

replicate pens of 12 male chicks. There was only one diet in each growth period and all groups were fed the same free medicine corn-soy based diet formulated according to NRC requirements. At 14 and 28 days of age, one chicken from each replicate was weighed and killed by cervical dislocation. Ileal contents were collected to use for microbial evaluation. At 49 days of age, one bird from each pen was killed to weigh gastrointestinal tract, liver, pancreas and abdominal fat. The total number of aerobes and coliforms were enumerated by using plate count methods. Adding citric acid in drinking water did not significantly affect feed intake, gain and feed to gain ratio in any period of production. Also no significant difference was observed for liver, pancreas and body weight ($p > 0.05$). However, treatment with citric acid at the levels of 0.1 and 0.2%, caused significant reduction in abdominal fat when compared with control birds ($p \leq 0.05$). There was no significant difference among treatments in total aerobic and coliform numbers ($p > 0.05$). Under the situations of this study, incorporation of citric acid into the drinking water could not affect the performance and ileal microbial counts of chickens, however it may reduce abdominal fat.

Key Words: Citric acid, Performance and ileal microflora, Broiler

54 Effect of acetic acid administration in the drinking water on performance, growth characteristics, and ileal microflora of broiler chickens. M. Reza Akbari*, J. Mahabadyani Nadaf, and H. Kermanshahi, *Ferdowsi University, Mashhad, Iran.*

This trial was conducted to evaluate the effects of adding acetic acid (10% acid acetic-vinegar) into the drinking water on the performance and ileal

microorganisms of broiler chickens. In a completely randomized design with 5 treatments and 5 replicates, 300 day-old commercial mail broiler chicks (Ross) were divided into 25 groups, 12 chicks per group. Each of the five levels of acetic acid added to drinking water (0.0, 0.1, 0.2, 0.3, and 0.4%) was given to five replicates for a period of 21 days. All groups were fed a practical corn-soy based diet formulated according to NRC requirements. During the experiment, chickens were not fed any antibiotics or coccidiostats. The diet formulation for all groups was the same. At 14 and 28 days of age, one chicken of each replicate was weighed and killed by cervical dislocation. Ileal contents were collected and used for microbial evaluation. At 49 d of age, one bird from each pen was killed to weigh gastrointestinal tract, liver, pancreas and abdominal fat. The number of total aerobes and coliforms per gram of ileal contents were enumerated on the appropriate bacteriological media. There was no significant difference in feed intake, weight gain, feed to gain ratio, and the weights of body, gastrointestinal tract, abdominal fat, liver and pancreas ($P > 0.05$). Also, the differences among treatments for total aerobic and coliform counts were not significant ($P > 0.05$). Under the conditions of this study, addition of acetic acid as an organic acid into drinking water at the used levels, could not affect the performance and ileal microbial counts of chickens.

Key Words: Acetic acid, Performance and ileal microflora, Broiler

Physiology

55 Differences in skeletal muscle fibre growth in broiler and layer chickens: Association with myopathy? V. E. Cooke*¹, S. Gilpin², M. Mahon², D. A. Sandercock¹, and M. A. Mitchell¹, ¹*Roslin Institute (Edinburgh), Midlothian, UK*, ²*University of Manchester, Manchester, UK.*

