

diets with no supplemental P and acidification of gastrointestinal tract may further improve this utilization.

**Key Words:** Organic Acid, Phytase, Laying Hen

**298 Effect of a thermo-tolerant phytase on performance and bone ash in broilers fed variable levels of dietary nutrients.** C. W. Wyatt<sup>\*1</sup>, M. R. Bedford<sup>1</sup>, T. Parr<sup>2</sup>, and S. Davis<sup>2</sup>, <sup>1</sup>Zymetrics Inc., Golden Valley, MN, <sup>2</sup>Colorado Quality Research, Wellington, CO.

Two studies investigated the effect of a thermo-tolerant E.coli-derived phytase (Quantum<sup>TM</sup>) on performance and bone parameters of broilers fed diets varying in content of available phosphorus (AP), metabolizable energy (ME) and total lysine (TLYS). A 48-day floor pen trial used 80 pens of 16 male broilers fed corn/soya/meat-bone meal based diets formulated to commercial averages (AS) or below. A four phase positive control (PC) diet was fed with .91, .82, .76, .69% calcium (CA) and .45, .39, .34, .30% AP, and two negative control (NC) diets were formulated to contain less AP(.09% or .12%), ME(26 or 45kcal/kg) and TLYS(.01 or .03%), respectively for each phase. 250, 500 or 1000 U phytase/kg diet were supplemented to each NC resulting in 10 treatments. At day 40 and 48, the initial reduction in AP, ME and TLYS did not affect performance, but further nutrient removal reduced performance compared to the PC. Supplementing all NC diets with at least 250U/kg phytase improved performance to equal or better than the PC. Lowering AP significantly reduced 48 day femur ash, but this was restored to equal or better than the PC on adding phytase. A second 50-day floor pen trial using 88 pens of 16 male broilers was conducted to determine the impact of phytase on growth, bone ash and breast yield in corn/soya/meat-bone meal based diets varying in ME. As above, a four phase PC diet was fed and three NC diets were formulated to contain less AP(.12%) and TLYS(.01%) and ME lowered by 30, 45 and 60kcal/kg, respectively. 500 or 1000 U phytase/kg of diet were supplemented to the three NC diets resulting in 8 treatments. At day 40 and 50, reducing the amount of dietary AP, ME and TLYS resulted in significantly poorer BWT and cFCR, but further reduction in ME did not result in additional losses in performance. Adding 500 U/kg and 1000U/kg phytase to the NC equilibrated performance with that of the PC for the 45 and 60kcal reduced diet respectively. In these studies. EC phytase was able to return NC performance to that of the commercial PC, although the level of phytase needed would depend on the reduction in dietary nutrient levels.

**Key Words:** Broiler Chick, Escherichia Coli Phytase, Performance

**299 The influence of an E.coli derived phytase on performance of turkeys fed phosphorus deficient diets.** M. R. Bedford\* and C. L. Wyatt, Zymetrics Inc., Golden Valley, MN.

The effect of a thermo-tolerant E.coli-derived phytase (Quantum) on performance of turkeys fed phosphorus deficient diets to 84d was investigated. In experiment 1, each treatment consisted of 6 replicates of 15 BUT T8 birds. A positive (PC) and negative control (NC) corn/wheat/soya based ration containing 0.7, 0.5 then 0.5 or 0.35, 0.2 then 0.2% AvP in the starter, grower and finisher was employed. All other nutrients met or exceeded NRC requirements. 0, 100, 300, 900 or 2700 units/kg of an E.coli derived phytase (Quantum<sup>TM</sup>) was added to the NC to give 6 diets. Performance was monitored at 28, 56 and 84 d of age. At 28 d of age, intake and gain were depressed on feeding the NC but were restored to values equivalent to the PC at 900 units/kg feed.

At 56d of age, intake, gain and FCR deteriorated on feeding the NC, and only on feeding 2700 U/kg was performance restored to PC levels. At 84 days of age, performance on the NC was poorer than the PC and was not restored even on the 2700U/kg treatment. In experiment 2, 11 male Nicholas turkeys were assigned to 6 replicate pens per treatment. There were 5 treatments, a PC, (meeting all NRC requirements) and a NC which contained 0.14% less AvP in each of the 4 phases fed supplemented with 0, 200, 500 or 1250 units of an E.coli derived phytase (Quantum). At 21d the NC performed significantly worse than the PC in terms of FCR only, and this was restored on use of 500 units of phytase or more. By 84 days gain was marginally depressed on feeding the NC and restored on use of even the lowest level of phytase. FCR was unaffected by treatment. Intake tended to drop on feeding the NC and was restored on feeding phytase. These data suggest that the use of moderate levels of phytase can easily compensate for moderate dietary reductions in AvP, but if severe reductions are made, then the subsequent losses in performance are not readily apparent until late on in the growth phase. As a result, short term tests on the bioequivalency of phytase for replacement of inorganic P are likely flawed in their conclusions when applied to full production periods.

