

ASAS/ADSA Food Safety and PSA Pathology

1053 Effect of dietary fiber on enterohemorrhagic *Escherichia coli* O157:H7 shedding in lambs. M. Lema* and L. Williams, *Alabama A & M University*.

E. coli O157:H7 is an emerging food-borne pathogen implicated in several outbreaks. Fecal shedding of the pathogen constitutes the mode of entry into the human food chain. We compared the influence of dietary acid-detergent fiber (ADF) concentration on fecal shedding of the pathogen and meat production efficiency of lambs. Fourteen growing-finishing Suffolk ram lambs averaging 28.13 kg in body weight were arranged in two 7 x 7 Latin squares with 1-week adaptation and 2-weeks of fecal sample collection and fed diets with 5, 10, 15, 20, 25, or 35 % ADF content, ranging from all-concentrate (5 % ADF) to all-forage diet (35 % ADF). Diets were fed daily at the rate of 1.4 kg/head. Fecal samples were collected weekly and analyzed for *E. coli* O157:H7 using modified tryptic soy broth with novobiocin as a pre-enrichment broth and cefixim-tellurite sorbitol MacConkey agar (CT-SMAC) as a selective media. *E. coli* O157:H7 was confirmed by its reaction with O157 and H7 antisera. Lamb growth rate and daily dry matter intake were determined and composite feed samples analyzed for nutrient content. When fed 10, 15, 20, 25, 30 and 35 % ADF in the diet, *E. coli* O157:H7 shedding was comparable among treatments suggesting that within 10 to 35 % ADF range dietary fiber does not impact *E. coli* O157:H7 shedding. However, lamb growth rate and feed efficiency were significantly lower ($P < 0.05$) for the 25, 30 and 35 % ADF diets. The 5 % ADF (100 % concentrate) diet resulted in significantly higher ($P < 0.05$) shedding of the pathogen (6.09 versus 5.12, 4.82, 5.21, 5.05 and 5.04 log₁₀ CFU/g of feces for the 10, 15, 20, 25, 30, and 35 % ADF diets, respectively) than the other treatments. The results suggests that increasing the ADF content of the concentrate ration between 10 and 20 % ADF results in significant reduction in the shedding of *E. coli* O157:H7 as compared to 5, 25, 30, and 35 % ADF diets without adversely affecting animal meat production efficiency.

Key Words: *E. coli* O157:H7, Fecal shedding, Dietary fiber

1054 Simultaneous detection of *Salmonella* sp. and *E. coli* O157:H7 using PCR on beef carcasses from a slaughterhouse in Mexico City. E Lopez, R Alonso, MS Rubio*, F Nuez, M Nicoli, and P Miranda, *Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autonoma de Mexico*.

The aim of this work was to develop a multiplex PCR assay to detect simultaneously *Salmonella* (S) and *E. coli* O157:H7 (Ec) on beef carcasses. Shiga like toxin I, II, eaeA gene and uidA primers were used to detect Ec. The invA gene was used to detect S. In order to have an internal control to distinguish between negative and false negatives results, we incorporated to the PCR mix pBR322 plasmid DNA and primers specific to amplify a characteristic fragment. PCR reaction conditions, which amplify DNA from both bacteria, and control plasmid, were as follow: a denaturalization step at 94C by 3 min followed by 30 cycles consisting of 94C during 30 s, 50C for 30 s and 72C by 30 s and finally a step of 72C for 3 min. Experimentally infected meat was used to evaluate the assay. Individual strains and the mixture of both (meat was previously washed using a physiological salt solution) were used. Non-infected washed pieces of meat were used as negative controls. The assay was evaluated in field specimens. Sixty beef carcasses from slaughterhouses were sampled on the shoulder region with sterile cotton swap on a delimited surface of 17x17 cm². To transport, the swap was kept in a plastic bag containing a peptone broth. Before DNA extraction, samples were cultivated in an enriched broth and incubated for 24 h. Results indicated that the positive experimental controls (individual strain and mixture) showed the expected amplified bands. Washed meat showed no band. Field results showed that none of the carcasses were contaminated with Ec, and only 10% of the carcasses were positive to S. Standards microbiology procedures of the samples showed a higher percentage of samples with S, which in comparison with the PCR results indicates that no all the S found were invasive.

Key Words: PCR, *E. coli* O157:H7, *Salmonella*, Meat

1055 Simple and Rapid Method for Screening Antimicrobial Activities of *Bifidobacterium* Species of Human Isolates. S.A. Ibrahim* and M.M. Salameh, *North Carolina Agricultural and Technical State University*.

Bifidobacteria constitute a major part of the human intestinal microflora. Many health benefits are associated with bifidobacteria including their antimicrobial activities. For this reason, there has been increased interest in the development of a rapid method for screening antimicrobial activities of *Bifidobacterium* isolates from human fecal samples. The objective of this research was to develop a rapid procedure for the screening antimicrobial activities of *Bifidobacterium* species of human isolates. A Bifidobacteria selective medium BIM-25 agar was modified by adding of 0.5g/l cysteine-hydrochloride, 1.5g/l lithium Chloride, 1.5g/l beef extract, and 5ml/l Tween 20. This medium was inoculated (45C) with diluted fecal material from 5 subjects and overlaid into 0.1% Tween 20 BHI agar plates. Plates were incubated in anaerobic chamber at 37C for 48 hours. Plates were then inverted to allow the two layers of agar to fall into the petri lid. BHI soft agar (0.45%) containing *Micrococcus luteus* (as indicator) was overlaid onto the other layers in the petri dish. Plates were incubated at 37C overnight and zone of growth inhibition was observed. This method is simple and rapid whereas the original method for screening of antimicrobial activities of bifidobacteria is a more time consuming and cumbersome procedure.

