

response to 1% change in CPI was  $0.05 \times \ln(\text{EBW})$ . Increased dietary ME exhibited negative effects on rate of feed intake, and the allometric relationship of breast muscle. Increased dietary CP exhibited negative effects on rate of feed intake, and positive effects on energy retention, and on the allometric relationship of breast muscle. This platform allowed dynamically evaluating nutritional effects on variables affecting broiler performance.

**Key Words:** empirical model, broiler chicken, breast meat, metabolizable energy, crude protein

**M70 Effects of feed formulation and level of feed intake on the energy partitioning of finisher broiler chickens.** S. Gomez\*<sup>1,2</sup>, M. L. Angeles<sup>1</sup>, E. Ramirez<sup>1,2</sup>, and V. Mondragon<sup>1</sup>, <sup>1</sup>CENIDFyMA - INIFAP, Ajuchitlan, Queretaro, Mexico, <sup>2</sup>FES-Cuautitlán-UNAM, Ajuchitlan, Queretaro, Mexico.

A balance trial and slaughter experiment were carried out to evaluate the true metabolizable energy (TMEn) and the efficiency of conversion from gross energy (GE) to TMEn and from TMEn to net energy (NE) for protein (p) and fat (f) deposition in broiler chickens from 39 to 49 days of age fed different amounts of diets based on sorghum (S) or corn (C) and with or without the inclusion of dry distillers grains with solubles

(DDGS). Forty eight Ross B308 male broilers allocated in individual pens were assigned to 12 treatments in a complete randomized design with a factorial arrangement of 2 grains (S or C), 2 levels of DDGS (0 and 10%) and 3 feeding levels (90, 120 and 150 g of feed/day). The last two days, total excreta were collected and in an additional group of birds the endogenous excretion of nutrients was determined. At the end, birds were killed to determine the energy retained in protein and fat. Chickens killed at the beginning of the trial were used to correct for the initial composition. There were four birds per treatment and results were subjected to analysis of variance. The nutrient excretion was lower and the nutrient retention, TMEn and the energy conversion from GE to TMEn were greater for C than for S ( $P < 0.05$ ). The TMEn was lower but the energy conversion from TMEn to NE<sub>f</sub> and to NE<sub>p+f</sub> ( $P < 0.05$ ) were greater for DDGS0 than for DDGS10 ( $P < 0.05$ ). As the FL increased, the retention of nutrients, the TMEn, the total protein and fat in the body, the energy retained in protein and fat, the conversion from GE to TMEn and from TMEn to NE<sub>p</sub>, NE<sub>f</sub> and NE<sub>p+f</sub> showed linear increments ( $P < 0.01$ ). Diets based on corn had a greater energetic value than diets based on sorghum; the inclusion of 10% DDGS reduced the energy retained in fat; broiler chickens fed at the highest feeding level showed the greatest efficiency of nutrient retention and conversion from gross energy, to true metabolizable energy and to net energy retained in protein and fat.

**Key Words:** broiler chickens, GE, TMEn, NE

## Physiology

**M71 Comparison of egg hatchability between two breeds of layers.** O. K. Awobajo\*<sup>1</sup>, A. A. Mako<sup>1</sup>, O. I. Abiola-Olagunju<sup>2</sup>, O. A. Ogunwole<sup>2</sup>, R. A. Hamzat<sup>3</sup>, A. O. Igboanu<sup>1</sup>, and R. O. Ettu<sup>1</sup>, <sup>1</sup>Tai Solarin, University of Education, Ijebu Ode, Ogun State, Nigeria, <sup>2</sup>Department of Animal Science, University of Ibadan, Ibadan, Oyo State, Nigeria, <sup>3</sup>Purdue University, West Lafayette, IN.