**Key Words:** Phytase, Turkeys, Performance

**300 Efficacy of Phyzyme<sup>®</sup> XP phytase in broiler diets containing different levels of calcium and non-phytate phosphorus: performance, bone ash and mineral retention.** D. R. Ledoux<sup>\*1</sup>, J. N. Broomhead<sup>1</sup>, and J. S. Sands<sup>2</sup>, <sup>1</sup>University of Missouri, Columbia, <sup>2</sup>Danisco Animal Nutrition, Marlborough, Wiltshire, UK.

A six-week floor pen study was conducted to determine the efficacy of Phyzyme<sup>®</sup> XP, in corn-soybean meal-based diets containing different Ca & P levels, on performance, tibia ash, and P retention in broiler chickens. A 3 X 4 factorial arrangement of dietary treatments from hatch to week 3 included 3 Ca & non-phytate P (npP) levels (0.80 & 0.25%, 0.85 & 0.30%, and 0.90 & 0.35%) and 4 levels of Phyzyme<sup>®</sup> XP (0, 250, 500, and 750 U/kg diet). From week 3 to 6, dietary phytase levels were kept the same but Ca & npP levels were reduced (0.70 & 0.15%, 0.75 & 0.20%, and 0.80 & 0.25%). The starter basal diet contained 21.74% CP and 2920 kcal ME/kg, and the grower basal diet contained 19.5% CP and 3010 kcal ME/kg. Six pens of 25 chicks each were assigned to each dietary treatment from day 1 to 42. Significant (P < 0.05) Ca & npP level by phytase interactions were observed for feed intake (FI) at weeks 3 and 6, and for body weight gain (BWG) at week 6. Feed intake, BWG, and FCR were also affected (P < 0.05) by phytase treatment at both weeks 3 and 6, with chicks fed phytase outperforming chicks fed no phytase. Chick performance was not affected (P > 0.05) by Ca & npP at 3 weeks, but were affected (P < 0.05) at 6 weeks, with the best performance observed in chicks fed the 0.90% Ca & 0.35% npP combination. Significant interactive and main effects were observed for tibia ash (%) at 6 weeks, with the response to phytase differing among the Ca & npP levels. Both 3 and 6 week P retention were improved (P < 0.05) by phytase supplementation. Combination of Ca & npP did not affect P retention (P > 0.05) at week 3, but did affect P retention (P < 0.05) at week 6. No Ca & npP by phytase interaction for P retention occurred at either weeks 3 or 6. Results indicate that Phyzyme<sup>®</sup> XP was effective in improving phytate P utilization, and efficacy was influenced by Ca & npP level.

**Key Words:** Phytase, Mineral Retention, Broilers

## Poultry Reproductive Physiology

**301 Effect of heat stress on production, reproduction hormone levels, acid-base status, and liver expression of heat shock protein-70 observed in three varieties of laying hens.** D. J. Franco\*, L. Robeson, and M. M. Beck, University of Nebraska, Lincoln.

Thirty-two hens of each strain (Brown, W98, W36) at 38 weeks of age, were allowed to acclimatize for two weeks at 22C, and then were exposed to heat stress (HS) at 35C for two weeks with two additional weeks at 22C to recover. Production parameters (PP) and mortality rates (MR) were collected for each phase. Reproductive hormone levels (RHL) were obtained from blood collected at each phase. Acid base status (AB), Intestinal calcium uptake rate (CaT), and liver expression of HSP70 data

were obtained from samples collected before and during HS exposure. The data for PP and RHL were analyzed using the SAS program 1999 version 8.0 as repeated measure in a 3x3 factorial experiment with a level of significance of 0.05. The data for liver expression of HSP70, AB, and CaT were analyzed in a 3x2 factorial experiment with a level of significance of 0.1. Differences among means were obtained based on LSD test. Brown birds performed least well during subsequent HS, with a higher reduction in egg production and a lower CaT. Most of the PP were reduced in all the birds during HS; however, the highest reduction in egg weight was observed in the W36 hens. AB was characterized by an increase in blood pH and a reduction in PCO<sub>2</sub> during HS. PO<sub>2</sub> showed a small positive increment during HS only for the W36 and HCO<sub>3</sub> levels

were reduced during HS except in W98. HSP70 expression was higher after HS exposure and estrogen was reduced during HS in all strains. Progesterone levels were not affected and LH levels were lower in the W98 birds. In general, the Brown hens fared worst in all parameters measured during HS, while W98 hens performed best; W36 was almost always intermediate. However, Brown birds had higher MR (16%), W36 the lowest (4%) with the W98 (8%) intermediate. Some physiological parameters responded differentially by strain, suggesting that further investigation in gene expression might yield markers that could be incorporated into breeding programs for increasing HS resistance in laying hens.

