
Salmonella Typhimurium is attracting world-wide attention as it continues to cause foodborne illness to human beings. The organism is resistant to a wide range of anti-infectious agents and as a result the illness is more difficult to treat. The objective of this study was to analyze the expression of specific virulence and stress genes (hlaA and rpsS) in Salmonella Typhimurium at various nutritional stress and pH in a continuous culture system. Salmonella Typhimurium cells were propagated in continuous cultures with a total volume of 0.50 liter LB minimal medium at 98% turnover rate. Two chemostats were used for adjusting dilution rates and pH levels. Dilution rates were 0.0125 h⁻¹, 0.025 h⁻¹, 0.05 h⁻¹, 0.1 h⁻¹, 0.27 h⁻¹, 0.54 h⁻¹, 1.08 h⁻¹, and 1.44 h⁻¹, while pH levels were 6.1 through 8.0. Results indicated that cell protein increases as dilution rates (D) increase (D > 0.0125 h⁻¹). To analyze gene expression, samples were stored in RnAprotect (Qiagen, Valencia, CA) in duplicate. Real-time PCR reactions were performed on an ABI Prism 7700 Sequence Detection System (Perkin-Elmer Applied Biosystems, Foster City, CA). Samples were analyzed using the Comparative Ct (ΔΔCt) method by normalizing to the endogenous control gene (rsmC). Both hlaA and rpsS expression appeared to change in response to corresponding pH and Yglc transitional changes in the continuous culture.

Key Words: Salmonella, Continuous Flow Culture, Virulence


Campylobacter is one of the leading causes of human gastroenteritis in the United States and epidemiological evidence has implicated raw poultry products as a significant source of human infection. Campylobacter frequently colonizes the avian intestine but recent research indicates that this organism can also colonize the oviduct of laying hens and broiler breeders. The present studies were undertaken to determine if Campylobacter is present in the reproductive systems of commercial turkeys. In the first study, the reproductive tracts of 11 hens and 17 toms were aseptically excised and the segments (female: vagina, shell gland, isthmus, magnum, and infundibulum; male: ductus deferens and testes) were swabbed with a dry cotton sterile swab. The swabs were incubated for 24 h in Campylobacter enrichment broth and 0.1 mL of the enriched sample solution was streaked onto Campy-line agar plates and incubated at 42°C for 48 h in a microaerophilic environment for detection of Campylobacter. Of the 11 hens sampled, Campylobacter was isolated from the vagina (10/11), the shell gland (7/11), the isthmus (8/11), the magnum (6/11), and the infundibulum (3/11). Of the 17 toms sampled, Campylobacter was isolated from the ductus deferens (8/17) and the testes (2/17). In a second study, pooled semen samples from 7 separate farms were randomly collected by abdominal massage over a period of 13 weeks. The pooled semen samples were serially diluted and 0.1 mL of each dilution was plated on Campy-line agar and incubated at 42°C for 48 h in a microaerophilic environment for enumeration. Campylobacter was isolated from 57 of the 59 pooled semen samples and levels ranged from <10³ to 1.58 x 10⁶ cfu/mL of semen. Naturally occurring Campylobacter is present in the reproductive tracts and semen of commercial turkeys and may enable vertical transmission of Campylobacter from the hen to the poult.

Key Words: Campylobacter, Turkeys, Reproductive Tract


While the contents of chicken crops are well known to play an important role in colonization of carcasses at processing, recent evidence has been presented suggesting that Salmonella may amplify and perhaps sometimes colonize the crop in vivo. Salmonella infections have been associated with the use of animal/fish meal supplementation, possibly because of the known increased contamination frequency of these feedstuffs. Presently, we evaluated the ability of Salmonella enteritidis (SE) to amplify in a simulated in vitro crop assay, using either an all-vegetable chick starter diet or a similar diet supplemented with fish meal (5%). In three replicate experiments, three different chick starter formulations, two with all vegetable protein sources, and one with fish meal (5%) supplementation, were compared for ability to support Salmonella growth in vitro. Briefly, for each experiment, 1.25 g of each feed type was measured into 13 x 100 mm borosilicate tubes (n=10 replicate) and autoclaved. Sterile saline (4.5mL) was added to each tube with 0.5mL of SE inocula at initial concentrations of 6.75x10⁵, 1.15x10⁶, and 8x10⁴ colony forming units (cfu)/mL for each experiment, respectively. The tubes were then incubated at 40°C for 1 or 2.5 hrs, and cfu/mL were determined by serial dilution and spread plate enumeration on selective medium. Each of the feed types, in each of three experiments, supported the apparent amplification of SE by >1 log10 cfu/mL during the 2.5 hr incubation. Inclusion of fish meal did not affect the ability of SE to grow in the feed substrates in any of the 3 experiments. Since inclusion of fish meal did not enhance the ability of Salmonella to grow in this in vitro crop model, these data suggest that fish meal may not be important for supporting Salmonella growth in the crops of chickens.