Key Words: Rapid Method, *Bifidobacterium* species

1056 D-value Determination of *Listeria monocytogenes* and *Salmonella typhimurium* in low fat ready-to-eat processed meat. Kevin McCormick*, Inyee Y. Han, and Paul L. Dawson, *Clemson University, Clemson, South Carolina/US*.

The research was conducted to determine the best processing temperature for inactivating *Listeria monocytogenes* and *Salmonella typhimurium* on packaged turkey bologna. To determine the best treatment temperature, the D-values of *L. monocytogenes* and *S. typhimurium* at various temperatures were tested. A low-fat turkey bologna was cut into 3cm² squares and irradiated to ensure that the sample was sterile before being inoculated. Each sterile sample was inoculated with approximately 10⁸ CFU/ml inoculum and aseptically placed into a LLDPE pouch and vacuum-sealed. Then, the samples were submerged in a water bath held at constant temperature, and removed at timed intervals. Different water bath temperatures between 60° and 85° C were used with processing temperatures measured and recorded using a CALPlex datalogger and Calsoft software. The number of cells on each sample was determined by plating on appropriate growth media after making serial dilutions. The plates were incubated for 48 hours, and the counts were used along with time and temperature data to determine the D-values at 60°C, 65°C, and 70°C. At 85°C water temperature, no *L. monocytogenes* cells were detected (< 10²) after 10 seconds submersion, while at 60°C cells were detected (> 10²) up to 10 minutes after submersion of the packaged meat in the heated water bath. Similar results were found for *S. typhimurium*. The data shows that significant reductions in bacterial counts can be achieved with these temperatures and that only a small increase in temperature greatly affects the D-values for both bacteria examined.

Key Words: D-value, *Listeria monocytogenes*, *Salmonella typhimurium*

1057 Molecular Subtyping and Tracking of *Listeria monocytogenes* in Hispanic Cheese Factories. D.Y. Kabuki¹, A.Y. Kuaye¹, M. Wiedmann², and K.J. Boor², ¹Faculdade de Engenharia de Alimentos-UNICAMP-Brazil, ²Food Safety Laboratory - Department of Food Science-Cornell University.

This research focused on identifying *L. monocytogenes* prevalence patterns to track sources of bacterial contamination in Hispanic-style fresh cheese manufacture, which represents a growing sector of the New York State dairy processing industry. A total of 357 samples were collected from three Hispanic cheese factories in New York City during 4 visits in a 6-month period. *L. monocytogenes* was found in 6.3% (7/111) of finished fresh cheeses and 11.0% (27/246) of environmental samples (food contact surfaces, floor drains, floors, walls and miscellaneous). Among

the environmental samples, 30% of drain and 20.6% of floor samples were positive for *L. monocytogenes*. A total of 80 representative isolates from all positive samples were subtyped for allelic polymorphisms using PCR-RFLP for virulence genes encoding listeriolysin O (*hlyA*) and ActA (*actA*). Allelic type 4 for *actA* and allelic type 1 for *hlyA* (lineage I) were predominant among the isolates. Isolates were classified to lineage I (80.0%) more frequently than to lineage II (20.0%) or III (0.0%). 23 *L. monocytogenes* isolates from the factory with positive cheeses and food contact surfaces were ribotyped using EcoRI with the Qualicon RiboPrinter. Ribotypes DUP-1044 (82.6%), DUP-1045 (13.0%) and DUP-1039 (4.4%) were identified among the isolates tested. A unique *L. monocytogenes* subtype (*actA* type 4, *hlyA* type 1, and ribotype DUP-1044) was isolated from finished products, drains, floors, a plastic connecting tube, and a polytetrafluorethylene table in one plant. This subtype was found during all 4 visits to this plant. Our subtyping data indicate persistent environmental contamination as a primary source of finished product contamination. Although the prevalence of *L. monocytogenes* positive samples decreased following some modifications in the plant's sanitation program during the sampling period, the unique, persistent *L. monocytogenes* strain still remained in the factory. We conclude that focused measures will be required to eliminate persistent *L. monocytogenes*, which are a likely cause of finished product contamination.

Key Words: *Listeria monocytogenes*, cheese, subtyping

1058 Characterization of isolated bacterial strains with antagonistic properties against food-borne pathogen *Listeria monocytogenes*. H. Roman*, E. T. Ryser, S. Rust, and M. T. Yokoyama, *Michigan State University, East Lansing, MI/United States*.