**Key Words:** Laying Hens, Heat Stress, Production Parameters

**302 Melengestrol acetate (MGA) as an alternative method to induce molting in hens.** J. M. Koch\*<sup>1</sup>, J. S. Moritz<sup>1</sup>, D. C. Lay Jr.<sup>2</sup>, and M. E. Wilson<sup>1</sup>, <sup>1</sup>WVU, WV, <sup>2</sup>USDA-LBRU, IN.

Inducing hens to molt increases egg quality, egg production and extends the productive life of hens. Molting is normally accomplished by feed withdrawal, which has received criticism, and alternatives described thus far have resulted in poor post-molt performance. The process of molting leads to cessation of lay, regression of large yellow follicles (LYF) and results in loss of steroidogenic support for the oviduct. MGA, an orally active progestin, may decrease gonadotropic support for the ovary and cessation of lay. Hyline W-36 laying hens (n=104) at 40 wk-of-age were fed either 0 or 8 mg/d MGA for 28 d in a balanced diet and then returned to a normal diet. Four birds on d 0 and 4 birds/treatment on d 1, 2, 4, 8, 12, 16, 20, 24, 28, 32, 36, 40 and 44 were euthanized. The weight (g) of ovary with LYF, magnum, isthmus, shell gland and the length (cm) and weight of the intact oviduct were determined. By d 19, egg production in the MGA treated hens (M) had decreased to 24% where it remained until d 28 compared to 88% in the controls (C). The weight of the ovary with LYF, the oviduct and the magnum decreased ( $p < .05$ ) by day 8 in M compared to C ( $33 \pm 15$  vs  $62 \pm 7$ ,  $39 \pm 10$  vs  $71 \pm 1$  and  $19 \pm 6$  vs  $41 \pm 2$ ). Furthermore, oviduct length, isthmus and shell gland weight decreased ( $p < .05$ ) by d 20 in M compared to C ( $54 \pm 7$  vs  $78 \pm 3$ ,  $1.2 \pm 0.5$  vs  $3.3 \pm 0.3$  and  $11 \pm 3$  vs  $23 \pm 2$ ). From d 28 to 40, egg production in M increased until similar to C at 83%. Oviduct length, isthmus weight and LYF recrudescence in 3 out of 4 M was similar ( $p > .05$ ) to C by d 32 ( $59 \pm 10$  vs  $70 \pm 3$ ,  $2.0 \pm 0.5$  vs  $2.8 \pm 0.4$ ,  $36 \pm 12$  vs  $54 \pm 5$ ). By d 36 the shell gland weight in M was similar ( $p > .10$ ) to C and 4 out of 4 hens had recrudescence of LYF ( $14 \pm 2$  vs  $21 \pm 1$ ,  $47 \pm 8$  vs  $40 \pm 10$ ); however, it was not until d 40 that the weight of the oviduct and magnum in M was similar ( $p > .05$ ) to C ( $61 \pm 8$  vs  $64 \pm 1$ ,  $33 \pm 5$  vs  $37 \pm 1$ ). MGA, when fed to hens in a balanced layer diet, can cause regression of LYF on the ovary, leading to loss of steroidogenic support for the oviduct. Removal of MGA led to recrudescence of LYF, leading to steroidogenic support for the oviduct and return to lay.

**Key Words:** Hen, Molting, Melengestrol Acetate

**303 Incidence of bone breakage of processed White Leghorn hens monitored for skeletal integrity during the second cycle of egg laying.** H. Mazzuco\*<sup>1,2</sup> and P. Y. Hester<sup>1</sup>, <sup>1</sup>Purdue University, West Lafayette, IN, <sup>2</sup>CNPQ, Brasilia-DF, Brazil, <sup>3</sup>EMBRAPA Concordia-SC, Brazil.

