

Physiology & Endocrinology - Livestock and Poultry: Poultry

313 Changes in zebra finch (*Taeniopygia guttata*) eggshell morphology after oral estrogen exposure as chicks. S. L. Westmoreland*¹, H. Pourarsalan¹, D. H. Hawkins³, J. R. Rochester², and J. R. Millam², ¹The University of Texas at Arlington, Department of Biology and The Center for Electron Microscopy, Arlington, ²The University of California, Department of Animal Science, Davis, ³The University of Texas at Arlington, Department of Mathematics, Arlington.

Environmental estrogens have been implicated in changes in the reproductive performance, song systems, oviduct histology, egg production, and shell breakage in Zebra Finch birds (*Taeniopygia guttata*); we investigated the relationship of estradiol benzoate (EB) to eggshell thickness and morphology. Zebra Finch birds from a breeding colony at The University of California at Davis were fed a mixed seed diet and water *ad libitum* and kept on a 16L: 8D photoschedule. Female chicks were treated for 7 days, from post-hatch days 5-11, with either orally administered estradiol benzoate in canola oil or canola oil alone (control) at 1 microliter/g body weight. (EB was 100 nmol/g body weight); males were treated only with canola oil. At approximately 110 days of age, 5 males and 5 females at a time were moved to a communal cage and provided with nest boxes and nest material to stimulate pair-formation and breeding. They were then removed to individual breeding cages, where eggs were collected. The 25 eggs for this study included 15 eggs from control females and 10 eggs from EB-treated females; all eggs were the second egg laid in the clutch. On the day of collection eggs were split open at the equator, emptied, rinsed with deionized water, and air dried. Three samples were taken from each egg at the equator region and were placed on aluminum stubs. The samples were oriented for a radial (cross section) view of the shell. Stubs were coated with gold and palladium and viewed using scanning electron microscopy. Digital micrographs were made for all shell samples at 500X. Micrographs of shell cross sections were analyzed for shell thickness using Image Pro Plus computer software. These data were statistically analyzed using SAS. The experimental shells were found to be significantly thinner than the control shells ($p = 0.02$, experimental mean = 70.45 μm , control mean = 76.90 μm , SE = 2.58). Future studies will include analysis of shell structure to determine the relationship of shell features to shell thickness and to determine the underlying physiology of shell formation and how it is impacted by estrogen exposure.

Key Words: Avian Eggshell, Estrogen, Endocrine Disruptor

314 Comparison of oral vs. injected dosing of the soy phytoestrogen genistein on the reproductive development of female broiler chicks. L. M. Stevenson*, C. R. James, S. S. Oates, J. B. Hess, and W. D. Berry, Auburn University, Auburn, AL.

Developmentally inappropriate exposures to estrogenic compounds are known to alter morphology and function of the reproductive tract in various species. Chickens are continually exposed to the relatively potent estrogenic soy isoflavones through the diet. Previous experiments in this laboratory have demonstrated that the primary soy isoflavone genistein induces proliferation of the chick oviduct. However, information is lacking as to specific reproductive tract developmental effects of genistein exposure in chicks. Experiments were done to compare the effects of oral exposure and injected

exposure to genistein. The oral and injected effects of genistein were also compared to a classical estrogen, diethylstilbestrol (DES). To avoid the effects of dietary soy isoflavones, the experimental diets were formulated with dried egg white as a protein source. Day old female chicks were assigned to treatments: egg white based diet with daily oral gavage or subcutaneous injection of sesame oil vehicle (CV); 1mg diethylstilbestrol (DES); 10mg genistein (G10) or 40mg genistein (G40). There was also a treatment fed a standard starter diet with daily oral gavage or subcutaneous injection of sesame oil vehicle (SV). At 15 days of age, the injected treatments increased the absolute oviduct weight and oviduct weight as a percentage of final body weight as compared to the oral treatments ($P < 0.05$). The DES treatments, both oral and injected, increased the absolute oviduct weight and oviduct weight as a percentage of final body weight as compared to all other treatments ($P < 0.05$). The injected treatments of G40 and DES showed adult hen behaviors, marked development the right oviduct, and increased the absolute oviduct weight and oviduct weight as a percentage of final body weight as compared to the CV and SV treatments ($P < 0.05$). The injected DES treatment showed partially developed right oviducts. The injected G40 treatment showed membranous cystic right oviducts. It was concluded that injected doses of genistein elicit a stronger estrogenic response in the developing female chick as compared to oral doses.