Listeriosis in ruminants is most frequently implicated with improperly fermented silage. The causative agent is *Listeria monocytogenes*. We hypothesize that bacterial strains, antagonistic against this foodborne pathogen, can be isolated directly from the farm environment. Three wild bacteria strains (designated Ba-E, Bs-3 and Bs-13) antagonistic towards strains of this pathogen (CWD246 Brick silage and DGallup CWD95), were isolated from fresh corn silage and rumen fluid. Isolates were identified as *Bacillus* spp. according to the API system, FAME and 16S rRNA. Ammonium sulfate precipitation of these isolates revealed the presence of bioactive protein factors. SDS-PAGE analysis of two of these preparations (Bs-3 and Bs-13) showed that only one peptide band displayed antimicrobial activity. These peptides were sensitive to protease and gastric peptidases (protease, proteinase K, chymotrypsin and lipase) tolerant of changes in heat (30-100C), organic solvents (acetone, acetonitrile, ethyl alcohol, methanol, chloroform and toluene) and pH (4 # 8). We speculate that at least one of these bioactive peptides may be a new bacteriocin which can potentially be used as a probiotic for silage and other feeds to control *Listeria monocytogenes*. These isolates survived and grew in TSB with yeast extract, containing either rumen fluid (50% v/v) or bile (0.3% w/v). An *in vitro* challenge study using co-cultures of *Listeria monocytogenes* CWD246 Brick Silage and strains of *Bacillus* spp (either wild type or ATCC 6633 strains) was conducted. Both the optical density (OD_{600nm}) and the viable counts (log cfu/0.1ml) of *Listeria monocytogenes* were monitored during a 24-h period. The treatment containing a mix of two wild type strains of *Bacillus* spp. (Bs-3 and Bs-13) was more effective in decreasing the viable counts of *Listeria monocytogenes*. We conclude that this combination might be a better treatment for controlling *Listeria monocytogenes* than either the ATCC6633 strain or the Ba-E wild strain. This research was partially supported by the National Food Safety & Toxicology Center, MSU.

Key Words: Listeriosis, Bacteriocin, Probiotic

1059 The effect of environmental and substrate factors on the growth and survival of *Salmonella agona*. S.A. Ibrahim*, T.A. Lloyd, M.M. Salameh, A. Shahbazi, R. Purcell, and C.W. Seo, *North Carolina Agricultural and Technical State University*.

Salmonella agona (*S. agona*) is one of approximately 2000 *Salmonella* serotypes that can cause illness in humans. *S. agona* has been linked to cereal food outbreak. This is the first time a commercial cereal product has been implicated in a *Salmonella* outbreak. *S. agona* is an uncommon serotype of *Salmonella*, it is found in a variety of animal reservoirs including poultry, cattle, pigs, and animal feed. Little information is

available about the physiology, growth and survival of this pathogen in foods. Therefore, the objective of this research was to determine factors affecting the growth and survival of *S. agona* in foods. Two strains obtained from the Center for Disease Control (CDC); *S. agona* H6115 and F 5567 were used in this study. Results indicated that 30C is optimum growth temperature for *S. agona*. Acetic acid is most inhibitory organic acid at pH value of 5.5. The addition of 3% sodium acetate or 3% sodium chloride showed significant reduction (P < 0.05) in microbial growth. Heat treatment at 55C for 20 min, 57.5C for 14 min, or 60C for 5 min was needed to achieve 5-log reduction in microbial populations. Thermal resistant of *S. agona* can be lowered by combining extrinsic factors such as pH, sodium chloride or sodium acetate. Cold shock at 2C for 25 min, prior to heat treatment, increased the thermal sensitivity of *S. agona*. In a model broth system, thermal death time at 57.5C was reduced by up to 1.5 log after the pathogen was cold shocked for 25 min. This indicates that cold shock has a potential to become a practical method to control pathogens in foods.

Key Words: *Salmonella agona*, Thermal

1060 Effects of Tasco® 14 on Prevalence Levels of Enterohemorrhagic *Escherichia coli* and *Salmonella* spp. in Feedlot Steers. A.R. Barham¹, B.L. Barham*¹, J.R. Blanton, Jr.¹, V.G. Allen¹, K.R. Pond¹, and M.F. Miller¹, ¹*Texas Tech University*.

The purpose of the study was to determine the effects of Tasco® 14 supplementation at 2% of a commercial grain based diet on *Salmonella* spp. (SAL) and Enterohemorrhagic *E. coli* (EHEC). Sixteen pens from a large commercial feedlot in West Texas were fed Tasco® 14 supplementation for 14 days starting at day forty-five of the feeding period and received a second supplementation for 14 days prior to shipment to slaughter facilities. Fecal and hide samples were collected from 13 animals in each pen for USDA approved microbiological assays prior to feeding Tasco® 14, prior to shipment for slaughter, and immediately following exsanguination. Prevalence levels were generated using the frequency procedures in SAS (1995). Changes in prevalence levels from the three sample times were analyzed using the T-test procedure in SAS (1995). Prior to Tasco® 14 supplementation, prevalence of EHEC and SAL was 18% and 39% respectively on hides and 34% and 30% in fecal samples. After the 14d feeding period prevalence of EHEC and SAL were 5% and 34% respectively on hides and 0% and 30% in fecal samples. Data reveals that at the packing facility, prevalence of EHEC and SAL were 0% and 70% respectively on hides and 2% and 57% in fecal samples. A significant reduction in EHEC on both hides and fecal samples occurred after the first feeding of Tasco® 14 compared to the pre-feeding samples (P < .05). The first Tasco® 14 supplementation did not significantly reduce SAL on hides but did significantly reduce prevalence of SAL in fecal samples. The second feeding period of Tasco® 14 significantly reduced EHEC in fecal samples when compared to both the pre-feeding and the first Tasco® 14 supplementation sample times. Samples taken at the packing plant indicated significantly lower prevalence levels of EHEC when compared to pre-feeding samples. SAL prevalence was reduced in fecal and hide samples at the packing plant when compared to samples taken after the second supplementation of Tasco® 14.