Hens housed in cages experienced a 29% broken bone incidence after transport and immediately before the water bath stunner (Gregory and Wilkins, 1989, Br. Poult. Sci. 30:555). The current study was carried out to determine the correlation between bone mineral density (BMD) and bone mineral content (BMC) measured in live birds (85 to 125 wk of age) and excised tibia (at 126 wk of age only) with the incidence of broken bones in carcasses of processed 126-wk-old White Leghorn hens. Twenty-seven hens out of a total of 207 White Leghorns of a pedigree line were monitored for BMD and BMC of the tibia and humerus using a dual energy X-ray absorptiometer during a second cycle of egg production. At 126 wk of age, the 207 birds were processed and the bones (femur, tibia, fibula, humerus, radius, ulna, keel, scapula, coracoid, ribs, clavicle, ischium and pubis) were examined for breakage. Values for in vivo BMD and BMC were averaged over the age of the hens and averaged over both bones (humerus and tibia). Correlation coefficients for the incidence of broken bones with the average in vivo BMD during the second cycle of lay and with the excised tibial BMD at 126 wk of age were  $-0.39$  ( $P < 0.05$ ) and  $-0.54$  ( $P < 0.01$ ), respectively. Correlation value for the incidence of broken bones with excised tibia BMC at 126

wk of age was  $-0.53$  ( $P < 0.01$ ) and with the second cycle in vivo BMC was not significant ( $r = -0.20$ ). The percentage of broken bones of the total bones examined per bird at the end of processing at 126 wk of age averaged 34% with a range of 0 to 61%. These results show that BMD monitored in live birds during a second egg laying cycle (85 to 125 wk of age) as well as in the excised tibia at 126 wk of age were negatively and significantly correlated with the incidence of bone breakage in processed carcasses. It is concluded that as BMD decreased in White Leghorns, the incidence of breakage increased.

**Key Words:** Spent Hens, Bone Mineral Density, Bone Breakage

**304 Impact of supplemental L-carnitine in broiler breeder diets on subsequent egg hatchability and progeny embryogenesis.** J. P. Tanksley\*<sup>1</sup>, E. D. Peebles<sup>1</sup>, M. T. Kidd<sup>1</sup>, C. D. McDaniel<sup>1</sup>, S. K. Whitmarsh<sup>1</sup>, H. M. Parker<sup>1</sup>, and P. D. Gerard<sup>2</sup>, <sup>1</sup>Department of Poultry Science, Mississippi State University, Mississippi State, <sup>2</sup>Experimental Statistics Unit, Mississippi State University, Mississippi State.

The impact of supplemental L-carnitine in broiler breeder diets on subsequent egg hatchability and progeny embryogenesis were investigated in this study. Beginning at 21 weeks of age, hens were fed diets supplemented with either 0 (control) or 25 ppm L-carnitine (treated) through 40 weeks of age. In each of 16 breeder floor pens, 20 hens were housed, with 8 replicate pens assigned to each of two treatment groups. At 27, 32, and 38 weeks of age 15 eggs from each replicate pen were set at random according to treatment in a single incubator for determination of set egg weight (SEW) and relative embryo (REMW; % of set EW), yolk sac (RYSW; % of set EW), and liver (RLW; % of embryo weight) weights at 18 days of incubation. Similarly, at 25, 30, 32, and 38 weeks of age, approximately 70 eggs from each replicate pen were set in a separate single incubator for determination of hatchability and embryonic mortality. Breeder hen age significantly affected percent hatchability of fertile eggs set, percent early dead mortality, REMW, and RLW, but did not influence percent late or piped embryo mortalities, and RYSW. Dietary treatment did not significantly affect any of the aforementioned parameters except SEW. At Week 27, supplemental carnitine reduced SEW by 3.1 %. In this study, 25 ppm supplemental dietary L-carnitine decreased SEW at 27 wk, but did not impact subsequent egg hatchability and progeny embryogenesis between 25 and 38 weeks of breeder hen age.

**Key Words:** Broiler breeder, Carnitine, Embryogenesis

**305 Chicken sperm motility and metabolism are altered immediately by semen dilution.** H. M. Parker\* and C. D. McDaniel, Mississippi State University, Mississippi State.

The Sperm Quality Index (SQI) is most predictive of rooster semen quality and fertility when semen is diluted no more than 10-fold prior to analysis. The present study was conducted to determine why the SQI was not as predictive of fertility at higher semen dilutions by examining the effects of semen dilution on sperm motility and metabolism. Semen from 15 roosters was collected, pooled and then diluted with 0.85% saline to achieve sperm concentrations from 30 to 1300 million sperm/mL. For each vial of diluted semen, oxygen and ATP content as well as the SQI and sperm viability were measured within one minute of dilution. To examine motility of individual sperm cells the SQI was expressed as SQI/million sperm. Viability was not affected by semen dilution. As expected, the SQI declined logarithmically with increasing semen dilution or decreasing number of live sperm. However, as semen dilution increased, motility of individual sperm cells was accelerated. Also, no free oxygen was present in the neat semen sample, but the partial pressure of oxygen for the fresh diluent was 218 mmHg. Therefore, as semen was diluted, the oxygen present/sperm increased exponentially resulting in a greater rate of sperm motility ( $r^2 = 0.93$ ). Additionally, as semen was diluted, the sperm utilized more oxygen resulting in a positive correlation ( $r = 0.93$ ) of the SQI/sperm with oxygen utilization. Furthermore, at lower dilutions, ATP present/sperm declined with dilution. This decline was most likely due to the sperms dependence on anaerobic metabolism, because of the lack of oxygen, and the slow increase in sperm motility. However, at higher dilutions ATP/sperm increased rapidly with dilution most likely due to greater available oxygen and aerobic metabolism. Apparently, diluting semen samples alters oxygen utilization, ATP metabolism, and therefore sperm motility. These

