Enzymatic hydrolysis of fumonisins in the gastrointestinal tract of broiler chickens. B. Grenier¹, H. E. Schwartz-Zimmermann¹, C. Gruber-Dorninger¹, and E. Vicuna⁎¹, ¹Purdue University, W. Lafayette, IN, USA, ²BIOMIN Research Center, Vienna, Tulln, Austria, ³University of Natural Resources and Life Sciences, Vienna, Tulln, Austria, ⁴University of Georgia, Athens, GA, USA.

Some of the most frequent mycotoxins found in finished feed and feed ingredients are Fumonisins (FB). FB levels between 10 to 20 mg/kg (concentrations that can be encountered in the field and below EU and US guidelines) can deteriorate gastrointestinal tract (GIT) functions in poultry species, as reported in recent studies. Therefore, it is necessary to develop new tools to reduce the impact of FB in the GIT and immune system of poultry. In the present study, we evaluated the efficacy of fumonisin esterase FumD (EC 3.1.1.87, commercial name FUMzyme) to cleave the tricaprylmycolic acid side chains of FB, leading to the formation of non-toxic hydrolyzed fumonisins in the GIT of broiler chickens. Broiler chickens were fed for 14 d (7 to 21 d of age) 3 different diets (6 birds/cage, 6 cages/diet), i) control feed (negative control group), ii) feed contaminated with 10 mg FB/kg (FB group), and iii) feed contaminated with 10 mg FB/kg and supplemented with 100 units of FUMzyme / kg (FB+FUMzyme group). Two biomarkers were used to determine the degree of reduction of FB. First, the sphinganine-to-sphingosine ratio in the serum and liver was determined as a marker of effect from exposure to FB. Second, the fumonisin B1 levels and its hydrolyzed forms in the gizzard, the proximal and distal parts of the small intestine, and the excreta were determined. Significantly reduced sphinganine-to-sphingosine ratios in the serum and liver of the FB+FUMzyme group (serum: 0.15 ± 0.01; liver: 0.17 ± 0.01) compared with the FB group (serum: 0.20 ± 0.01; liver: 0.29 ± 0.03) proved that supplementation of broiler feed with FUMzyme was effective in partially counteracting the toxic effect of dietary FB. Likewise, FB1 concentrations in digesta and excreta were significantly reduced in the FB+FUMzyme group compared with the FB group. FUMzyme furthermore partially counteracted FB-induced upregulation of cytokine gene expression in the jejunum. The FB group showed significantly higher gene expression of IL-8 and IL-10 compared with the negative control group, whereas IL-8 and IL-10 mRNA levels were not significantly different in the FB+FUMzyme group compared with the other 2 groups. In conclusion, FUMzyme is able to reduce the damage caused by FB in the GIT of chickens and maintain gut functions.

Key Words: fumonisin, fumonisin esterase, sphinganine, sphingosine, gut

Evaluation of the overall impact of antibiotics growth promoters on animal health and productivity during medication and withdrawal period. H. Hamid⁎¹, L. H. Zhao, G. Ma, W. X. Li, H. Shi, J. Zhang, C. Ji, and Q. G. Ma, China Agricultural University, State Key Laboratory of Animal Nutrition, College of Animal Science and Technology, China Agricultural University, Beijing, China.

The efficacy of some commonly used combination of antibiotic growth promoters (AGP) was re-evaluated during medication and withdrawal period in the broiler, through its effects on growth performance, immune organ index, gut health, and meat quality. A total of 540 one-day-old male broilers (Arbor Acre) were randomly assigned to 5 treatments, with 6 replicates of 18 chicks per replicate. Broilers received diets as follows during 0–42d: NC (control diet without AGP), EN (control diet + enduracinid 8 ppm + colistin sulfate 8 ppm), BZ (control diet+ bacitracin zinc 40 ppm + colistin sulfate 8 ppm), CT (control diet + chlorotetracycline 50 ppm + colistin sulfate 8 ppm), and VG (control diet+virginiamycin 20 ppm + colistin sulfate 8 ppm). After 42d medication period, all broilers were switched to the same finisher diets without AGP. Data were analyzed by one way ANOVA and means were compared by Duncan’s multiple comparison tests, considered significantly different at P < 0.05. Results showed that diets supplemented with antibiotics significantly decreased the feed/gain (F: G) of broilers during 1–42d (P < 0.05), but the F: G of birds in antibiotics group in withdrawal period was significantly increased (P < 0.05). There was no significant effect on duodenal digestive enzyme activity and cecal bacteria of broiler on d 42 and 49 (P > 0.05), the histomorphological study revealed increased jejunum villus height (P < 0.05) and reduced 42d crypt depth (P < 0.05) in antibiotic groups compared with control. At the end of the feeding trial, meat quality was measured. In pectoralis major evaluation, higher 24 h pH was recorded in BZ group and higher shear force was observed in EN group. Adding antibiotics cooking loss increased significantly in CT group in pectoral and thigh muscle. In summary, Antibiotics promote the growth of broiler chickens but withdrawal period resulted in reduced growth performance. So no effect was found in overall growth performance. Moreover, meat quality has also been negatively affected by the antibiotics in the diet of broilers. These findings help to persuade more farmers to choose diets without AGP in the whole period in the strictly supervised market.

