(8.93% *vs.* 9.18 and 9.12%). Phytase significantly increased the breaking strength of the humerus, but not the tibia, measured after 45 wk of production. These results indicate that early photostimulation decreases egg weight and feed intake and increases per cent shell, and that the levels of available P, Mn, Zn and Cu in the basal diet are adequate to support layer performance. However, the increased humerus breaking strength due to phytase suggests that the level of P was marginal for maintaining bone strength.

Key Words: Phosphorus, Phytase, Photostimulation

1366 Sources and levels of total phosphorus in the diet of broilers from 2 to 28 days of age. L. F. Araujo*[1], O. M. Junqueira[1], D. Mucke[2], R. Knoop[2], and C. S. S. Araujo[1], [1]*Universidade Estadual Paulista - UNESP/Jaboticabal - Brazil,* [2]*Burge Fertilizantes S/A - Sao Paulo - Brazil.*

One experiment was conducted to evaluate the performance of broilers receiving various sources and levels of phosphorus from 2 to 28 days of age. Four hundred chickens, 2 days of age, were distributed in a 4 x 2 factorial design (4 sources of phosphates - fine dicalcium phosphate (FDP); granulate dicalcium phosphate (GDP); a mixture of dicalcium phosphate + sodium (DPS); defluorinated phosphate (DFP) and two total phosphorus levels - 0.60% and 0.70%) with 8 treatments and 5 replications of 10 birds each. At the end of the experiment, two birds of each pen were slaughtered to obtain the tibia bone for phosphorus, calcium, magnesium and fluorine. There were no interactions between the treatments. The best weight gain and feed conversion were found in the birds receiving the diet containing GDP in the presence of 0.70% total phosphorus. The worst weight gains and poorest feed conversions were from birds fed the diet with DPS and 0.70% total phosphorus. The highest feed intakes were from diets containing GDP and 0.70% total phosphorus. There was no difference in the tibia calcium, phosphorus and magnesium between studied treatments. However, broilers fed the diets containing DPS and DFP showed, respectively, higher and lower tibia fluorine. According the results obtained, it is concluded that the best performance was from broilers fed diet with GDP and the level of 0.70% total phosphorus. The tibia fluoride level was lower in DFP diets. Acknowledgements: Burge Fertilizantes S/A Brazil for technical support.

Key Words: Phosphorus Sources, Phosphorus Levels, Tibia Minerals

1367 Total phosphorus (TP) requirements of meat chickens from 3 to 7 weeks of age. A. Abudabos*, D. V. Maurice, S. F. Lightsey, and W. C. Bridges, Jr.[1], *Animal & Veterinary Sciences,* [1]*Department of Experimental Statistics, Clemson University, Clemson, SC.*

We previously reported that dietary phytate P is utilized and TP could be decreased without impairment in productivity and substantial reduction in P excretion. However, the variation in body weight between birds on the same treatment prompted us to examine the response of birds, sorted by weight, to dietary TP. The aim of this experiment was to determine the effect of dietary TP in birds sorted into 2 groups: heavy (H) and light (L) at 3 wk. of age; then each group received 4 different diets. Corn-soybean diets were formulated, based on assayed TP values checked with standard reference materials, to contain 0.5, 0.55, 0.6 and 0.65% TP from 3-6 weeks and 0.38, 0.55, 0.6, and 0.65% TP from 6-7 weeks of age. Each diet was fed to 3 pens of each weight group. We measured the effects of TP, gender, and weight groups on growth, breast muscle yield, serum P, bone ash and measures of bone integrity, and intestinal phytase activity. Interactions were not detected and neither dietary TP or weight group had a significant effect on feed intake, body weight gain, feed conversion, breast muscle yield, and intestinal phytase at 6 weeks. Serum P was decreased in birds fed 0.5 % TP when compared to those fed the other three diets ($P< 0.001$). The responses at 7 weeks were similar to those observed at 6 weeks except that gender and weight group effects were detected. Serum P was a function of the amount of inorganic P in the diet ($P<0.0001$). Phytase activity was dramatically affected by diet ($P<0.02$); birds that received the 0.38% TP diet (no inorganic supplement) had the highest activity. Bone ash decreased ($P<0.0001$) as dietary TP was lowered but femoral and tibial length, weight, cross-sectional area, polar moment of inertia, and area moment of inertia were not affected by diet. The results show that dietary P can be lowered from 3 to 7 weeks without loss in performance and reduction in mechanical properties of long bones.

