M84 The effect of supplemental guanidino acetic acid in Brazilian type broiler diets at summer conditions. J. Ringel*[1], A. Lemme[1], and L. F. Araujo[2], [1]*Evonik Degussa GmbH*, *Hanau, Germany*, [2]*University of Sao Paulo*, *Sao Paulo, Brazil*.

Guanidino acetic acid (GAA) is naturally occurring in animal tissues playing a major role in the energy metabolism as a precursor of creatine. The objective of the study was to examine potential effects of GAA (CreAMINO™) supplementation in purely vegetable diets compared to a positive control. Therefore a total of 780 male ROSS 308 broiler chickens were assigned to one of the following three diets: positive control (6% meat and bone meal), negative control (vegetable diet) and a vegetable diet supplemented with 0.6 g CreAMINO™/kg feed. Performance parameters were recorded from day 14 to day 35 and at the end of the experiment 5 chickens per pen (26 pens) were utilized for carcass quality determination in terms of carcass percentage, breast yield, leg yield, wing yield and abdominal fat yield. Additionally, meat quality in breast meat samples including pH, color, drip loss was measured. Overall weight gain of the broilers did not differ between positive control and the CreAMINO™ supplemented diet while broilers fed the purely vegetable diet showed significantly lower weight gain ($p<0.05$). Feed conversion was significantly lower in the treatment with CreAMINO™ supplementation compared to both positive and negative control ($p<0.05$), while mortality remained unaffected by treatment. Only numeric differences between treatments for carcass quality could be determined. Furthermore pH, drip loss, tenderness evaluation and lightness (L*), yellowness (b*) did not show any effect, only redness (a*) was found to be lower in the CreAMINO™ treatment. It can be concluded that supplementation of CreAMINO™ in purely vegetable diets improves broiler performance to levels achieved with a diet containing meat and bone meal, while carcass and product quality are not affected by supplemental CreAMINO™.

Key Words: Guanidino acetic acid, Broiler, Vegetable diets, Feed conversion, Meat quality

M85 Productive parameters in broiler chicks vaccinated against coccidiosis and with a diet that has yeast cell walls (Saccharomyces cerevisiae) added. R. Morales*[1], A. García[1], F. García[1], S. Solorio[1], and J. Arce[2], [1]*Safmex S.A. de C.V.*, *Toluca, México*, [2]*UMSNH. Morelia*, *Michoacán, México*.

Two-thousand two-hundred, one-day-old broiler chicks were maintained in production until 45 days of age, completely randomized in seven treatments with six replicates (treatments 1, 4, 5, 6 and 7) and seven replicates (treatments 2 and 3) of 50 birds each: T-1) Negative control without coccidiostat (NC); T-2) Positive control with coccidiostat (PC); T-3) NC + coccidiosis vaccine; T-4) NC + S. cerevisiae cell walls (YCW or Saf-Mannan®) (600 mg/kg feed); NC + YCW (750 mg/kg feed); T-5) NC + YCW (600 mg/kg feed) + coccidiosis vaccine; T-6) NC + YCW (750 mg/kg feed) + coccidiosis vaccine. Final results at 45 days, showed that broilers that received YCW, at different doses, with and without vaccine had similar body weights when using coccidiostat ($P<0.05$) (2433b; 2626[a]; 2397b; 2583[a]; 2604[a]; 2646[a]; and 2587[a]g). Vaccine application caused a reduction in feed consumption when compared to group NC, while treatments with coccidiostat and YCW (vaccinates and not vaccinates), showed feed consumptions that were similar among themselves and higher than the groups NC and NC + coccidiosis vaccine ($P<0.05$) (4379b; 4626c; 4012[a]; 4626c; 4736c; 4717c; and 4634cg). The lowest feed conversion index corresponded to group NC + coccidiosis vaccine that was similar to the groups with coccidiostat and with YCW (600 mg/kg feed) + coccidiosis vaccine (1.81b; 1.78ab; 1.70[a]; 1.81b; 1.81b; 1.78ab; and 1.81b g/g). Results showed that YCW added to chicken feed, with or without vaccine against coccidiosis, may have effects on the productive parameters, similar to those obtained with the use of coccidiostat.

Key Words: Yeast cell walls, S. cerevisiae, Coccidiosis vaccine, Broilers, Coccidiostat

M86 Coccidiosis control with hyper immune, egg yolk immunoglobulins compared to a traditional coccidiostat program, nicarbazine plus salinomycine. D. Marrufo, R. Alejo, G. Parra, E. Lucio, and G. Victoria*, *Investigación Aplicada S. A.*, *Tehuacán, Puebla, Mexico*.