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Key Words: antibiotic growth promoter, broiler, performance, gut, health, meat quality

Nutribiosis feeding strategies: DFM supplementation of NE-challenged birds. L. P. Barnard⁎¹, M. Bernieau¹, and S. Athanasiadou², ¹Danisco Animal Nutrition, DuPont Industrial Biosciences, Marlborough, United Kingdom, ²SRUC, Roslin Institute, Easter Bush, Midlothian, United Kingdom.

Nutribiosis is the interaction between nutrition, the microbiome and the gut and immune function of the animal. An unfavorable nutribiotic state can be induced by a disease challenge, such as in a necrotic enteritis outbreak. The aim of this study was to demonstrate the importance of a DFM on achieving a favorable nutribiotic state within the gastrointestinal tract (GIT) of the broiler chicken in the presence of a disease challenge. There were 4 treatments arranged in a 2x2 factorial challenge +/- and DFM supplementation +/- . The challenge was achieved by oral gavage of a NetB producing Clostridium perfringens strain (1 x 10⁸ cfu/ml). The DFM was a commercially available 3-strain Bacillus product applied to provide 150,000 cfu/g feed (Enviva PRO; from Danisco Animal Nutrition, DuPont Industrial Biosciences). Each treatment was replicated 8 times with 10 male Ross 308 birds/ replicate; test diets were fed from d0–21. All birds received the same feed, which was based on wheat and soybean meal. DFM supplemented birds showed a significant decrease (P < 0.05) in and necrotic enteritis B-like (NetB) toxin-specific serum antibodies. DFM supplemented birds showed increased innate immunity, the levels of transcripts encoding interleukins (IL); IL1, IL12 and interferon (P < 0.01) and induced changes in transcriptional expression in intestine and bio functional analysis related with the inflammatory.
response. Importantly birds supplemented with the DFM also gained significantly more weight than birds not receiving the DFM ($P < 0.05$), this is likely through modulation of the microbiome and management of the immune status of the animal. Achieving a favorable nutritriotic state enabled maximum absorption of nutrients which could be used for growth rather than excessive immune modulation. It is important to consider the effects a product has in the context of nutribiosis to optimize performance.

Key Words: nutribiosis, microbiome, DFM, gut and immune function, necrotic enteritis

78 Chitosan nanoparticles are superior to mineral oil to confer mucosal protection on inactivated vaccines against infectious bronchitis virus. P. D. Lopes*,1,2, F. S. Fernando1, C. H. Okino3, L. F. Dalmolin4, G. Schaefer5, R. F. V. Lopez4, M. F. S. Montassier5, and H. J. Montassier2, 1Animal Health Laboratory, Seara Foods, Nuporanga, São Paulo, Brazil, 2Department of Veterinary Pathology, Universidade Estadual Paulista (UNESP), Jaboticabal, São Paulo, Brazil, 3Embrapa Pecuária Sudeste, Empresa Brasileira de Pesquisa Agropecuária, Jaboticabal, São Paulo, Brazil, 4Department of Pharmaceutical Sciences, Universidade de Sao Paulo (USP), Ribeirão Preto, São Paulo, Brazil.

Vaccination is the most efficient way to prevent infectious bronchitis virus (IBV), which invades the respiratory mucosal surfaces. Conventional inactivated IBV vaccines are formulated with oil adjuvant and have been routinely used in chickens; however, are not efficient for inducing protection at the site of invasion of the IBV. The aim of this study was to compare the efficacy of the inactivated vaccine formulated with IBV BR-I genotype encapsulated in chitosan nanoparticles (IBV-CS) administered by the mucosal route with an inactivated vaccine of the same virus incorporated into oily adjuvant (IBV-O), administered intramuscularly. The humoral and cellular (CMI) mediated immune responses in the mucosal and local compartments induced by IBV-CS and IBV-O vaccines were evaluated in SPF chickens (Protocol Number of Animal Ethics Committee: 010140/14), when these vaccines were associated or not with a live attenuated H120 serotype vaccine. The protection induced by the different vaccine protocols was evaluated after challenge with the virulent homologous strain of BR-I genotype. For this, the levels of IgG and IgA anti-IBV antibodies in the lachrymal secretion and the expression levels of CMI genes in the trachea were evaluated. In addition, the histopathology and viral load in the trachea were evaluated after challenge. IBV-CS vaccine used alone or associated with a live attenuated vaccine induced higher levels of IgG and IgA anti-IBV antibodies in mucosal and the expression of CMI genes in trachea. The vaccine protocol using IBV-O vaccine in conjunction with the live attenuated vaccine induced IgG anti-IBV antibodies in lachrymal secretion, and upregulated expression of CMI genes in trachea. The IBV-O vaccine was unable to induce alone at mucosal compartment a robust antibody and cellular immune responses and did not confer effective protection to vaccinated chickens against the challenge. On the contrary, both vaccine protocols using IBV-CS induced effective protection for chickens against challenge, as demonstrated by the significantly lower intensity of the pathological alterations in the trachea, and this immune status can be associated with humoral and cellular immune responses induced at the primary site of IBV replication. These vaccine protocols proved to be effective against the challenge with a Brazilian variant strain of IBV.