Genetic selection for improved production traits in poultry may be associated with an increased incidence of spontaneous and stress induced myopathies. Increased muscle fibre diameter and reduced oxidative capacity of specific muscles may be linked with increased incidence of myopathology in broilers. This study compared the growth of skeletal muscles of differing fibre type composition in commercial broilers (B) and layers (L). In the latter, selection strategies have not focused upon increased muscle growth and altered conformation. 4 birds per line were weighed and euthanised at intervals between 4 and 22 weeks of age. Samples of *Pectoralis major* (*Pm*) (breast) and *Biceps femoris* (*Bf*) (thigh) muscles were frozen for cryostat sectioning and stained using histochemical techniques. The minimum fibre diameter (MFD) of 100 fibres was measured for each muscle sample. Qualitative analysis of fibre type composition demonstrated that in both lines and at all ages *Pm* consisted almost entirely of glycolytic fibres, whereas *Bf* had a large population of oxidative fibres. Body weight and mean MFD of both muscles increased with age ($P \leq 0.05$) in both lines, and were greater in broilers at all ages ($P \leq 0.05$). In B, mean MFD of *Pm* was greater than that of *Bf* at all ages ($P \leq 0.05$) except for at 20 wks ($P = 0.064$). In L there was no difference in mean MFD of the two muscles at any age. The larger muscle fibre diameters in B compared to L may reflect increased rates of hypertrophy in B from hatch or before. Previous studies in turkeys have demonstrated a threshold muscle fibre diameter, above which further hypertrophy is associated with an increased incidence of spontaneous myopathy. Genetic selection for increased breast meat yield in broilers may have caused the divergence in *Pm* and *Bf* fibre size and the commercially desirable high degree of muscle fibre hypertrophy may be associated with a predisposition towards spontaneous or idiopathic myopathy.

Key Words: Broiler, Muscle fibre growth, Myopathy

56 Enhanced growth and muscle development of broilers by in-ovo photostimulation of green light. I. Rozenboim*, Y. Piestun, and O. Halevy, *Hebrew University of Jerusalem.*

Posthatch green monochromatic photostimulation enhanced body weight and muscle growth of broilers. The effects were observed already after 3 days of illumination, suggesting that photostimulation during embryogenesis may cause the same phenomenon. In experiment 1, the effect of

green light photostimulation on embryo development was studied. 250 fertile eggs were divided into 2 groups. Groups were placed in a commercial incubator. The first group was illuminated from the fifth day until hatch by LED green light (560nm, half band 15nm, intensity of 0.1 W/m² at egg shell level). The second group was incubated under dark conditions and served as control. Green light significantly increased embryo weight at days 14, 15, 17 and 20 of incubation. The percentage of breast muscle was significantly higher in the illuminated embryos on days 11 and 13, and from day 17 to hatch. Experiment 2 tested posthatch growth and muscle development of broilers in ovo photostimulated by green light, and the proliferation and differentiation of skeletal muscle satellite cells were tested. 60 fertile eggs were divided into 2 groups and treated as in experiment 1. Body weight was recorded at days 0, 1, 3 and 6 of age. Significant increase in body weight and percent of breast muscle was observed in birds photostimulated in ovo by green light. Satellite cells were separated from the breast muscle at various days posthatch. The number of satellite cells/gram of muscle was significantly higher in the illuminated group compared to the control on days 0, 1 and 3 posthatch. Myogenin levels were higher in the illuminated group on day 1 and 3 posthatch, suggesting that cell differentiation in the illuminated group began earlier than in controls. Indeed, the muscle tissue levels of IGF-I, which plays a pivotal role in muscle cell proliferation and differentiation, were two fold higher in the illuminated group related to those in the control group on day 3. Taken together, we suggest that embryonic green light illumination enhances body and muscle weight of embryos and chicks at early days posthatch. Satellite cell proliferation and differentiation are affected probably due to the local increase in IGF-I levels during the short-time period posthatch in which new muscle fibers are added.

Key Words: Broiler, Photostimulation, Muscle, Satellite cell

57 The involvement of erythropoiesis in the development of ascites in broiler chickens - the role of corticosterone and triiodothyronine. D. Luger and S. Yahav*, *ARO The Volcani Center, Bet Dagan, Israel.*

