31 Can the dietary probiotic recover gut microbiome after of chickens submitted to antibiotic therapy? Rafaela Pereira¹, José Fernando M. Menten¹, Adriana A. Pedros², Cristiano Bortoluzzi³, Naíara S. Fagundes¹, Thiago Gumiere¹, Diogo P. Costal¹, Glacius S. Napt¹, Kelen Zavarize¹, and Fernando D. Andreote¹, ¹University of São Paulo, Piracicaba, SP, Brazil, ²University of Georgia, Athens, GA, USA.

The purpose of this study was to verify the ability of a probiotic in the feed to maintain the stability of gut microbiota in chickens after antibiotic therapy. The probiotic Bacillus subtilis was supplemented or not in the feed based on corn and soybean meal from 1 to 42 d of age. At 21, 22, and 23 d of age the chickens received or not antibiotic treatment (bacitracin methylene disalicylate + neomycin sulfate) in the drinking water. At 26, 28, and 30 d of age (2, 4, and 6 d after the therapeutic treatment), birds were euthanized and the contents of the ceca of 3 birds/pen were collected and pooled in a total of 3 replicates. The trial was conducted in floor pens (40 birds/pen) in a 2 × 2 × 3 factorial arrangement: with or without probiotic (+P / -P) × with or without antibiotic therapy (+A / -A) × 2, 4, and 6 d after the antibiotic therapy (T). DNA was extracted and the 16S rRNA gene was analyzed by terminal restriction fragment length polymorphism (T-RFLP) through multivariate statistical analyses to study microbial profile and the real time PCR (qPCR) technique was used to quantify the number of 16S rDNA gene copies per gram of cecal content. T-RFLP analysis showed that each treatment modulated individually the microbial profile in the gut. In addition, at 30 d there was higher similarity in the cecal microbiome profile of birds from the 2 treatments that received probiotic (+P-A and +P+A); on the other hand, the bacterial community from treatments that did not receive probiotic (-P-A and -P+A) were more distanced. The qPCR analysis revealed a main effect of days after the antibiotic therapy on the number of copies 16S rRNA gene in cecal contents, with a lower bacterial population at 28 and 30 d in relation to 26 d. These results indicated changes on the microbial profile and lower quantity of microorganisms with the time after the antibiotic therapy that possibly were related to recovering of the microbiota 6 d after the therapeutic treatment promoted by dietary probiotic.

Key Words: probiotic, antibiotic therapy, microbiota, broiler chicken, cecum

32 Use of stable isotopes to assess intestinal quality of broilers supplemented with Baccharis dracunculifolia extract. Everton M. Muro*, Antonio C. Pezzato, Guilherme A. M. Pasquali, Daniella A. Berto, Mônica M. Aoyagi, and Nathália M. G. Causso, Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista, Botucatu, São Paulo, Brazil.

Due to growing ban on performance enhancer antibiotics (PEA) it is necessary to search for substitutes to maintain both productivity and animal health. In this context plant extracts appear as a natural and safe alternative. Therefore, this study aimed to evaluate the effects of Baccharis dracunculifolia’s extract (BDE) in broiler diets on intestinal health. Two studies in a completely randomized design with 6 treatments were performed: control diet (CD) without inclusion of PEA and BDE; CD + 0.1% BDE; CD + 0.2% BDE; CD + 0.3% BDE; CD + 0.4% BDE and CD+PEA (virginiamycin, 10 ppm). In the first experiment 1,080 male broilers were allotted to 6 replicates per treatment. Histomorphometric measurements of intestine were performed at 21 and 42 d of age, and the data collected were analyzed by GLM procedure of SAS. In the second experiment we used 720 male broilers to evaluate the carbon turnover of the intestinal mucosa with the same treatments in 2 phases: from 1 to 21 d of age and from 21 to 49 d of age. The mucosa was analyzed by isotope-ratio mass spectrometry, and the weights of birds were recorded, the data were plotted in OriginPro software, and the turnover constant obtained was derived for metabolic and growth rate. At 21 d of age, birds treated with CD + 0.2% BDE showed an increase in duodenal crypt depth ($P < 0.05$) compared with controls, but no stimulation of villi development was observed at 42 d of age. In the second experiment we observed, at 21 d, decreased carbon half-life and increased metabolic rate of the intestinal mucosa for the treatment with CD + 0.2% BDE; the effects disappeared in the second phase of the study, also the growth rate did not differ between treatments. The high metabolic rate and shorter half-life, combined with increased crypt depths in the first period, especially at the 0.2% level, seemed to indicate a possible irritant effect of the extract on the intestinal mucosa, an effect that would cause the need for greater tissue replacement. The inclusion of BDE in any levels studied did not result in improved intestinal health compared with PEA.