Key Words: Genistein, Oviduct, Phytoestrogen

315 Analysis of plasma serotonin levels and hemodynamic responses following chronic serotonin infusion in broilers challenged with bacterial lipopolysaccharide and microparticles. M. E. Chapman*¹, R. L. Taylor², and R. F. Wideman¹, ¹University of Arkansas, Fayetteville, ²University of New Hampshire, Durham.

There has been much interest in the role of serotonin (5-hydroxytryptamine, 5-HT) in the pathogenesis of pulmonary hypertension due to episodes of pulmonary arterial hypertension (PAH) in humans linked to serotonergic appetite suppressant drugs. In this study, we investigated the role of serotonin in the development of pulmonary hypertension induced by intravenously injecting bacterial lipopolysaccharide (LPS, endotoxin) and cellulose microparticles. In experiment 1 we employed a 5-HT ELISA kit for the in-vitro quantitative determination of 5-HT in plasma during the development of pulmonary hypertension induced by injecting 1 mg LPS and 0.35 mL cellulose microparticles suspended at 0.02 g/mL in saline i.v. in broilers ($n = 240$). In experiment 2 broilers were either chronically infused with 5-HT (10 mg/mL) via surgically implanted osmotic pumps designed to deliver 5 $\mu\text{L/hr}$ for 14 d or received sham surgery as a control ($n = 40$). After a period of 10 d, the pulmonary arterial pressure (PAP) was recorded during challenge with injected LPS or microparticles. Microparticles elicited plasma levels of 5-HT more than two-fold higher than those elicited by LPS from 15-45 min post injection ($P \leq 0.05$). This indicates that 5-HT is an important mediator in the pulmonary hypertensive response of broilers to microparticles, but 5-HT may not play a prominent role in the pulmonary hypertensive response to LPS. Furthermore, chronic 5-HT infusion via osmotic pumps caused an increase in the duration of the pulmonary hypertensive response of broilers to microparticles indicating that the infused 5-HT was sequestered by circulating thrombocytes and then released upon

microparticle mediated thrombocyte activation. Serotonin appears to play a less prominent role in the pulmonary hypertensive response of broilers to LPS indicating that other mediators within the innate response to inflammatory stimuli may also be involved. These results are consistent with our hypothesis that PAH ensues when vasoconstrictors such as 5-HT overwhelm the dilatatory affects of vasodilators such as nitric oxide (NO), thereby effectively reducing the pulmonary vascular capacity of PAH-susceptible broilers.

Key Words: Hypertension, Broiler, Serotonin

316 Chicken visfatin: The leaner side of an adipokine.

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The adipokine visfatin (VFN), also known as pre-B-cell colony-enhancing factor 1, has been shown in mammals to be preferentially secreted by visceral fat, and elicits insulin-mimetic effects on glucose metabolism. Increased visceral fat and hyperglycemia have been correlated with higher plasma VFN levels in mammals. However, the expression of VFN in chickens, a naturally occurring hyperglycemic model, has not yet been described. The objective of the present study was to determine the level of VFN gene expression in the primary metabolic tissues, adipose, liver, and skeletal muscle, as well as to determine how this expression is altered with age in male broilers. We hypothesized that VFN gene expression in broiler chickens would be greatest in the abdominal fat pad and would change with age and/or fat accretion. Using RT-PCR and western blotting, we detected VFN mRNA and protein, respectively, in adipose, liver and skeletal muscle of male broilers. Further analysis with real-time quantitative PCR revealed that skeletal muscle had significantly greater amounts of VFN mRNA ($P < 0.05$; $n = 5$) compared with adipose and liver. To determine the influence of body composition and adiposity on VFN mRNA expression, adipose, liver and skeletal muscle were collected from 4 week and 8 week old male broilers. Quantification of VFN mRNA revealed significantly greater expression in skeletal muscle, regardless of age, with the greatest level being found in 8 week old broilers ($P < 0.05$; $n = 5$). No significant difference, however, was found in blood glucose levels at either age. Collectively, these findings indicate that skeletal muscle is one of the primary sources of VFN in chickens, in contrast to mammals where VFN is primarily expressed in visceral adipose tissue. Our data indicates that skeletal muscle VFN gene expression may be influenced by body composition and fat accretion. We propose that VFN is involved in regulating energy metabolism in broiler chickens.

