512 Effects of dietary calcium formate inclusion on broiler growth performance, bone ash, and tibia breaking strength. S. Pohl*, D. Caldwell, J. Lee, J. Coppedge, K. Stringfellow, S. Dunn-Horrocks, K. Jessen, and M. Farnell, *Texas A&M University*, *College Station*.

Calcium formate (CaFo; Rovelan–Lanxess Corporation, Germany) is a commonly used source of calcium in poultry and swine feed in the EU. Due to increased cost, US interest in alternate feedstuffs has grown. The objective of this study was to evaluate CaFo in broiler diets as an alternate calcium source during a 49d pen trial. A total of 2,160 male Cobb 500 broilers were randomized and placed in 40 floor pens with 50% used litter and 50% fresh pine shavings. Four experimental groups (0.0, 0.5, 1.0, and 1.5% CaFo) were evaluated with 10 replicate pens per treatment. Bulk pen weights were determined at the conclusion of each feeding phase (d15, 28, 42, and 49). Additionally, at d15, 28, and 42, one bird was removed from each pen and tibia removed for determination of bone strength and ash. At termination (d49) an additional 3 birds were removed from each pen for tibia sampling. Parameters evaluated for each phase included average BW, FCR, bone strength, bone ash content, and mortality. With regard to BW, significant differences ($P \leq 0.05$) were seen at d15 with 1.0% CaFo showing decreased BW. At d28 the 0.5% CaFo diet showed increased BW with respect to the 1.0 and 1.5% CaFo diets. At d42 and 49 all diets yielded statistically equivalent BW. For the duration of the trial, no differences were observed with regard to FCR among diets. No significant differences were seen among treatment groups with regard to mortality, tibia weight, or bone ash percentage. With respect to tibia breaking strength, a significant increase in breaking force was observed in the 1.0% CaFo treatment group in comparison to the control and 1.5% CaFo groups. These data suggest that CaFo may be used as a calcium alternative in broiler diets and may potentially increase bone strength in larger broilers when included at the appropriate concentration.

Key Words: broiler, calcium formate, bone ash

513 Broiler breeder age and dietary Cu, Zn and Mn source affect chick bone development at hatch. C. A. Torres* and D. R. Korver, *University of Alberta*, *Edmonton, AB Canada*.

Organic sources of dietary trace minerals (OTM) can have higher bioavailability than inorganic sources (ITM). OTM in the hen's diet might positively impact bone development in the embryo as Cu, Zn and Mn are involved in bone development. We investigated the effects of maternal dietary Cu, Zn and Mn source and level on chick bone traits at hatch from early (EEP; 33 wk), mid (MEP; 46 wk) and late (LEP; 60 wk) hen age. Broiler breeders (n = 18/diet), were housed in individual cages, and fed a basal ration low in Cu, Zn and Mn. Trace minerals were added as: 1) Control: ITM; mineral sulfates at industrial levels (100 ppm Zn, 120 Mn, 10 Cu); 2) OTM: Zn, Mn and Cu chelated by 2-hydroxy-4-(methylthio) butanoic acid (HMTBA) at NRC (1994) levels (50 ppm Zn, 60 Mn, 10 Cu); 3) OTM+ITM: Trt 1 plus an additional 40 ppm Zn, 40 Mn and 20 Cu as OTM; 4) High ITM: Trt 1 plus 40 ppm Zn, 40 Mn and 20 Cu as ITM. Weekly egg production, and chick bone weight, thickness and length data were analyzed using repeated measures analysis (PROC MIXED). Total and settable eggs, egg weight and hatchability were analyzed as a 1-way ANOVA (PROC MIXED). Significance was set at $P \leq 0.05$. Total and settable egg production, egg weight and hatchability to 60 wk of hen age were not affected by diet. Femur weight (with chick weight as a covariate) decreased 12% from EEP to LEP. At EEP, chicks from the OTM hens had femurs and tibias that were thicker than chicks from Control (both by 5%) and High ITM (both by 6%) hens. At MEP, chicks from the OTM and High ITM hens had thicker femurs (by 4 and 7%, respectively) than chicks from the OTM+ITM group; none of the treatment chicks were different from the Control chicks. At LEP there was no diet effect on femur and tibia thickness. Dietary TM affected chick bone thickness at early and mid hatches even though bone development appeared to decrease with hen age. Despite a lower level of supplemental TM, tibia development at hatch was increased in the OTM group relative to all other treatments at EEP; OTM resulted in greater femur development relative to the ITM and High ITM at EEP, and relative to OTM + ITM at MEP.