Key Words: Beef Cattle, *E. coli*, *Salmonella*

1061 A challenge trial testing the effects of Acid Pak 4-Way on *Salmonella* cecal colonization in broiler chicks. J.W. Evans* and M.S. Plunkett, *Alltech Biotechnology, Inc., Nicholasville, KY*.

An experiment was conducted to evaluate the effectiveness of a product containing a combination of organic acids, electrolytes, bacteria and enzymes (Acid-Pak 4-Way, Alltech, Inc.) at reducing cecal colonization in day-old chicks challenged with *Salmonella typhimurium* 29E (1 x 10⁶ CFU/g). Day-old chicks were randomly assigned to a control chamber with an untreated water supply or to a chamber supplied with water containing 1g/L Acid-Pak 4-Way. The experiment consisted of five control groups and five treatment groups with ten chicks in each group. Each chick was inoculated by oral gavage with a standard inoculum to provide similar microflora at the beginning of the experiment. On d3, each chick received 0.25ml *S. typhimurium* (4 x 10⁶/ml) by oral gavage. On d10 chicks were sacrificed and both ceca removed. The content of one cecum was diluted and plated on Brilliant Green Agar (BGA) to determine salmonella concentrations. The content of the other cecum

was placed in deionized water to measure pH levels and to determine VFA concentrations. The cecum was then placed in lactose broth to be used as a confirmation in samples that did not grow on the BGA. Cecal VFA concentrations and pH in the treatment group were not different from those in the control group. However, salmonella concentrations were lower ($P < 0.01$) in the treatment group ($6.87 \log_{10} \text{CFU/g}$) than in the control ($7.5 \log_{10} \text{CFU/g}$). The results from this experiment indicate that adding Acid-Pak 4-Way to the drinking water of young chicks reduces the cecal colonization of chicks exposed to salmonella.

Key Words: Water acidification, Challenge trials, Salmonella

1062 Effect of an essential oil blend on coccidiosis in broiler chicks. J.W. Evans*, M.S. Plunkett, and M.J. Banfield, *Alltech Biotechnology, Inc., Nicholasville, KY.*

The effects of a dietary essential oil blend on an *Eimeria acervulina* and *Clostridium perfringens* infection was investigated in broiler chicks. Two hundred, day-old chicks were housed in ten isolation chambers. At 15d old, one hundred and fifty were allotted to ten isolation chambers with 15 chicks per chamber. Chicks in five chambers received a starter poultry diet as a control. The chicks in the remaining five chambers received the same diet containing an essential oil blend (1.0% clove oil, 0.1% thyme oil, 0.1% peppermint oil, and 0.1% lemon oil). All chicks received 0.25ml *Cl. Perfringens* ($4 \times 10^8 \text{CFU/ml}$) for three days by oral gavage on d19-21. They received 0.25ml oral gavage of 2.5×10^5 *E. acervulina* oocysts on d22. Five chicks per chamber were sacrificed on d15, 22 and 36, and the entire gastrointestinal tract (GIT) was removed for *Cl. perfringens* enumeration. The chicks were housed on raised wire mesh floors in isolation chambers. From d21-35 feces were analyzed for oocysts. Performance parameters were measured on d15, 22, 29 and 36. The control chicks live weight was greater than the live weight of the treatment chicks on d36 (1575 vs. 1429g, $P < 0.05$). Feed intake was lower in the treatment chicks than in the control chicks from d15-21 (80 vs. 75g/bird/d, $P < 0.01$). FCR was not affected by treatment. Fecal oocyst yields were lower in the treatment chicks than in the control chicks at the peak of the infection (d26) (5.24×10^8 vs. 6.86×10^8 oocysts/bird/d, $P = 0.028$). The average of the total oocyst yields were also lower over the whole infection (9.94×10^8 vs. 1.17×10^8 oocysts/bird/d, $P = 0.032$). No differences in GIT *Cl. perfringens* populations were observed between treatments. The results of this trial indicate that the addition of essential oils to a normal poultry diet reduced the oocysts excreted at peak infection, however the addition of the oils also appeared to reduce the overall performance of the birds.

Key Words: Coccidiosis, Essential oils, *Eimeria acervulina*

1063 Apramycin resistance of *E. coli* isolated from cold-stressed swine. D.B. Arnett*, P. Cullen, P.D. Ebner, and A.G. Mathew, *University of Tennessee, Knoxville, TN.*

To determine if the development of resistance to apramycin in *E. coli* isolated from cold-stressed swine could be linked to plasmids, six weaned pigs were administered apramycin in the feed (150 g/ton for 14 d) and was exposed to cold stress in the form of an 8C reduction in recommended daily temperature throughout the wean-to-finish period. Fecal swabs were taken on days 0, 2, 7, 14, 28, 64, 148, and 149 for recovery of *E. coli*, and isolates were tested for sensitivity to apramycin using microdilution minimum inhibitory concentration (MIC) analysis. One resistant and one sensitive isolate were randomly chosen from each sampling day, and plasmids separated from each isolate. Plasmid profiles of resistant and sensitive isolates were compared based on plasmid number and size. Arbitrarily primed PCR (AP PCR) was used to identify resistant plasmids. PCR fingerprints were made of all plasmids using primers 23L and OPB-17. Pulsed-field gel electrophoresis (PFGE) was performed on chromosomal DNA using *SpeI* and *XbaI* restriction endonucleases. Profiles from AP PCR and PFGE were compared separately for similarity by eye and by using a molecular analyst fingerprinting software that created dendrograms for comparisons. Resistant plasmids were not identified, and the source of resistance could not be linked to plasmids by these methods. Other methods, including southern blot analysis, may be needed to identify resistance plasmids.