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Key Words: infectious bronchitis virus, chitosan nanoparticles, mucosal adjuvant, inactivated vaccine

79 Broiler chickens fed with yellow mealworm: Effects on the gut health. R. T. Pereira*,1, M. A. N. Filho1, D. Suckeveris1, D. B. Moretti1, and A. B. S. Oliveira1, D. V. Costa2, and J. F. M. Menten1, 1Escola Superior de Agriculura Luiz de Queiroz ESA/LQ-USP Piracicaba, São Paulo, Brazil, 2Universidade Federal de Minas Gerais UFMG/ICA, Montes Claros, Minas Gerais, Brazil.

Chickens have natural behavior of feeding themselves from a variety of insects during their entire lifecycle. This study aimed to evaluate the functional properties of yellow mealworm on the gut of broiler chickens following voluntary intake. A total of 60 14-d-old male broiler chickens of commercial line were divided into 2 dietary treatments: a control (C) group, and a Tenebrio molitor meal (TM) group. Group C was fed with standard corn-soybean diet and the group TM had the choice to feed among yellow mealworm larvae, corn, extruded semi-whole soybean, supplement mixture (vitamin-mineral premix) separately. All with the same granulometry. Each group consisted of 6 replicate floor pens (5 birds/pen) assigned in a completely randomized design. Feed and water were available ad libitum. Following a preference and voluntary feed intake trial, at the d 32, samples of duodenum and ceca were collected. Lysozyme activity was determined in tissue extracts and content of duodenum homogenized in Tris/HCl Buffer. After centrifugation, the samples were mixed with Microoccus luteus and suspended in PBS at pH 6.0 and absorbance read in a spectrophotometer at 450 nm. The lysosome activity (unit mL−1) was defined as the amount of enzyme producing a decrease in absorbance. The antioxidant capacity in the duodenum tissue was assessed by the determination of superoxide dismutase (SOD). The SOD activity was measured using the tetrazolium salt to detect superoxide radicals generated by xanthine oxidase and hypoxanthine. One unit of SOD (U mg−1) was defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical. The short-chain fatty acids (SCFA, acetic, butyric, isobutyric, isovaleric and valeric) in ceca were quantitated by chromatography. Ceca content were homogenized in Mili-Q water and centrifuged. To the supernatant was added metaphosphoric and formic acids at 3:1. Through gas chromatography compared with a standard column, the concentration of the SCFA were determined. All data were analyzed through the SAS version 9.4. After Shapiro-Wilk analysis, Student’s t-test was performed at $P < 0.05$. The yellow mealworm was the most consumed ingredient accounting for 70–80% of the daily feed intake. Lysozyme and SOD activities were higher in TM group. Dietary intake of mealworm influenced the gut physiology of broiler chickens. Further investigations of the yellow mealworm functional properties are required.

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Key Words: insect, functional properties, gut health, voluntary intake

80 Effects of varium on intestinal barrier integrity of broiler chickens. H. Xue*,1, S. Ching1, S. Johnston1, D. Wang1, B. Beirão2, C. Fávaro3, and M. Ingberman2, 1Amlan International, Chicago, IL, USA, 2Imunova Análises Biológicas, Curitiba, Paraná, Brazil.

The avian gastrointestinal tract is the first line of defense against enteric pathogen invasion. Intestinal integrity describes how well the intestinal barrier inhibits paracellular translocation of undesirable substances, such as bacterial toxins and microorganisms. The role of tight junctions in pathogenic infections has been widely demonstrated. Tight

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juncture disruption leads to increased paracellular permeability that enables entry and spread of bacteria, toxins and metabolites. Previous studies have shown Varium binds bacterial toxins and improves broiler performance. Varium also has resulted in significantly improved necrotic enteritis-induced intestinal lesion scores (P < 0.05) and tended to reduce translocation of Clostridium perfringens-produced α- and Net-B toxins in an experimental necrotic enteritis model. Two studies were conducted to evaluate effects of Varium on intestinal barrier integrity of broilers. During Study 1, day-old Ross 224 chicks (8 pens/treatment, 7 chicks/pen) were randomly allotted to 1 of 4 treatments: 1) control; 2) challenged (CH) control; 3) CH-control + BMD (55 ppm); and 4) CH-control + Varium (0.1%). Chicks were orally challenged with ~5,000 Eimeria maxima oocysts on d 3 and 10⁸ cfu of C. perfringens on d 7. On d 9, intestinal sections were scored for necrotic enteritis-induced lesions and mRNA expression of intestinal tight junction proteins was measured. Results showed chickens fed Varium had lower lesion scores (P < 0.05) than those of the CH-control group. Varium treatment also increased intestinal mRNA expression of occludin and zonula occludens-1, 2 key tight junction proteins, compared with CH-controls (P < 0.05). In Study 2, day-old Cobb broiler chicks were randomly assigned to 1 of 3 groups: 1) control; 2) Salmonella-infected control; and 3) Salmonella-infected + Varium (0.1%). Two chicks/group were tested to confirm they were negative for Salmonella. Chicks were then orally challenged with 10⁸ cfu of Salmonella sv. Enteritidis on d 1. On d 4, 8, 14 and 28, intestinal permeability was assessed following oral administration of fluorescein isothiocyanate (FITC)-dextran. Results showed Salmonella infection induced increased intestinal permeability to FITC-dextran 3 d and 7 d after challenge, indicating intestinal integrity was disrupted. Compared with the Salmonella-infected control, Varium effectively prevented increased intestinal permeability on d 4 and d 8 (P < 0.05). In conclusion, Varium can preserve the structural and functional integrity of the intestinal barrier of chickens during necrotic enteritis and Salmonella sv. Enteritidis challenges.