SUMMARY. A 100,000 broiler flock was divided in two groups in order to compare the hyper immune, egg yolk immunoglobulins anticoccidial efficacy, compared to dual program, nicarbazine plus salinomycine in-feed anticoccidial program. Powdered egg yolk immunoglobulins were administered at 200 ppm during the production cycle.The anticoccidial program consisted on 125 ppm nicarbazin (days 0â€“14) and 66 ppm salinomycin (SAL) (days 15â€“35). Group B was considered as a control.
The weight gain was used as an evaluation parameter in this experiment.
Weight gain at 35 days was greater in birds that received egg yolk immunoglobulins as compared with the nicarbazine plus salinomycine group. This difference caused a significant ($P< 0.05$) results.
The performance data, lesion scores, and oocyst counts showed that the immunoglobulins treatment was successful.
Immunoglobulins can thus be used as an alternative program instead of a dual program salinomycin and nicarbazin.

Key Words: Coccidiostat, Immunoglobulins, Coccidiosis, Eimeria, Anticoccidial drug

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SYMPOSIA AND ORAL SESSIONS

SCAD II

T87 Identification of infectious bursal disease virus field isolates with unusual antigenicity using reverse genetics. A. Icard, H. Sellers, C. Hofacre, and E. Mundt*, *University of Georgia*, *Athens*.

Currently, commercial and autogenous IBDV vaccines are used in the field to provide protection in poultry from humoral immunosuppression caused by circulating variant IBDVs. Recently, it has been observed that during the last third of the rearing period, chickens exhibited an increase in clinical respiratory disease. However, antibody response to vaccinations was low as measured by ELISA. This finding leads to the conclusion that viruses causing immunosuppression might play a role. To investigate the antigenicity of currently circulating IBDV field strains, the reverse genetics approach was used as a diagnostic tool. To this end, the coding region of VP2 encompassing the complete antigenic variable region of VP2 was amplified by RT-PCR and ligated into a full length plasmid of IBDV segment A. The insert of the resulting chimeric plasmids was sequenced. The chimeric segment A was subsequently used for co-transfection experiments employing the reverse genetics approach. Antigenicity was evaluated by monoclonal antibody (mAb) reactivity patterns obtained using immunofluorescence to characterize antigenic subtypes of IBDV. 50% of the analyzed constructs resulted in a reaction pattern specific for E/Del subtype. In contrast, the remaining constructs resulted in no reactivity with any of mAbs in the panel. This indicates IBDV strains with an unknown antigenic subtype are co-circulating in the field. Interestingly, most of the nucleotide sequences of the unknown IBDV field isolates grouped with the E/Del subtype in phylogenic analysis indicating that use of phylogenic analysis alone would lead to an incorrect conclusion regarding the field isolate subtype. Alignment of the deduced amino acid (aa) sequence and the localization of aa in the crystal structure of VP2 of IBDV was performed. Most of the observed aa exchanges in the unknown phenotype VP2s

were localized adjacent to the projection loop FG and in the projection loop HI of VP2. This indicates that the selection pressure on the antigenicity is focused on two hydrophilic loops localized outside of the capsid protein VP2.

Key Words: IBDV, Reverse genetics, Antigenicity, Phylogeny, VP2

T88 Interaction of cellular dsRNA binding proteins with viral proteins of infectious bursal disease virus. R. Stricker* and E. Mundt, *University of Georgia*, *Athens*.

A better understanding of viral replication is the basis for discovery of new ways to interfere with viral replication. Thus, there is an increasing interest in dissecting the molecular pathways and identifying cellular proteins involved in viral replication. It is known that cellular proteins operate as functional elements during viral replication. To gain more insight into viral-host-interaction we focused on dsRNA binding proteins NF45 and p68, which are physiologically localized in the nucleus. To this end RNA was isolated from chicken cells, RT-PCR was performed and the genes of both proteins were cloned and sequenced. By using the recombinant Baculovirus technology recombinant proteins were subsequently purified by affinity chromatography. The identity of the purified proteins was confirmed by MALDI/TOF MS-MS. Sera were raised against both proteins in rabbits. In studies employing immunofluorescence the localization of both proteins was investigated in cells infected with infectious bursal disease virus (IBDV). Interestingly, in infected cells the proteins NF45 and p68 were present in the cytoplasm. In non-infected cells the proteins were observed only in the nucleus of the cell. In double-labeling studies using monoclonal antibodies raised against IBDV proteins, a co-localization of cellular proteins was observed only with VP3. This indicates that the investigated cellular proteins interact with VP3. It is known that VP3 interacts with the viral RNA-dependent RNA-polymerase VP1. Thus, our findings indicate an involvement of the investigated cellular proteins in the viral replication machinery. To investigate whether VP3 alone or the presence of VP1 is necessary for the observed phenotype, transfection experiments with cRNA of only the full length segment A or in combination with segment B of IBDV were conducted. The results showed that the presence of VP3 alone was not sufficient to observe the presence of the investigated cellular proteins in the cytoplasm of the double-transfected cells. This is an additional sign that VP1 needs to be present for the observed phenotype of the cellular proteins.

