M1 Immunotherapeutic effects of different extracts (hot water, methanolic and polysaccharide) of mushroom (Lentinus edodes) against coccidiosis in chicken

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1-Purpose for the experiment:

Immunotherapeutic effects of mushroom extracts have been widely studied in various experimental models including mice, rats and rabbits against several diseases with encouraging results; though limited studies in this regard has been conducted in avian birds. Keeping in view, the present study reports the immunotherapeutic effects of different extracts including conventional extracts (hot water and methanolic) and purified extract (polysaccharide) of mushroom Lentinus edodes and their subsequent therapeutic efficacy against Eimeria infection in chicken.

2-Experimental Design

- Mushrooms (Lentinus edodes) were processed for hot water (HWE), methanolic (ME) and polysaccharide (PSE) extracts. Polysaccharides were isolated through ion exchange (DEAE cellulose) and size exclusion (Sephadex G-100) chromatography. Monosaccharides including maltose (0.282 %), glucose (0.113 %) and mannose (0.451 %) were qualitatively and quantitatively identified from isolated polysaccharides through HPLC. These extracts (HWE, ME and PSE) were administered to their respective groups in chicken (5 days old). Cell mediated and humoral immune responses were demonstrated through lymphoproliferative response to Phytohaemagglutinin-P (PHAP) and antibody response to sheep RBCs, respectively. Cell mediated immune responses observed at 48 and 72 hours post administration of PHAP in all three extracts (PSE, ME and HWE) were statistically significant (P<0.05) in comparison to control. Statistically significant antibody titers (Ig, IgG and IgM) were observed at day 7 and 14 post administration of sheep RBCs. After experimental infection of mixed species of Eimeria, birds were monitored for oocyst per gram (OPG) of droppings, lesion scoring and percent protection against Eimeriasis. Significantly higher (P<0.05) OPG and lesion scorings were observed in control as compared to extract administered groups (HWE, ME and PSE). Significantly higher (P<0.05) per cent protection against Eimeriasis was also observed in all groups administered with different extracts (HWE, ME and PSE) of Lentinus edodes.

4-Conclusion

The results revealed that different extracts (HWE, ME and PSE) of mushroom (Lentinus edodes) have immunotherapeutic potential and can be used as a potent immunomodulator in chicken.

Key Words: Mushroom, Lentinus edodes, chicken, polysaccharides, Eimeriasis

M3 The Development of a Transgenic Chicken Line by Sperm-Mediated Gene Transfer

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An experiment was conducted to develop a transgenic chicken line using the sperm-mediated gene transfer (SMGT) technique. The plasmid pUC18 (2686 bp) was used as the exogene and two random-bred local chicken lines were used as the parental flocks. The experiment was designed, so that the plasmid DNA was incubated with the rooster semen in the presence of lipofection at different concentrations. Lipofectin was used to facilitate the sperm cell uptake of the plasmid. Hens were inseminated with the semen incubated with the plasmid DNA and lipofectin and their eggs were hatched. Polymerase chain reaction (PCR) was performed on spermatozoa genomes of the parental flocks and on blood genomes of the parental and progeny flocks of both chicken lines. The results indicated that the plasmid DNA was highly fused into the sperm cells in the presence of lipofectin (5% concentration) and this was shown in both chicken lines. Also, the plasmid DNA band was highly amplified in the progeny that have been derived from sperm cells incubated with the plasmid and lipofectin (5% concentration). The results revealed the success of the development of F1 chickens by SMGT. The positively detected SMGT-derived offspring in both chicken lines formed 40.0 to 50.0% of the F1 generation, and were mated to obtain the F2 generation. PCR was applied to the blood genomes of the F2 individuals and the plasmid DNA was successfully recognized announcing the formation of the transgenic chicken line.

