24 DNA barcoding and qPCR for identification and enumeration of *Eimeria* species of chickens based on mitochondrial cytochrome c oxidase subunit I sequences. M. E. Ogedengbe*1, A. Naum2, R. Hannen3, and J. R. Barta1, 1Department of Pathobiology, University of Guelph, Guelph, ON, Canada, 2Department of Integrated Biology, University of Guelph, Guelph, ON, Canada.

_Eimeria_ species are responsible for causing coccidiosis, a globally important disease found wherever chickens are reared commercially. Analysis of more than 100 partial and 6 whole mt genome sequences from 7 closely related _Eimeria_ species that can infect chickens have suggested that the mitochondrial cytochrome c oxidase subunit I (mt COI) sequences could be used as species-specific barcode markers. In addition, there appears to be sufficient sequence variation among these species for the development of a quantitative PCR assay for diagnosis and perhaps enumeration of _Eimeria_ species of chicken. We have developed a pair of _Eimeria*-specific primers that amplify a 206 bp fragment of the mt COI gene. This primer pair was successful in amplifying all of the _Eimeria_ species that infect chickens; in addition, the sequence variation within the amplified region was sufficient to differentiate pure lines of parasites solely using melting curve analysis at the end of a qPCR reaction. Using a fixed quantity of parasite DNA per qPCR reaction, mean melting peaks identify single-species isolates of the various _Eimeria_ species of chickens. The detection limit of this qPCR assay is approximately 1–5 oocysts worth of DNA. This is a rapid means of confirming the identity and quantity of parasites in single-species samples; however, mixed parasite samples cannot be analyzed in this manner (although the total number of genome copies of the combined parasite species found in the sample can be approximated using this qPCR method). Sequence variation within the mt COI sequences of _Eimeria_ species infecting chickens is now being exploited to generate a multiplexed, probe-based qPCR assay for the identification and enumeration of up to 4 _Eimeria_ species simultaneously within a single qPCR reaction.

Key Words: coccidiosis, replacement layer pullets, caged-rearing, antimicrobial free, _Eimeria_

25 User-friendly method to enhance live *Eimeria* vaccine efficacy for pullets. K. R. Price*1, M. Petrik2, M. T. Guerin1, L. Newman3, and J. R. Barta1, 1University of Guelph, Guelph, ON, Canada, 2McKinley Hatchery, St. Marys, ON, Canada, 3Merck Animal Health, Summit, NJ.

Consumption of poultry products are on the rise and to meet consumer demand, yet remain profitable, commercial production must be intensive and cost-effective; balancing performance and health with environment and management. Coccidiosis, a disease caused by _Eimeria_ parasites, continues to be an economic burden on the poultry industry. With increased prophylactic drug-use concerns, alternate methods of coccidiosis control have been developed, yet innovation to enhance these techniques has been limited. Live _Eimeria_ vaccines stimulate immunity from the first small dose of vaccinal oocysts and are improved through fecal-oral cycling of the parasites. This immune response exhibits as no pathogenesis with minimal parasite reproduction. Only limited immunity may be acquired without cycling, thus birds risk coccidiosis when challenged. Poultry management can influence _Eimeria_ cycling. Pullets are often reared in tiered cages with mesh floors to allow feces to fall away. Some producers have used paper to cover the mesh floor for the first 10 d; but coccidiosis occurred in the layer barn (suggests inadequate cycling). To improve cycling, we tested durable fiber trays (lasting ~5 weeks) to cover part of the cage floor. Birds were gavaged orally with live vaccine and reared with 0, 20, 40 or 60% tray coverage from hatch to 6 weeks, then challenged with _Eimeria_ species. A significant difference was found between the oocyst output of birds housed with or without coverage. Pullets on 40% coverage had significantly reduced output compared with the other birds (>99% lower than birds with no coverage). No difference was found between 20 and 60% coverage. At 6 d post challenge infection, lesion scores mirrored oocyst output results. Increased access to oocysts using 40% cage floor coverage with fiber trays over 5 weeks allowed for enhanced vaccine efficacy in live _Eimeria_ vaccinated caged pullets.