Key Words: broiler breeder age, organic trace mineral, chick bone development

514 Use of the broiler (*Gallus gallus*) as an in vivo screening tool for Fe bioavailability in maize-based diets. E. Tako*[1], M. Lung'aho[1], L. V. Kochian[2], O. A. Hoekenga[2], and R. P. Glahn[2], [1]*Cornell University*, *Ithaca, NY,* [2]*Robert W. Holley Center for Agriculture and Health*, *Ithaca, NY*.

Iron biofortification of staple food crops such as maize (*Zea mays*), is a strategy that alleviates Fe deficiency. By using in vitro cell culture model, 2 maize varieties were developed for high and low Fe bioavailability. In vitro observations should be tested in animals before human efficacy studies. Therefore, the maize varieties were tested for Fe bioavailability by using the broiler chicken as a model. Diets were made with 75% w/w maize of either the low (Low) or high (High) Fe bioavailability maize; Fe content did not differ between varieties (both 24 µg/g). In vitro analysis showed lower cellular ferritin formation (ie. Fe uptake, $P \leq 0.05$) in cells exposed to the Low (20 ng/mg) vs. High (37 ng/mg) diets. One-week-old broiler chicks (n = 6) were fed the maize based diets for 4 weeks. Hemoglobin (Hb), body weight, feed consumption, liver ferritin and gene expression were measured. Duodenal DMT1, Dcytb and ferroportin were higher ($P < 0.05$) in the Low group vs. the High group, indicating a response to lower dietary Fe availability. Hb concentrations, hemoglobin maintenance efficiency, Hb-Fe and liver ferritin were higher in the High group vs. the Low group ($P \leq 0.05$), indicating greater Fe absorption from the High diet and improved Fe status. We conclude that the in vivo results support the in vitro observations, i.e., the High variety contains more bioavailable Fe than the Low and that maize shows promise for Fe biofortification. In addition, the results indicate that, the broiler model can serve as an intermediate screening tool for Fe bioavailability before human efficacy trials.

Key Words: broiler, bioavailability, maize

515 Relationship between expression of sodium-dependent phosphate transporter type II-b gene and phosphorus utilization in broilers. O. A. Olukosi*, S. A. Adedokun, K. M. Ajuwon, and O. Adeola, *Purdue University*, *West Lafayette, IN*.

Broiler chicks at 21 d old were used to study the relationship between the level of expression of sodium-dependent phosphate transporter type IIb gene (NaPi-IIb) in 3 sections of the small intestine (duodenum, jejunum or ileum) and P utilization. Birds were allocated at 1-d old to 4 treatments in a randomized complete block design, each treatment had 7 replicate cages with 8 birds per replicate cage. Corn-soybean meal diets formulated to meet all nutrients requirements except for P were fed throughout the study. The ratio of Ca to total P was kept constant in all

diets. Non-phytate P levels in the diets were 2.5, 3.5, 4.5 and 5.5 g/kg. Twenty-eight birds, one bird from each cage, with weight closest to the average weight of the cage, were killed at d 21. Mucosa scraping was collected from the duodenum, jejunum and ileum of the selected birds and the expression level of NaPi-IIb relative to GAPDH was determined by RT-PCR. Ileal digesta was collected on d 21 from the remaining birds in the cage. Expression level of NaPi-IIb was negatively correlated with P level and total P intake but positively correlated with P digestibility ($P < 0.05$) at the jejunum. Correlation of expression level of NaPi-IIb at duodenum and ileum were mostly opposite to that at jejunum. Expression level of NaPi-IIb was similar in duodenum and jejunum but lowest ($P < 0.05$) at the ileum. Dietary P level had no influence on NaPi-IIb expression at any section of the small intestine. Ileal P digestibility was not affected by P supply but both total and digestible P intake were increased ($P < 0.01$) with increasing level of P in the diets. The data indicate that all the sections of the small intestine contribute to prececal P disappearance but duodenum and jejunum contribute the most. In addition, the data indicate that manipulation of NaPi-IIb expression level especially at the jejunum will strongly influence P utilization and excretion in broilers.