Key Words: Visfatin, PBEF1, Adipokine

317 Transpulmonary pressure gradient verifies pulmonary hypertension is initiated by increased arterial resistance in broilers.

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Previous hemodynamic evaluations demonstrated that pulmonary arterial pressure (PAP) is higher in broilers that are susceptible to pulmonary hypertension syndrome (PHS, ascites) than in broilers that are resistant to PHS. We compared key pulmonary hemodynamic parameters in broilers from PHS-susceptible and PHS-resistant

lines (selected under hypobaric hypoxia), and in broilers from a relaxed (control) line. Data were compared using one-way ANOVA by group/anatomical segment to compare data between and within lines, respectively. In experiment 1 the PAP was measured in male broilers in which a flow probe positioned on one pulmonary artery permitted the determination of cardiac output (CO) and pulmonary vascular resistance (PVR). The PAP and relative PVR were higher in the susceptible (34.3 ± 2.3 mm Hg and 0.11 ± 0.01 resistance units, ru) than in relaxed (22.4 ± 1.4 mm Hg and 0.08 ± 0.01 ru) and resistant broilers (24.4 ± 1.1 mm Hg and 0.08 ± 0.01 ru), whereas CO did not differ between lines. In experiment 2 male and female broilers from the three lines were catheterized to measure pressures in the wing vein, right atrium, right ventricle, pulmonary artery and pulmonary veins (WP, wedge pressure). The transpulmonary pressure gradient (TPG) was calculated as (PAP-WP), with PAP quantifying precapillary pressure and WP approximating post-capillary pulmonary venous pressure. When compared with resistant and relaxed broilers, PAP values in susceptible broilers were >10 mm Hg higher, TPG values were >8 mm Hg higher, and WP values were <2 mm Hg higher, regardless of sex. The combined hemodynamic criteria (elevated PAP and PVR combined with a proportionally elevated TPG) demonstrate that susceptibility to PHS can be attributed primarily to pulmonary arterial hypertension associated with increased pre-capillary (arteriole) resistance rather than to pulmonary venous hypertension caused by elevated post-capillary (venous and left atrial) resistance.

Key Words: Broiler, Pulmonary Arterial Hypertension, Transpulmonary Pressure Gradient

318 Cloning and characterization of chicken nucleobindin-2 (NUCB2) cDNA: The precursor for a putative anorexigenic peptide, nesfatin-1.

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Nucleobindin-2 (NUCB2) is a secreted protein that regulates intracellular Ca^{2+} and promotes bone maturation in mammals. Recently, NUCB2 has been characterized as a precursor molecule for three peptides (nesfatin 1-3), of which nesfatin-1 has been shown to suppress feed intake in rats through a leptin-independent melanocortin signaling pathway in the hypothalamus. However, a chicken homologue of NUCB2 has not been previously described. The objectives of the present study are to clone NUCB2 cDNA, characterize the deduced protein sequence for identifying nesfatin 1-3, and quantify NUCB2 gene expression in the diencephalon and adipose tissue in male broiler and leghorn chickens. Using RT-PCR, we found that NUCB2 mRNA is expressed in broiler diencephalon, myocardium, pituitary gland, skeletal muscle, liver, spleen, kidney, testes, and adipose tissue. For further characterization, we cloned and sequenced the full-length NUCB2 cDNA from broiler adipose tissue and found its deduced amino acid sequence to be 76.7% similar to human NUCB2 protein. The chicken NUCB2 protein sequence contained three cleavage sites, similar to human NUCB2 protein that may yield nesfatin 1-3. The putative chicken nesfatin-1 and nesfatin-2 amino acid sequences are 78% and 85% similar to that of rat, respectively. Using real-time quantitative PCR, significantly greater NUCB2 mRNA quantities were found in the abdominal fat of 8 week-old male broilers compared with male leghorns ($P < 0.05$; $n = 4$). No significant difference, however, was found in NUCB2 mRNA quantity in the diencephalon of broiler versus leghorn chickens. Based on these findings, we conclude that the

NUCB2 is expressed in multiple chicken tissues, which may possibly yield nesfatin as in mammals, and that NUCB2 gene expression is influenced by fat accretion in the adipose tissue. However, a physiological role of NUCB2 or nesfatin on feed intake in broilers remains to be studied.