This study was carried out to compare hatchability between Rhode Island Red and White Leghorn. The hatchability records were collected for one year on each breed selected from the hatchery farms in Ijebu-Ode local Government. The result were subjected to statistical analysis. The result of the analysis (including T-test) revealed no significant differences ( $P < 0.001$ ) between rejected eggs (3.97%) from Rhode Island Red and (3.93%) from White Leghorn breeds. There was no significant difference ( $P < 0.001$ ) between the infertile eggs of Rhode Island Red (29.04%) and White Leghorn (24.82%). Significant differences ( $P < 0.001$ ) occurred in the egg hatchability of Rhode Island Red (64.29%) and White Leghorn breeds (68.61%). The White Leghorn had higher egg hatchability percentage than Rhode Island Red.

**Key Words:** hatchability, layers, breeds, rejected eggs, infertile eggs

**M72 Effect of pre-warming profile on hatchability and chick quality.** I. A. M. Reijrink\*<sup>1</sup>, D. Berghmans<sup>2</sup>, R. Meijerhof<sup>1</sup>, B. Kemp<sup>2</sup>, and H. Van den Brand<sup>2</sup>, <sup>1</sup>HatchTech Incubation Technology, Veenendaal, The Netherlands, <sup>2</sup>Wageningen University, The Netherlands.

Pre-warming of hatching eggs prior to incubation is to prevent condensation and to reduce variation in egg temperatures. The pre-warming

profile might affect embryo viability, as it might affect cell death especially when cell viability is reduced after prolonged storage. The aim of this research was to investigate the effect of storage time and pre-warming profile on hatchability and chick quality. Eggs from a Ross broiler breeder flock with an age of 41 to 50 weeks were used. The experiment was a 2\*3 factorial design: 2 storage times (4 and 14 d), and 3 pre-warming profiles (in 30 minutes, 4 h, or 24 h from 17°C to 37.8°C). All eggs were stored at 17°C. Eggs pre-warmed in 30 min were warmed in a water bath with water of 37.8°C. The other eggs were pre-warmed during 4 and 24 h in air. During incubation egg shell temperature was maintained at 37.8°C in all treatment groups. Infertility and embryonic mortality was determined macroscopically. Chick quality was evaluated 12 h after hatch by measuring chick length and yolk free body mass. No interaction was found between storage time and pre-warming profile for hatchability and chick quality. Although no significant interaction was found, there was a numerical difference in first week embryonic mortality between 24 h of pre-warming and 30 min and 4 h of pre-warming in eggs stored for 14 d (3.4%, 11.1%, and 9.4%, respectively,  $P = 0.34$ ). Storage time and pre-warming profile did not affect hatchability. Pre-warming profile did not affect chick quality. Fourteen days storage resulted in 0.1 cm shorter chick length ( $P = 0.003$ ) and 0.4 g lower yolk free body mass ( $P = 0.006$ ) compared with 4 d storage. In this experiment no effect of pre-warming profile on hatchability or chick quality was found.

**Key Words:** storage time, pre-warming profile, hatchability, chick quality

**M73 Number and distribution of sperm-storage tubules in four strains of broiler breeders.** M. R. Bakst<sup>\*1</sup>, S. M. Whipple<sup>2</sup>, R. K. Bramwell<sup>2</sup>, D. E. Yoho<sup>2</sup>, J. R. Moyle<sup>2</sup>, G. Liu<sup>3</sup>, and A. M. Donoghue<sup>4</sup>, <sup>1</sup>ABBL, ARS, USDA, Beltsville, MD, <sup>2</sup>Department of Poultry Science, University of Arkansas, Fayetteville, <sup>3</sup>Yangzhou University, Yangzhou, Jiangsu, P.R. China, <sup>4</sup>PPPSRU, ARS, USDA, Fayetteville, AR.