Key Words: broilers, phosphate transporter, small intestine

516 Effects of HMTBA chelated zinc, manganese and copper on performance, mineral status and immunity of broilers. Y. Ruangpanit*, S. Attamangkune, S. Rattanatubtimthong, and C. Khomkamon, *Kasetsart University*, *Nakhon-Pathom, Thailand*.

Two studies were conducted to investigate the effects of HMTBA chelated Zn, Mn and Cu on performance, mineral status and immunity of broilers. In Exp. 1, the effects of HMTBA Zn, Mn and Cu on performance and mineral status of broiler were evaluated. Two thousand, day-old, Ross 308 were divided into 2 dietary treatments including, 1) Corn-soy basal diet containing inorganic Zn, Mn and Cu at the recommended levels of the Thai standards (ITM) and 2) Corn-soy basal diet containing HMTBA Zn, Mn and Cu (Mintrex) at 25% of the inorganic levels used in ITM diet (CTM). Both diets were calculated to be isonitrogenous and isocaloric and were offered in pellet form. Each treatment consisted of 20 replications with 50 broilers per replication (25 males and 25 females). All birds were raised in environmental controlled house for 42 d. At the end of the experiment, carcass trail was evaluated. Liver and bone were collected for mineral analysis. Birds received CTM diet had significantly lower feed intake during 1–17 and18–35 d, and lower body weight gain during 1-17 d ($P < 0.05$). However, no significant difference in overall broiler performance and carcass trait were observed. The Zn, Mn and Cu concentrations in serum at d 20 were similar, but serum Zn and Cu concentrations of birds fed CTM diet were lower at d 42 ($P < 0.05$). Liver Zn, Mn and Cu concentrations and Zn and Cu concentrations in bone were not significant different. However, birds received CTM diet had lower bone Mn concentrations ($P < 0.05$). In Exp. 2, the effects of HMTBA Zn, Mn and Cu on immunity, oxidative stress status and mineral excretion were evaluated. One hundred and twenty, 12 d old, male Ross 308 were assigned into 2 dietary treatments as in Exp. 1. Each treatment consisted of 6 replications with 10 birds per replication. All birds were raised in metabolic cages. Birds fed CTM diet had higher secondary IgG responses against 7% SRBC antigen ($P < 0.05$). Both ITM and CTM birds had similar serum MDA concentration. Expectedly, broiler fed CTM diet had lower Zn, Mn and Cu excretion ($P < 0.05$). From this study, it can be concluded that chelated trace minerals have higher bioavailability and lower levels can be fed in the diet.

Key Words: broiler, organic mineral, mineral excretion

517 Effect of organic zinc supplementation on growth performance and carcass quality of broilers. H. M. Salim*, H. R. Lee, C. Jo, S. K. Lee, and B. D. Lee, *Chungnam National University*, *Yuseong, Daejeon, South Korea*.

The effect of supplementing diets with organic zinc on growth performance and carcass quality of female broiler chickens was investigated. A total of 3,200 1-d-old female broiler chicks were randomly assigned to 4 floor pens, 800 birds per pen, with 4 replicates (200 birds/replicate). A corn-wheat-soybean meal basal diet (Control) was formulated, and 20 ppm organic zinc (20 OZ), 40 ppm organic zinc (40 OZ), and 80 ppm organic zinc (80 OZ) were added to the basal diet to form 4 dietary treatments. During the 5-wk experimental period, feed and water were provided ad libitum. Body weight, feed intake, feed conversion and mortality were measured. At the end of the feeding trial, 2 birds per replicate pen were selected according to average body weight, slaughtered, defeathered and carcass evaluation was performed. For histology analysis, about 1 cm2 of skin samples from the thigh and back region of each bird were collected, embedded, sectioned, stained, and thickness of skin layers were examined under light microscope. Results showed no significant difference between the treatments in growth performance. A significant increase ($P < 0.05$) of thigh skin epidermis and dermis thickness were shown in the organic zinc supplementation groups; however, no effect of the zinc on the thickness of back skin epidermis and dermis was found. Collagen content in breast and thigh muscles was not influenced by organic zinc supplementation but a significant increase of collagen content was found in the back and thigh skin ($P < 0.05$). This increase of collagen content was significantly higher in the back and thigh skin of OZ 80 compare with OZ 20. Shear force of back skin and muscles was not significantly influenced by the dietary supplementation of zinc. Water holding capacity in breast muscle increased significantly ($P < 0.05$) when birds were fed OZ 40 and OZ 80; however, organic zinc supplementation had no adverse effect on over all consumer acceptability of broiler meat. It has been concluded that dietary organic zinc does not affect growth performance of broilers but increases collagen content in skin, thereby improving carcass quality of broilers.