Key Words: coccidiosis, vaccination, live vaccine, pullets, _Eimeria*

Previous studies have shown that egg shell temperatures (EST) exceeding 102 F can cause decreased hatchability and post-hatch growth performance. A project to determine the optimum temperature for single and multi-stage incubation was conducted to minimize embryonic and grow-out mortality, and improve growth performance and carcass yield. Three phases were investigated: Pre-incubation (2 h (P) and 12 h (S) to incubation temperature), Incubation (EST from 7 to 18 d at 100 F (7C) or 102 F (7H)), and Hatcher (EST from 18 to 21 d at 100 F (18C) or 102 F (18H)). Twelve cases of Ross × Cobb eggs were randomized into these 6 incubation patterns by using 3 different incubators (Chickmaster – single stage). After hatch, embryonic mortality (EM) patterns were assessed by evaluating hatch residue. Fifty chicks per treatment were evaluated for yolk-sac mass (YSM) and heart size (HS), and 864 chicks were randomized into 48 pens and reared to 35 d of age (6 pens per treatment). Feed conversion, growth, EM and ascesites incidence was analyzed by incubation treatment. Carcass yield was determined for 20 broilers/treatment (10 males and females). Late dead EM was 1.5% lower when eggs took 12 h. to obtain an EST of 100 F (S) compared with 2 h. treatment (P). EST was more critical for late dead EM (2%) and hatchability (4%) for 7C eggs than for 7H eggs. The 7C treatment resulted in significantly greater (SG) YSM (4.1 vs. 3.8 g), HS (0.3 vs. 0.2 g) and YSM and HS as a % of chick weight than 7H chicks. Carcass weight of 7C birds was SG than 7H (1559 vs. 1479 g). Hatcher EST showed that 18C chicks had SG YSM (4.2 vs. 3.7 g), HS (0.29 vs. 0.27 g), and YSM and HS as a % of chick weight than 18H chicks. BW of 18C chicks was SG at hatch (39.8 vs. 38.2 g) and 21 d (0.864 vs. 0.850 kg). The combination of S-pre-incubation warming, 7C incubation treatment of 100F EST and 18C hatcher treatment of 100F EST appears to give the best results for reducing EM and promoting bird growth.

Key Words: egg shell temperature, incubation, pre-heating

28 Improving broiler live performance and carcass yield. 1. Thermal treatments before and up to 5 days of incubation improve performance of Cobb 500 broilers to 35 days of age. Y. Piestun1, S. Druyan1, J. Brake2, and S. Yahav*1, 1Institute of Animal Science, ARO the Volcani Center, Bet Dagan, Israel, 2Department of Poultry Science, North Carolina State University, Raleigh.

Embryo development during pre-incubation storage and incubation largely depends upon temperature. The potential beneficial impact of manipulating temperature in a programmed manner during the pre-incubation and incubation periods of broiler chickens has been realized during the past several decades. Furthermore, convincing evidence that temperature could influence the sex ratio of avian offspring has recently become available. This study was designed to elucidate the effect of temperature that was elevated above standard conditions before and/or during the sex determination period of incubation on hatchability, sex ratio, growth and development post-hatching, and secondary sexual phenotypic characteristics. Two experiments were conducted using Cobb 500 broiler hatching eggs that had been stored for 4 and 9 d, respectively. Four treatments were applied: Control maintained at standard conditions of no pre-heating and 37.5 C throughout incubation; pre-heating (Pre) for 12 h before incubation; heating the embryos to 38.5 C between E0 and E5 (M) of incubation; and a combination of both (Pre+M). All 3 thermal treatments increased early embryonic deaths, but significantly improved hatchability. The point of 50% hatchability was achieved earlier in the thermal treatments. The BW of males and females at 35 d of age in both experiments was greater from the thermal treatments. This was coincident with increased relative breast muscle weight. The secondary sexual phenotypic characteristics of comb and wattles were also affected by the thermal treatments, being heavier in most cases. The tests were larger in thermal treated males, which may be associated with increased plasma testosterone concentration in both genders and experiments. It was concluded that thermal treatments pre-incubation and/or during the sex determination period of incubation had, in general, positive effects on hatchability, growth, carcass yield, and secondary sexual characteristics of broilers.