Key Words: gut health, Clostridium perfringens, Salmonella


This study was conducted to investigate the optimum dietary level of tryptophan (Trp) supplementation at which broiler chickens have better growth with efficient immune system and antioxidant status. One hundred twenty (n = 120) 1-d-old broiler chicks were fed a common commercial diet from d 1 to 7. On d 7, the chicks were randomly divided in 3 treatment groups i.e., Trp 0.2 (NRC recommended level of tryptophan), Trp 0.3 (Tryptophan supplemented at 0.3%) and Trp 0.5 (Tryptophan supplemented at 0.5%). All the experimental diets were iso-caloric (ME: 3000 kcal/kg) and iso-protein (CP: 18.5%). On d 19, avian tuberculin was injected to note the cellular immunity. Blood serum samples were collected for analysis of humoral immune response against sheep red blood cells, total oxidant and antioxidants by spectrophotometric method. Feed intake, carcass and visceral organs weights remained unaffected by dietary treatments while BWG and FCR tended to improve (P < 0.05) in broiler chicks fed the Trp 0.3 and the Trp 0.5 diets. Total oxidant status was also improved (P < 0.05) in broiler chicks fed the Trp 0.5 diet. Likewise, broiler chicks fed the Trp 0.3 and the Trp 0.5 diets tended to have better (P < 0.05) total antioxidant status, catalase, glutathione peroxidase, glutathione reductase and arylstearase. The overall antibodies response and IgG improved (P < 0.05) by the Trp 0.3 ad Trp 0.5 diets compared with control. However, IgM level remained similar across the treatment. The cellular immunity against avian tuberculin improved at 24 h post injection but its effect disappeared at 48 h. The results of present study revealed that Trp above the NRC recommended level may give better growth, immune response and antioxidant status in broiler chickens.

Key Words: broiler, tryptophan, growth performance, immune response, oxidant and antioxidant status

82 The effect of a novel Bacillus-based multi-strain probiotic on performance under sub-clinical necrotic enteritis conditions. A. B. Kehlet1, F. Rudeaux2, C. L. Hofacre2, and M. Lang3, 1Chr. Hansen A/S, Harsholm, Denmark, 2Southern Poultry Research Group, Athens, GA, USA.

The aim of this study was to determine the responses in body weight gain, feed efficiency, mortality and necrotic enteritis lesions of broilers supplemented with a new 3-strain direct fed microbial (DFM) when exposed to a mild C. perfringens (CP) challenge during a 42-d floor pen trial. A total of 1800-d-old Cobb male chicks were distributed in 36 floor pens (50 birds/pen), 4 dietary treat. (9 rep/treat). No challenge (T1), CP challenge (T2), 500 ppm DFM + CP challenge (T3), 500 ppm DFM (T4). Diets were provided by age of bird (starter, 0–14 d; grower, 15–35 d; and finisher 35–42 d). On d 0 all birds received a coccidia vaccine. On d 18 and 19, a one-hour feed and water fast was observed before infecting birds with a CP isolate by oral gavage (~10⁸ cfu/mL). Body weight, feed intake, and FCR were assessed at each feed change. Total mortality and NE mortality were documented throughout the study. On d 21 3 birds per pen were randomly selected for NE lesion score evaluation by a 0–3 scoring system, 0 being normal and 3 most severe NE lesions. Results were tested for dietary treatment effect in a Randomized Complete Block Design. If the effect was significant (P < 0.05), means were separated using Tukey’s test. At d 14 before NE challenge the DFM treated group (T3) had a significant lower FCR (1.43b) than control (T2) (1.56a) with the 2 other groups intermediate (T1 = 1.48ab and T4 = 1.49ab). Shortly post challenge the DFM group (T3 = 1.65ab) appears to recover more quickly than the challenged control (T2 = 1.70a) with a FCR closer to the none- challenged groups (T1 = 1.63ab and T4 = 1.60b). At d 42 T4 (DFM, no challenge) continued to have the lowest FCR (1.71b) whereas T3 (DFM w. challenge) had the same FCR as T1 (none challenged control) (1.75ab) and T2 (challenged control) had the highest (1.78a). There was no statistical difference in body weight at any of the measuring periods. Total mortality at d 42 was significantly higher in the challenged groups (T2 = 12.22a and T3 = 14.67a) compared with the none challenged groups (T1 = 4.44b and T4 = 3.78b). NE mortality was also higher in the challenged groups (T1 = 0.22b, T2 = 6.22a, T3 = 9.33a, T4 = 0.22b). Despite the numerically higher NE mortality T3 had a numerically lower NE lesion score (0.63a) compared with T0 (0.85a) whereas the none challenged groups both had a lesion score of 0. The findings in this study indicate that birds exposed to a sub-clinical NE infection can faster recover when supplemented with a direct fed microbial compared with none-supplemented birds.