Key Words: swine, antibiotic resistance, plasmids

1064 The effect of antibiotics on broiler body weight, feed conversion and tibial dyschondroplasia scores. T.L. Peters*, K.D. Roberson, R.M. Fulton, and M.W. Orth, *Michigan State University, East Lansing, MI/USA.*

One of the major metabolic skeletal disorders that effect the long bones of fast growing birds is tibial dyschondroplasia (TD). Disrupted bone metabolism allows an avascular plug of cartilage to accumulate in the metaphyseal region of tibiae, which can weaken bones. The etiology of TD remains unclear. Recently, we found that doxycycline, oxytetracycline, enrofloxacin, ceftiofur, and salinomycin inhibited in vitro cartilage degradation of embryonic chick tibiae. Therefore, the objective of this study was to determine if these five antibiotics inhibited cartilage degradation and induced TD in growing broilers. Day-old male broiler chicks were randomly assigned to a treatment group and placed in a heated wire-floored battery brooder at 8 birds per pen with 3 pens per treatment. Chicks had ad libitum access to feed and water and were raised under continuous fluorescent light. Oxytetracycline, chlortetracycline, doxycycline, salinomycin, enrofloxacin, and ceftiofur were administered appropriately to chicks. A non-antibiotic control and thiram (a fungicide known to induce TD) group were also included in the experiment. Weekly growth rates and feed consumption were measured. Both proximal tibiotarsi were visually inspected for TD lesions at 22 days of age. Oxytetracycline, chlortetracycline, doxycycline, and ceftiofur did not differ from the control for any parameter tested. All salinomycin treated birds were lighter than controls; however, feed conversion only differed at the highest dose and TD incidence was not different for any dose tested. The lowest dose of enrofloxacin treated birds were heavier, but there was no difference for any dose in feed conversion or TD incidence. Control birds had a 15% incidence of TD while the 20 ppm thiram treated birds had a 92% incidence rate. Body weight and feed conversion were not different for thiram treated birds. Therefore, the results of this study show that the antibiotics tested are likely not involved in the etiology of TD in broilers.

Key Words: Tibial dyschondroplasia, Cartilage degradation, Antibiotic

1065 Haematological and histological findings in experimental Newcastle disease. F Galindo¹, N Calderon¹, M Charles¹, G Tellez¹, and T Fortoul², ¹Departamento de Produccion Animal Aves, FMVZ, UNAM, ²Departamento de Biologia Celular y Tisular, Facultad de Medicina, UNAM.

In an attempt to obtain more information about the pathogenesis of haemorrhages in Newcastle disease, blood cells counts with special emphasis on thrombocytes were performed in 25 specific pathogen-free chickens experimentally infected by ocular instillation of 10^6 embryolethal doses 50% of a velogenic viscerotropic strain of Newcastle disease virus (Chimalhuacan strain); five control chickens were also included. Birds were killed at 24 hours followed by 12 hours intervals. Whole blood and tissue samples were collected. The previous studies were complemented with the histological evaluation of bone marrow, brain, kidney, lung and proventriculus of the same animals. In this study a significant reduction of thrombocyte counts was detected within 72 hours post infection (hpi). A typical lymphopenia was detected; also an increase in heterophils and a decrease in monocytes at 60 hpi was observed. The histological findings consisted in swelling and vacuolization of capillary endothelial cells, mainly in the bone marrow and lung at 60 hpi. In the bone marrow early necrosis of haematopoietic islands was noted within 48 hpi. At 72 hpi there was a multifocal necrosis with the loss of cellular details and abundant debris. The thromboplasts at 60 hpi showed cytoplasmic vacuolation, nuclear vacuolation and basophilia. The thrombocytopenia and the endothelial damage are considered the main causes of haemorrhages. In this study, it is presumed by our findings that thrombocytopenia resulted from a direct mononuclear precursor cell viral damage of the thrombocytes in bone marrow.

Key Words: Newcastle disease, haemorrhages, vascular endothelium, thrombocyte, bone marrow

1066 Pathogenesis of thrombocytopenia in Newcastle disease: ultrastructural study. F Galindo¹, N Calderon*¹, G Tellez¹, and T Fortoul², ¹*Departamento de Produccion Animal Aves, FMVZ, UNAM*, ²*Departamento de Biología Celular y Tisular, Facultad de Medicina, UNAM.*

The ultrastructure of the bone marrow in 25 chickens experimentally infected with the Newcastle disease virus, Chimalhuacan strain and five control chickens were studied in order to determine if the virus caused direct damage to thrombocytes or their precursors. Light microscopy observations of semithin sections in the bone marrow revealed cell depletion and marked cell degeneration, principally of granulocytes and erythrocytes. At 24 hours post infection (hpi) electron microscopy showed cells with dense cytoplasm and chromatin associated with cellular death. At 36 hpi, decreases in erythrocytes and in some granulocytic cells were observed, and spherical electron-dense structures like viral particles were detected in dilated vesicles, as well as dilation of Golgi complex and endoplasmic reticulum. At 48 and 60 hpi an important depletion of hematopoietic cells was found and several cells presented perinuclear edema with necrosis or cytoplasmic degeneration. Apoptotic cells also were observed. At 72 hpi in the hematopoietic islands, few cells were found which showed necrosis and numerous cellular debris. Our results indicate that the depletion and necrosis of hematopoietic cells, including thrombocyte precursors, were responsible for thrombocytopenia detected in the early stage of the disease.

