 1 basal diet; 2 basal + 0.5% Myco-Ad; 3 basal + 2.8 ppm AFB; 4 basal + 2.8 ppm AFB + 0.25% Myco-Ad and 5 basal + 2.8 ppm AFB + 0.5% Myco-Ad. AFB was obtained from a culture material containing 96.4% AFB; 1.61% Aflatoxin B2 and 1.99% Aflatoxin G1 produced in LAMIC. Myco-Ad adsorption capacity of 1 ppm AFB was above 95% and 97% at 0.25 and 0.5%, respectively. Results at 21 d of age indicated that broiler fed 2.8 ppm AFB contaminated diet presented significant ($P \leq 0.05$)lower feed intake (31%), smaller body weight (29%), heavier liver weight (56%) and lower plasma protein levels (54%) than chicks fed the control diet. The addition of Myco-Ad (0.25–0.5%) significantly($P \leq 0.05$) improved feed intake (32–40%), body weight (24–33%), liver size (17–30%) and plasma proteins (19–33%) observed in chicks fed the AFB contaminated diet. The addition of 0.5% Myco-Ad to the chick diet did not show any statistical difference in performance, relative liver weight

or total plasma proteins compared with the control diet, demonstrating its lack of interference with the absorption of nutrients. These results indicated that 0.25% Myco-Ad was effective in preventing the toxic effects of AFB in broiler chicks; and therefore met the requirements for AMA registration in Brazil.

Key Words: Myco-Ad, aflatoxin

987 Efficiency of feed additives to reduce the effects of chronic exposure to aflatoxin and deoxynivalenol on growth and immune status of pigs. A. C. Chaytor*[1], M. T. See[1], J. A. Hansen[2], A. L. P. de Souza[2], D. C. Kendall[2], T. F. Middleton[3], and S. W. Kim[1], [1]*North Carolina State University*, *Raleigh*, [2]*Murphy-Brown LLC*, *Rose Hill, NC*, [3]*AgProvision LLC*, *Kenansville, NC*.

Three feed additives with potential ability to detoxify mycotoxins were tested to determine the effects on growth and immune responses of pigs fed diets containing aflatoxin (AF, 180 μg/kg) and deoxynivalenol (DON, 900 μg/kg) for 42 d. Gilts (n = 225, 8.8 ± 0.4 kg BW) were allotted to 5 treatments: PC (positive control without AF and DON); NC (negative control with AF and DON); A (NC + a clay based additive); B (NC + a clay and yeast cell wall based additive); and C (NC + a clay and enzyme based additive). Each treatment had 15 replicates with 3 pigs per pen. Feed intake and BW were recorded weekly, and blood was sampled on d 28 and d 42. On d 42, pigs were killed to obtain liver, kidney and spleen. Pigs in NC had smaller ($P < 0.05$) body weight (24.6 kg) and ADG (374 g) than PC (26.6 kg and 423 g) but were not different from others (25.4 kg and 393 g). Pigs in NC tended to have a smaller ADFI (753 g, $P = 0.090$) and gain:feed (0.495, $P = 0.052$) than PC (816 g and 0.520) but were not different from others (775 g and 0.507). On d 42, pigs in NC had a greater ($P < 0.05$) monocyte count (1432/μL) than PC (968), A (1053), and B (952). Pigs in NC tended to have a greater basophil count (158/μL) than PC (91, $P = 0.088$), but were not different from others. Pigs in NC tended to have a greater serum IgG (1.02 mg/mL) than PC (0.89, $P = 0.074$), and A (0.83, $P = 0.010$). Pigs in NC had a greater ($P < 0.05$) serum IgM (0.2 mg/mL) than PC (0.17) and A (0.17), and tended to have a greater (0.093, $P = 0.099$) serum IgM than B (0.18) and C (0.18). Serum TNF-α was not affected by dietary treatments. Pigs in NC had a greater ($P < 0.05$) % liver weight (14.9%) than PC (12.3%) and B (12.7%). Collectively, feeding 180 μg/kg AF and 900 μg/kg DON to pigs for a 42 d period reduced growth performance and increased immune challenges. Use of these feed additives tended to ameliorated the immune challenges but without affecting the growth performance.

Key Words: alfatoxin, deoxynivalenol, pigs

988 Discrepancies between in vitro and in vivo fumonisin binding with organoclays. J. N. Broomhead*, *Amlan International*, *Chicago, IL*.

An in vitro binding and an in vivo chicken study were conducted to test the efficacy of 2 experimental and 1 commercial organically modified clays (OMC) in binding fumonisin B_1 (FUM). In vitro mycotoxin binding was conducted at physiological conditions of the stomach (pH 3.0) followed by the intestine (pH 6.5) at 1000:1 binder-to-toxin ratio. The in vivo study consisted of 200 d-old male broiler chicks assigned to 8 treatments with 5 replicate pens of 5 chicks each. The 3 OMC were either fed alone (0.5% dietary inclusion) or in combination with 70 ppm FUM supplied from naturally contaminated corn. Chicks were placed in battery-brooders and fed experimental diets for 21 d. On d 21, 3 birds per replicate were killed with CO_2, weighed, and livers removed and weighed from 3 birds per pen for determination of relative liver weight. After weighing, the livers were frozen and then 2 livers per pen were later analyzed for sphinganine (SA) and sphingosine (SO) concentration. In vitro FUM binding results were high and did not vary greatly between OMC (85 to 96% FUM bound). Replacing the normal dietary corn with FUM contaminated corn (70 ppm dietary FUM) reduced ($P < 0.005$) body weight gain (BWG) and feed intake, and increased ($P < 0.0001$) liver SA and SA:SO ratio. Relative liver weight and feed conversion were not significantly affected by treatments ($P > 0.05$). Inclusion of one of the experimental clays to the FUM diet significantly decreased ($P < 0.0005$) BWG and increased ($P < 0.0001$) SA and SA:SO ratio compared with the FUM, alone, treatment. In conclusion, none of the OMC ameliorated the toxic effects of FUM to the broilers. Also, the in vitro FUM binding procedure used may not be a good predictor of in vivo efficacy and in vivo studies should always be conducted to validate in vitro results.

Key Words: fumonisin, in vitro, in vivo