Key Words: Transgenic chickens, Sperm-mediated gene transfer (SMGT), Rooster sperm, SMGT-derived offspring, Lipofectin

M4 Composition of mid-cycle turkey eggs as a function of weight and progression through the day

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During egg formation, the maximal mobilization of protein occurs early after lighting, while the greatest requirement for calcium occurs during shell assembly in the uterus prior to darkness. A complete feed attaining requirements was used to examine possible alteration of egg composition as day length progressed. Caged 42- wk-old Diamond White hens at 55% production supplied one day’s egg collection while receiving corn-soy pellets (16.6% CP, 2.75 kcal ME/g, and 2.35 % Ca) and 16 hrs of 70 lux light. The first 12 eggs appearing at the beginning of each 4 hr light period were individually weighed and partitioned into heavy, medium and light wt relative each group’s median. Manual separation of yolk and
shell conducted after 1 hr at 15°C was followed by 1 min sieving (20mm) of albumen to separate outer thin from thick and inner thin. A temperature compensated refractometer measured protein while yolk was lyophilized for Kjeldahl N, EE and ash. Differences between heavy (88.0), medium (80.3) and light (74.0) g of all eggs arose by their wt selection. Yolk decreased as a proportion of egg as wt increased (32.6, 31.2, 29.3% L, ***), while albumen increased (56.1, 59.1, 60.6% L, *). Increasing albumen with egg wt was largely due to outer thin (22.1, 20.1, 15.9% L ***), while the converse for inner thin (14.5, 16.7, 18.2% L, ***) and thick (23.7, 22.5, 26.0% L, *). Albumen protein increased with egg wt for outer thin (10.8, 10.9, 11.3% L *), inner thin (11.6, 11.7, 12.1% L *) and thick (11.2, 11.3, 11.8% L *). Yolk moisture increased with time laid (46.8, 47.0, 47.7, 47.7% L **), whereas all other dry wt analyses were similar among wts and times of collection. Although average egg wt decreased as time of collection progressed (80.9, 80.8, 80.7, and 80.6 L *), differences with time could be detected. Differences in eggs selected to be light, medium or heavy wt were apparent, but modifications arising with time of day could not be discerned when offered a complete pellet.

**Key Words:** albumen, turkey hen, egg composition, yolk

M5 Interaction of resident sperm with sperm-storage tubule (SST) epithelial cell microvilli in the turkey hen Murray Bakst*, Charles Murphy Agricultural Research Service, USDA, Beltsville, MD

Little is known regarding the cellular and molecular mechanisms responsible for sperm subsistence in the lumen of the SST. Using transmission electron microscopy (TEM) and differential interference contrast microscopy (DIC) we examined the relationship between resident sperm the microvilli on the apical surface of the SST epithelial cell. For TEM, UVJ mucosa containing SSTs were isolated from Hybrid turkey breeders at 46 (n=6) and 51 (n=2) wks of age and fixed in 2% glutaraldehyde/glutaraldehyde-Kar- nosky’s fixative (paraformaldehyde/glutaraldehyde-mix, respectively). Sections of plastic embedded tissue from both age groups were examined by bright field microscopy while squash preparations of unfixed UVJ from the 46 wk old hens were examined by DIC. Although the older hen group had fewer sperm in their SSTs, observations of the SSTs with and without resident sperm were generally consistent with previous reports. However, in the younger group, TEM cross sections of SSTs revealed two remarkable features: the microvilli were intimately associated with resident sperm; and, blebbing of the apical tips of some microvilli. Furthermore, small, membrane bound vesicles, possibly originating from the blebs, were in contact with the plasmalemmae of resident sperm. While less prevalent in the older hen group, microvilli blebbing was observed in SSTs with and without sperm. We do not believe the microvilli blebb- bing was an artifact as the mitochondria, which are highly susceptible to preparation artifacts, in the same cells as the blebs appeared normal. We hypothesize that the blebbing represents a form of apocrine secretion providing lipid material via the small membrane bound vesicles for sperm sustenance and maintenance during storage. We are currently inves- tigating whether the sperm-microvilli interactions in the SST lumen is a receptor mediated function similar to that seen in mammalian oviduct.