Acknowledgments: To FAPESP for the scholarship grant

Key Words: carbon, metabolic rate, phylogenetic additive, plant extract, turnover rate

33 Age-related energy values of bakery meal determined using the regression method for broiler chickens. Catarina Stefanello¹, Olayiwola Adeola², Sergio L. Vieira³, Cristina T. Simoes³, and Heitor V. Rios¹, ¹Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil, ²Purdue University, West Lafayette, IN, USA.

A study was conducted to determine the ileal digestible energy (IDE), ME, and MEi contents of bakery meal using the regression method and to evaluate if the energy values are age-dependent in broiler chickens from 0 to 21 d post hatching. A total of 780 male Ross 708 chicks were fed 3 experimental diets in which bakery meal was incorporated into a corn-soybean meal-based reference diet at 0, 100, or 200 g/kg by replacing the energy-yielding ingredients. A 3 × 3 factorial arrangement of 3 ages (1, 2, or 3 wk) and 3 dietary bakery meal levels was used. Birds were fed the same experimental diets in these 3 evaluated ages. Birds were grouped by weight into 10 replicates per treatment in a randomized complete block design. Data were analyzed using the GLM procedures of SAS Institute. The effects of increasing levels of bakery meal in assay diets were compared using linear and quadratic contrasts. Apparent ileal digestibility and total-tract retention of DM, N, and energy were calculated. Expression of mucin (MUC2), sodium-potassium linked transporter (NaPi-IIb), and sodium-glucose linked transporter (SGLT1) genes were measured at each age in the jejunum by real-time PCR. Inclusion of bakery meal to the reference diet resulted in a linear decrease in retention of DM, N, and energy; and a quadratic reduction ($P < 0.05$) in N retention and ME. There was a linear increase in DM, N, and energy as birds’ age increased from 1 to 3 wk. There was no effect of dietary bakery meal on jejunal gene expression. Expression of genes encoding MUC2, NaPi-Iib, and SLC7A2 linearly increased ($P < 0.05$) with age. Regression-derived MEi of bakery meal linearly increased ($P < 0.05$) as the age of birds increased, with values of 2,710; 2,820, and 2,923 kcal/kg DM for 1, 2, and 3 wk, respectively. Based on these results, utilization of energy and
34 Effect of oil level, enzyme, and emulsifier in diets of broiler chickens. Levy V. Teixeira1,2, Cristiano Bortoluzzi1,2, Rosana C. Maia1,2, Antonio G. Bertechni1,2, and Todd J. Applegate1,4, 1Purdue University, West Lafayette, IN, USA, 2Federal University of Lavras, Lavras, MG, Brazil, 3Federal University of Viçosa, Viçosa, MG, Brazil, 4University of Georgia, Athens, GA, USA.

The objective of this study was to evaluate the effects of oil level, enzyme, and emulsifier on performance, nutrient digestibility, and nitrogen-corrected apparent metabolizable energy (AMEn) for broiler chickens. A total of 480 1-d-old male Ross 708 were weighed and randomly assigned to 8 treatments in a factorial arrangement with 2 levels of soybean oil (1.5% and 4.5%), 2 levels of amylase (0 and 80 KNU/kg) and 2 levels of emulsifier glyceryl polyethylene glycol ricinoleato (0 and 350 g/ton) with 10 replicates of 6 birds each. Total excreta were collected from d 5 to 7 and from d 19 to 21 to determine AMEn, apparent retention coefficients of dry matter (ARCMD), crude protein (ARCCP), and fat (ARCF). Feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR) were recorded at d 7 and 21. The data were analyzed as a 3-way ANOVA (PROC GLM/SAS), and the means were compared using Tukey’s test with P < 0.05 and tended values lower than P < 0.1. There was no interaction (P > 0.05) among oil level, enzyme, and emulsifier. A high level of soybean oil increased (P < 0.05) DM, CP, and fat retention and improved the performance in both ages evaluated. The emulsifier improved (P < 0.05) fat retention and tended to improve AMEn (P = 0.056) and ARCMD (P = 0.096) at 21 d. In addition, amylase tended to increase AMEn (P = 0.068) and ARCMD (P = 0.086) at 21 d of age. From performance results, amylase tended to increase BWG (P = 0.063) at the d 7 and BWG (P = 0.089) and FCR (P = 0.07) at 21 d. Emulsifier tended to increase BWG (P = 0.053) at 7 d and FCR (P = 0.055) at 21 d. There was no effect of factors on FI. In conclusion, the effects of amylase and emulsifier were not additive; however, both tended to improve the AMEn of the diets and the performance of the birds.