Significant development in the genetic selection of broiler chickens, coupled with inferior response of the cardiovascular system, has led to a relatively low capacity to balance energy expenditure under cold conditions, resulted in ascites syndrome development. The present study examined a. the association between the blood system and the accumulated fluid in the abdominal cavity. b. The efficacy of erythropoiesis process in relation to corticosterone and triiodothyronine (T₃) (inducers of pluripotent stem cells proliferation and erythroblast differentiation, respectively)

plasma concentration. An experiment includes ascites induced treatment was conducted. At weekly intervals venal blood samples were taken for hematocrit, hemoglobin and hormones analysis. Plasma, packed cells and whole blood volume were determined, and the maturation level of red blood cells was analyzed. Plasma volume was similar in the ascitic and control chickens. Osmolality, albumin and total protein concentration was similar in the plasma and the abdominal cavity fluid, suggesting a dramatic deleterious effect of the syndrome on the selective permeability capacity of the blood vessels. The increase in hematocrit was related to a significant increase in erythrocyte number, which resulted in the enlargement of the packed cell volume. Hemoglobin concentration increased significantly too, but its concentration per amount of red blood cells declined significantly. This decline was related to a significant increase of immature erythrocytes (23.5%) in the blood stream of ascitic broiler's compared to non-ascitic one (7%). The elevation of immature erythrocytes can be explained by the significant and continuous increase of corticosterone plasma concentration, coincided with a significant decline in plasma T₃ concentration. It can be concluded that increase in the level of immature red blood cells leads to inferior blood oxygen carrier capacity, which contributes to the development of the ascitic syndrome.

Key Words: Broiler, Ascites, Red blood cells, Triiodothyronine, Corticosterone

58 Relationship between glutathione and respiratory chain complex activities in duodenal mitochondria in broiler breeder males with low and high feed efficiency. C. Ojano-Dirain^{*1}, M. Iqbal¹, T. Wing², M. Cooper², and W. Bottje¹, ¹Dept. of Poultry Science, Center of Excellence for Poultry Science, Univ. of Arkansas, Fayetteville, ²Cobb-Vantress Inc., Siloam Springs, AR 72761.

We have observed increased reactive oxygen species (ROS) production in intestinal mitochondria obtained from broiler breeder males with low feed efficiency (FE, gain to feed). As antioxidants are critical for combating ROS-mediated oxidative stress and preserving mitochondrial function, objectives of this study were a) to determine levels of reduced glutathione (GSH), a major antioxidant in mitochondria, and activities of GSH recycling enzymes: GSH peroxidase and GSH reductase, and b) to establish relationships between antioxidants and respiratory chain complex activities (Complex I, II, III, IV and V [ATP synthase]) in broiler breeder males. Duodenal mitochondria were isolated from broilers with low (0.62 0.02, n = 5) and high (0.80 0.01, n = 7) feed efficiency. Activities of respiratory chain complexes (Complex I to V), GSH peroxidase and GSH reductase, and levels of GSH were measured by UV spectrophotometry. Although there were no differences in GSH peroxidase or reductase activities, or in individual complex activities between groups, GSH levels were higher (P < 0.08) in broilers with high FE. Regression analysis revealed significant correlations (P < 0.05) between the mitochondrial GSH and activities of Complex II, IV and V with R-square values of 0.39, 0.59, and 0.51, respectively. These data suggest that reduced GSH may be important in maintaining or enhancing the activity of certain respiratory chain complexes, and could be important in the phenotypic expression of feed efficiency in broilers.

Key Words: Broilers, Feed efficiency, Respiratory chain complex activity, Oxidative stress, Glutathione

59 Incubation temperature manipulation alters turkey muscle development. V. M. Maltby* and N. C. Stickland, *The Royal Veterinary College, London, UK.*

Incubation temperature is the most important factor affecting hatching success. Previous studies have considered the effects of changing the incubation temperature for short periods. Hatchability is not affected when a temperature of 38.5°C is applied for 3 days or less, however for 5 or more days a significant reduction is seen. Few studies focus on the effect of incubation temperature on tissue development. Previously in our laboratory, temperature has been shown to affect muscle development in fish with a consequential effect on post hatch growth. The aim of this study was to ascertain if manipulating turkey incubation temperature for short periods affects muscular development.

Previous research (Somaiya A-PhD thesis 2002) has shown that manipulating the incubation temperature (4 days at 38.5°C or 35.5°C) causes a change in the posthatch muscle phenotype. Deviations from the control temperature (37.5°C) in the early days of incubation had most effect. Eggs incubated at 35.5°C from 5-8 embryonic days (ed) and 9-12ed produced poults (16days posthatch) with significantly fewer myofibres.