**Key Words:** SST, ultrastructure, turkey, oviduct

M6 Detection of parthenogenesis in mated quail hens and its impact on fertilization Priscila Santa Rosa*, Holly Parker, Aaron Kiess, Chris McDaniel Mississippi State University, Poultry Science Department, Mississippi State, MS

Parthenogenesis, embryonic development in unfertilized eggs, resembles very early embryonic mortality in fertilized eggs. Parthenogenesis alters egg albumen ionic and gas concentrations in virgin quail (PV) hens genetically selected for parthenogenesis. Also hatchability of these PV hens once mated (PM) is reduced compared to control mated (CM) hens that have not been genetically selected for parthenogenesis. However, it is unclear if parthenogenesis is actually occurring in PM hens and therefore reducing hatchability or if this reduction is due to infertility. Sperm-egg penetration (SEP) holes are indicative of fertilization and may be useful in identifying if un-hatched eggs from PM hens are unfertilized eggs exhibiting parthenogenesis or early dead embryos from fertilized eggs. Also, SEP may be useful in determining if genetic selection for parthenogenesis impacts the ability of the egg to be fertilized. Therefore, the objectives of this study were to determine if parthenogenesis occurs in mated hens by comparing albumen from PV and PM eggs as well as to determine if SEP is different between PM and CM hens. Daily, PV and PM eggs were incubated for 10 d then broken out to determine parthenogenesis, embryonic death and albumen characteristics. Also, fresh PM and CM quail eggs were macroscopically examined and classified as fertile, no development or parthenogen and then microscopically examined for SEP. Incubated eggs classified as parthenogens from both PV and PM hens yielded similar ionic and gas albumen concentrations. Also, for both PV and PM incubated eggs, parthenogenesis decreased albumen pH, O2 and protein yet increased Ca2+ and CO2 when compared to eggs with no development. For incubated PM eggs, albumen pH and O2 were lower, yet CO2 was higher for eggs containing parthenogens or early dead em- bryos versus eggs with no development. Also fresh eggs from PM hens classified as no development or parthenogen had similar SEP holes and only about one sixth as many SEP holes as eggs classified as fertile. On average, fresh CM eggs had 3.5 times as many SEP holes as PM eggs. In conclusion, parthenogenesis is apparently occurring in mated quail hens and is in fact reducing sperm penetration. Additional research is needed to determine if this reduction in SEP is due to the male, female or both.

**Key Words:** Parthenogenesis, pH, fertilization, sperm-egg penetration, embryonic development

M7 Body Weight and heterosis of Parental Lines and F1 and F2 Crosses of Japanese Quail Selected for 28-Day Body Weight James Mason*, Nick Anthony University of Arkansas, Fayetteville, AR

Experiential learning methods are designed to build knowledge and problem solving skills by placing the students in an unfamiliar environment outside of the classroom. To utilize this teaching method four students from the University of Arkansas Poultry Breeding Class were tasked with generating a data set to quantify non-additive genetic variation from diverse populations of Japanese quail. Since a semester is only 16 weeks long it was necessary to stage populations of Japanese quail prior to the start of the semester. The parental populations used in the study originated from the University of Georgia, selected by Dr. Henry Marks and The Ohio State University, by Dr. Karl Nestor. Pure line populations were maintained, while sire x dam crosses were made resulting in two F1 populations, Ohio x Georgia, and Georgia x Ohio. These populations were then maintained and crossed resulting in all four F2 offspring combinations. The students prepared the respective matings, pedigree collected and hatched the eggs. Birds were banded and chick weights were collected. Although the semester was not long enough to collect reproduction data the students were able to obtain weekly body weights. Parental lines and F1 offspring were used to assess heterosis while F2 offspring were used to calculate percent recombination from the F1 as well as maternal and paternal effects. Heterosis for body weight was present with a high of 18.21 percent at one week and a low of -5.02 percent at hatch. In general, heterosis behaved as expected with increased heterosis at early ages, then decreasing as age increased. Recombination peaked at 9.85 percent at hatch, with a low of -4.15 percent at two weeks of age. Recombination did not appear to follow any general trend. Significant maternal effects were present at hatch, but were not significant from week one onward. No significant paternal effects were found. The results of this project provided the students with a better understanding of the topic since there was a clear understanding of the data origin.