Key Words: broilers, embryo, pre-heating, sex determination, broiler live performance

29 Improving broiler live performance and carcass yield. 2. Thermal treatments before and up to 5 days of incubation after development of Ross 708 embryos and increase hatched chick length. Y. M. Lin*1, S. Yahav1, and J. Brake1, 1Department of Poultry Science, North Carolina State University, Raleigh, 2Institute of Animal Science, ARO the Volcani Center, Bet Dagan, Israel.

Elevated temperature during the pre-incubation period and up to E5 of incubation has been shown to improve broiler live performance and carcass yield. This study delineated the changed observed at E15 of incubation and in hatched chicks in a 2 × 2 factorial design involving pre-incubation temperatures of 23.9C and 29.4C followed by incubation temperatures of 37.5C and 38.1C to E5 of incubation. Incubation temperature was thereafter 37.5C or slightly reduced beyond E12. The relative humidity was maintained at 53% at all times during incubation. From a 41-wk-old Ross 708 breeder flock, 90 weight-matched pairs of eggs were produced by individually weighing a large number of eggs laid the same day. These eggs were then subjected to the above detailed treatments in Natureform I-14 incubators. A total of 2880 eggs were set to balance the air flow in the machines. Eggs were weighed and embryos necropsied at E15 to determine egg weight loss, embryo weight, yolk sac weight, embryo length. Embryo weight and length were increased by 29.4C and 38.1C temperatures while only the 38.1C temperature increased egg weight loss. At hatching, chick BW was decreased by the 29.4C and 38.1C temperatures, probably due to slightly earlier hatching, while yolk sac weight was decreased and chick length increased by the 38.1C temperature. The longest chicks were produced by the 29.4C and 38.1C temperature combination. Greater chick length has been associated with increased carcass yield, which may explain increased carcass yield due to these thermal treatments.

Key Words: broilers, embryo, pre-heating, sex determination, broiler live performance

30 Improving broiler live performance and carcass yield. 3. Thermal treatments before and up to 5 days of incubation affect Ross 708 carcass yield at 36 and 50 days of age. Y. M. Lin*1, S. Yahav2, and J. Brake1, 1Department of Poultry Science, North Carolina State University, Raleigh, 2Institute of Animal Science, ARO the Volcani Center, Bet Dagan, Israel.

Elevated temperatures during pre-incubation and up to E5 of incubation have improved BW and breast muscle of Cobb 500 broilers at 35 d of
31 Improving broiler live performance and carcass yield. 4.
Thermal treatments from 7 to 16 days of incubation improve performance of Cobb 500 broilers to 70 days of age. Y. Piestun1, S. Druyan1, J. Brake2, and S. Yahav3, 1Department of Poultry Science, North Carolina State University, Raleigh, 2University of Georgia, Athens, 3Merial Select, Gainesville, USA.