Key Words: emulsifier, amylase, nutrient retention, dietary oil, AMEn

35 Effects of canthaxanthin and 25-hydroxycholecalciferol on broilers meat physical and chemical characteristics. Douglas V. Bonamigo1, Alexandre P. Rosa1, Daniele P. Rosa1, Sandro J. Paixão1, Alexandre B. Mariani1, Adrian S. Ertmann1, Isaac Bittar2, and Leticia C. Bittencourt2, 1Universidade Federal de Santa Maria, Santa Maria, Rio Grande do Sul, Brazil, 2DSM Nutritional Products, São Paulo, São Paulo, Brazil.

The purpose of this study was to evaluate the effect of MaxiChick and its active ingredients, canthaxanthin combined with 25-hydroxycholecalciferol (25-OH-D3) in broilers feed on the physico-chemical characteristics of the meat. Two experiments were made to evaluate the effects on male and female chicks: 1500 and 1680 male and female one-day old Cobb-500 broilers were used, distributed in a completely randomized design, with 2 treatments of 15 replicates of 50 birds each for male chicks and 56 birds each for female chicks. Male broilers were raised during 42 d and female broilers during 43 d. Treatments were: T1: Control Diet and T2: Control Diet + 0.1% MaxiChick until 21 d of age. Samples for breast meat physical and chemical characteristics’ measurement were harvested at 28 and 43 d of age in female chicks and 42 d of age in male chicks. Three birds with average weight per replicate were selected. Variables measured were: pH, color (L*, a* and b*), water-holding capacity, sheare force and cook loss. Leg color pigmentation levels were evaluated in 6 birds per replicate, at 42 d of age in male chicks and 43 d in female chicks. Data were subjected to ANOVA. Significant results (P < 0.05) were observed in yellow color strength (b*) in breast meat of male broilers supplemented with MaxiChick (7.76 vs. 7.06) and in leg color pigmentation in male broilers evaluated at 42 d of age (4.68 vs. 3.11). In female chicks, significant results (P < 0.05) were observed in red color strength (a*) (3.26 vs. 2.83) and yellow color (b*) (6.48 vs. 5.70) in breast meat of birds evaluated at 28 d of age supplemented with MaxiChick. No differences were observed at the other variables. It is therefore concluded that the addition of MaxiChick to the diet, based on Canthaxanthin and 25-hydroxycholecalciferol (25-OH-D3), had a direct influence on color, with differentiated results among sexes. Better pigmentation of meat results in greater acceptance of the product by the consumer market.

Key Words: Vitamin D, color, nutrition, pigmentation, meat quality

36 Effect of supplementation of vitamin E in laying hens diets on egg quality with different storage periods. Mariane O. Fernandes1,2, Alexandre P. Rosa1, Daniele P. Rosa1, Catiane Orso1, Angelica Londero1, Adriano S. Ertmann1, Alexandre B. Mariani1, and Janaina S. Moura, Universidade Federal de Santa Maria, Santa Maria, Rio Grande do Sul, Brazil.