**Key Words:** experiential learning, Japanese quail, body weight, selection, nonadditive variance
M8 Pretreatment primary macrophages (Mφ) with Bacillus subtilis but not mannan oligosaccharide increases nitric oxide production after Salmonella enteritidis challenge. Marta Viguie\textsuperscript{1,2}, Kimberly Livingston California Polytechnic State University, San Luis Obispo, CA

Direct-fed microbials (DFM) including probiotic and prebiotic additives have been shown to improve growth and feed conversion when incorporated into animal diets. Additionally improvements in the ability of the animal to withstand challenges from bacteria have been observed. However, the mechanisms by which the additives improve immunological response have not been fully elucidated. The objective of this experiment was to better understand how DFMs may alter macrophages (Mφ) ability to clear a Salmonella enteritidis (SE) challenge. Primary Mφ were isolated from chickens and pretreated for 24 h with Bacillus subtilis (BS; 4500 CFU/ml), and/or mannan oligosaccharide (MOS; 0.075 ug/ml), extracted from the cell wall of Saccharomyces cerevisiae. Mφ were then challenged with SE for 1 h. Nitric oxide(NO) in the supernatants was evaluated. In addition culture lysates were evaluated to determine Mφ killing ability of SE. Pre-treatment of Mφ with BS or MOS did not improve SE killing as determined by plating the lysates and counting CFU. However, Mφ pretreated with BS did have greater NO production than Mφ pretreated with MOS or left alone (P<0.001). In conclusion, BS does appear to have a direct effect on Mφ when co-cultured in-vitro, which leads to increased NO production when challenged with SE.

Key Words: Direct fed microbial, Macrophage, Salmonella enteritidis, Nitric Oxide

M9 Physiological responses of broiler hatchlings to commercial in ovo coccidiosis vaccine administered on days 18.5 and 19.0 of incubation Adebayo Sokale\textsuperscript{2,3}, E. David Peebles\textsuperscript{1}, Wei Zhai\textsuperscript{1}, Timothy S. Cummings\textsuperscript{2}, Christopher J. Williams\textsuperscript{1} Mississippi State University, Mississippi State, MS; \textsuperscript{2}Zoets, Durham, NC

The physiological responses of broiler hatchlings to the EM-1 coccidiosis vaccine delivered on 18.5 or 19.0 days of incubation (DOI) were determined. Commercial EM-1 vaccine containing live oocysts of E. acervulina, E. maxima, and E. tenella was injected in ovo using an automated multiple-egg injector. Fertile Ross × Ross 708 broiler hatching eggs from a 48-wk-old breeder flock were subjected to one of the following 3 treatments (Trt) administered on 18.5 and 19.0 DOI. Trt 1: non-injected control; Trt 2: diluent-injected control; and Trt 3: EM-1 vaccine-injected group. The main and interactive effects of Trt and DOI on hatchability of fertile eggs (HF), hatching BW (HBW), yolk-free BW (YFBW), yolk sac weight (YSW), and relative intestine weight (RIW) were determined. Site of injection (SOI) and embryonic stage (ES) scores were also determined on 18.5 and 19.0 DOI. Data were analyzed by the MIXED procedure of SAS 9.3. There were no significant Trt or DOI effects on HF. There was a significant main effect due to DOI on HBW (P = 0.002) and YSW (P = 0.001), with HBW and YSW being higher in the 18.5 DOI group, but there were no significant Trt or DOI effects (P = 0.06) on YFBW. There was a significant Trt × DOI interaction for RIW (P = 0.05), with RIW being highest in chicks injected with diluent on 19.0 DOI. Mean ES scores of 18.5 and 19.0 DOI embryos were 2.44 and 3.24, respectively, and were found to be significantly different (P = 0.001). The SOI result showed that vaccine deposition occurred in the amnion in 88.2% and 73.1% of embryos injected on 18.5 and 19.0 DOI, respectively. In conclusion, the vaccine was more precisely deposited in the amnion on 18.5 DOI when compared to 19.0 DOI and developmental (e.g., HBW and YSW) differences between 18.5 and 19.0 DOI broiler embryos can affect the subsequent response of hatchlings to the EM-1 coccidiosis vaccine.