Key Words: Nucleobindin, Nesfatin, Broiler

319 Gene expression in the lateral septal organ, mediobasal hypothalamus and septal-pituitary-gonadal axis following activation of the photoneuroendocrine system. H. Li^{*1}, J. A. Proudman², S. Jin¹, and W. J. Kuenzel¹, ¹University of Arkansas, Fayetteville, ²USDA/ARS/BGL, Beltsville, MD.

Long day stimulation releases gonadotropin-releasing hormone (GnRH-1), gonadotropins, and induces testes growth in some wild and domestic avian species. Encephalic photoreceptors (EPRs) have been posited to detect photoperiodic changes. Two distinct groups of cerebrospinal fluid contacting neurons (CSFcn) residing in the mediobasal hypothalamus (MBH) and lateral septal organ (LSO) have been proposed to serve as EPRs with the former site thought to be the most likely candidate due to the presence of clock genes shown in quail. The current study examined possible roles for both the MBH and LSO in the avian photoneuroendocrine system. The hypothesis tested was that brain areas first showing expression for genes associated with photoreceptors should be the most likely region/s for housing EPRs. Gene expression profiles analyzed included vasoactive intestinal polypeptide (VIP), phosphodiesterase (PDE), type II deiodinase (D2), GnRH-1 and gonadotropins. Sulfamethazine (SMZ) mixed in the feed at 0.2% plus a long-day photoperiod was utilized to stimulate rapid gonadal development. Real time qRT-PCR (SYBR Green or TaqMan) was used to quantitate mRNA transcripts. SMZ augmented and temporally advanced the stimulatory effects of long day on gene expression of VIP, PDE, and GnRH-1. The first elevated expression of genes occurred in the LSO including VIP at 4h and PDE at 6h, followed by GnRH-1 in the bed nucleus of the pallial commissure (NCPa) at 4-8h after SMZ administration. Treatment of SMZ had no effect on VIP gene expression in the MBH within the first 8h. The long day treatment stimulated VIP gene expression at 12h in the LSO and MBH, GnRH-1 in the NCPa and D2 in the MBH. Taken together, the current study suggests that the LSO contains neurons displaying the earliest gene expression characteristic of EPRs and is followed by a neuroendocrine cascade responsible for rapid gonadal development.

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Key Words: Encephalic Photoreceptors, Sulfamethazine, Chicks

320 Study of the effects of blindness on sexual maturation in Smoky Joe roosters. J. Perttula* and G. Bedecarrats, University of Guelph, Guelph, ON, Canada.

In domestic chickens, sexual maturation is generally induced by increasing the photoperiod. Stimulation of hypothalamic photoreceptors triggers the secretion of gonadotropin releasing hormone which in turn induces the synthesis and release of gonadotropins by the pituitary gland. Increasing levels of gonadotropins then stimulate the development and maturation of the gonads. In addition to this stimulatory axis, it has recently been proposed that an inhibitory

pathway involving melatonin produced by the retina and pineal gland may exist. Using a genetically blind line of chickens, we previously showed that the lack of retinal stimulation advances egg-laying in hens. To determine if blindness also influences sexual maturation in males, 54 Smoky Joe roosters (24 blinds and 30 sighted) were raised under an 8 h photoperiod until 17 weeks of age, then stimulated with a 14 h photoperiod. At 14, 17, 18, 19, 21, and 23 weeks of age, 4 blind and 5 sighted birds were sacrificed, body weight as well as comb length were measured, and blood and various tissue were collected. Relative testicular weight was then calculated and total testosterone concentrations were measured in plasma by ELISA. With the exception of week 21, no difference in body weight was observed between blind and sighted roosters or between collection dates. In blind birds, comb length significantly increased between 14 and 17 weeks of age ($p < 0.001$) while growth was more gradual in sighted ones. Similarly, testes were significantly larger in blind than sighted roosters at 17 weeks of age (sighted: $2.92 \pm 0.58\%$ of body weight ; blind: $6.43 \pm 1.43\%$; $p < 0.05$). However, following photostimulation, no significant difference could be observed. Interestingly, no significant difference in testosterone plasma concentration was observed between blind and sighted roosters or between collection dates. However, this could be explained by the small number of individuals used and the large standard deviations obtained. In conclusion, our study shows that blindness advances sexual development in roosters before photostimulation. In addition, these results suggest that signals originating from the retina may inhibit the reproductive axis in normally sighted birds.