Restricted to the utero-vaginal junction (UVJ) in the hen's oviduct are tubular invaginations of the surface epithelium collectively referred to as the sperm-storage tubules (SSTs). One would expect that a larger number of SSTs would be positively correlated with longer, sustained fertility. However, only two studies have reported SST numbers. Goodrich-Smith & Marquez (1978) estimated between 20,000-24,000 SSTs for the turkey while Birkhead & Hunter (1990) observed 1000-2000 SSTs in finches. We report our preliminary results of SST numbers, number of mucosal folds lining the UVJ, and the length (mm) of the mucosal fold with SSTs present in four strains of broiler breeder hens. Two hundred commercial pullets from each strain were reared according to industry standards. Hens were light stimulated at 21 wk and at 33-38 wk, 6 hens per group were randomly selected and euthanized. The vagina was dissected free of connective tissue, UVJ mucosa exteriorized and the number of mucosal folds counted. Six UVJ folds per hen were isolated, placed on a stereomicroscope and the portion of the UVJ fold containing SSTs was measured. The fold was then placed on a slide and a coverslip pressed firmly over the fold. The squash preparation was photographed and SSTs were counted off the images. Preliminary data (2 hens per strain) revealed no statistical variation between strains. However, the following was observed: total number of SSTs, 2,923-5,765; number of UVJ mucosal folds per hen, 16-19; length of UVJ folds containing SSTs, 14-27mm. Interestingly, the total number of SSTs for these broiler strains fell between that reported for turkeys and finches. Future analyses may reveal correlations between the SST numbers, strains, and sustained fertility as the study will be repeated when the hen flock reaches 60 wk of age.

**Key Words:** broiler breeder, oviduct, reproduction, birds, sperm-storage tubule

**M74 Stability of added enzymes during gastrointestinal tract passage: In vitro versus in vivo data.** M. F. Isaksen<sup>\*1</sup>, S. Dalsgaard<sup>1</sup>, L. Salmon<sup>2</sup>, and C. Gilbert<sup>2</sup>, <sup>1</sup>Genencor, Brabrand, Aarhus, Denmark, <sup>2</sup>Danisco Animal Nutrition, Marlborough, Wiltshire, United Kingdom.

Feed enzymes are used extensively and are tested for several parameters when they are evaluated, such as pH profile, temperature profile, thermostability. A new, important parameter to test is stability during passage through the gastrointestinal tract. This is crucial to check if the enzymes can remain active after contact with the endogenous proteases and low pH in the digestive system.

An experiment was run to test if in vitro stability corresponds to stability measured in vivo. Several enzymes were tested. The pig was used as model animal, because it is possible to collect bigger samples, which is needed to study the enzymes stability. Furthermore, it was evaluated, that the challenge in the pig digesta was higher due to lower pH in the stomach compared to a broiler digesta system. Three male cannulated pigs were fed a basal diet, formulated using barley/wheat/SBM and including a marker. The diet was heated treated to remove all endogenous enzyme activity. Test enzymes were added to the basal feed. Blank

samples were taken at day 0 before the animals were fed the enzyme test diets. Digesta samples were collected at two time points post AM feeding. Enzymes were tested in triplicate and enzyme treatments were randomised between the three animals. Feed, duodenum and ileum samples were analysed for marker and enzyme activity. The stability of the enzyme in the digestive system was then calculated. The in vivo stability was compared to the stability found in an in vitro experiment. In the in vitro test each enzyme was incubated at low or neutral pH with increasing level of pepsin or pancreatin, to mimic the Gizzard/stomach region or ileum/small intestine region, respectively. After two hours of incubation, enzyme activity was determined. The stability of the enzymes varied from total resistance towards the challenging environment to no detected activity after incubation. Results show that there are differences between the stability of the tested enzymes depending of the origin of the enzymes.

**Key Words:** enzymes, stability, pH, in vitro, in vivo

**M75 Development of a quail embryo model for the detection of botulinum neurotoxin activity.** R. J. Buhr<sup>\*1</sup>, D. V. Bourassa<sup>1</sup>, N. A. Cox<sup>1</sup>, L. J. Richardson<sup>1</sup>, R. W. Phillips<sup>2</sup>, and L. C. Kelley<sup>2</sup>, <sup>1</sup>USDA-ARS Russell Research Center, Athens, GA, <sup>2</sup>USDA-FSIS Russell Research Center, Athens, GA.