Key Words: organic zinc, skin quality, broilers

518 Effect of dietary copper source and level on GI copper levels and ileal *E. coli* survival in broiler chicks. K. C. Klasing* and A. Naziripour, *University of California*, *Davis*.

Pentahydrate copper sulfate (CS) and tribasic copper chloride (TBCC) are the primary Cu sources fed at high levels to improve the health and growth of animals. TBCC is less soluble in water than CS but is more bioavailable so our goal was to examine their biological properties along the GI tract. These 2 Cu sources were fed at 150 ppm to 6 pens per trt of 3 broiler chicks per pen from d 3 to 14 of age. Intestinal luminal content was collected to determine total Cu, water extractable Cu, Cu that could be extracted using the strong complexing agent, ethylenebis-hydroxyphenylglycine (EHPG), and Cu that could not be extracted (presumed to be unavailable for nutritional and microbicidal purposes). Bacteriostatic activity to *E. coli* spiked into ileal intestinal contents was greater for TBCC than for CS ($P < 0.05$). Total Cu in luminal contents was not affected by Cu source. CS increased duodenal luminal soluble Cu and epithelial metallothionein more than TBCC ($P < 0.05$), indicating that it was taken up by epithelial cells at a greater rate in the upper small intestine, but this effect did not occur in the lower intestines. TBCC resulted in more EHGP-extractable Cu in all regions of the intestines ($P < 0.05$) and less unremovable Cu ($P = 0.05$), which may be related to its greater bioavailability and anti-coli activity.

Key Words: copper, intestines, broilers

519 Effects of dietary iron and age on cellular copper metabolism in liver of weanling pigs. R. S. Fry*, J. W. Spears, S. L. Hansen, H. C. Liu, and M. S. Ashwell, *North Carolina State University*, *Raleigh*.

Thirty-six weanling, male pigs were used in a 2 × 3 design to determine the effect of dietary iron (Fe) and age on hepatic cellular copper (Cu) metabolism. Pigs received diets containing 97 mg/kg Fe (control) or 797 mg/kg Fe (H-Fe) for either 21, 42, or 63 d. On each of these d 6 pigs per treatment were harvested and liver and bile were collected for Cu analysis and mRNA analysis of hepatic Cu transporters and chaperones. On the day before harvest jugular blood was obtained for plasma Cu and ceruloplasmin (Cp). Liver Cu was not affected by diet, but was affected by age. Pigs harvested on d 21 had higher ($P < 0.01$) liver Cu than pigs harvested on d 42 and 63. Plasma Cu and Cp were not affected by diet but both increased ($P < 0.01$) with age. Bile Cu was affected by a treatment × day interaction ($P < 0.05$). On d 21, bile Cu tended to be higher ($P < 0.10$) in H-Fe vs. control pigs, but did not differ on d 42. However, by d 63, bile Cu tended to be lower ($P < 0.10$) in H-Fe vs. control pigs. Messenger RNA of Ctr1, Atox1, and Atp7b were affected ($P < 0.05$) by a treatment × day interaction, and Cp mRNA tended ($P < 0.10$) to be affected by a treatment × day interaction. On d 21 and 63, Ctr1 was not affected by diet, but on d 42 mRNA of this Cu-importer was lower ($P < 0.01$) in H-Fe vs. control pigs. On d 21, Atox1, a chaperone that delivers Cu to the Cu-exporter, Atp7b, was markedly higher ($P < 0.01$) in H-Fe vs. control pigs, but did not differ on d 42 and 63. On d 21, Atp7b tended ($P < 0.10$) to be higher in H-Fe vs. control pigs. By d 42, Atp7b was lower ($P < 0.01$) in H-Fe vs. control pigs and was numerically ($P = 0.11$) lower in H-Fe pigs on d 63. On d 21 and 42, Cp, a Cu-dependent ferroxidase, was not affected by diet, but by d 63 it was lower ($P < 0.05$) in H-Fe vs. control pigs. In conclusion, age did not affect mRNA of Cu transporters and chaperones. However, high dietary Fe affected bile Cu and Cu transporters and chaperones involved in the secretory pathway of Cu metabolism. These data provide a better understanding of how Fe antagonistically affects Cu metabolism.