Key Words: probiotic, necrotic enteritis, broiler, Bacillus, C. perfringens

83 Influence of different Clostridium perfringens and Eimeria maxima isolates on necrotic enteritis. K. Chasser1, K. Wilson1, W. Briggs1, A. Duff1, J.-D. Latorre2, B. Hargis3, J. Barta3, and L. Bielke*, 1Ohio State University, Columbus, OH, USA, 2University...
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Necrotic enteritis (NE), caused by *Clostridium perfringens* (CP), in broilers is often associated with a concurrent infection of *Eimeria maxima* (EM). However, outbreaks of this disease have been reported to occur independent of EM, and to be related to CP strains that produce NE toxin B (NetB). Presently, multiple methods of inducing NE in broilers were compared with evaluate the role of various toxins in development of clinical disease. Six CP strains were evaluated by PCR for toxin associated genes, including α-toxin and NetB, followed by inclusion of some strains, NetB + and NetB - , for 2 in vivo experiments. Experiment 1 consisted of non-challenged control (NC), EM + non-NetB CP (EMCP), non-NetB CP (nonNetB), NetB strain CP 1 (NetB1), NetB strain CP 2 (NetB2) or NetB strain CP 3 (NetB3). All groups, except NC, were challenged with 10⁴ cfu of *Salmonella* Enteritidis on day of hatch. On d16, EMCP birds were weighed and challenged with 2x10⁴ EM oocysts. On d20–22, nonNetB and NetB1–3 groups were challenged with 50mL of 10⁴ to 10⁶ cfu/mL CP directly onto the feed once daily, all birds were weighed d22. On d17, EMCP birds were challenged with 10⁶ cfu CP via oral gavage. On d22, all birds were weighed for body weight gain (BWG) and lesion scores (LS). BWG was analyzed by ANOVA and lesion scores by Proc Mixed procedure, ANOVA. At D22, % change in BWG was significantly different (P < 0.05) between NC, EM, and EMCP, but not NetB1–3 treatments. Lesion scores were significantly higher (P < 0.05) than NC for only EMCP, with all others statistically similar to NC. The second experiment tested an increased dose of NetB1 CP, 1 L of 10⁵ cfu/mL 2x/d on d17–20, against NC, nonNetB, and EMCP. By d22, EMCP had the greatest decrease (P < 0.05) in BWG and was lower than both NC and NetB1. NetB1 also had decreased BWG compared with NC (P < 0.05), but not EMCP. Lesion scores reflected BWG results. Through d57, EMCP had the greatest influence on BWG, while in all other groups BW after NE challenge was no different than NC within 2 wk. These studies suggest that Net-B positive strains of CP induce dysbiosis, when administered in extremely high doses over a prolonged period, and that predisposing factors, such as *Salmonella* and EM play an important role in the pathogenesis of NE in nonNetB strains.

**Key Words:** necrotic enteritis, *Clostridium perfringens*, Net B, *Eimeria maxima*, lesion scores

84 Use of chestnut and quebracho tannins to control *Clostridium perfringens* induced necrotic enteritis in poultry.

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Necrotic enteritis in poultry is mainly caused by *C. perfringens*. Traditionally, this disease has been controlled by antibiotics in feed as growth-promoting factors (AGP). In the last years, the use of GPF was banned and alternatives should be evaluated. Tannins from different sources have antibacterial or antitoxic activity and they are able to prevent the development of clostridial enteric diseases when added to feed. The aim of this study was to determine the effect of the addition of tannins to assess whether they can be used to control the development of enteric diseases under experimental and commercial conditions. For the experimental trial, we used a model of *C. perfringens* necrotic enteritis in broiler chickens. Three days before the challenge, birds were fed with regular feed supplemented with condensed tannins from quebracho (*Schinopsis lorentzii*), hydrolysable from chestnut (*Castanea sativa*) and a blend of both. Animals were challenged at d 14 after birth and euthanized at d 19 and 30. Presence and severity of lesions was recorded using a score ranging from 0 to 5 (0: no lesions; 5: massive necrosis). The commercial trial was carried out in commercial farms from Argentina. In each trial chickens from treated farms were provided commercial feed added with a blend of tannins (NP) while in control farms chickens only received regular feed (COM). Different parameters were analyzed including mortality, weight gain and presence of undigested feed in feces. Animals randomly selected from 1 house of each farm were necropsied, intestinal gross lesions were scored as described before and histomorphometry was done in different sections of small intestine. In the experimental trial, only the blend group shows a significant reduction in gross lesions in jejunum (38.5%, P < 0.05) and ileum (7.7%, P < 0.05), compared with the control (J:57.1%; I:48.5%). no significant differences were observed in the quebracho and chestnut group, although in this last one no lesions were observed in ileum. Lesion score was reduced only in the blend group (P < 0.05). In the commercial trial, the number of animals with gross duodenal lesions were not statistically different between treatments. Significant differences (P < 0.05) were observed in number of animals with jejunal lesions (COM: 18.8%; NP: 12.2%) and ileal lesions (COM: 6.6%; NP: 3.3%). Intestinal morphology was improved, as NP treated birds show a higher villus height/ crypt depth ratio (P < 0.05). These results demonstrate that addition of tannins in the diet of poultry is a promising alternative to AGP and can be used to control necrotic enteritis in chickens by reducing clinical signs and impact on production parameters.