*Clostridium botulinum* is a ubiquitous microorganism that under anaerobic conditions produces botulinum neurotoxins. In regards to both food-borne illness and the potential use of botulinum toxin as a biological weapon, the capability to assess the amount of toxin in a food or environmental sample efficiently is critical. Currently the mouse toxicity and neutralization assay is used for assessment of botulinum toxin activity and can detect 20 pg/mL. There is growing pressure to replace the mouse LD<sub>50</sub> assay for ethical concerns over the use of death by asphyxiation of the mice as the test endpoint. The objective of this study was to develop and evaluate a screening assay for detecting biologically active botulinum neurotoxins using Japanese quail embryos. Quail embryos at day 15 of incubation were injected into the neck/shoulder area with botulinum toxin type A (0.05 to 250 ng / 0.05 mL) and types B, E, and F (10 to 80 ng). At 3 d post-injection, embryos injected at 0.1 ng or higher with type A toxin had significantly more embryos than the control determined to be non-viable (21.5 vs. 2.5%). Neurotoxins B, E, and F were all detected at 10 ng/embryo with 40, 90, and 70% of embryos determined to be non-viable. Premixing of the toxin type A with type A specific antibody demonstrated that the depression in the ability of the quail embryos to pip and hatch was indeed attributable to biologically active toxin (80 vs. 14%). These experiments demonstrate that the Japanese quail embryo is an effective vertebrate animal model to detect the biological activity of botulinum neurotoxins A, B, E, and F. The minimal detectable dosage by the quail embryos of type A toxin is 100 pg (14 µg/kg of body weight). Utilization of a quail embryo may be a beneficial model for the analysis of botulinum toxins activity by enhancing personnel safety and since BoNTs does not kill the quail embryo, but restrict the ability of the embryo to progress to pipping into the air cell and through the eggshell, non-viable embryos can be presumptively euthanized upon detection.

**Key Words:** botulinum toxin, toxin types A B E and F, quail embryo, embryo viability, bioassay

**M76 Genetic selection increases parthenogenesis in Chinese Painted Quail (*Coturnix chinensis*).** H. M. Parker\* and C. D. McDaniel, *Mississippi State University, Mississippi State.*

Parthenogenesis, embryonic development of an unfertilized egg, occurs naturally in turkey, chicken, and quail species. In fact, parthenogenesis in turkeys and chickens can be increased by genetic selection for this trait. However, it is unknown if genetic selection for parthenogenesis is effective in quail or if parthenogenesis affects hatchability of fertilized eggs. Therefore, the objectives of this study were to determine if the incidence of parthenogenesis in quail could be increased by genetic selection, and if parthenogenesis and hatchability characteristics are correlated. To prevent fertilization, 917 females were caged separately from males at 4 wk of age and then caged individually at 6 wk of age to monitor egg production. Eggs were collected daily, labeled with hen number and date, and stored for 0 to 3 d. After 10 d of incubation, 20 unfertilized eggs from each hen were examined to determine the occurrence of parthenogenesis and embryo width. In the first generation (P) and subsequent generations (F<sub>1</sub> to F<sub>3</sub>), hens laying eggs containing parthenogenetic development and males whose sisters or mothers exhibited parthenogenesis were used for breeding. Weekly, eggs from breeding stock were set, and hatch residue analyses were performed on unhatched eggs. When compared to the P generation, the percentage of hens exhibiting parthenogenesis was greater as generation of selection increased. With each successive generation, percentage of eggs positive for parthenogenesis increased linearly ( $r^2=0.99$ ). Parthenogenesis was 72% greater among eggs from the F<sub>3</sub> generation (7.9%) when compared to the P generation (4.6%). There was a quadratic increase in embryo size as generation increased ( $r^2=0.99$ ), and embryonic size for each generation was greater than the P generation. Percentage of eggs exhibiting parthenogenesis was negatively correlated with egg production yet positively correlated with percentage of early dead embryos from breeding stock. In conclusion, genetic selection for parthenogenesis increased the incidence of parthenogenesis and embryonic size and also influenced early embryonic mortality.