Key Words: amnion, broiler, coccidiosis, hatchlings, in ovo injection

M10 Avian Coccidiosis: Have we managed the antiooccidials to optimize their effectiveness? Steve Fitz-Coy* Merck AH, Salisbury, MD

Summary: Birds of all ages are susceptible to one or more species of coccidia, unless complete immunity is established. The prevalent Eimeria species in commercial broiler houses are E. acervulina, E. maxima, E. tenella and E. mivati and occasionally other species such as E. praecox are found. Although the concept of using live coccidia vaccine for the control of coccidiosis was introduced in 1950’s it was the pharmaceuticals that became the preferred choice. The synthetic class of drugs was used extensively during the early period; however, the development of resistant coccidia became the major issue. The ionophores followed, they were efficacious and the development of resistance was slower. Over time the ionophores became the core products for the control of coccidiosis. With extensive usage of the ionophores there is clear evidence that drug resistant populations are being developed. With a decline in the availability of highly effective antiooccidials there has been a renewed interest in vaccines for the control of coccidiosis. Reports on vaccinating birds with drug sensitive strains of coccidia have shown to restore antiooccidial efficacy. Mathis et al, 2003, demonstrated that there are benefits for using Coccivac-B® in a rotational program with Clinacoxx™ (diclazuril). More recently the use of a live coccidia vaccine concomitantly with an ionophore has shown promise. It is believed that the ionophore aid in the transition from a non-vaccine program to a vaccine program with a lowered occurrence of necrotic enteritis.

Key Words: coccidiosis, antiooccidials, control


Bacterial Chondronecrosis with Osteomyelitis (BCO) is one of the most common forms of lameness and is associated with opportunistic bacterial infections in the proximal long bones, often occurring in microfractures in the bone. Some of the bacterial species identified from BCO lesions originate in the gut, indicating that the disease is associated with gut barrier failure. One strategy to reduce incidence of BCO is to improve gut health via probiotics, presumably by enhancing barrier function. A wire flooring model has been developed to reliably induce BCO and lameness in broilers (Wideman et al., 2012). We used this model to test the efficacy of SPORULIN®, a direct-fed Bacillus spore-based probiotic, in reducing BCO symptoms including femoral and tibial head necrosis in broilers reared on wire flooring. Ross 308 male broiler chicks were assigned to two treatment groups, one with .5lbs/ton SPORULIN® and one without. There were 12 replicates per treatment with 50 chicks per pen. All chicks were reared on 4′X12′ wire flooring pens with waterers and feed on the opposite ends, and fed corn-soy starter diet on day 0-35 and grower diet on day 36-58. On days 57 and 58, 2 non-lame and 2 lame broilers per pen were scored for femoral and tibial head lesions. Lameness birds exhibited a significantly greater severity of femoral head lesions (p=0.008), tibial head lesions (p=0.0001) and total lesions (average score of femoral and tibial head lesions, p<0.0001) than non-lame birds, suggesting that the broilers became lame due to femoral and tibial head necrosis. The broilers fed SPORULIN® exhibited numerically lower incidence of total tibial head lesions (p=0.08) and significantly lower severity of right femoral head lesions (p=0.0403), tibial head lesions (p=0.0223) and total leg lesions (p=0.0135) than broilers without SPORULIN®. These results suggest that SPORULIN® is a potential intervention strategy to reduce BCO in the poultry industry. SPORULIN® is a trademark of Pacific Vet Group-USA, Inc. and is registered in the U.S. and other countries.