Key Words: IBDV, dsRNABP proteins, Interaction, Replication, Colocalization

T89 Rapid detection and monitoring of avian reovirus shedding in broiler chicken cloacal swabs using real-time RT-PCR. K. Guo*, T. Dormitorio, and J. Giambrone, *Auburn University*, *Auburn, AL*.

Avian reoviruses (ARV) cause a variety of diseases, such as tenosynovitis, malabsorption syndrome, chronic respiratory disease, and immunosuppression in young commercial poultry. Early detection is critical for choosing proper vaccines and for the prevention of ARV related diseases. Sigma NS Taqman probe real-time PCR was developed and used to detect and monitor ARV virus shedding from cloacal swabs using the Roche LightCycler®. Virus shedding was monitored and quantified for three weeks. Results indicated that viral RNA can be detected as early as the first day post-infection (dpi) using the enteric ARV strain 2408. Reovirus-infected commercial broilers with maternal immunity against reoviruses displayed a peak release of virus in fecal material at 2 dpi, and thereafter, it decreased rapidly throughout the study. On the other hand, specific pathogen free broilers showed much higher and fluctuating amounts of virus secretion in their feces. Results confirmed the high sensitivity of this test, and that maternal immunity greatly reduced viral replication and shedding in chickens.

Key Words: Avian reovirus, Real-time RT-PCR, Detection, Cloacal swab sample

T90 Distinguishing infectious bronchitis vaccine viruses from field viruses in commercial poultry. E. T. McKinley*, D. A. Hilt, H. Moscoso, and M. W. Jackwood, *Georgia State University*, *Athens*.

Infectious bronchitis vaccine viruses have been implicated in causing upper-respiratory disease in poultry flocks because viruses isolated from outbreaks are often found to be the same serotypes used to vaccinate the birds. Unique sequences in the S1 gene of vaccine viruses from the field were not known; therefore, it was not possible to differentiate vaccine viruses from true field isolates. Infectious bronchitis vaccine viruses recovered from vaccinated and contact exposed SPF chicks have several amino acid changes in the S1 gene sequence that are unique to re-isolated vaccine viruses. In our analysis, we compared the S1 sequences of thirty-nine IBV field isolates, collected over a five-year period, to the S1 sequence from re-isolated vaccine viruses. Our data showed that vaccine viruses were re-isolated from flocks experiencing increased mortality.

Key Words: Infectious bronchitis viruses

T91 Challenge study to evaluate vaccine protection against infectious laryngotracheitis virus (ILTV). A. Rodriguez* and M. Garcia, *University of Georgia*, *Athens*.

A challenge study was conducted to evaluate the protection elicited by the tissue culture origin (TCO) vaccine against a currently circulating ILTV field strain. Protection was evaluated by scoring clinical signs, mortality, body weight gained, virus isolation and shedding of vaccinated/challenge as compared to non-vaccinated/challenge chickens. Shedding and transmission of the challenge virus was evaluated by virus isolation and real-time PCR using sentinel chickens. Briefly, a total of 80 four-week-old specific pathogen free (SPF) chickens were placed in four negative pressure HEPA filtered isolator units. At 4 weeks of age twenty chickens were vaccinated via eye-drop with TCO vaccine with the recommended full dose. At 8 week of age the twenty vaccinated and ten non-vaccinated chickens were challenged intra-occularlly and intra-tracheally with 200 µl of 2.7 log10 TCID50 of the challenge virus. Twenty and ten sentinel chickens were placed in the same units to vaccinated challenge and non-vaccinated/challenge chickens respectively. A third group of twenty chickens was kept un-inoculated as negative control. Eye conjunctiva, trachea, and cloacal swabs were collected from four sentinel chickens from day 2 to 12 post-challenge. Significant differences in clinical signs score, mortality, and body weight gained were observed among vaccinated/challenge, non-vaccinated/challenge, and sentinel chickens. Viral DNA was detected and virus isolated only from sentinel chickens in the non-vaccinated/challenge group. Results showed that the utilized vaccine protected against clinical signs, mortality, weight losses, and viral shedding.