The objective of this study was to evaluate the effect of inclusion of vitamin E on egg quality of laying hens. We used 128 laying hens Novogen Brown, 40 to 55 wk of age, distributed in a completely randomized design with 2 treatments, 8 replicates of 8 hens each. The treatments were: DC - negative control diet and DVitE - diet with addition of 200 mg/kg of vitamin E (acetate dl-α-tocopherol). The parameters evaluated were: weight of stored eggs (WSE), percentage of yolk, albumen and shell (% Y, %A, and %S), Haugh unit (HU), pH of albumen (pH), yolk color (YC), vitelline membrane resistance (VMR) and lipid oxidation. To determine the quality parameters were selected 3 eggs per replicate for each storage time (0, 14, 21 and 28 d) within a range of 2.5% variation from the average egg weight. To determine VMR was used TA.XT Texture Analyzer 123 and to the lipid oxidation was used TBars method (reactive substances to the Thiobarbituric Acid on μg of Malondialdehyde/kg of yolk). Data were subject to ANOVA (ANOVA) using the SAS statistical program. Supplementation with vitamin E provided a better %Y and %A for eggs stored for 21 (P = 0.0581 and P = 0.0392) and 28 d (P = 0.0470 and P = 0.0530). Also WSE (P = 0.0711) and pH albumen (P = 0.0085) on 14 d of storage had better results. The DvitE showed lowest lipid oxidation (P = 0.0268), highest VMR (P = 0.0132) and better UH (P = 0.0889) in eggs stored on 21 d. No differences were observed between treatments for other variables in different times of storage (P > 0.10). The addition of Vitamin E in diets of laying hens is an alternative to improve the internal quality of stored eggs with up to 28 d.

Key Words: laying hen, internal egg quality, storage time, lipid oxidation.
The aim of this study was to evaluate the effects of canthaxanthin (CTX) supplementation on internal egg quality of laying hens, storage at room temperature or refrigerated. We used 2,400 eggs, distributed in randomized design with a 2 × 2 factorial arrangement [supplementation 0 or 6 mg of CTX (CAROPHYLL Red 10%, DSM Nutritional Products)/kg diet; 2 types of storage: room temperature (25°C) or refrigerated (4°C)], with 10 replicates of 60 eggs each. The eggs were stored for 0, 7, 14, 21, and 28 d (d). The parameters analyzed were: egg weight (EW), yolk and albumen percentage (%Y and %A), yolk index (YI), Haugh unit (HU), vitelline membrane resistance (VMR), lipid oxidation (TBARS), and milligrams of total carotenoids per kilogram of yolk (C). To determine internal quality, 3 eggs were selected per repetition according to EW (within an interval for variance of 25%). To determine the C we used an iCheck portable photometer, VMR was measured by a TA.XT Texture Analyzer 123, and TBARS measured the amount of thiobarbituric acid-reactive substances. All the data were subjected to ANOVA. Tukey’s test was used for significant interaction at the 10% level. Statistical procedures were performed using SAS software. In the interactions study only was observed that storage eggs at 4°C from hens fed with different diets had higher Y1 to 21 d (P = 0.0061) and 28 d (P = 0.0738) than eggs stored at 25°C. Hens fed with 6 mg CTX/kg of diet had highest C in all storage periods (P < 0.10), VMR at 21 d (P = 0.0918), HU at 0 d (P = 0.0335) and lowest %A at 14 d (P = 0.5664) and 21 d (P = 0.0405). However, hens fed with 0 mg/kg CTX had highest Y1 to 0 d (P = 0.0086). Eggs stored at 4°C had highest HU from 7 to 28 d (P < 0.10), Y1 and lowest TBARS at 7 to 10 d (P < 0.10). Eggs from hens fed with 6 mg CTX/kg of diet, stored at refrigerated temperature (4°C) had better shelf life.

**Key Words:** corn, carotenoid, refrigerated, yolk index

38 Lipid profile and susceptibility to peroxidation on semen of roosters supplemented with canthaxanthin. Catiane Orso*, Alexandre Rosa, Angélica Londero, Janaina Moura, Adrian Ertmann, Alexandre Mariani, Ana Cougo, Pedro Feltrin, and Ademir Bach, Universidade Federal de Santa Maria, Santa Maria, Rio Grande do Sul- Brazil.