Key Words: lameness, wire flooring, probiotics, SPORULIN, BCO

Necrotic enteritis (NE) in commercial broilers most often occurs between the ages of 14 to 30 days-of-age and is caused by Clostridium perfringens. An Eimeria maxima infection is commonly cited as a predisposing factor for NE as it results in primary damage to the intestinal mucosa. Poultry producers are increasingly limiting or eliminating the use of antibiotics in the control of NE. Alternative products to control NE infections are being investigated. The objective of this study is to examine the effectiveness of Calsporin® (Bacillus subtilis C-3102) against an NE challenge. In this trial, NE was reproduced by challenging broilers at 14 days-of-age with Eimeria maxima, followed by an oral gavage of C. perfringens starting at 19 days-of-age. NE lesion scores were assessed at 21 days-of-age. Mortality and production parameters were assessed at 28 days-of-age. Varying dosages of Calsporin® were analyzed to determine corresponding capabilities of NE amelioration. The effectiveness of Calsporin® in this NE challenge model was compared to a non-treated control group and one that received therapeutic levels of Stafac®. Significant reductions in mortality and lesion scores were attained with Calsporin® against an NE challenge compared to the non-treated/infected group. All lesion score results for the treatments groups receiving Calsporin® were statistically equivalent to the groups fed the Stafac® diets. The results indicate that there is evidence of dose response with the Calsporin® treatments in regard to the reduction of NE lesions, mortality and production influences, however these data were not significantly different. The NE challenge model in this trial resulted in disease reproduction that compares with field reports. In this trial, the Calsporin® treatments reduced the disease severity of NE and its adverse effect on production parameters.

Key Words: DFM, Bacillus subtilis, necrotic enteritis, Calsporin, broiler


Statement Purpose: Salmonella Heidelberg is the third leading cause of human food borne illness from Salmonella. Intestinal flora modulation has been known to reduce Salmonella colonization since Dr. Nurmi in 1961 showed that a single strain of Bacillus subtilis could be used to reduce colonization in broilers. The aim of this trial was to examine the effectiveness of a probiotic that could be consistently and uniformly administered to all chickens in a production complex to reduce Salmonella colonization in broilers.

Experimental Design: This study consisted of 24 pens with 50 male broilers per pen with 8 replicates per treatment. The treatments were: No treatment (coccivaccine Day 1); Optibac-L (Bacillus licheniformis) – 1 lb/ton (plus coccivaccine); and BMD (50g/1 ton (starter grower) + Optibac-L (1 lb/ton all feeds) + Salinomycin 50g/ton (all feeds). The Salmonella heidelberg nalidixic acid resistant strain was challenged to 25 chicks/pen at 1 day of age by oral gavage.

Drag swabs in all pens were taken at 1, 14 and 42 days and 10 ceca collected at 42 days for S. Heidelberg prevalence and enumeration by MPN (most probable number). Feed consumption and body weights were also measured.

Results: The birds receiving Optibac-L or Optibac-L plus BMD had significantly lower FCR and higher average body weight gain to 42 days. The univariate analysis of the Salmonella data found there was a significant difference between treatments with respect to Salmonella prevalence (P < 0.001); the Optibac-L treatment and the BMD + Optibac-L treatment both had significantly lower prevalence than the control. The odds of Salmonella detection were 60% lower in birds receiving the Optibac-L treatment and 77% lower in birds receiving the BMD-Optibac treatment. In conclusion, this study has demonstrated that a pelleting temperature tolerant Bacillus licheniformis can be used to successfully reduce the prevalence of Salmonella Heidelberg colonization in broilers to 42 days of age.

Key Words: Salmonella, Colonization, Bacillus licheniformis, Probiotic food safety

M14 Evolutionary relationships between known serotypes and unique variants of Salmonella enterica as determined by ISR secondary structure Jean Guard1, Prerak Desai2, Michael McClelland3, 1U. S. Department of Agriculture, Athens, GA; 2University of California, Irvine, CA