Poults from eggs incubated at 38.5°C from 5-8ed and 9-12ed exhibit a significantly increased nuclei number and fibre number respectively. Subsequent trials in the laboratory have focused on the time period of 5-8ed and have considered embryonic parameters. Myogenin, expressed during muscle fibre differentiation, showed a peak at ed16 for controls, whereas in treated embryos the levels peaked at ed18. Myogenin levels were significantly lower in 38.5°C and 35.5°C embryos at ed16. At ed18, 38.5°C (5-8ed) treated embryos exhibited a significant increase in expression. All results in comparison to controls.

This work has shown a delay in muscle differentiation in temperature manipulated embryos. We suggest that this delay produced a longer period of proliferation for the increase of muscle fibre and nuclei number. From previous research in mammals we hypothesise that the improved muscle parameters will produce better posthatch growth and meat quality.

Key Words: Incubation temperature, Muscle

60 A comparison of pore size in avian eggshells measured using three methods: water vapor gas conductance, computer image analysis, and Vitalscan. Sandra Westmoreland*, *The University of Texas at Arlington.*

Gas exchange, which occurs by diffusion through the eggshell pore system, is critical to the optimum growth of the avian embryo. Eggshell porosity must be appropriate to accommodate the embryo's needs, allowing for adequate gas exchange but preventing the occurrence of desiccation. This study compared data collected with three methods of pore measurement to determine if the values obtained were consistent. Eggshell of White Leghorn chickens of the Hyline-19 breeding line was used for this study. The most commonly used method of measuring eggshell porosity for the last 30 years has been that developed by Ar, et al. (1974). Ar, et al. quantified shell porosity by studying the loss of water from eggs stored under conditions of known humidity. Ar, et al. stated that water vapor gas conductance (G_{H_2O}) through pores is a function of both the total functional pore area (A_p) and shell thickness (L). Using Ficke's first law of diffusion, Ar, et al. derived a formula for calculating functional pore area using G_{H_2O} values. The method used in this study for determining eggshell porosity as measured by water vapor gas conductance was that described by Arad and Marder (1982). Water vapor conductance (G_{H_2O}) and functional pore area (A_p) were calculated according to the method of Ar, et al., 1974. Pore surface area openings were measured using an image analysis computer program developed using Image Pro Plus software. The outer surfaces of shell fragments, which had been treated for cuticle removal, were digitally imaged using a Vanox light microscope and Olympus camera attachment. The pore surface area data were collected and analyzed. In addition, pore dimensions were taken from the cross-sections of eggshell fragments using scanning electron microscope images and Vitalscan computer software, which has a calibrated measuring feature. The pore measurements from the three different techniques were found to be complementary. The functional pore area, as determined with the gas conductance method, was found to be approximately equivalent to the narrow pore width at the shell interior. Direct measurement of the pore dimensions using Vitalscan provided data that were consistent with that collected by the other two methods of pore measurement.

Key Words: Avian eggshell porosity, Gas conductance, Image analysis

61 Liver glycogen relationships to somatic, visceral, and physiological changes during the first 48 hours of broiler brooding under nutrient restriction. R. W. Keirs*, E. D. Peebles, L. W. Bennett, P. D. Gerard, and S. K. Whitmarsh, *Mississippi State University.*

The time dependent changes of liver glycogen content and its correlation with select somatic, visceral, and physiological parameters at 6, 24, and 48 h post-hatch in broiler chicks from young (29-wk-old) breeders were determined. The selected parameters determined included BW, body fat loss score (BFLS), relative liver weight (RLW), kidney pathology score (KPS), rectal temperature, hematocrit, plasma refractive index, and plasma glucose. After hatch (0 h), chicks from 16 trays were held for 6 h at the hatchery prior to a 2 h transport to a laboratory brooding facility. Therefore, all chicks were deprived of nutrients (feed and water) through 8 h post-hatch. From that time through 48 h post-hatch, 6 birds each from 2 trays were provided feed and water ad libitum (controls), whereas 6 chicks from each of 16 trays were deprived of nutrients (restricted) through 48 h post-hatch. All chicks were provided normal