Key Words: Salmonella, Crop, Fish Meal

PSA-Nutrition: Amino Acids and Vitamin/Mineral Nutrition I


Guar (Cyamopsis tetragonoloba) is a drought-tolerant annual legume grown for its high concentration of galactomannan gum. Guar seeds are grown for its high concentration of galactomannan gum. Guar seeds are harvested at 30% moisture, with the 2.5% guar meal-fed group higher than the other groups. Feeding guar did not affect yolk color or shell quality (shell thickness, egg breaking force and specific gravity), but decreased Haugh units. A guar fraction x concentration interaction was detected with respect to FCR which decreased in birds fed guar meal as concentrations increased from 2.5 to 5%. The results showed that both guar gum and guar meal can be fed to high production laying hens at up to 5% without adverse effects on laying hen performance and egg quality.

Key Words: Guar, Laying Hen, Egg Production
The objective of this research was to examine the main and interactive effects of dietary nutrient density (2800, 2950, 3100 kcal/kg ME), feed form (mash, crumble/pellet) and lighting program (20L:4D, 12L:12D) on production characteristics of broilers raised to a young age (35 days). Diets (starter, grower and finisher) were formulated so that amino acid levels were in proportion to diet energy level. Lighting programs were initiated at 4 days of age and maintained until trial end. Body weight was not affected by nutrient density when diets were fed in a crumble/pellet form but decreased in a linear fashion with density when fed as a mash. Final body weight for birds fed mash diets were smaller than for those fed crumble/pellet diets. Feed to gain ratio decreased with increasing nutrient density but was not affected by feed form. Feed intake decreased with increasing nutrient density and was lower for birds fed mash. The effect of nutrient density on feed intake was less when birds were fed mash in contrast to crumble/pellet diets as indicated by a significant interaction. Diet nutrient density did not affect mortality but feeding a mash diet decreased death loss (1.83%) in comparison to feeding processed feed (5.63%). The increase in mortality can be attributed to an increase in the number of culls due to leg abnormalities. Mean gait scores suggested an increase in leg abnormalities for birds fed processed feed (0.756), as compared to those fed a mash (0.342). Use of 12L:12D reduced body weight, feed to gain ratio, feed intake and mortality in comparison to the use of 20L:4D. Overall there were no interactions between lighting program and diet main effects. The data demonstrate the importance of treatment effects and their interactions on broiler performance and provide data that permits selection of nutrition and management programs on the basis of economic and animal welfare considerations.

Key Words: Energy, Mash, Photoperiod

Myofiber growth is dependent upon the contribution of new nuclei from the mitotically active satellite cell population. The effect of starvation or feeding with different levels of amino acids within different ideal protein levels on satellite cell mitotic activity was studied using 3-day old male broiler chicks. The experiment consisted of one starved group and 4 fed groups (n=10 per group) receiving differing levels of digestible lysine 0.82%, 0.99%, 1.16% and 1.33%. The feeds were formulated to contain 0.82%, 0.99%, 1.16% and 1.33%. The feeds were formulated to contain these levels on satellite cell mitotic activity was studied using 3-day old male broiler chicks. The experiment consisted of one starved group and 4 fed groups (n=10 per group) receiving differing levels of digestible lysine 0.82%, 0.99%, 1.16% and 1.33%. The feeds were formulated to contain 2.950 ME kcal/kg and an ideal balance for essential amino acids. All other nutrients met NRC recommendations (1994). Birds were housed in battery cages, receiving feed and water ad libitum, except for the starved group, from hatch to 3 days of age. All chicks were injected with 5-Bromo-2'-deoxyuridine (BrdU) 2 hours before being killed. By the end of experiment, each bird was killed and the Pectoralis thoraci- cuscus was removed, fixed, dehydrated, cleared and embedded in paraffin. Mitotically active satellite cells were identified in the Pectoralis thoraci- cuscus using BrdU immunohistochemistry and enumerated using computer-based image analysis. Mitotic activity in the starved group was significantly lower than the fed groups. Within the fed groups the satellite cell mitotic activity was highest for the treatment with 0.82% digestible lysine. Myofiber cross sectional area was reduced on the fed group. Myofiber cross sectional area was reduced in the starved group compared to fed treatments. The higher satellite cell mitotic activity observed for the 0.82% group, at day 3, may reflect a longer period for satellite cell activation. This activation delay may be a result of a deficiency, because a level of 0.82% of digestible lysine can be insufficient for early pectoral muscle growth. The results of the current study suggest that a satellite cell pathway may be sensitive to early nutritional supplementation.