Intergenic Sequence Ribotyping (ISR) near the dkgB gene of Salmonella enterica subspecies I genome is now used to assign serotype in a manner that is largely concordant with the historical Kauffman-White-LeMinor (KWL) antibody-based scheme. ISR has a number of advantages over the KWL scheme, and it can be used to i) assign serotype to strains lacking O- and/or H-antigens, ii) detect mixtures of serotypes, and to iii) find strain variation within a single serotype as designated by the KWL scheme. Other DNA-based methods detect genomic variation within a single serotype, but ISR is more suitable for use in larger scale projects that continuously monitor environments due to its simplicity of application. A limitation of ISR is that sequence cannot be evaluated by phylogenetic analysis for evolutionary relatedness, because there is a disproportionate impact of size of sequence on clade formation and no parameters have been found to circumvent inappropriate clustering. We wanted to know if comparing secondary structures generated from ISR sequences was a better approach for understanding the evolutionary relationship within Salmonella enterica serotypes. To explore this issue, secondary structures were generated from a database of ISR sequences and then compared for similarity. Results were that strains with ISRs of different sizes but with similar backbone. Serotypes Newport and the Group D poultry-associated serotypes Gallinarum, Pullorum and Enteritidis were revealed to have new variants that maintained structural similarities regardless of ISR size. We suggest that the roadblock preventing assignment of unique ISR variants within an evolutionary framework of existing serotypes can thus be overcome by recognizing that blocks of sequences rather than individual bases are better for assessing evolutionary relatedness. ISR secondary structure to assign serotype relatedness within Salmonella enterica subspecies I may be adequate to recognize new variants within serotypes, but other approaches for studying blocks of nucleotides as individual elements should also be assessed.

Key Words: Salmonella, Evolution, Ecology, Serotype, Food Safety
M15 Identification of risk factors and causes of persistence of Salmonella Gallinarum in laying hens farms from Colombia

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The presence of Salmonella Gallinarum (SG) was recently identified in brown egg layers in Colombia. During 2013, twenty isolates were analyzed using Intergenic Sequence Ribotyping. Eighteen (90%) were SG and two (10%) were Salmonella Enteritidis (SE). SG was isolated mainly from organs of sick birds (liver, spleen, bone marrow, and ovary follicles) and from environmental samples (feces, feeders, manure belts, cages). SE was isolated from organs. In the affected houses, the mortality distribution pattern was mainly focal and severe egg drops were not observed. Some risk factors were identified; they were related not only with the widespread of the disease, but also with its persistence. The top ten risk factors were as follows: kind of housing, improper operation of the manure belts, improper handling of the manure, delay of the removal of dead birds, high iron contents in water, high water turbidity, no removal of biofilm and sediments from water pipes, inadequate traffic of the staff within the farm, high stocking density in houses, and multiage farms. Fowl typhoid is a very difficult and costly disease to eradicate. Contrary to other reports, our results showed that SG can often survive in the environment. Although in many countries the successful control was achieved with the elimination of positive birds, this practice is frequently not economically viable in large multi-age companies or in developing countries. Therefore, it is necessary to identify the potential persistence of the risk factors and to implement measures to avoid or prevent the widespread of the disease.

Key Words: Salmonella Gallinarum, Fowl Typhoid, Risk factors, Intergenic Sequence Ribotyping, Salmonella persistence

M16 The Effects of Coarsely Ground Corn Inclusion on Female Broiler Live Performance, Gizzard and Proventriculus Weight, Litter Characteristics and Ammonia Emission