Key Words: Blindness, Rooster, Reproduction

321 Dopamine-melatonin neurons in the turkey hypothalamus controlling seasonal reproduction. S. Kang*, A. Thayananuphat, T. Bakken, and M. El Halawani, University of Minnesota, St Paul.

The neural and neurochemical substrate mediating the reproductive photoperiodic time measurement (PTM) in birds has not been definitively established. Our previous studies have shown that a 30 min light pulse induced c-fos mRNA expression in dopamine (DA) neurons within the premammillary nucleus (PMM) of the turkey caudal hypothalamus, as well as in GnRH-I neurons of the anterior hypothalamic/pre-optic area, where GnRH-I mRNA was also found. Double-label immunocytochemistry (ICC) showed these PMM neurons to be immunoreactive (ir) to both tyrosine hydroxylase (TH; the rate limiting enzyme in DA biosynthesis) and melatonin (MEL). Moreover, the intensity of MEL staining appeared greater in brain sections obtained during night than day. We have shown that mRNA expression of TH and tryptophan hydroxylase 1 (TPH1; the first enzyme in MEL biosynthesis), cycle rhythmically and with opposite phases in the PMM neurons of birds kept under a diurnal illumination cycle (12-h light: 12-h dark; LD). These neurons can generate 24 hr TH and TPH1 mRNA expression rhythms in constant light (LL) and constant dark (DD). In addition, the expression patterns and amplitudes of TH and TPH1 mRNAs were different between long and short photoperiods. It is suggested that endogenous oscillators within PMM neurons appear to be important in regulating the DA and MEL rhythms which are required to drive the circadian system controlling reproductive seasonality in turkeys.

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Key Words: Dopamine Melatonin, Avian Photoperiod, Seasonal Reproduction

322 Lipoic acid-induced changes in food intake in chickens. D. M. Denbow* and P. B. Siegel, *Virginia Polytechnic Institute and State University, Blacksburg.*

The enzyme AMP-activated protein kinase (AMPK) is believed to serve as an "fuel gauge" monitoring the energy level in the body. It may function both within and outside the central nervous system. Using a line of chickens selected for either low- or high-eight week body weight, we investigated whether altering the activity of this enzyme affected food intake, and whether genetic selection for high or low body weight altered the effect of lipoic acid. Lipoic acid is known to inhibit AMPK. Therefore, lipoic acid was injected either intraperitoneally (IP) or intracerebroventricularly (ICV) to determine its effect on food intake in both lines of birds. Food intake was monitored for 3 or 24 hours postinjection following ICV or IP injections, respectively. The ICV injection of 0, 12, 24 or 48 µg lipoic acid dose-dependently increased (P<0.05) food intake in 7 week-old high weight male birds while having no effect in 15 week-old low weight male birds. The IP injection of 0, 50, or 100 mg/kg BW of lipoic acid decreased (P<0.05) food intake in 11-15 week-old males of both lines. Therefore, altering AMPK activity can affect food intake in chickens.

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Key Words: AMP-Activated Protein Kinase

323 Clock gene expression in the premammillary nucleus (PMM) and the pineal gland of turkey hens. B. Leclerc*¹, S. Kang¹, A. Thayananuphat¹, C. Howell¹, S. Kosonsiriluk², Y. Chaiseha², and M. E. El Halawani¹, ¹University of Minnesota, St. Paul, ²Suranaree University of Technology, Thailand.