**Key Words:** tannins, poultry, production, enteritis, *C. perfringens*

85 Evaluation of the intestinal health of broilers chickens challenged with *Clostridium perfringens* and treated with *Herbanoplex* and/or Lactobacillus spp. M. F. M. Costa1, M. Souza1, L. Justino1, A. Oba2, F. Matté3, F. L. Gazoni3, F. Chiarelli3, R. K. T. Kobayashi4, G. Nakazato4, and A. A. S. Baptista1, 1Department of Preventive Veterinary Medicine, Laboratory of Avian Medicine, State University of Londrina, Londrina, Paraná, Brazil, 2Department of Zootecny, CCA, State University of Londrina, Londrina, Paraná, Brazil, 3Vetanco Company, Chapeçó, Santa Catarina, Brazil, 4Department of Microbiology, CCB, State University of Londrina, Londrina, Paraná, Brazil.

The present study was conducted to evaluate the profile intestinal health of broilers chickens challenged with *Clostridium perfringens* (CP) and treated with Herbanoplex and/or *Lactobacillus* spp. Fifty 1-d-old male broilers (Ross) were placed in 5 different groups. We adopted a completely randomized design, consisting of 5 groups: T1-negative control; T2-positive control - challenged with CP; T3-birds that received Herbanoplex and were challenged with CP; T4-birds that received *Lactobacillus* spp. and were challenged with CP; T5-birds that received *Lactobacillus* spp. and Herbanoplex and were challenged with CP. Birds were fed wheat-based diet since the seventh days old (do). The Herbanoplex additive was added in the diet 1 kg / ton from the 1st do in the T3 and T5 groups. At 10th do the T4 and T5 birds received *Lactobacillus* spp. (10⁸ cfu/ml) via oral gavage for 3 consecutive days. The challenged birds at 12th do received a cociddiosis vaccine, 10x the dose, via oral gavage, and vaccine against the infectious bursal disease (10x dose). Between 15th and 19th do the birds were challenged via oral gavage with CP type A, netB (10⁸ cfu / mL). At 20th, 21st and 23rd do were performed the euthanasia by cervical dislocation and intestine were analyzed to determine the profile of macroscopic intestinal lesion.
The lesions observed were classified as: (0) normal; (1) mild - small intestinal wall is thin and flaccid with thickened mucus covering mucus membrane; (2) minor necrotic enteritis: 1 to 6 necrotic enteritis pocks; (3) moderate - necrotic enteritis pocks: more than 6 necrotic enteritis pocks; (4) severe- extensive areas of necrosis and ulceration of the small intestinal membrane, significant layer of fibrin and necrotic debris on the mucus membrane. Data were submitted to ANOVA and analyzed through the Scott-Knott test at 5% probability. The lesions observed from the different treatments ranged from 1 to moderate 3. It was observed the following lesions scores in the guts of the chickens under the different treatments: 1.45 (T1), 2.25 (T2), 1.65 (T3), 2.2 (T4) and 1.5 (T5). There was a significant difference ($P < 0.05$) between T1, T3, T5 and T2 and there was no significant difference between T4 and T2. In this study it was possible to demonstrate the broilers that received the Herbanoplex additive associated with *Lactobacillus* spp. were able to reduce the intestinal lesions caused by *Clostridium perfringens*.

**Key Words:** *Clostridium perfringens*, Herbanoplex, intestinal lesions, *Lactobacillus* spp., necrotic enteritis

### Egg quality of commercial laying hens not vaccinated or vaccinated with a *Mycoplasma synoviae* strain

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A study was conducted to evaluate the effects of vaccination against *Mycoplasma synoviae* on egg and eggshell quality of commercial laying hens. A total of 360 eggs from Bovans White laying hens were randomly collected in 2 commercial poultry houses and quality was evaluated. Each poultry house had 80,000 hens fed a common mash diet, the same management and facilities. Hens were allocated in metallic cages and egg quality was evaluated in 3 periods of 28 d from 35 to 46 weeks. Treatments were considered the laying hens positively diagnosed with *Mycoplasma synoviae* without vaccination or laying hens vaccinated at 5 weeks against *Mycoplasma synoviae* (Micovax MS-H) according to the commercial product recommendation, being negatively tested for this disease. Sixty eggs from each treatment were randomly collected in the last week of each period and eggshell quality was analyzed. Data were analyzed using the GLM procedures of SAS Institute and Test t was used to compare means ($P < 0.001$). At 38 and 42 weeks, laying hens vaccinated against *Mycoplasma synoviae* had higher ($P < 0.0001$) produced eggs weight, albumen and shell weight, Haugh unit, specific weight as well as higher shell thickness ($P < 0.0001$) compared with birds that were not vaccinated. In the third period, Haugh unit, yolk index of eggs were higher ($P < 0.0001$) in hens vaccinated against *Mycoplasma synoviae* compared with birds not vaccinated. Shell thickness and shell strength were also higher ($P < 0.0001$) when hens were vaccinated. Finally, in the present study data from all evaluated periods showed that vaccination was able to prevent losses in egg quality providing improvements on egg, albumen and shell weights as well as the shell strength was increased or guaranteed when hens were diagnosed as negative for *Mycoplasma synoviae*. In conclusion, laying hens in a commercial poultry house vaccinated against *Mycoplasma synoviae* and diagnosed as negative for this disease had higher parameters of egg and eggshell quality. This knowledge can be useful to help the poultry industry and prevent egg losses.