Key Words: Laryngotracheitis, Tissue culture origin, Real-time PCR, Virus isolation, Sentinel chickens

T92 Development of a recombinant avian influenza vaccine in yeast. J. J. Giambrone*[1], H. Wu[2], T. V. Dormitorio[1], and N. Singh[1], [1]*Auburn University*, *Auburn, AL*, [2]*Alabama State University*, *Montgomery*.

Vaccines are part of a program to prevent avian influenza virus (AIV) outbreaks in poultry flocks in Southeast Asia, where the virus is endemic. AIV vaccines can reduce virus shedding and transmission. Inactivated AI vaccines, and recombinant fowl pox (FP) and Newcastle disease (ND) H (hemagglutinin) vectored vaccines against H5N1 viruse are licensed and used in these countries. However, the first two vaccines must be injected and the later two are adversely affected by maternal immunity and immunity derived from prior vaccination against fowl pox or NDV. Yeast-expressed recombinant protein provides advantages over viral vectored vaccines. Yeast are used in poultry to control bacteria as probiotics in feed or water, are not affected by maternal immunity, and can be administered by mass application in the field. Our previous work confirmed a yeast-derived sigma C protein induced immunity against avian reovirus, when given orally to young chickens. We designed primers based on the 15 and 21 terminal segment specific nucleotides of the genomic RNA, which are conserved in all AIVs and are unique for each fragment. We amplified the H gene from a non-pathogenic

H10N7 isolate from Alabama aquatic wild birds. The resulting gene (1.8 kb) was cloned into Schizosaccharomyces (S) pombe. The H gene expressed a 62.2 KD protein as demonstrated by Western blotting. This protein was purified and its expression efficiency was 0.1% of the total soluble protein. We plan to optimize protein expression to increase its immunogenicity prior to work in chickens.

Key Words: AIV, Poultry, Yeast, Recombinant vaccine

T93 Role of different genes in the pathogenesis of H5N1 avian influenza virus in chickens. J. L. Wasilenko*[1], C. W. Lee[2], L. Sarmento[1], E. Spackman[1], D. L. Suarez[1], and M. J. Pantin-Jackwood[1], [1]*Southeast Poultry Research Laboratory*, *Athens, GA*, [2]*Department of Food Animal Health Research Program*, *Wooster, OH*.

The determinants of pathogenicity of Avian Influenza (AI) virus are not totally defined. Previous studies have pointed towards the importance of different influenza virus genes in determining virulence in various hosts. We used reverse genetics to generate recombinant viruses in order to better understand the role of recombination in AI pathogenesis and to determine which viral genes contribute to the virulence of AI viruses in chickens. A recombinant virus, Ck/Indonesia/03, displayed 50-75% mortality in chickens. Single genes in this virus were then replaced with those from a rEgret/HK/02 virus which displays 100% mortality and high viral titers in tissues. Exchange of the HA gene considerably affected virulence which was reflected in increased mortality, increased viral replication and spread in tissues, demonstrating the importance of the HA gene in pathogenesis of the virus. The HA genes had identical cleavage sites however there were 11 amino acid differences, 5 located in the receptor binding site of the glycoprotein, which could explain the difference observed in pathogenicity of the viruses. Exchange of the NS, NP or M genes also resulted in decreased mean death times (MDT), increased viral replication and spread of the virus in tissues compared to the rIndonesia/HK/02 parent virus. Replacing the NP gene resulted in a virus with the shortest MDT and increased viral spread in tissues, surpassing that of the HA gene recombinant. Exchanging either the PB2 or PB1 genes did not result in increased virulence suggesting these polymerase genes may function more effectively as a unit derived from the same viral strain.

Key Words: H5N1, Influenza, Pathogenesis, Virus, Chickens

T94 *In ovo* administration of a novel vaccine expressing putatively universal and protective epitopes for avian influenza. G. Gaona-Ramírez*, S. L. Layton, A. D. Wolfenden, R. E. Wolfenden, N. Pumford, G. Tellez, Y. M. Kwon, W. G. Bottje, and B. M. Hargis, *University of Arkansas*, *Fayetteville*.