Key Words: Newcastle disease, pathogenesis, thrombocytopenia, thromboplast, electron microscopy, chickens

1067 Organochlorine pesticide residues in cow's milk from tropical region of Veracruz (Mexico). V.T. Pardo*¹, K.N. Waliszewski², and A. Ramirez¹, ¹*Universidad Veracruzana, Veracruz, Veracruz/Mexico*, ²*Instituto Tecnológico de Veracruz, Veracruz, Veracruz/Mexico.*

The purpose of the study was to assess the organochlorine pesticide levels in cow's milk produced in the central agricultural region of Veracruz State. These pesticides are intensively used in Mexico, causing exposure to significant levels of contamination to consumers and livestock. A hundred and twenty milk samples were collected bi-weekly at random during six weeks from farms located in three areas. Samples were cooled down, the fat layer was separated by centrifugation and stored in glass vials at -25°C until analysis. Organochlorine pesticide levels were determined by GLC/ μ ECD according to USEPA Method 608. A recovery study was performed on ten replicate spiked blank milk fat samples. Analyses were conducted for α -, β -, and γ -HCH, pp'-DDT, op'-DDT, pp'-DDD and pp'-DDE. The residue levels were reported as ng/g on lipid basis and the estimated dietary exposure as ng/day. Results were analyzed by analysis of variance with Minitab 10.5. Milk samples from Tlalixcoyan area showed a mean level of γ -HCH 128 ng/g which was significantly higher than Lindane residues in Jamapa (49 ng/g) and Paso San Juan (22 ng/g) milk samples. The mean value of Σ -HCH 230 ng/g was two times the FAO/WHO tolerance level of 100 ng/g. Concerning to DDT, mean levels of op'-DDT 26 ng/g, 39 ng/g of pp'-DDE, and 89 ng/g of pp'-DDT were found in milk samples collected from Jamapa area. The mean value of pp'-DDT was significantly higher than the levels detected in samples collected in the other two areas and four times the FAO/WHO tolerance level of 20 ng/g. For all chlorinated pesticide residues the estimated mean daily intake was 6.9 ng/d. Results indicate direct cattle exposure to HCH and DDT due to the fact that Lindane is still used to a limited extent for animal husbandry and the spray of DDT in vector control programs against malaria in Veracruz. This fact causes special concern since milk plays a central role in human nutrition and these pesticides have provided compelling evidence of hormone disruption activity in humans and lipid profile-disrupting activity in laboratory animals.

Key Words: Organochlorine pesticides, cow's milk, dietary exposure

1068 Detection of ochratoxin A in sorghum grain using various methods. J.H. Franco de la Torre*, W.P. Reyes, R. Nuo, and A. Taylor, *Centro Univ. de Los Altos, Universidad de Guadalajara.*

Ochratoxin A (OA) is a secondary metabolite, produced by *Aspergillus ochraceus* and *Penicillium viridicatum*. It is found as a frequent contaminant in various grains, cereals and animal feeds. The purpose of this

trial was to compare the efficiency of three immunoassay techniques (Veratox, Ridascreen, and Agraquant), and two immunoaffinity techniques (Afinitest and Ochratest), with HPLC in the detection of OA in sorghum grain, artificially and natural contaminated. Also two extraction methods (acetonitrile 60% and methanol 70%) were validated for the OA determinations with HPLC. The OA data were analyzed with a random and an angular transformation for arcsine of the CV for precision, the exactitude with the quadratic error, and the linearity with simple lineal correlation. The results showed that Agraquant gave a high linearity, precision, and exactitude, similar to the HPLC ($P > 0.05$), while Veratox, Ridascreen, Afinitest, and Ochratest; overestimate the detected levels of OA ($P < 0.05$). The extraction methods were similar ($P > 0.05$) in detecting OA, giving an additive constant of recuperation with acetonitrile, while the methanol has given a proportional recuperation (85%) as a multiplicative constant. Therefore, the immunoassay are a choice compare to HPLC in the detection of Ochratoxin A in animal feedstuffs.