Key Words: pigs, copper, iron

520 Effect of level and source of dietary copper on copper metabolism in the small intestine of weanling pigs. R. S. Fry*, M. S. Ashwell, W. L. Flowers, K. R. Stewart, and J. W. Spears, *North Carolina State University*, *Raleigh*.

Thirty weanling pigs were used to determine the effect of level and source of dietary copper (Cu) on performance and Cu metabolism in the duodenum, proximal jejunum, and ileum. Dietary treatments consisted of 1) control (no added Cu), 2) 225 mg supplemental Cu/kg from Cu sulfate ($CuSO_4$) or 3) 225 mg supplemental Cu/kg from tribasic Cu chloride (TBCC). Feeding regimen consisted of 3 phase diets. Phase 1 diets were fed from d 0 to 6, phase 2 diets were fed from d 7 to 21, and phase 3 diets were fed for the remainder of the study. Prior to harvest on d 35 and 36, pigs were fasted for 8 h then re-fed for 8 h. Digesta and mucosal scrapings were collected from each section of the intestine for determination of soluble Cu and mucosal Cu concentrations. Digesta pH was obtained upon collection. During phase 1, TBCC pigs had higher ($P < 0.05$) average daily gain and gain:feed than $CuSO_4$ and control pigs. However, overall performance for the 35 d study was not affected by Cu level or source. Digesta pH increased ($P < 0.01$) as digesta descended down the small intestine, but did not differ by Cu level or source. Soluble Cu in the digesta and mucosal Cu in duodenum, proximal jejunum, and ileum were higher ($P < 0.05$) in Cu supplemented vs. control pigs. In the duodenum, soluble Cu in digesta tended ($P < 0.10$) to be lower while mucosal Cu was lower ($P < 0.05$) in TBCC vs. $CuSO_4$ pigs (104.3 vs. 130.3 mg/kg Cu). Soluble Cu in digesta from proximal jejunum and ileum was not different between Cu sources. However, pigs fed TBCC had higher ($P < 0.01$) Cu concentrations in mucosa of the proximal jejunum than $CuSO_4$ fed pigs (44.7 vs. 22.1 mg/kg Cu). In the ileum, mucosal Cu tended ($P < 0.10$) to be higher in TBCC vs. $CuSO_4$ pigs. In conclusion, pigs supplemented with Cu had much higher concentrations of soluble Cu in the digesta and intestinal mucosa than control pigs. Furthermore, Cu source affected Cu uptake differently throughout the small intestine. Markedly lower water solubility of TBCC compared with $CuSO_4$ may explain these differences.

Key Words: pigs, copper, metabolism

521 Dietary calcium and phosphorous and organic and inorganic trace minerals on nursery pig growth performance. J. S. Jolliff* and D. C. Mahan, *The Ohio State University*, *Columbus*.