**Key Words:** eggshell, egg quality, hen, mycoplasma

### Effect of *in ovo* lactic acid bacteria and gram-negative bacterial inoculation on the intestinal microbiota of chicks

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Initial intestinal microbial colonization is thought to have a major influence on early animal health. Commercial hatchery practices may delay appropriate colonization of the gastrointestinal tract (GIT), because neonatal chicks are primarily exposed to bacteria within a hatchery, rather than chicken-specific microbiota, until on-farm placement. Recent studies of gut microbial establishment suggest that initial inoculation and colonization of chicken GIT microbiota can have a major influence on performance and health of birds. However, the impact of different types of pioneer colonizers on the development of the microbiome community and GIT are not well-studied, especially direct comparison of gram-negative and lactic acid producing (LAB) strains. The objective of this study was to compare impact of 2 apathogenic gram-negative chicken isolates or LAB as pioneer colonizers on the microbial community immediately post-hatch (DOH) and evaluate the treatments influence through 10d of age on different sections of the GIT. At ED18, embryos were inoculated with either saline (S), or ~10^2 cfu of *Citrobacter freundii* (C), *Citrobacter* sp (C2) or LAB (L) in the amnion. DOH whole GIT, plus ileum and ceca at 10d were collected from microbiome analysis. Once the DNA was isolated from mucosal and digesta contents, samples underwent 2 × 300 paired-end Illumina MiSeq preparation, targeting the V4-V5 region of the 16S RNA gene for microbiome analysis. An increased abundance of *Lactobacillaceae* ($P < 0.05$) and *Lactobacillus* genus were observed in the L group at DOH (41.74% vs 10.38% C; 6.78% C2; 15.82% S; $P < 0.05$), while the abundance of *Enterococcaceae* and *Enterococcus* were numerically decreased. While *Enterobacteriaceae* was the dominant family in C group at DOH (57.15% vs 37.10% C2; 27.89% L; 29.95% S; $P < 0.05$), by 10d, *Enterobacteriaceae* populations decreased (0.01% ileum and 8.15% in ceca) and was negatively correlated with age in the upper ileum ($r = −0.36$) and ceca ($r = −0.45$). While at 10d there was no difference in *Lactobacillus* populations, an evident increase in ileal, *Clostridiiaceae, Blautia* and *Candidatus Savagella* genus ($P < 0.05$) only occurred in the L group. Minimal changes in the composition occurred in the ceca as only *Streptococcus* was higher in S and C2 group. This suggests that different isolates in *ovo* can have a strong impact on pioneer colonizers of the provided isolate by DOH, and also affect the host intestinal environment, where providing a LAB may increase colonization of desirable bacterial groups in the ileum by 10 d of age.

**Key Words:** *Candidatus Savagella*, microbiome, pioneer colonizers

### Field variants of avian reoviruses are associated to tenosynovitis and carcass condemnation in poultry flocks from Brazil

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Avian reoviruses (ARVs) can infect birds of different species worldwide. Chicken can present stunting syndrome, respiratory and/or enteric diseases, immunosuppression, malabsorption, tenosynovitis and even secondary infections by other microorganisms. Flaws in conventional vaccines and the increase in the diagnostic rate of these diseases in the last 5 years suggest the emergence of pathogenic ARVs in the poultry flocks worldwide. This study aimed to detect ARVs and to perform a
89 Immunity, performance and carcass analysis of broilers vaccinated against coccidiosis and fed commercial diets with salinomycin. I. Alvarado*1,2, G. Pesti2, and M. DaCosta2, 1Merck Animal Health, DeSoto, KS, USA, 2University of Georgia, Athens, GA, USA.