Key Words: Muscle, Satellite Cell, BrdU

Synthetic methionine has received scrutiny in organic production due to its extraction process and a belief that it is used for growth promotion rather than bird health. Organically reared poultry have access to forage that may supply additional nutrients. The objective of the current study was to evaluate the extent in which birds will utilize nutrients, especially methionine, from forage and the subsequent effects on performance and carcass quality. Three hundred and eighty 1-day old Ross 308x344 broilers were reared from 0-3-weeks in floor pens. On day 21, 240 birds were randomly selected and moved into houses with access to pasture for five weeks. Experimental diet consisted of two different methionine levels (including or excluding crystalline methionine) arranged in a factorial structure with two different levels of feed access (ad libitum or 50% intake of ad libitum). In addition, fifty-six birds were withheld pasture access and fed similar diets. Birds with access to forage and comparable feed access had similar live weight gain (P=0.476) and feed intake (P=0.5182) despite variation in dietary methionine. These birds also had similar hot carcass weight (P=0.0850), fat pad weight (P=0.6806), and breast weight (P=0.0345). A comparison of birds with and without access to pasture showed that birds without access to pasture had an increase in LWG (P=0.0001) and FI (P=0.0001). Feed efficiency did not differ between groups. Car- cass weight (P=0.0001) and fat pad weight (P=0.0001) were larger for birds without access to pasture. However, these birds had small breast weight (P=0.0001). These results demonstrate that birds given access to pasture during the fall, with no synthetic methionine supplementation, can adequately meet their nutrient requirements. Furthermore, providing access to forage may improve carcass quality by decreasing fat pad weight and increasing breast yield.

Key Words: Organic Production, Broiler Production, Methionine

Organic broiler production addresses consumer concerns with drug/animal by-product use and bird welfare. Synthetic methionine is typically added to corn-soybean based diets to meet broiler methionine requirements. However, the use of synthetic methionine in organic feed has recently become a concern to consumers. Research focusing on the removal of synthetic methionine from corn-soybean based diets for organically-reared birds does not exist. The objective of the current study was to determine the extent of synthetic methionine use as well as feed restriction on performance and meat quality of organically-reared broiler chickens. Diets consisted of two different methionine levels (excluding and including synthetic methionine) each provided for ad libitum or restricted access. Diets were fed to four replicate pens of 15 straight-run 308x344 Ross broilers, each having free access to pasture during the day. Additionally, broilers were reared without pasture access, but given similar diets. Birds given ad libitum feed and pasture access had similar live weight gain (LWG), feed intake (FI), and feed efficiency (FE) (P=0.88050.05) despite variation in dietary methionine. In addition, these birds did not differ in carcass characteristics (P=0.88050.05). Birds without pasture access, had increased LWG (P<0.05), decreased FI (P<0.05) and increased FE (P<0.05) compared to pastured birds fed similar diets. Pastured birds had decreased fat weights relative to carcass weight (P=0.88040.05), while all other carcass characteristics remained similar to birds without pasture access. Birds given restricted feed and pasture access had similar LWG and FI despite methionine level; however FE varied (P=0.88040.05) based on period of measurement (21-38d) or (38-56d). These results suggest that birds fed diets without synthetic methionine could compensate for the methionine deficiency if diets are provided for ad libitum consumption and summer forage is accessible.