Genetic selection of broilers for rapid growth rate has coincided with inferior development of visceral systems, which has contributed to difficulties coping with environmental stressors, especially at higher BW. In many countries the average marketing age is 5 to 6 wk, but the US, French, and other markets demand heavier broilers (greater than 4 kg). In previous studies we had shown that elevating incubation temperature by 1.7 C for 12 h/d from E7 to E16 improved post-hatching thermotolerance as well as feed conversion (FCR) and carcass yield at 35 d of age. The objective of the present research was to elucidate the effect of a similar elevation of incubation temperature on BW, FCR, body temperature (Tb) and carcass yield of broilers raised to 70 d of age. Two treatments were applied to Cobb 500 hatching eggs: Control of 37.5C; and thermal manipulation (TM) where incubation temperature was elevated to 39.5C from E7 to E16 for 12 h/d. After hatching, males and females were reared to 70 d of age while monitoring BW, feed intake, FCR, and Tb. Relative breast muscle and abdominal fat pad were then measured on 160 birds. TM did not affect hatchability or male BW, but female BW was reduced at hatching. This trend was maintained to 70 d of age. Broiler Tb was significantly reduced throughout the growth period by TM, which was evidence of a reduced metabolic rate that was responsible for an improved FCR of males and females of 8 and 10 points, respectively, and improved relative breast muscle weight by 1.0 and 0.7%, respectively. A reduced abdominal fat pad was also observed in females. It can be concluded that TM during embryogenesis had a long-lasting effect on performance of broiler chickens.

Key Words: broilers, embryo, thermal manipulation, broiler live performance, breast muscle

32 Improving broiler live performance and carcass yield. 5.
Beneficial effect of thermal manipulations before and up to 5 days of incubation on Ross 708 carcass yield at 36 and 50 d of age may be modified by turning frequency due to altered vascular development and weight loss during incubation. J. Brake*, Y. M. Lin, and S. Yahav, 1Department of Poultry Science, North Carolina State University, Raleigh, 2Institute of Animal Science, ARO the Volcani Center, Bet Dagan, Israel.

Elevated temperatures during pre-incubation and up to E5 of incubation has improved breast muscle yield of broilers. This study investigated the effects of early thermal manipulation (TM) in combination with turning frequencies of 24 or 96 times (96X) daily on the carcass yield of Ross 708 broilers at 36 and 50 d of age. A total of 2880 broiler hatching eggs from 41-wk-old breeders were subjected to a 2 x 2 factorial design involving pre-incubation temperatures of 37.5C and 38.1C to E5 of incubation. Incubation temperature was thereafter 37.5C or slightly reduced beyond E12. The relative humidity was maintained at 53% at all times during incubation. The chicks were placed in a 64-pen facility with 12–18 birds per pen as determined by hatchability and sex ratio. At 36 and 50 d of age 2 broilers from each replicate pen that were within 250 g of the average male and female broiler BW at each respective age were selected for processing and carcass deboning. At 36 d of age the weight of the Pectoralis minor of the male was increased by the 38.1C temperature while at 50 d of age the weight of the Pectoralis minor of the female was increased and the Pectoralis major weight of the male was increased by the 38.1C temperature. The experimental design of this experiment differed from previous experiments in that there was no treatment that involved moving eggs directly from an egg cooler to the incubator without going through a pre-incubation period. This was concluded to be the reason for the less evident, but still apparent, effects of thermal treatments on breast meat yield.

Key Words: broilers, embryo, pre-heating, sex determination, broiler live performance

33 Effects of breeder feed restriction programs and temperature incubation profiles on progeny bone development. E. O. Oviedo-Rondón*, M. J. Wineland, M. J. Da Costa, K. Claassen, J. L. Wilson, and E. R. Montiel, 1North Carolina State University, Raleigh, 2University of Georgia, Athens, 3Merial Select, Gainesville, GA.