Dietary Ca and P levels were evaluated for their effect on trace mineral usage as measured by postweaning growth performance and plasma minerals. Two levels of Ca and P (Ca:P) and 5 trace mineral (TM) treatments (2 × 5 factorial) were analyzed as an RCB over 6 blocks with 240 total nursery pigs (6.43 kg BW) for 35 d. The 2 Ca:P levels were 0.80% Ca and 0.65% P (Low) and 1.10% Ca and 0.91% P (High). Of the 5 TM treatments, 4 comprised a 2 × 2 factorial between 2 TM sources (organic or inorganic) and 2 TM levels. The first TM level (1x) provided 15 ppm Cu, 15 ppm Fe, 10 ppm Mn, and 140 pp Zn while the second level (2x) provided twice the amount of TM of the first level. The fifth TM treatment was no TM supplemented to the diet, i.e., all minerals were considered indigenous (basal). Pigs were weighed and feed disappearance recorded once per week postweaning. On d 35, all pigs were bled and hemoglobin, hematocrit, and plasma minerals were analyzed. TM treatment affected pig BW ($P < 0.05$) from d 21 onwards with the Basal TM treatment resulting in lighter weight pigs. Furthermore, pigs fed the Basal TM treatment had lower ($P < 0.05$) ADG, ADFI, and G:F by 21 d postweaning. Inorganic TM resulted in greater ADFI for the entire 35 d trial. There were Ca:P x TM interactions ($P < 0.05$) for BW, ADG, ADFI, and G:F in the later half of the nursery period. TM treatment affected ($P < 0.05$) pig hemoglobin with Basal having the lowest concentrations, inorganic TM having greater concentrations than organic, and the 2x TM level having greater concentrations than the 1x level. Pig hematocrit showed a similar pattern to hemoglobin, although TM level had no effect ($P > 0.05$). Low Ca:P resulted in greater ($P < 0.05$) plasma P and Zn than High Ca:P. Pigs in the Basal treatment had lower ($P < 0.05$) plasma P, Fe, and Zn than pigs receiving supplemental TM. There were no Ca:P interactions for any blood constituents. In summary, pigs fed inorganic TM had greater feed intake, hemoglobin concentrations, and hematocrit while High Ca:P reduced plasma P and Zn.

Key Words: pig, calcium, minerals

522 Effect of organic and inorganic trace mineral source and preslaughter deletion on tissue mineral content of pigs. Y. L. Ma*, M. D. Lindemann, G. L. Cromwell, and G. Rentfrow, *University of Kentucky*, *Lexington*.

Crossbred pigs weaned at 21 ± 3 d (n = 144; BW = 7.4 kg) were used to assess an organic form (ORG) of several trace minerals to standard inorganic forms (IN) on tissue mineral content when those minerals were deleted for various times preslaughter. Pigs were allotted to 24 pens (6 pigs/pen) based on gender and BW and fed a diet containing either IN ($CuSO_4 \cdot 5H_2O$, ZnO, $FeSO_4 \cdot H_2O$, MnO) or ORG (Bioplexes; Alltech Inc., Nicholasville KY) trace minerals (Cu, Zn, Fe, Mn) at the NRC (1998) requirement for each of 5 dietary phases of BW (equivalent to

14, 14, 42, 28, 42–d periods). Two pigs were removed from each pen at the end of phase 4 (BW = 82.6 kg), and at the end of phase 5 (BW = 126.6 kg) for the collection of tissue samples. After phase 4, 3 pens from each treatment were switched to a common diet without trace mineral supplementation in 2–wk intervals. This resulted in 4 groups within the IN and ORG source in which supplementation was deleted for 0, 2, 4, and 6 wk preslaughter. All data are reported on an mg/kg tissue DM basis. At the end of phase 4, ORG Mn content was greater ($P < 0.03$) in heart (0.77 vs. 0.68), liver (9.46 vs. 8.30), and longissimus dorsi (LD; 0.30 vs. 0.23). ORG Cu was greater ($P < 0.03$) in LD (2.12 vs. 1.89). ORG Fe was greater ($P < 0.03$) in LD (21.8 vs. 19.4) but lower in liver (466.1 vs. 564.4). Zn source did not affect tissue content. At the end of phase 5, increased length of deletion period (from 0 to 6 wk) resulted in a decrease (linear and quadratic, $P < 0.01$) in liver Zn (ORG – 209.2 to 118.8; IN – 183.8 to 124.8) and an increase (linear, $P < 0.01$) in heart Mn (ORG – 0.76 to 1.12; IN – 0.64 to 1.03) and liver Mn (ORG – 8.21 to 12.68; IN – 7.27 to 13.23). The only mineral source by deletion period interaction ($P < 0.04$) was in LD Zn where increasing deletion was associated with less Zn in IN–fed pigs (63.9, 55.7, 56.8, and 49.8) but not in ORG–fed pigs (56.4, 59.5, 56.7, and 62.0). The results demonstrate differential effects of mineral deletion on tissue mineral content depending on both mineral assessed and source of the mineral.

Key Words: trace minerals, pigs