A long-term evaluation of the inclusion of Salinomycin in diets of coccidiosis vaccinated broilers starting on immunity, performance and carcass composition was performed. Broilers were vaccinated with a non-attenuated vaccine containing E. acervulina, E. tenella, E. mitivari and E. maxima (Fortegra) at day of age by coarse spray. Chickens in each of the 3 consecutive pen trials were randomly assigned to 4 treatment groups according to the age at which Salinomycin was initially added to the feed (basal diet without Salinomycin and diets with Salinomycin starting around 13, 21 or 28 d of age). At 21 and 28 d of age, all the broilers in the treatment groups were administered a pre-established dose of E. acervulina, E. tenella and E. maxima in the feed. Body weights and adjusted feed conversions were obtained around d 13, 21, 28 and at different processing ages (35, 42 and 48 d of age for the 3 consecutive trials, respectively). In the first 2 trials, an increase in body weight gain and lower adjusted feed conversion were observed in broilers fed diets with Salinomycin since d 13. When compared with broilers fed Salinomycin free diets, broilers fed Salinomycin starting at d 13 showed a decrease of 6 and 4 points of feed conversion in the first 2 trials, respectively. With respect to carcass composition, a significant difference was observed in leg weight (P < 0.05). No other differences in carcass composition were observed. In the third trial, higher body weight gain and lower adjusted feed conversion were observed in the groups fed diets with Salinomycin since d 13 and 21, respectively. However, no difference in carcass composition was observed. Development of immunity against the 3 Eimeria species was observed starting on d 28 during the 3 consecutive trials. In summary, improved performance was observed with the incorporation of Salinomycin in the diets of coccidiosis vaccinated broilers starting at 13 and 21 d of age. However, such improvement seems to diminish overtime and with a higher processing age.

Key Words: coccidiosis, intestinal health, salinomycin, Fortegra, carcass analysis

90 Probiotic, symbiotic and essential oil effects on the necrotic enteritis lesion score in broiler chickens. M. Souza1, A. M. Saito1, M. F. M. Costa1, L. Justinbo*, R. N. Reis2, A. Oba3, and A. A. S. Baptista1, 1State University of Londrina, Londrina, Paraná, Brazil, 2Biomin Company, Piracicaba, São Paulo, Brazil.

Necrotic enteritis (NE) is a costly disease for the poultry industry, as a result of growth delay and bird death. The aim of the study was to compare the intestinal lesion score caused by Clostridium perfringens (CP) in broilers chickens fed different commercials additives. One day male broiler chickens (120) were housed in experimental cages at the Londrina State University - UEL. The animals received ad libitum water and feed. The chickens were divided into 6 groups and received specific feed according to the treatments: T1 - Negative control of unchallenged birds; T2 - Positive control of birds challenged with CP; T3 - Digestaron [100g/ton] + challenge with CP; T4 - Poultrystar [1kg/ton] + challenge with CP; T5 - Digestaron [200g/ton] + challenge with CP; T6-Poultrystar [1kg/ton] + Digestaron [100g/ton] + challenge with CP. The experimental diet was based on maize and soybean until 5 d of age (DA), followed by a diet based on wheat bran (62.75%) and soybean bran (29.6%). The additives (Poultrystar and Digestaron) were added to the diet and provided to the birds since the first DA. At 12 DA the broilers were challenged, by gavage, with vaccine Eimeria spp. oocysts. At 14 DA they received, by gavage, Bursal infection disease vaccine 10X the recommended manufacturer dose. Between 15 and 21 DA the broiler chickens were challenge, by gavage, with CP (107 cfu/mL). At 23 DA, the broilers were euthanized to determine the macroscopic lesion profile. The lesion score followed a methodology described in the literature, with modifications (0 – normal intestine; 1 – mild; 2 – minor necrotic enteritis; 3 – moderate necrotic enteritis pocks; 4 – severe). The lesion scores observed in the treatments were: T1 – 1.2; T2 – 2.80; T3 – 1.65; T4 – 1.50; T5 – 1.55; T6 – 1.65. Birds belonging to negative control (T1) presented lesions with mean score of 1.2. These findings can be explained by the provided diet characteristics, which may have contributed to the development of dysbacteriosis. A decrease in the lesion score of groups T3; T4; T5 and T6 was observed, differing significantly (P < 0.05) from the positive control. The treatments provided an improvement in intestinal health, regardless of the product used (symbiotic or essential oils). All treatments exhibited at least a mild lesion score. The applied products demonstrated effects broiler chicken intestinal health by minimizing the lesion score.

Key Words: chicken, intestinal health, necrotic enteritis, probiotics, essential oils

91 Protection conferred by an rHVT/ILT vaccine in broilers under intense ILT infectious pressure (a field case report). J. F. Rios Cambre* and E. M. Trejo Martinez, MSD Salud Animal Mexico, Ciudad de Mexico, Mexico.

A broiler flock was vaccinated with a recombinant HVT/ILT vaccine at one day of age at the hatchery and placed in a 17 house broiler farm located in an area with recent history of infectious laryngotracheitis (ILT) virus circulation. Around 41 d of age some of the birds in one house...
started to show mild to moderate respiratory signs that were consistent with ILT, and lab diagnostic tests confirmed an ILT infection. Control measures were taken for the birds in that particular house, while the rest were under strict observation, while no other measure were undertaken. At the end of the grow-out period at 56 d of age, clinical signs in the affected house had subsided, while in the rest of the farm no signs were detected, even though lab test confirmed the exposure to an ILT virus in those birds, and although the production parameters in the affected house were not satisfactory, the entire flock ended within the expected results for the broiler company. After intense scrutiny it was concluded that some of the birds placed in the affected house had been vaccinated improperly, while the rest were adequately immunized. The overall conclusions were that it is imperative to verify that this kind of vaccine has to be properly reconstituted and applied, and that by accomplishing this, the vaccinated birds can withstand an intense field virus exposure.

Key Words: broiler, ILT, recombinant vaccine