Breeder feed restriction programs influence lipid metabolism with carryover effects on progeny. Incubation temperature profiles (TEMP) have been reported to affect broiler bone development. Two experiments were conducted to evaluate the carryover effects of skip a day (SAD) and every day feed (EDF) restriction programs and 2 incubation TEM profiles on progeny bone development. Eggs of Cobb 500 fast feathering breeders subjected to either SAD or EDF programs during rearing, were collected at 54 and 60 wk of age. Eggs from each treatment were incubated following 2 TEM profiles to either maintain eggshell TEM at 38.1°c (standard) or have eggshell TEM similar to the ones observed in multistage machines. This second profile had low (36.9°C) eggshell TEM for the first 3 d, and standard TEM until the last 3 d when eggs...
endured elevated (38.9°C) eggshell TEM. In both experiments, 15 chicks per treatment combination were sacrificed at hatch. Relative weights (RW), length and relative asymmetry (RA) of leg bones were calculated. In experiment 2, 72 chicks per treatment were identified at hatch and placed in 12 battery cages with 6 chicks per cage. Bone parameters were evaluated on one chick per cage at 7 and 21 d of age. Data were analyzed as a 2x2 factorial design with breeder feed restriction program and TEM as main factors. No significant interaction effects were observed at hatch in both experiments. Incubation TEM affected RW of tibias and metatarsus length in both experiments, and RA of all bones in experiment 2. In experiment 2, interaction effects of treatments were observed at 7 and 21 d on femur, tibia and metatarsus length and on femur and metatarsus RW only at 21 d. Broiler progeny bone development from SAD breeders were generally affected by incubation TEM profiles, while progeny of EDF breeders were not. Breeder feed restriction effects were only observed on RA of bones, but results were not consistent. It was concluded that deviations from optimum incubation may affect bone development and this negative effect is more evident in progeny of breeders fed using the SAD program.

**Key Words:** breeder restriction, incubation profiles, breeder, bone development


Feed restriction programs affect breeder metabolism with effects on progeny development. Incubation temperature profiles (TEM) have been reported to affect progeny gastrointestinal (GTI) development. Two experiments were conducted to evaluate the carryover effects of skip a day (SAD) and every day feeding (EDF) restriction programs and 2 incubation TEM on progeny GTI development. Eggs of Cobb 500 fast feathering breeders subjected to either SAD or EDF programs during rearing, were collected at 54 and 60 wk of age. Eggs from each treatment were incubated following 2 TEM profiles to either maintain eggshell TEM at 38.1°C (standard) or have eggshell TEM similar to the ones observed in multistage machines. This second profile had low (36.9°C) eggshell TEM for the first 3 d, and standard TEM until the last 3 d when eggs endured elevated (38.9°C) eggshell TEM. In both experiments, 15 chicks per treatment combination were sacrificed at hatch. Relative weights (RW) of liver, gizzard+proventriculus (GP), and intestines were calculated and intestinal length (IL) was measured. In experiment 2, 72 chicks per treatment were identified at hatch and placed in 12 battery cages with 6 chicks per cage. RW of similar GTI parts were recorded at 7 and 21 d of age and gizzard pH was measured. Data were analyzed as a 2x2 factorial design with breeder feed restriction program and TEM as main factors. No significant interaction effects were observed at hatch in both experiments. In experiment 2, no effects of treatments were detected on IL or on gizzard pH. Incubation TEM affected the development of all GTI parts evaluated at hatch in both experiments and IL in experiment 1 at hatch. Chicks incubated with standard TEM had bigger GTI. In experiment 2, similar effects of TEM were observed at 7 d, and at 21 d only RW of intestines were affected by TEM. It was concluded that these breeder feed restriction programs did not have consistent carryover effects on progeny GTI development produced at 54 and 60 wk of age. It was corroborated that incubation TEM profiles affected broiler GTI development.

**Key Words:** breeder restriction, incubation profiles, broiler, gastrointestinal development

35  **Effect of hen age and late incubation temperature on embryo development. 1. Embryo metabolism.** J. A. Hamidu,* C. A. Torres, M. L. Johnson, and D. R. Korver, University of Alberta, Edmonton, AB, Canada.