Key Words: Organic Production, Broiler Production, Methionine
Dietary copper sulfate (CS) and Tri-Base copper chloride (TB) were examined to test their effects on broiler body weight (BW) and immune responses. Experiment 1 was a 6 x 2 factorial design using 6 diets containing a basal diet with no additional copper (Neg. control), 125ppm CS or TB, 188ppm CS or TB, or a basal diet + bacitracin (Pos. control). Birds were housed in Petersime batteries at 8 chicks/rep, 8 reps/diet. At 3 wk all diet groups were weighed, then placed into 1 of 2 treatments; chicks were either vaccinated with lipopolysaccharide (LPS, 100 μg/kg BW) or unvaccinated and sampled 24 h later. BW was significantly affected by diet at 3 wk (P < 0.05). Broilers fed Pos. control diet weighed significantly less than those fed 125ppm CS, 188ppm TB, and 188ppm CS (P < 0.05). Plasma Zn was significantly reduced by LPS (P < 0.01) for all birds, except for birds fed 125 ppm CS or TB (P < 0.05). There was a significant increase in plasma Cu and liver weights in LPS versus unvaccinated birds (P < 0.01 for each). Experiment 2 was a completely randomized block design with four diets using a basal diet containing no additional copper (Neg. control), 188ppm TB or CS, or basal diet + bacitracin and roxarsone (Pos. control). Birds were housed in floor pens at 65 chicks/pen, 8 pens/diet. At 13 d TB and CS diets had significantly increased BW compared to Neg. control (P < 0.05). At 31 d CS diets had significantly increased BW compared to Neg. control (P < 0.05). Diet significantly affected carcass weight (P < 0.01); specifically, birds that had significantly increased in weight compared to Neg. control (P < 0.03, P < 0.01, respectively). Finally, TB diet had greater plasma Cu compared to Neg. or Pos. control (P < 0.05). These data demonstrate that dietary copper sources have some positive effects on broiler performance, similar to bacitracin control. Effects on inflammation are variable, but 125ppm CS or TB demonstrate some anti-inflammatory properties. Further effects on immune response will be discussed in relation to gut histology and antibody titers.

**Key Words:** Copper, Inflammation, Broiler

121 Effects of copper source and concentration on phytate phosphorus hydrolysis by Phytase in vitro. Y. Pang*, and T. Apelage, Department of Animal Science, Purdue University, West Lafayette, IN.

Higher concentrations of copper (Cu) in the diet may decrease phytate phosphorus (Pp) hydrolysis because of the chelation of Cu with the phytin molecule. Different sources of Cu may affect the activity of phytase at different pH conditions. Therefore, five Cu sources (Cu sulfate (Cu Sul), Cu chloride (Cu CL), tri-basic copper chloride (TBCC), Cu lysinate (Cu Lys) and Cu citrate (Cu CIT)) were studied in vitro at pH 2.5, 5.5 and 6.5 to determine how Cu from each of these sources affects PP hydrolysis by phytase. Five Cu concentrations were used for these studies (0, 62.5, 125, 250 and 500 ppm), and were incubated at 40-41°C for 60 min. The values were expressed by the relative percentage of PP hydrolysis of the 0ppm Cu treatment from separate assays. At pH2.5, 500ppm Cu Sul inhibited PP hydrolysis (≤0.05), whereas, both 250 ppm and 500ppm Cu from Cu CL inhibited PP hydrolysis. No concentrations of Cu from TBCC, Cu Lys or Cu CIT inhibited PP hydrolysis. At pH 5.5, addition of either Cu Sul or Cu CL between 62.5 and 500 ppm inhibited PP hydrolysis from 23.1 to 78.0% (≤0.05). Increasing pH to 6.5 increased the extent of inhibition for Cu Sul and Cu CL treatments such that 62.5ppm to 500ppm caused an 89.8 to 95.4% inhibition (≤0.05). Plasma Cu was significantly increased by LPS (P < 0.01) for all birds, except for birds fed 125 ppm CS or TB (P < 0.05). There was a significant increase in plasma Cu and liver weights in LPS versus unvaccinated birds (P < 0.01 for each). These data demonstrate that dietary copper sources have some positive effects on broiler performance, similar to bacitracin control. Effects on inflammation are variable, but 125ppm CS or TB demonstrate some anti-inflammatory properties. Further effects on immune response will be discussed in relation to gut histology and antibody titers.