Recent findings from our laboratory have implicated the PMM as a site of putative photoreceptive neurons. These neurons are shown to express both dopamine (DA) and melatonin (MEL), with DAergic activity up regulated during the light phase and MELergic activity during the dark phase of the light-dark illumination cycle. These neurons reach threshold activation (as indicated by c-fos mRNA expression) when a light period is provided during the photosensitive phase (14hr after light on). And, this is coincided with the activation of gonadotropic releasing hormone-I (GnRH-I) and the upregulation of GnRH-I mRNA expression. It is hypothesized that PMM DA-MEL neurons may be a component of a biological clock involved in reproductive photoperiodic time measurement (PTM), controlling seasonal reproduction in turkeys. In this study we cloned turkey's clock genes including Clock, Per2, Per3, Bmal1, Cry1 and Cry2 and examined their expression in the PMM which was compared to that expressed by the pineal gland. Turkey hens maintained on short photoperiod (6L:18D) were subjected to a 30 min light pulse at circadian times (CT) 8, 14 and 20. Tissues were collected 30 min, 1

hour and 3 hours following the onset of the light pulse. In the pineal gland, Per2 mRNA expression level was highest followed by mRNA expression of Cry1, Cry2, Per3, Clock and Bmal1. However, Per2 gene was not significantly modulated by light (one-way ANOVA; P>0.05) across all CTs. The expression of Per2, Cry1 and Cry2 genes was also examined in the PMM of turkeys following the 30 min light exposure. The expression of Cry1 and Per3 transcripts was enhanced 2-3 fold by the 1 hour light pulse at CT14 and CT20 and both were statistically significant by the ANOVA (P<0.05) and confirmed by the Tukey-Kramer test. It is not clear at this time whether clock genes are involved in mediating photic information to the reproductive neuroendocrine system of turkeys.

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Key Words: Clock Genes, Turkey, Hypothalamus

324 The expression patterns of HIF 1 α , HYOU1, HO1, and cTnT during embryonic development in the chicken heart. S. Druyan*¹, A. Cahaner², and C. M. Ashwell¹, ¹North Carolina State University, Raleigh, ²Hebrew University, Rehovot, Israel.

Oxygen is one of the critical determinants of appropriate embryonic and fetal development including cardiogenesis. When tissues demand for oxygen exceeds oxygen supply, hypoxic conditions develop. In the developing embryo, hypoxia is associated with increased fetal mortality, cerebrovascular anomalies, cardiovascular dysfunction and altered angiogenesis. In this study 4 genes: hypoxia inducing factor subunit a - 1 (HIF 1 α), hypoxia up regulated protein 1 (HYOU1) also known as ORP150, heme oxygenase 1 (HO1) and cardiac troponin T (cTnT), were examined in the embryonic heart of the chicken to determine the expression patterns throughout development. The effect of embryonic age on gene expression was determined by real-time quantitative PCR normalizing to the level of GAPDH. On embryonic day 7 (E7) all three hypoxic induced genes were expressed at their highest levels evaluated, likely due to the fact that the yolk sac is the principal gas exchange organ and the origin of the primitive red blood cells. All three hypoxic induced genes expression significantly decreased from d E7 to E19 (internal pipping) followed by a significant increase in expression between internal pipping and external pipping (E20). During this period a gradual hypoxia and hypercapnia develop due to the decline in the allantois gas exchange the embryo metabolic requirement depends on the lungs, as the new breathing organ, thus limiting the O₂ supply. As expected cTnT expression increased with embryonic development in correlation with the cardiovascular system development. It appears that tissue hypoxia is a necessary component of normal embryonic development.

Key Words: Gene Expression, Hypoxia, Incubation

Production, Management & the Environment - Livestock and Poultry: Broiler and Broiler Breeder Production and Management

325 Dosing with the fatty acid, sodium caprylate in the water did not reduce enteric *Campylobacter* concentrations in broilers. J. H. Metcalf*¹, K. Venkitanarayanan², F. S. de los Santos¹, A. M. Donoghue³, M. L. Dirain¹, I. Reyes-Herrera¹, V. Aguiar¹, P. Blore¹, and D. J. Donoghue¹, ¹University of Arkansas, Fayetteville, ²University of Connecticut, Storrs, ³PPPSRU, ARS, USDA, Fayetteville, AR.

Campylobacter is a leading cause of foodborne disease and poultry is an important vector for this pathogen. Water additives are one possible method to reduce Campylobacter concentrations within preharvest poultry. Previous research in farm animals using the medium chain fatty acid, sodium caprylate, demonstrated the potential to reduce enteric pathogens. To determine the ability of sodium caprylate to