changes in sperm metabolism and motility are most likely the reason the SQI is not very predictive of fertility at dilutions greater than 10-fold.

**Key Words:** Sperm Quality Index, Semen Dilution, Sperm Metabolism

**306 Expression of the mRNA for zona pellucida proteins 1 and 3 in two genetic lines of turkey hens that differ in fertility.** A. P. Benson<sup>\*1</sup>, A. J. Davis<sup>1</sup>, B. D. Fairchild<sup>1</sup>, and V. L. Christensen<sup>2</sup>, <sup>1</sup>University of Georgia, Athens, <sup>2</sup>North Carolina State University, Raleigh.

The inner perivitelline layer of avian species contains zona pellucida protein-1 (ZP1) and zona pellucida protein-3 (ZP3) and these two proteins may be involved in sperm binding. ZP1 is produced by the liver and transported to the developing follicle, while ZP3 is synthesized and secreted by granulosa cells of the preovulatory follicle. The expression of mRNA for ZP1 and ZP3 was investigated in two lines of turkey hens selected for over 40 generations for either increased egg production (E) or increased body weight (F). Total RNA was extracted from the liver and from 1 cm<sup>2</sup> sections of the granulosa layer around the germinal disc (GD) or a nongerminal disc (NGD) area of the F<sub>1</sub> and F<sub>2</sub> follicles of six, 48 week-old hens from each genetic line. In order to obtain enough RNA for subsequent Northern analysis, the granulosa samples had to be pooled for two birds for each follicle size (n = 3). Northern analysis for ZP1 and ZP3 was performed using chicken cDNA probes. Equality of RNA loading and transfer was verified with a cDNA probe of chicken GAPDH for ZP1 and with a cDNA probe of mouse 18S ribosomal RNA for ZP3. Hepatic expression of the mRNA for ZP1 tended (P = 0.07) to be greater in turkey hens from the E-line than the F-line. The expression of the mRNA for ZP3 was equal between the two genetic lines of turkeys and within each line between the GD and NGD regions of the F<sub>1</sub> and F<sub>2</sub> follicles. The results suggest that the higher rates of fertility previously observed for eggs from the E-line versus the F-line of turkeys may be related to the higher expression of the potential sperm binding protein ZP1.

**Key Words:** Zona Pellucida Proteins, Turkeys, mRNA Expression

**307 Follicular development and expression of the mRNA for the inhibin/activin subunits in two genetic lines of turkey hens that differ in total egg production.** J. B. Hoffman<sup>\*1</sup>, A. P. Benson<sup>1</sup>, A. J. Davis<sup>1</sup>, B. D. Fairchild<sup>1</sup>, and V. L. Christensen<sup>2</sup>, <sup>1</sup>University Of Georgia, Athens, <sup>2</sup>North Carolina State University, Raleigh.

Inhibin and activin appear to play key roles in follicular maturation in avian species. The expression of mRNA for the inhibin/activin subunits and the activin binding protein follistatin was investigated in the developing follicles of two lines of turkey hens selected for over 40 generations for either increased egg production (E) or increased body weight (F). Individual bird weights and follicle weights were obtained from six birds of each genetic line at 46 and 58 weeks of age. Total RNA was extracted from individual granulosa layers of the F<sub>1</sub> through F<sub>4</sub> follicles and from the combined theca and granulosa layers of the small yellow follicles (SYF, 5-10 mm) and large white follicles (LWF, 2-5 mm). Chicken cDNA clones were used in the Northern analysis of the inhibin  $\alpha$ -subunit, inhibin/activin  $\beta_A$ - and  $\beta_B$ -subunits and follistatin. Average body weight was 3 times greater for the F-line birds, however, total follicular weight relative to body size was significantly less for these hens compared to the E-line hens. Although total follicular weight was less in the F-line hens, the preovulatory hierarchy for these birds contained 5-7 more follicles than the hierarchy for the E-line hens. Furthermore, the total relative mass of both the SYF and LWF was the same for both genetic lines of turkeys, however, the number of these follicles was significantly greater for the F-line hens. Thus, the decrease in total relative follicular mass in the F-line hens results from significantly smaller relative to body weight F<sub>1</sub> through F<sub>4</sub> follicles when compared to the E-line. The inhibin/activin  $\beta_B$ -subunit and follistatin were expressed very strongly in the large white follicles. Expression of the  $\beta_A$ -subunit was limited to the hierarchical follicles. The inhibin  $\alpha$ -subunit was expressed in all follicles examined. The lack of a well defined preovulatory follicular size hierarchy in the F-line hens contributes to the decreased production and fertility observed in these birds compared to the E-line hens.