The impact of maternal flock age and incubation (machine temperature; MT) on embryo metabolism was studied to understand the biological reasons for late incubation overheating and mortality. Fresh broiler hatching eggs (n = 88; average weight eggs within each parent flock) were obtained simultaneously from 3 parent flock ages: Young (Y): 26 to 34 wk, Peak (P): 35 to 45 wk, Old (O): 46 to 54 wk of age), stored for 2 to 4 d at 18°C and 73% RH, then incubated for 14 d at 37.5°C and 56% RH. At 15 d of incubation (E15), 12 fertile eggs per flock age were placed in individual metabolic chambers at one of 4 incubation temperatures (36.0, 36.5, 37.0, or 37.5°C) until E21.5. O2 consumption, CO2 production and eggshell temperatures (EST) were recorded 6 times/d and embryonic heat production (EHP) determined as an indication of embryonic metabolism. Data were analyzed by Proc Mixed (SAS Inc.) with initial egg weight as a covariate. Differences were considered significant at P < 0.05. Relationships among incubation temperature, parent flock age, EHP and EST were determined by regression or correlation. Embryos from O flocks consumed less O2 at E20 and produced less CO2 at E15 and compared with Y and P embryos. The O2 consumption at E16 and E17 was lower for 36.0°C than other treatments; at E19 and E20, 37.0°C resulted in the greatest O2 consumption. The CO2 production at 36.5°C and 37.0°C was greater than at other temperatures, but only at E20. Incubation at 37.0°C increased EHP from E15 d to E20 relative to 36.0°C. The EST was highest at 37.0°C compared with 36.0, 36.5 and 37.5°C from E15 to E20. The EST was highest at 37.5°C compared with 36.0 and 36.5°C at E21. There was a linear relationship (R2 = 0.46) between incubation temperature and mean EST, whereas mean EHP had a significant but small relationship (R = 0.33) to EST. The MT of 37.0°C resulted in higher embryonic metabolism leading to higher EHP and EST which can increase the risk of overheated embryos and increase late incubation embryo mortality.

**Key Words:** breeder flock age, incubation temperature, embryo metabolism, heat production, eggshell temperature

36  **Effect of hen age and late incubation temperature on embryo development. 2. Chick quality.** J. A. Hamidu,* C. A. Torres, M. L. Johnson, and D. R. Korver, University of Alberta, Edmonton, AB, Canada.

The impact of maternal broiler breeder (Ross 708) flock age and incubation (machine temperature; MT) on embryo development and early chick quality was determined. Fresh broiler hatching eggs (n = 88, of average weight within each parent flock) were obtained from each of 3 parent flock ages: Young (Y), 26 to 34 wk; Peak (P), 35 to 45 wk; Old (O): 46 to 54 wk of age), stored for 2 to 4 d at 18°C and 73% RH, then incubated for 14 d at 37.5°C and 56% RH. At 15 d of embryo development (E15), 12 fertile eggs per flock age were placed in individual metabolic...
chambers at one of 4 incubation temperatures (36.0, 36.5, 37.0, or 37.5°C) until E21.5. After 452 h of incubation, eggs were checked every 6 h to record the approximate time to external pipping and hatch. After 518 h of incubation, chicks were removed, weighed, length determined and dissected. The yolk sac was removed and the yolk-free body mass (YFBM) determined. Data were analyzed by Proc Mixed (SAS Inc.) with initial egg weight as a covariate. Differences were considered significant at $P < 0.05$. Relationships among incubation temperature, breeder age, chick weight and length were determined by regression. Breeder age and MT did not affect chick weight, wet or dry yolk sac weight or wet YFBM; chick weight was more related with maternal flock age ($R^2 = 0.45$) than MT ($R^2 = 0.03$). The Y flock had longer chicks than M and O flocks and chicks from 37.0°C were longer than from 36.0, 36.5 or 37.5°C. The chicks from 37.0°C pipped and hatched in a shorter time compared with 36.0°C and 37.5°C. Although chicks from 37.0°C MT may be of higher quality (longer length), the early hatch would result in longer residence time in hatchers and may reduce overall chick quality at placement due to the potential for dehydration. Chick length was longer in Y eggs incubated at 37.0°C MT compared with all other interactions between breeder age and MT except Y*36.0°C, Y*36.5°C and O*37.0°C. Y chicks may be less affected by lower and higher MT than M and O chicks.