Previously, an attenuated ΔaroA *Salmonella enteritidis* strain (ΔSE) expressing two M2e epitope sequences with an immune-enhancing CD154 sequence on the cell surface provided high and persistent titers against several influenza serotypes and produced neutralization titers in embryos and cell culture when administered post-hatch. Presently, we evaluated *in ovo* (air cell) administration of this vector on hatchability, seroconversion and performance. Embryos (240, 18 dE) from a commercial broiler hatchery were divided into 2 groups: .25 mL PBS containing $3.25x10^3$ cfu ΔSE/embryo into the air cell or vehicle alone (control). Liver/spleen and cecal tonsils were aseptically cultured for ΔSE strain recovery, and blood samples were obtained for determination of M2e and ΔSE specific IgG antibody response. The hatchability for the treated group was 91.52% vs. 86.55% for vehicle-treated controls. ΔSE (positive/total) recovery was as follows: liver/spleen: Day 1 20/20 (100 %); d 7 10/20 (50 %); d 14 5/20 (25 %); d 21 2/10 (20 %); and d 28 0/10 (0 %). For cecal tonsils: Day 1 20/20 (100 %) with an average of $1.87x10^{11}$ ΔSE/gram of cecal contents; d 7 20/20 (100 %); d 14 18/20 (90 %); d 21 3/10 (30 %); d 28 4/10 (40 %). No ΔSE was recovered from control chicks. The M2e serum antibody levels (S/P ratio) were higher at day 7 and decreased weekly (day 7, .8; day 14, .2; day 21, .15; and day 28, .1). The ΔSE serum antibody levels showed a low response at 7 d in the vaccinated group and decreased during the 4 weeks of the study. Weekly body weights during the experimental period did not show any statistical difference between the vaccinated embryos when compared with control embryos (P>.05). These data suggest that *in ovo* ΔSE administration does not affect the hatchability of broiler embryos nor the performance of the chickens. A strong immunological response was observed during the first week of age against the M2e. The clearance of the ΔSE in liver and spleen was observed at day 28 (0/10). Further studies to evaluate secondary immune response and clearance from the cecal tonsils are currently being conducted.

Key Words: Embryo, Vector, Avian Influenza, M2e

Nutrition V

T95 Utilization of poultry production information among poultry farmers in Ibadan metropolis, Nigeria. J. O. Oladeji* and E. O. Uwagboe, *University of Ibadan*, *Ibadan, Oyo State, Nigeria*.

Ibadan is a prominent area for poultry production in Nigeria. Increase in poultry production information in this era of Information Communication Technology (ICT) is an issue that needs to be addressed. A multistage random sampling technique was used to select 3 Local Government Areas (LGAs) out of the 9 LGAs in Ibadan Metropolis while systematic random sampling technique was used to select 120 poultry farmers out of a population of 200 registered farmers and questionnaire was used for data collection. Frequency counts and percentages were used for descriptive statistics while Chi-Square was used for data analysis. The result shows that all (100%) of the respondents are educated and majority (82%) has above secondary school level. Few (14%) of the respondents obtained information from Extension agents and all (100%) rear less than 1,500 birds. The Chi-Square result revealed that significant relationship exists between education (X^2= 21.66, P≤0.05), level of production (X^2= 11.870, P≤0.05) and level of information utilization. In conclusion, poultry farmers in the study area are educated but have inadequate poultry information hence operate at small scale level. There is a need for an intensive animal husbandry extension services to facilitate adoption of improved poultry production practices and enhance farmers income generation.

Key Words: Utilization, Information, Poultry, Farmers, Nigeria

T96 Influence of endogenous phytase activity on exogenous phytase pellet stability evaluation. S. Dalsgaard*[1], M. F. Isaksen[1], M. Hruby[2], and T. Gravesen[1], [1]*Genencor*, *Danisco, Denmark*, [2]*Danisco Animal Nutrition*, *St. Louis, MO*.

Today, a large proportion of monogastric feed is enriched with microbially derived phytases to improve the digestibility of phosphorus. Phytate is the major phosphorus source in wheat and corn, and approximately 75% of all phosphorus in the grains is bound within phytate molecules. Phytate cannot be degraded by the animal itself, and the animal needs exogenous phytase to degrade the phytate in vivo.

The exogenous phytase pellet stability is an important topic for the feed industry. The primary way of evaluating exogenous phytase thermostability is by running pelleting tests in commercial feed mills. However, endogenous phytase from the raw materials can influence the outcome of the test results. The endogenous phytase originates mainly from wheat or wheat by-products, which are, in many markets, ingredients used at high levels in animal feed. The presence of endogenous phytase can then cause an incorrect interpretation of thermostable phytase product thermostability.

The evaluation dealt with two aspects of how endogenous phytase influences the outcome of pelleting trials. First, a commercial pelleting trial, where the endogenous phytase is inactivated at 90°C and second, a test of endogenous phytase thermostability over a temperature range of 75°C to 95°C. The results show 85% inactivation of the original endogenous phytase activity at 95°C. The