**Key Words:** Inhibin, Activin, Turkey Hens

**308 Progesterone injections induce a polycystic ovarian follicle syndrome (PCOF) in young turkey hens.** W. L. Bacon<sup>\*</sup> and H.-K. Liu, *The Ohio State University, Wooster.*

An arrest in laying associated with a PCOF syndrome has been reported in turkey hens photostimulated with constant light. The ovaries of PCOF hens contained a normal number of hierarchical follicles (7 to 10), and 5 or more larger follicles, some of which were cystic. Comparing PCOF hens to laying hens, the oviduct was of equal weight, plasma concentrations of estradiol-17 $\beta$  (E<sub>2</sub>) were equal, luteinizing hormone (LH) slightly lower, and progesterone (P<sub>4</sub>) several fold higher. We hypothesized that constant lighting overstimulated the ovary, inducing a high level of P<sub>4</sub> secretion which blocked ovulations but not entrance of follicles into the hierarchy, resulting in the PCOF syndrome. Experiments to examine effects of P<sub>4</sub> injections (0.17 to 1.50 mg kg<sup>-1</sup>d<sup>-1</sup> for 7 to 14 d), duration of egg production (6 to 38 wk), and photoperiod (14L:10D or constant light) on egg production, ovarian follicles, and oviduct weight were conducted. In 6 experiments, hens were necropsied immediately after the last P<sub>4</sub> injection. Egg production was decreased by about 50% with injection of 0.17 mg kg<sup>-1</sup>d<sup>-1</sup>, and ceased with injection of 0.33 mg kg<sup>-1</sup>d<sup>-1</sup> or greater. In hens of < 15 week of production ovarian follicle number was slightly increased, oviduct weight was unaffected, and atretic follicle number increased. At 38 wk of production, ovarian follicle number and oviduct weight decreased, and atretic follicle number increased. No hens presented with the PCOF syndrome. Two additional experiments were conducted with young hens, with the hens necropsied 3 wk after last P<sub>4</sub> injections. In these experiments most of the hens given constant lighting and P<sub>4</sub> (0.33 mg kg<sup>-1</sup>d<sup>-1</sup>) presented with the PCOF syndrome at necropsy. The PCOF presenting hens had several fold higher P<sub>4</sub> levels at necropsy, slightly lower levels of LH, and normal levels of E<sub>2</sub> in comparison to laying hens. We concluded that P<sub>4</sub> injection for 12 d of hens that had been photostimulated with constant light and were early in the reproductive period could initiate the development of the PCOF syndrome at necropsy 3 wk later.

**Key Words:** Progesterone, Ovary, Turkey

**309 Programming of photorefractoriness: The turkey breeder hen is not like a tree sparrow.** J. A. Proudman<sup>\*1</sup> and T. D. Siopes<sup>2</sup>, <sup>1</sup>USDA, ARS, Biotechnology & Germplasm Laboratory, Beltsville, MD, <sup>2</sup>Department of Poultry Science, North Carolina State University, Raleigh.