**Key Words:** breeder flock age, incubation temperature, embryo metabolism, chick quality, chick size


The broiler chick hatches with a functional, but immature innate immune system. We have previously shown that early chick innate response decreased as hens aged. However, it was unclear whether hen age and incubator temperature interacted to cause this. Eggs (n = 88 from broiler breeder hens of 3 different ages: 26 to 34 wk; 35 to 45 wk; and 46 to 54 wk (young (Y), peak (P) and old (O), respectively) were stored for 2 to 4 d at 18°C and 73% RH, then incubated for 14 d at 37.5°C and 56% RH. At 15 d of embryo development (E15), 12 fertile eggs per flock age were individually placed in metabolic chambers and incubated at 36.0, 36.5, 37.0, or 37.5°C. Chick innate immunity indices (ex vivo *E. coli* bacterial-cidality (killing) and phagocytosis) on d of hatch were measured. Phagocytosis was a measure of the number of active phagocytes (PA) and the phagocytic capacity (PC) was a measure of the number of *E. coli* each cell phagocytized as measured by fluorescence. Pearson correlation analysis (SAS, Inc.) was used to determine relationships between immune indices and cumulative embryonic heat production (eEHP, in mW). eEHP was determined by summing the average heat production per day from E15 to E20. Differences due to hen age and incubation temperature were determined using PDIFF of LSMeans and considered significant at $P < 0.05$. *E. coli* killing was greatest in Y chicks (60.8%) compared with P (35.4%) and O (30.9%) chicks and was greater when incubator temperature was 36.5°C (72.1%) than 36.0°C (23.0%). PA was weakly correlated with eEHP ($R = 0.18$); neither PA nor PC were affected by hen age or incubation temperature. PC in the 36.5°C group was lower than other temperature groups. Efficacy of the chick innate immune function decreased as hens aged. Optimal incubator temperature for overall innate immune function was 36.5°C; however, PC was lowest at this temperature suggesting other immune mechanisms of *E. coli* killing may be more prominent at hatch.

**Key Words:** incubation temperature, broiler breeder age, embryo metabolism, phagocytosis, innate immunity


We have previously shown that, regardless of hen age, newly-hatched chicks from broiler hatching eggs incubated at 37.0°C had wider tibias but reduced mineralization relative to those incubated at 36.0°C or 36.5°C. Additionally, eggs incubated at 37.0°C produced embryos with higher embryonic heat production (EHP) at embryo age 19 (E19). The influence of broiler breeder (Ross 708) age (Young (Y): 26 to 34 wk, Peak (P): 35 to 45 wk, and Old (O): 46 to 54 wk) and incubation temperature (36.0, 36.5, 37 or 37.5°C) from E15 to E21.5 on embryo metabolism and tibia development at hatch were investigated. Additionally, the relationships among embryonic metabolism (i.e., egg shell temperature (EST) and EHP) on bone development were studied. Correlations between average EST (from E15 to E19), cumulative EHP from E15 to E19 (cEHP; determined by summing the average heat production (mW) per day from E15 to E20) and tibia thickness, length, % of calcified tissue (mineralization) and breaking strength at hatch were determined using Pearson correlation analyses of SAS. Significance level was set at $P < 0.05$. Tibia thickness was moderately and positively correlated with cEHP and EST ($R = 0.49$ and 0.45, respectively). Tibia length was also correlated with cEHP ($R = 0.45$). Even though cEHP was moderately associated with bone size, it did not influence tibia mineralization and strength at hatch. Additionally, EST correlated with bone length ($R = 0.26$) but not to the same extent as cEHP possibly due to the negative correlation between EST and mineralization ($R = −0.28$). Embryos with higher metabolic rate (heat production) between E15 to E19 might accumulate more heat relative to embryos with lower metabolism and reduce bone size; Additionally, increased EST may result in heat stressed embryos which in turn could decrease bone mineralization. Overall, high embryonic metabolism (cEHP and EST) increased bone growth without compromising its mechanical strength at hatch.

**Key Words:** breeder flock age, incubation temperature, embryo metabolism, heat production, bone development