Key Words: Ochratoxin A, Immunoassay, Immunoaffinity, HPLC

1069 Interaction between ochratoxin and aluminosilicate on the histopathologic aspect of organs and the humoral immunity against Newcastle disease virus in broilers. Elizabeth Santin*, A.C. Paulillo, A.C., Alessi, E.L. Krabbe, and A. Maiorka, *Faculdade de Ciências Agrárias e Veterinárias - UNESP, Jaboticabal, SP, Brasil.*

This study aimed at evaluating the effect of ochratoxin in the diet, in the presence or absence of aluminosilicate, on the histopathological aspect of the liver and the kidneys and the humoral immune response of broilers vaccinated against Newcastle disease virus (NDV). The exposure of 200 broilers to 2 ppm of ochratoxin, either in the presence or absence of aluminosilicate, reduced their humoral immune response and the number of mitotic cells in the bursa, but aluminosilicate did not influence ($P > 0.05$) these parameters. All groups reacted to antigenic stimulation by NDV at 11 days of age, but the best ($P < 0.05$) hemagglutination-inhibiting antibody titers were obtained in birds not exposed to ochratoxin (GMT 8.04, 8.24, 6.59 and 6.89 to group without ochratoxin and aluminosilicate, group with aluminosilicate, group with ochratoxin, and group with ochratoxin and aluminosilicate, respectively), which demonstrated the interference of this mycotoxin in the humoral immune response of broilers vaccinated against Newcastle disease. The liver of the birds exposed to this toxin showed an increase in its relative weight, and histopathological lesions of vacuolisation, megalocytosis in hepatocytes and proliferation of bile duct cells, whereas the kidneys had hypertrophy of proximal tubule cells and megalocytosis, with thickening of the glomerule membrane. The presence of aluminosilicate did not reduce the deleterious effects promoted by ochratoxin in broilers. Although several prior studies showed the efficacy of aluminosilicates in cases of aflatoxicosis, recent research demonstrated that these minerals do not inactivate other types of mycotoxins. This is probably due to the variation in the structure of adsorbent substances, as the adsorption is a function of ion and/or molecule exchange between these compounds and mycotoxins.

Key Words: Ochratoxin, Aluminosilicate, Immunity

1070 Surveillance programme of the microbiological safety and hygiene of meat in South Africa. AE de Jesus¹, EM Buys*¹, RP Greebe¹, J Kruger¹, L Kgosana¹, and WH Giesicke², ¹*Animal Nutrition and Animal Products Institute, Agricultural Research Council, Irene 0062, South Af.*, ²*Department of Agriculture North West Province, Mmabatho 2735, South Africa.*

The Directorate of Veterinary Services (DVS) of the Department of Agriculture in the North West Province recognised the need for information on the microbiological status of the meat supply as an essential supplement to veterinary efforts of improving hygiene management during the harvesting and post-harvesting stages of the meat supply chain. The Animal Nutrition and Animal Products Institute (ARC-ANPI) was contracted to design and implement a surveillance scheme to monitor the microbiological quality of meat after the final stages of slaughtering and at the retailer. The present project was not just to establish the microbiological quality of meat in the NWP. It was also designed as a model to be used to improve meat quality and safety in the whole of the RSA. From a practical point of view, the "bacterial scores" generated by the MeatStats programme developed show how each abattoir performed while the "abattoir ranking system" shows where abattoirs

stand compared to other abattoirs. A particularly attractive feature of the bacterial score and rating systems is that the scoring is entirely based on sample data and that subjective scoring is eliminated. With such systems and assistance at hand, the present surveillance system can only benefit the whole meat industry for many years to come.

Key Words: Base line study, Microbiological safety, Meat

1071 Fermentation of whey permeate to lactic acid by *Lactobacillus helveticus* in a spiral-sheet bioreactor. M.M. Salameh*, A. Shahbazi, S.A. Ibrahim, M. Mims, and V. Shirley, *North Carolina Agricultural and Technical State University.*

The United States generates nearly 325 billion gallons of wastes and by-products during the processing of foods. Whey is a by-product produced in cheese industry and it is estimated that as much as 40-50% of the whey produced is disposed of as sewage or applied to agricultural lands. The remaining 50-60% is being used primarily for animal feed or for human food. Whey contains high quantity of lactose and other nutrients, which can be easily utilized by lactic acid bacteria to produce organic acids and value added products. The objectives of this work were to determine the ability of spiral-sheet to immobilize *Lactobacillus helveticus* (*L. helveticus*) and to determine the performance membrane for the continuous production of lactic acids using cheese whey as by-product. Active culture of *L. helveticus* (ATCC15009) was weekly transferred into fresh MRS and maintained at 4C. This culture was inoculated into 9 liters of MRS and incubated at 37 C for 16-18 hours. Fermented broth was transferred into the spiral-sheet bioreactor and was allowed to immobilize for 24 hours. After successful immobilization, MRS broth was withdrawn and replaced by fresh 4.8% lactose-MRS broth under aseptic conditions. Bioreactor was connected to Bioflo III (2-liter fermentor which has a control loop for temperature and pH) with continuous medium circulation at 37 C and agitation rate at 100rpm. Samples were withdrawn every 6 hours for lactose and lactic acid analysis using HPLC. Changes in pH were monitored during fermentation process. During fermentation the pH value was dropped from 6.2 to 3.8, which inhibited the bacterial activity. During the following experiments, we adjusted the pH to 6.50 by neutralizing the acid with 5-N ammonium hydroxide. Under the controlled pH condition, lactose conversion rate was 59.00%. These results showed that immobilization of *L. helveticus* on spiral-sheet reactor was a successful operation for the continuous production of lactic acid.