Photostimulation (PS) with a long photoperiod is thought to both initiate egg laying and to program the onset of the photorefractory (PR) response by the presence of thyroid hormone, probably thyroxine (T<sub>4</sub>), in the brain during the early weeks following PS. We have conducted three experiments to estimate when programming of the PR response by long days occurs in turkey hens. In Experiment 1, we tested the hypothesis that hormone levels (T<sub>4</sub>, T<sub>3</sub>, or prolactin) during the first week of lighting may program PR. We retrospectively compared hormone levels at 7, 0, 1, 3, and 7 days from PS of hens that did or did not become PR during 50 wk of lay. Results showed no differences in any hormone considered likely to be involved in programming PR. In Experiments 2 and 3, we attempted to estimate when programming for PR occurs by subjecting hens to long days for differing lengths of time and then returning them to a photoperiod (12L:12D) that is known to support egg production but not induce PR. Each experiment included a long-day control and a control group that would remain photosensitive (constant 12L:12D). All hens were tested for PR by an increase in photoperiod at the end of a lay cycle. Hens that had been programmed for PR should not respond to the increased photoperiod. In Experiment 2, treated hens received 1, 14, or 28 d of 16L:8D followed by 12L:12D until 20 wk of PS. Programming for PR was then assessed by providing 20L:4D for 8 wk. All treatment groups (but not the long day control group) responded to the light change, indicating that programming for PR does not occur within the first 4 wk following PS. In Experiment 3, treated hens received 1, 5, or 9 wk of 18L:6D followed by 12L:12D to 18 wk of PS. We then tested for photosensitivity by restricting light to 8L:16D for 2 wk and then stimulating with 22L:2D for 6 wk. Only 6 of 18 control (18L:6D) hens remained photosensitive at the end of this experiment, while all hens in the 12L:12D control group and all treated hens remained photosensitive. We conclude from these experiments that programming for PR in the commercial turkey hen does not occur within the first 9 wk following photostimulation.

**Key Words:** Photorefractoriness, Turkey, Photoperiod

**310 Photoperiod effects on spontaneous ovarian adenocarcinoma in the domestic turkey breeder hen.** C. B. Moore and T. D. Siopes\*, *Department of Poultry Science, College of Agriculture & Life Sciences, North Carolina State University, Raleigh.*

The effect of photoperiod or melatonin treatments on ovarian adenocarcinoma in turkey breeder hens was investigated. In Experiment 1, photoperiod effects were tested by exposing turkeys with ovarian tumors to 8 wks of short days (8:16LD) followed by a 12 wk period of long days (16:8LD). In Experiment 2, exogenous melatonin was administered to turkeys during long day-induced development of ovarian tumors. In both experiments, the stage of tumor growth was scored weekly on a subjective scale of 0 to 4. It was clear that exposure to short days produced complete regression of tumors, with a mean time to score 0 of 4.4 wks. Following re-exposure to a long photoperiod, all of the same birds showed re-growth of the ovarian tumor with a mean time to first palpable detection of 5.4 wks. When melatonin was administered daily during the long photoperiod (Experiment 2), there was a significant delay in the re-growth of tumors. It was concluded that the growth of solid ovarian tumors in the turkey breeder hen was promoted by long photoperiods and ceased, to the point of remission, on short photoperiods. Thus, ovarian adenocarcinoma in turkeys can be completely manipulated by photoperiod. In addition, treatment with melatonin attenuates tumor growth in the turkey hen. The results suggest that the domestic turkey hen may be a useful *in vivo* model for studying spontaneous ovarian adenocarcinoma.

**Key Words:** Cancer, Photoperiod, Ovary

**311 The distribution and change in the number of gonadotropin-releasing hormone neurons in chicks following an increase in photoperiod plus administration of sulfamethazine.** W. J. Kuenzel\* and C. D. Golden, *University of Arkansas, Fayetteville.*

Gonadotropin-releasing hormone-1 (GnRH-1) neurons are well known to be critical for the regulation of gonadal development due to their stimulation of luteinizing hormone and follicle stimulating hormone release from the adenohypophysis. One consistent finding for GnRH-1 neurons is that their cell number remains relatively stable over the life cycle of a particular avian species. In addition, many studies have suggested that the principle location of GnRH neurons occurs in the medial preoptic hypothalamic region. A study was designed to determine whether significant increases in GnRH-1 neurons could be induced after treatment with a compound, sulfamethazine (SMZ), known to stimulate early sexual maturation. A two-by-two factorial [two diets (SMZ and control) and two photoperiods (shortday, LD8:16 and longday, LD16:8)] experiment was designed to determine whether photoperiod and/or SMZ could alter the number of GnRH neurons in male chicks. A set of sections extending from the septopallio-mesencephalic tract (TSM) to the end of the septal region was completed for each bird (n=5/treatment). Results showed that regarding the distribution of GnRH neurons, a low percentage were actually found to occupy the preoptic, hypothalamic region. In

contrast, the vast majority of the neurons (75%) occurred in the subpallial brain region while the remainder was partitioned among three subgroups found in the diencephalon. No significant changes were noted in total GnRH-1 neurons among the four treatment groups, however, in one of the largest sub-groupings of GnRH neurons, the bed nucleus of the pallial commissure (NCPa), a significantly larger number of neurons were found in long-day SMZ fed birds compared to chicks raised in a short photoperiod (LD8:16) and fed a standard ration. Results suggest the importance of the NCPa, located in the subpallial region, as containing GnRH neurons that appear most responsive to manipulations of photoperiod in birds. Supported in part by NSF Grant # IBN-0315793 to W.J.K.