**Key Words:** parthenogenesis, quail, fertility, hatchability

**M77 Effects of lithocholic acid and cholecalciferol on plasma Ca and expression of calbindin and Ca ATPase in commercial broiler chickens.** A. Liem\*, G. M. Pesti, R. B. Beckstead, and H. M. Edwards Jr., *University of Georgia, Athens.*

Lithocholic acid (LCA) is one of the main bile acid found in mammals and birds. While bile acids are predominantly known for their function in digestion, LCA has been reported to act as a signaling molecule on the vitamin D pathway in mice by increasing expression of calbindin, TRPV6 (a calcium channel), and ATP2B1 (CaATPase) in intestinal mucosa. Since there are similarities between mammalian and avian vitamin D receptor, we tested the hypothesis that LCA would have similar D<sub>3</sub> signaling capacity in chicken.

To investigate if LCA has vitamin D activity in chicken, 240 Heritage broilers were randomly assigned to 6 treatments and raised from d1-16 in a battery brooder. The first 3 experimental diets were administered

from day 1 to 16 : basal diet deficient in vitamin D (B), B+ 0.2% LCA, B + vitamin D<sub>3</sub>. The next 3 experimental diets were fed from day 8-16 (birds in these treatments received B diet from day 1-7) : B + 0.1% LCA, B+0.2% LCA, B + vitamin D<sub>3</sub>. Each treatment had 4 replicate pens, with 10 mixed sex chicks in each pen. Body weight, liver weight, bone ash, leg score (incidence of vitamin D deficiency rickets), and P deficiency rickets), and plasma Ca were measured. Intestinal mucosa samples were collected to determine the expression of calbindin, and CaATPase.

As expected, vitamin D<sub>3</sub>, added since day 1 or day 8 increased body weight gain (BWG), increased plasma Ca, reduced the incidence of Ca rickets, and increased expression of calbindin and ATP2B1. At the levels fed, LCA did not affect BWG, linearly increased plasma Ca and liver weight, and reduced incidence of Ca rickets. At 0.1%, LCA increased expression of calbindin, but did not significantly affect expression of ATP2B1. At 0.2%, LCA had no effect on the genes tested.

In conclusion, similar to mice, vitamin D<sub>3</sub> increased plasma Ca and expression of calbindin and ATP2B1 in chicken intestinal mucosa ( $p<0.05$ ). At 0.1%, LCA appears to have some vitamin D activity in chickens. While at 0.2%, LCA is toxic to the birds and might cause an overall disruption of metabolic processes.

**Key Words:** vitamin D, lithocholic acid, calbindin, ATP2B1, plasma calcium

**M78 Haematology of egg-type chickens fed fermented and enzyme-treated cocoa bean testa based-diets.** M. D. Olumide\*<sup>1</sup>, R. A. Hamzat<sup>2</sup>, and A. O. Akinsoyinu<sup>3</sup>, <sup>1</sup>*Kolmat Farms Limited, Erunmu, Ibadan, Nigeria*, <sup>2</sup>*Purdue University, West Lafayette, IN*, <sup>3</sup>*Department of Animal Science, University of Ibadan, Nigeria.*

One of the limiting factors to the growth of poultry industries in Nigeria is high cost of feed ingredients. This is why animal nutritionists are therefore investigating into some agro allied wastes that have potential as feed ingredients. Cocoa bean testa (CBT) is one of such that constitutes economic waste in all cocoa industries in Nigeria. Measurements of haematologic parameters is an important part of evaluating health status in avian species. This study hence focused on evaluating the haematology of layers fed cocoa bean testa based diets.

Two hundred and ten (210) six – week –in –lay hens were used for this trial with thirty birds, randomly allotted to seven experimental diets containing ten birds per replicate in a 3 X 3 factorial design. These diets were: A (0% CBS – control); B (5% raw CBS); C (10% raw CBS); D (5% CBS with enzyme); E (10% CBS with enzyme); F (5% fermented CBS); and G (10% fermented CBS). The layers on each diet were offered feed and water ad – libitum throughout the experimental period. The hematological parameters studied were the red blood cell, hemoglobin, white blood cell, packed cell volume, erythrocyte mean cell haemoglobin concentration, erythrocyte mean cell volume and erythrocyte mean cell haemoglobin. Differences in the hematological parameters of the layers in the different treatments were not appreciable ( $p > 0.05$ ).

**Key Words:** fermentation, enzyme treatment, cocoa bean testa, egg-type chickens, haematology