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Coarsely ground corn (CC) inclusion has been reported to improve broiler feed conversion ratio (FCR) and decrease litter nitrogen and moisture, so it was hypothesized that ammonia production would be reduced as well. The objectives of this study were to evaluate the effect of CC inclusion on female broiler live performance, gizzard and proventriculus weight, litter characteristics, and litter ammonia emission. The study was a single factor experiment of two CC inclusion (0% CC to 42 d or 10% CC from 1 to 21 d and 50% CC from 22 to 42 d). There were 270 1-old female broiler chicks brooded in floor pens to 21 d with dietary treatments of 0% CC or 10% CC. Thereafter, 90 female broilers from each treatment were randomly distributed into 3 environmental chambers and each fed either 0% CC or 50% CC diets to 42 d of age. There were 30 birds in each of the 6 chambers with 3 chambers assigned per dietary treatment. At 21 d of age, birds fed the 10% CC diet had higher BW (P < 0.05) compared with 0% CC diet (787 g versus 767 g) without effect on feed intake and FCR. At 42 d of age, the birds fed the 50% CC diet had numerically lower feed intake (2587 g versus 2707 g) and BW gain (1917 g versus 2011 g) (P = 0.09) with a similar FCR from 22 to 42 d as compared with those fed 0% CC. Meanwhile, 50% CC increased gizzard weight (P < 0.01). The chambers with birds consuming the 0% CC diet exhibited significantly higher NH3 concentration (P < 0.01), ammonia emissions rate (P < 0.01), and litter nitrogen (P < 0.01) and moisture content (P < 0.01) as compared with the birds consuming the 50% CC diet. Feed intake was not significantly correlated with ammonia concentration (P = 0.48), ammonia emissions rate (P = 0.27), litter nitrogen (P = 0.64) or moisture content (P = 0.33). The results of this study supported the hypothesis that dietary CC inclusion resulted in decreased litter nitrogen, litter moisture, and ammonia emission.

Key Words: broiler, corn particle size, litter nitrogen, ammonia emission, litter moisture

M17 Division of Feed Ingredients to Minimize Crude Protein Variability and Maximize Savings of Feeds Formulated by Linear and Stochastic Methods

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Feed ingredients such as corn and soybean meal (SBM) vary in their nutrient content due to a variety of reasons. Such inherent variability can result in under or over-feeding of nutrients when feeds are formulated by linear methods, leading to reduced bird growth, added input costs, and increased environmental pollution. This research considers a simple feasible method to reduce nutrient variability and costs by employing rapid nutrient testing methods (e.g., NIRS) to separate feed ingredients. Thereafter, 90 female broilers were randomly divided into two separate bins of below- and above-average contents prior to feed formulation (2-bin method) in lieu of the traditional method of no separation (1-bin method). This study quantifies the effect of separating corn and SBM into below- and above-average batches on 1) crude protein (CP) variability of finished feed; and 2) the cost of providing CP at specified probability levels. To assess the impact of feed separation simulation analysis of feeds meeting NRC requirements for a broiler starter (e.g., CP ≥ 23%) was conducted. The simulation method was based on statistics of corn and SBM samples collected from various regions of North America. Simulations demonstrate that linear and stochastic feed formulation with the 2-bin method resulted in a considerable reduction in the coefficient of variation (CV) and standard deviation (SD) of CP in the finished feed (CV ≈ 1.20; SD = 0.28) compared to the 1-bin method (CV = 2.81; SD = 0.65). For the linear feed formulation, 96% of the batches of feed formulated by the 2-bin method have CP above 22.5% compared to 78% for the 1-bin method. On the other hand, only 3.5% of the batches of feed for the 2-bin method have CP above 23.5% compared to 22% for the 1-bin method. Further, stochastic feed formulation with the 2-bin method results in substantial savings ($3.44/ton for feed with a 90% probability of achieving target CP levels). Results of this study suggest that dividing feed ingredients into below- and above-average batches could be useful in improving performance due to reduced under-feeding and minimizing waste from reduced over-feeding. With stochastic feed formulation methods, the 2-bin method can maximize savings.

Key Words: Nutrient variability, Protein, linear programming, stochastic programming, savings

M18 Nutrient utilization of broilers fed corn-soy diets having a Thermomyces lanuginosus xylanase

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A study was conducted to evaluate the effects of a thermostable endo-1,4-β-xylanase (EC 3.2.1.8), RONOZYME® WX, DSM Nutritional Products) supplemented in corn-soybean meal diets fed to a total of 480 Cobb 500 slow feathering male broilers. Birds were fed a basal (Basal) diet using industry nutrient levels (21.0% crude protein (CP), 1.15% dig. Lys, 0.86% dig. TSAA, 0.75% dig. Thr and 3.100 kcal apparent metabolizable energy (AME/kg) and also the same formulated diet replaced with

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