brooding temperatures through 48 h post-hatch. In comparison to controls, the liver glycogen content in restricted birds was significantly lower at 24 and 48 h post-hatch. Furthermore, at 48 h RLW was lower and BFLS was higher in restricted birds compared to controls. The KPS of restricted birds was not different from that of controls at either 24 or 48 h. In restricted birds, liver glycogen content at 6 h was negatively correlated with RLW and BFLS at 24 h; however, RLW at 24 h was positively correlated with liver glycogen content at 48 h. Liver glycogen content in post-hatch chicks was depressed by 24 h due to nutrient restriction and was related to body fat content and RLW, but was not associated with BW, plasma glucose level, or KPS.

Key Words: Broiler, Glycogen, Restriction

62 Chemosensory responses to gaseous pollutants and Carbon dioxide: implications for poultry welfare? D.E.F. McKeegan^{*1}, T.G.M. Demmers², C. M. Wathes², R. B. Jones¹, and M. J. Gentle¹, ¹*Roslin Institute, Roslin, Midlothian, UK*, ²*Silsoe Research Institute, Silsoe, Bedford, UK*.

There was little understanding of olfactory perception in the domestic fowl, particularly for those gases that are pollutants of poultry houses or are used in gaseous stunning. This study combined electrophysiological and behavioural approaches to identify detection, aversion and pain thresholds for ammonia (NH₃), hydrogen sulphide (H₂S) and carbon dioxide (CO₂). Olfactory responses were investigated by recording the activity of single neurones in the olfactory bulb in response to precise presentations of different gas concentrations to the olfactory epithelium. These showed that the chicken has a well-developed olfactory system with detection thresholds of 2.5ppm for NH₃ and 1ppm for H₂S. There was no olfactory response to CO₂. Recordings from trigeminal nerve fibres arising from the nasal epithelium allowed nociceptor (pain) thresholds for NH₃ to be determined, these ranged from 195ppm to 6250ppm (mean 232ppm). In the behaviour study, hens were exposed to short pulses of gas in a series of ascending concentrations and their responses were classified in terms of those likely to indicate detection or aversion. Detection thresholds for NH₃ and H₂S were similar to those predicted by electrophysiological findings. Aversion to NH₃ was seen at and above 28ppm, well below levels believed to be painful (200ppm). H₂S was avoided above 4ppm. CO₂ was detected at 11% and aversion was seen at 24%. These studies provide physiological and behavioural evidence that chickens can detect gaseous stimulants at concentrations encountered in commercial production systems. At higher concentrations, evidence of aversion was seen in response to all the gases but this did not depend on the stimulus being perceived as painful. These results inform the welfare debate and should enable us to specify those concentrations of gases that could be considered as acceptable exposure limits for poultry.

Key Words: Pollutant gases, Ammonia, Olfaction, Pain, Welfare

63 Growth of broiler breeder males reared on a phytoestrogen-free diet using dried egg white as a source of protein. C. A. Pietsch^{*}, J. B. Hess, R. J. Lien, and W. D. Berry, *Auburn University, Auburn, AL/USA 36862*.

Diets used for rearing broiler breeders in the industry contain soy meal as a protein source. Soy is a source of phytoestrogens that may adversely affect the growth and development of the male reproductive tract. Alternate dietary sources of protein without estrogenic compounds could increase the overall reproductive performance of breeder males by eliminating any negative influence these compounds have on sexual development. The objective of this study was to explore potential developmental differences in development in males reared to sexual maturation on soy free diets. In this study, breeder males were reared to 3 weeks on a normal corn-soy starter diet and then divided into 3 treatments with 2 reps containing 15 birds. The control birds (Con) were fed a normal corn-soy developer diet, the egg white (EW) males were fed an egg white-corn diet, and the mix males (MX) were fed a diet where half the protein was from soy and half from egg white, with a small amount of protein provided by corn. Birds were reared on an accelerated growth program to 16 weeks and then lighting was increased to induce sexual maturation. All treatments were switched to experimental breeder diets at 17 weeks. The study terminated at 20 wk. Plasma samples, organ weights (heart, liver, and testes), and growth measurements (shank and keel) were taken during the study. The EW and MX birds had a higher testes weight as % of body weight at 20 wk. Shank and keel length were similar for all treatments during the trial. Body weight uniformity was significantly higher