**Key Words:** Copper Source, Phytate Phosphorus, Phytase


An experiment was conducted to determine the relative bioavailability (RBV) of 25-hydroxycholecalciferol (25(OH)D3) in comparison to cholecalciferol for hen day egg production (HDEP), hatchability (HAT), embryo mortality during the early (1-10 days of incubation EEM) and late stages (11-21 days LEM). The study was conducted with 77 to 90 week old Ross broiler breeders in an environment excluding UV light. A basal D3 deficient diet and this diet supplemented with three levels of D3 (125, 500, and 2,000 IU/kg of diets) and two level of 25(OH)D3 (125 and 500 IU/kg of diets) were fed. Three repetitions of seventeen hens were used for each treatment group. No D3 source was used in the vitamin premix and no animal byproduct was used in the corn, soybean meal and wheat middlings basal diet to guarantee that no unintentional vitamin D activity was present in the experimental diets. Data were collected weekly for HDEP, HAT, EEM and LEM. Slope ratio analyses were performed using the logarithm of 0, 125 and 500 plus 1 with the data from 81 to 88 weeks of age. The RBVs found were 140, 132, 122 and 127% for HDEP, HAT, EEM and LEM, respectively. The HDEP during the 14 weeks of the experiment was 2.09 times higher for 25(OH)D3 when compared with D3 at the 125 IU/kg level. However, at the 500 IU/kg level the difference was only 1.05 higher for 25(OH)D3. Hatchability was also 1.67 times higher for 25(OH)D3 at 125 IU/kg level than D3; however, no difference was observed between the sources at 500 IU/kg. Late embryo mortality was reduced 4 times by 25(OH)D3 when compared with D3 at 125 IU/kg level and only 1.26 times at 500 IU/kg level. The potency of 25(OH)D3 in relation to D3 depends on the levels tested. When comparing vitamin D sources, 25(OH)D3 has greater potency than D3 only at very low levels of supplementation.

**Key Words:** Broiler Breeders, Vitamin D3, 25-Hydroxycholecalciferol

123 Decreasing the time required for the auxotroph Lactobacillus rhamnosus assay by adaptation to microtiter plates. J. L. Golbach*, C. L. Woodward, V. I. Chalova, and S. C. Ricle, Texas A&M University, College Station.

Riboflavin is an essential part of animal diet by playing a significant role in the metabolism of carbohydrates, fatty acids, and amino acids. Riboflavin is also required for the utilization of oxygen, red blood cell formation, antibody production, and growth. Some specific sources of riboflavin are organ meats, nuts, cheese, eggs, milk, lean meat, green leafy vegetables, fish, legumes, whole grains, and yogurt. The objective of this research was to reduce time of the assay by scaling it down into a microtiter plate while still maintaining the precision of the original tube assay. The organism that is used most commonly to quantitate riboflavin is Lactobacillus rhamnosus ATCC 7469 formerly known as Lactobacillus casei. The riboflavin used in the Bacto Riboflavin Assay Medium was used as a guideline for transferring the riboflavin assay from a tube assay to a microtiter plate assay. Micro Inoculum Broth was inoculated with L. rhamnosus and grown overnight at 37°C. The bacteria were washed three times with sterile 0.85 percent saline solution. Riboflavin Assay Medium was prepared and riboflavin standard was added to achieve concentrations that range from 2.5 ng/mL to 30 ng/mL. The solutions were autoclaved and transferred to a microtiter plate. The microtiter plates were inoculated and incubated at 37°C for 8 hours and values were read by a Spectra Fluor Plus microtiter plate reader. The standard growth curve for the riboflavin assay was linear from 0 ng/mL up to 10 ng/mL with an average R2 value of 0.99. The average variance for the optical density Auo values between 0 ng/mL and 10 ng/mL was 3.448 x 10^-2. The microtiter assay reduced the amount of time required for sufficient bacterial growth response to generate linear standard curves from 22 hours down to 8 hours. This scaled-down assay can be utilized for the determination of riboflavin in more samples of potential nutritional sources such as poultry feeds and poultry products.

**Key Words:** Lactobacillus Rhamnosas, Riboflavin, Microtiter Plate Assay