Key Words: spiral-sheet bioreactor, *Lactobacillus helveticus*, immobilization

1072 Molecular certification in chicken meat channel. V. Haezebroeck¹, R. Renaville*¹, I. Parmentier¹, S. Fontaine¹, S. Hetzel¹, and D. Portetelle¹, ¹*Animal and microbial biology Unit, Gembloux Agricultural University, Gembloux, Belgium.*

Adulteration of meat products is prohibited for fair trading, consumer protection, religious reasons or public health. As DNA can be extract from many different sources (blood, raw or cooked meat, milk,), DNA fingerprinting methods find an interesting applicability in species identification and meat traceability. Microsatellites markers are very polymorphic loci constituted by a variable number of a tandemly repeated motif of 1 to 6 base pairs, more commonly (CA)_n/(TG)_n. There are

abundant, multi-allelic, codominant and uniformly distributed throughout the genome of numerous species. The aim of this study was to find specific chicken microsatellites markers and to verify their ability to authenticate chicken products from other major meats. Nine microsatellites markers (MCW180, MCW135, MCW68, LEI161, MCW239, MCW264, LEI162, HUJ2, LEI78) were selected in the chicken genome and were combined in three triplex reactions. No cross-amplification were observed with bovine and pig DNA. All the nine microsatellites were also tested for cross-species amplification with other avian genomes. We observed that the microsatellite LEI161 give amplification product only for chicken. LEI161 is associated in a triplex PCR reaction with the markers MCW239 and MCW264. These three markers were revealed to easily distinguish chicken or turkey meat products. In conclusion, this triplex could be used to authenticate chicken or turkey in food and to detect falsification. Such a mean of analysis give new and alternative approaches of animal and species characterisation in meat products (Grant S-5876, Belgian Ministry of Small Enterprises, Traders and Agriculture DG4 and DG6 / and Belgian Public Health Ministry).

Key Words: Certification, Chicken, Meat

1073 Nutritional evaluation of Bt-corn in pigs . Tim Reuter, Karen Aulrich*, Andreas Berk, and Gerhard Flachowsky, *Institute of Animal Nutrition, Federal Agricultural Research Centre .*

The increasing cultivation of genetically modified plants opens new questions to animal nutritionists. One of them is to compare the nutritional value from parental and transgenic plants, another one is consumer safety. Analytical investigations to check the nutritional value are in progress at our institute, also investigations on the fate of "foreign"-DNA. The present project deals with a balance study to check the substantial equivalence, a grower-finisher trial to measure the pig performances and the fate of foreign-DNA in different tissues of pigs. The pigs were supplied with a 70% corn of the parental or the Bt-line containing diet. In a three collection period containing trial with twelve pigs we compared the feeding value of the Bt and the parental corn. All measured parameters were similar for both corn lines as shown in Table 1 (p > 0.05). The pig performances were measured with 48 pigs in a second trial. 12 pigs were fed a diet containing the parental corn, 36 pigs a diet containing the Bt corn. There were no significant differences (parental vs. Bt) in daily weight gain g/d (81593 vs. 80464), feed consumption kg/d (2.060.10 vs. 2.040.16) and Feed:gain kg/kg (2.550.27 vs. 2.590.18) between both groups (p > 0.05). The calculated results base on a growing period of 91 days. To investigate the fate of foreign DNA pigs were divided into different groups of six pigs each and slaughtered on different times after feeding. Samples were taken after slaughtering from different tissues.

Table 1. Digestibility of crude protein (dP), N-free extracts (dX) and metabolizable energy (ME) of diets

	1.period (grower)		2.period (finisher)		3.period (finisher)	
	control	Bt	control	Bt	control	Bt
dP %	83.33.1	86.21.8	85.62.4	85.02.0	85.71.5	87.01.6
dX %	91.60.8	92.40.7	93.20.4	93.30.5	93.40.5	93.70.6
MJ ME/kg DM	15.40.3	15.70.2	15.80.2	15.60.2	15.90.1	15.90.2

Key Words: Bt-corn, Genetically modified plants, pig

PSA Environment and Management

1074 The Effect of Lighting Program and Light Intensity on the Performance and the Incidence of Leg Abnormalities of Broiler Chickens . A. Kamyab*¹, S. Raja-Abadi², K. Yousefi³, and A. Taghipour Farshi⁴, ¹*University of Tehran, Animal Sci. Dept.*, ²*University of Mazandaran*, ³*Mobark Andish, Co.*, ⁴*Telavang, Co.*

This experiment was conducted in a 2x2x2 factorial arrangement, using two different lighting regimens, continuous (23L:1D) vs. intermittent (1L: 3D), two light intensities, high (30 Lx) vs. low (8 Lx) and two sexes. Some 864-day old chicks were randomly distributed by sex among 24 pens of 36 chicks each. In the first 2 days, all birds received 23 hours of light per day with 20 Lx intensity. After that chicks were subjected to an intermittent or continuous lighting program, combined

with two different intensities. At the end of study, day 42, body weight gain was affected by lighting treatments (P<0.05), but feed intake was not affected by lighting programs and intensities, and the only difference was found among males and females which had reflected the sex effect (P<0.05). Cumulative feed conversion among lighting programs, intensities and genders was significant (P<0.05). Comparison of feed conversions revealed the superiority of the intermittent lighting program relative to continuous one, low intensity compared to that of high intensity and males in comparison with females (P<0.05). The interactions of lighting programXintensity, lighting program x sex and intensity x sex were significant in some cases (P<0.05). Abdominal fat was affected by lighting treatment and was much lower in broilers reared under the intermittent lighting regimen than that of the broilers reared under the continuous one. Also males deposited less fat than that of the fe-