The study evaluated the effect of dietary supplementation of canthaxanthin (CTX) on the oxidation and lipid profile of semen from roosters at different ages. 32 males White Plymouth Rock from 52 to 72 wk of age were distributed in a completely randomized design with 16 replicates. The treatments were males supplemented with 6 mg/kg/feed CTX or without CTX (negative control). Semen samples were collected at 52, 56, 60, 64, 68 and 72 wk of age, by abdominal massage, for analysis of lipids. Lipid oxidation was determined by Thiobarbituric Acid reactive-substances (TBARS) method, quantified by spectrophotometry. The fatty acid composition was determined using gas chromatography. The results were obtained as a percentage of the identified fatty acids. Data were submitted to ANOVA. No difference where observed between treatments for the TBARS (P > 0.05) in all weeks analyzed. There was an increase in the proportion of total polyunsaturated fatty acids, with a significant increase in Docosadienoic Acid C22:2 (P = 0.0001) and Nervonic acid + acid cervonic (C24:1+C22:6) (P = 0.0454) at 52 wk of age. At the 56th week we observed a significant increase of dihomo-γ-linolenic acid C20:3n-6 (P = 0.0152). At the 64th week there was an increase of eicosapentaenoic acid (EPA) C20:5n-3 (P = 0.0442) and dihomo-α-linolenic acid C20:3n-3 (P = 0.0240). At the 68th week there was an increase in the proportion of C20:3n-6 (P = 0.0163), C20:5n-3(P = 0.0291) and (C24:1 + C22:6) (P = 0.0358). At the 72nd week there was considerable increase in the arachidonic acid C20:4n-6 (P = 0.0385). The oleic acid, C18:1n-9c, was highest in semen of males feed with CTX (P < 0.005) in all age groups. The addition of canthaxanthin did not decrease lipid peroxidation. The increase in the proportion in fatty acids in the sperm phospholipids that resulted from dietary supplementation of the males with the canthaxanthin, may have beneficial consequences for fertility.

**Key Words:** male, oxidation, carotenoid, spermatozoa

39 Effect of supplementing exogenous protease in poultry by-product-meal based diets on growth, carcass characteristics, and nutrient digestibility in broilers. Tahir Mahmood*1, Muhammad A. Mirza1, Haq Nawaz1, Muhammad Shahid2, Tariq Mushtaq1, and Mubashar Hussain1,1 Institute of Animal Sciences, University of Agriculture, Faisalabad, Punjab, Pakistan, 2Department of Biochemistry, University of Agriculture, Faisalabad, Punjab, Pakistan, 3AgroVisions (Pvt) Ltd., Faisalabad, Punjab, Pakistan.

A 3 × 2 factorial experiment was conducted using 3 levels (0, 3, and 6%) of poultry by-product meal (PBM) and 2 levels of exogenous protease (with and without) in broilers to check the effect on growth, carcass characteristics and nutrient digestibility from d 1 to 35. The data were statistically analyzed in 3 × 2 factorial design by generalized linear method of ANOVA using MINITAB. Significant means were separated by Tukey’s test. The birds (n = 240) were randomly divided into 24 replicates (10 birds/replicate) and fed equi-caloric and equi-nitrogenous (ME 2850; CP 20) diets ad libitum throughout the experiment. The exogenous enzyme was supplemented at 200 g/t of feed. Enzyme supplementation increased feed intake and body weight gain (P < 0.01) but feed:gain remained unaffected (P > 0.05) from d 1 to 21. Increasing level of PBM decreased feed intake (P < 0.05). However, body weight gain and feed: gain was improved (P < 0.05) at 3% PBM level from d 1 to 21. A strong interaction effect (P < 0.01) of enzyme and PBM on feed intake was noted during d 1 to 21. No interaction was recorded for body weight gain and feed: gain. Percent carcass yield (P < 0.01) was improved by the addition of enzyme. Percent breast meat yield was depressed (P < 0.001) with addition of PBM in the diet. No main effect or interaction effect was noted for all other carcass characteristics. The AME (P < 0.001), nitrogen retention (P < 0.01), AME (P < 0.001), and apparent digestibility coefficient for nitrogen (P < 0.01), all were improved by the supplementation of protease; however, the reverse was noticed for birds fed diets containing only PBM. In conclusion, improved performance and nutrient digestibility were recorded in birds fed diets containing 3% PBM with protease supplementation

**Acknowledgments:** This study was funded by Higher Education Commission of Pakistan under Indigenous Scholarship Scheme for PhD in Agriculture and Veterinary Sciences.

**Key Words:** poultry by-product meal, broiler, exogenous protease, nutrient digestibility