**Key Words:** GnRH, Subpallium, Reproduction

**312 Changes in eggshell of Japanese quail during embryogenesis.** S. Westmoreland\*<sup>1</sup>, P. Hester<sup>2</sup>, and T. Halupnik<sup>1</sup>, <sup>1</sup>*The University of Texas at Arlington, Arlington,* <sup>2</sup>*Department of Animal Science, Purdue University, West Lafayette, IN.*

The avian eggshell is a source of calcium for the developing embryo. Movement of calcium from the shell to the embryo occurs once the chorioallantois is in place and functional. At the blunt end of the egg the chorioallantois has no contact with the shell due to the airspace and no calcium loss occurs. The purpose of the current study was to investigate changes that occur in shells of fertile Japanese quail eggs during incubation in order to have a baseline study for comparison to eggs that were incubated at the MIR space station. It was hypothesized that when eggs reached the stage at which the chorioallantois was in place, shell samples from the equator region (largest shell diameter) would show signs of calcium loss and reduced shell thickness, while samples from the airspace would not. Fertile Japanese quail eggs were collected from a breeding colony at Purdue University. Incubation of the eggs was interrupted at six different intervals: 3, 7, 10, 12, 14, and 16 days. Eggshells were shipped to The Center for Electron Microscopy at The University of Texas at Arlington for analysis. Shells of three eggs for each incubation date were sampled in two regions, the airspace and equator. From each region five radial samples and one inner shell surface sample were prepared for viewing on the scanning electron microscope. Digital micrographs were taken to document shell changes. Measurements were collected of shell thickness for each radial sample. No changes were observed in shell samples from either airspace or equator in eggs incubated 12 days or less. In eggs incubated 14 or 16 days voids were seen in the mammillary cones of equator samples; no voids were found in airspace samples. For any given day of incubation, the equator region shell samples were consistently and significantly thicker than airspace samples. In eggs that had been incubated 14 and 16 days, the equator samples approached the thickness of the airspace samples. This trend is interpreted to indicate that shells are normally thicker at the equator than the airspace region, but the relative shell thickness changes at day 14 of incubation when the equator region becomes thinner due to calcium loss.

**Key Words:** Japanese Quail, Eggshell, Embryogenesis

## PSA-Processing & Products: Microbiology & Egg Quality

**313 Bacterial load of the crop of turkeys offered a feed supplement during preslaughter feed withdrawal.** B. M. Rathgeber\*<sup>1</sup>, M. E. MacKenzie<sup>1</sup>, and J. L. Maclsaac<sup>2</sup>, <sup>1</sup>*Nova Scotia Agricultural College, Nova Scotia, Canada,* <sup>2</sup>*Atlantic Poultry Research Institute.*

Normally, feed is withdrawn from broiler turkeys several hours prior to the initiation of catching and shipping, allowing contents of the gut to pass through. This reduces the chance of bacterial contamination due to gut breakage during the evisceration process. During the starvation period there is an increased incidence of litter consumption increasing the bacterial load of the upper digestive tract. The objective of this study was to evaluate the use of a highly digestible supplement during preslaughter feed withdrawal as a means to reduce litter consumption and the bacterial load in the crop of turkeys. For each of two trials 480 female turkeys were grown in 8 pens (60/pen) at a density of 0.18m<sup>2</sup> / bird. At 9 weeks of age 80 birds from half of the pens were shipped for processing (20 birds/pen). At 10 weeks of age 40 birds from the other four pens were shipped (10 birds/pen). Commercial feed was withdrawn

6 h prior to catching from each pen. A highly digestible nutritive supplement was offered in half of the pens for 5 h in duration. Water only was provided for the last hour before shipping. All birds were slaughtered 9 h after conventional feed was withdrawn. At 9 weeks, after feather removal the crops of 5 birds/pen were removed, placed in sterile bags and placed on ice. At 10 weeks 3 crops per pen were sampled. The number of aerobic bacteria as well as E. Coli, coliformes and salmonella were determined for each crop sampled. There was no difference between the numbers of bacteria in these groups for crops at 9 weeks old compared to 10 weeks. Salmonella was not found in any of the crops in these trials. The total number of aerobic bacteria in the crop was not influenced by the use of the nutritive supplement. Both E. coli and total coliformes were reduced in crops from birds offered the nutritive supplement prior to slaughter (p<0.01). This reduction was more than one log for each of these two categories. The results of this project indicate that populations of bacteria in crop of commercial turkeys can be re-