for the EW birds, followed by the MX birds, with the Con group having the lowest uniformity. It was concluded that substituting egg white for soybean meal as a protein source in the diet positively influenced uniformity and testes weight. Effects on levels of hormones and LH in the blood are currently being determined.

Key Words: Phytoestrogen, Breeder, Male

64 Reproductive and immunological sensitivity of chickens to phytoestrogen. W. D. Berry^{*}, A. R. Peterson, J. B. Hess, and S. S. Oates, *Auburn University, Auburn, AL*.

Poultry species are exposed to the phytoestrogen genistein in soy diets. Phytoestrogens are known to alter reproductive and immune development in some mammals. Little is known about avian species sensitivity to phytoestrogens. To address this question, experiments were done to determine whether the soy estrogen genistein alters chicken reproductive and immune systems. To examine trophic effects of genistein on the chick oviduct, day old female chicks were assigned to treatments: soy based chick diet with daily subcutaneous injection of sesame oil vehicle (SV); soy-free diet/ vehicle injection (V); daily injection of 0.5 mg diethylstilbestrol (DES); 1.0 mg genistein injection (G1); and 10.0 mg genistein (G10). At 12 days of age, DES treatment increased oviduct and liver weight, 76x and 2x respectively, as compared to vehicle injected control. Oviduct weights of G10 chicks were significantly increased compared to SV. To examine possible immunological effects of genistein in comparison with a known estrogen and known androgen, five groups of day-old chicks received daily oral dosings of diethylstilbestrol (DES), testosterone (T), genistein (G), or corn oil vehicle (V). Control (Con) chicks received a corn/soy chick starter diet. All other chicks received a soy-free corn/casein based starter. 16 day organ weights were recorded. Lymphocyte populations were analyzed by flow cytometry. Chicks receiving genistein had largest % liver weight. DES chicks had the largest spleens. Genistein treatment resulted in the smallest bursa and thymus mass. Flow cytometry indicated a lower B cell population in the G and DES groups. The percent of T- helper cells was lowest in the G group and highest in the testosterone group. Total percentage of T- cells was highest in the V and T group and lowest in the G and DES groups. The results of these studies indicate that soy phytoestrogen can alter both reproductive tissue and the immune system. (Supported by the Alabama Agricultural experiment Station and USDA AD-421 project S-233).

Key Words: Phytoestrogen, Genistein, Oviduct, Immune

65 Age related changes in concentration of skin tissue pentosidine in the Ruffed grouse (*Bonasa umbellus*). J. A. Fallon^{*}, R. L. Cochrane, and H. Klandorf, *West Virginia University, Morgantown, WV/USA*.

There is no reliable marker of age in birds beyond onset of adult plumage. This investigation examines the correlation between age and the accumulation of the advanced glycation endproduct pentosidine (Ps) in skin tissue of captive raised ruffed grouse. Skin samples were taken from the breast of 52 ruffed grouse of known ages ranging from a few days to 10 years. Skin was cleaned of feathers and adipose tissue, acid hydrolyzed and measured for collagen content via hydroxyproline spectrophotometric assay. Ps concentrations were determined using reverse phase HPLC. Fluorescent detection (excitation 310nm, emission 385nm) revealed concentrations of Ps/mg collagen to increase linearly with age ($R^2 = .79$, $p < .0001$). No significant sex differences were detected. These results suggest that this technique can be used to reliably estimate the age of a bird. Accurate age information could aid in understanding population demographics and could greatly improve success rates of species survival programs.

Key Words: Ruffed grouse, Pentosidine, Aging