Key Words: Dietary Phosphorus, Intestinal Phytase, Growth, Bone Integrity

ASAS/ADSA Animal Health

1368 *In vitro* aflatoxin binding characteristics of an esterified glucomannan product. J.W. Evans* and M. Kudupoje, *Alltech Biotechnology Inc., Nicholasville, KY.*

Mycotoxin binders have been shown to reduce the deleterious effects of aflatoxin in animals. A series of experiments were conducted to examine the binding characteristics of an esterified glucomannan (EGM) derived from the yeast cell wall of *Saccharomyces cerevisiae*. The EGM has been shown to reduce the toxic effects of aflatoxin in livestock. In the first study, an *in vitro* binding assay was used to determine the saturation point of aflatoxin binding by EGM in water or phosphate buffer. The binder (0.1%) was mixed with increasing concentrations of toxin (2,4,6,8 and 10 ppm in water/18, 20, 22, 24 and 26 ppm in buffer), centrifuged and unbound toxin concentrations were determined in the supernatant. The saturation point was defined as the minimum binder concentration at which maximum binding is reached. The saturation point for aflatoxin binding was greater in phosphate buffer than in water (8 vs. 24 ppm). In addition, aflatoxin binding responded to phosphate concentrations in a quadratic fashion ($P<0.001$). Another trial was conducted to determine binding strength in water and phosphate buffer. Aflatoxin (1 ppm) and EGM (0.1%) were mixed, incubated at 37°C for 1 h, and centrifuged. Toxin concentrations were determined in the supernatant and pellet after three methanol extractions. Binding was stronger in the phosphate buffer than in water as indicated by lower aflatoxin recovery in the buffer (568 vs. 279 ppb, $P<0.001$). A third study compared the saturation points of the EGM and a clay binder (CB). Both binders were mixed in water with increasing toxin concentrations until saturation was reached. The binding capacity of EGM in water was more than four times higher than CB (5.2 vs.1.2 μg/mg binder, $P<0.001$). This series of studies showed that EGM is able to bind aflatoxin and not mask or destroy it. In addition, aflatoxin binding by EGM is phosphate dependent and more efficient at binding aflatoxin than binding by the CB tested.

Key Words: *In vitro* binding, Aflatoxin, Esterified glucomannans

1369 Growth and immune function of calves fed milk replacer with added nitrate. S. T. Franklin, R. O'Carra, R. J. Harmon, D. M. Amaral-Phillips, and J. A. Jackson, *University of Kentucky, Lexington, KY.*

A concentration of 10 - 20 ppm nitrate-N in water is considered a health risk for humans and may cause methemoglobinemia. Little is known, however, of the effects of nitrate-N on health and immune function of neonatal dairy calves. Thus, 24 calves (4 Jersey and 20 Holstein; male = 12 and female = 12) were assigned to treatments of 0, 10, 20, or 40 ppm nitrate-N (as potassium nitrate) added to reconstituted milk replacer. Calves were moved to individual calf hutches and assigned to treatments at an average of approximately 5 d of age. Milk replacer (20% protein and 20% fat) was reconstituted in water to provide 0.45 kg of solids daily and fed in two equal feedings. Nitrate-N was added to reconstituted milk replacer at each feeding. Milk replacer intake was recorded at each feeding and starter consumption was determined daily. At 5 wk of age, milk replacer was fed once daily and calves were weaned at 6 wk of age. Calves were weighed when moved to hutches and weekly thereafter. Blood samples were obtained at 2 wk intervals for total white blood cell counts, neutrophil function assays, and proliferation assays. There was no effect ($P > 0.05$) of nitrate-N addition to milk replacer on

body weights of calves, total white blood cell counts, ingestion or killing of *Staphylococcus aureus*, or proliferation of mononuclear leukocytes in response to mitogen stimulation. Both ingestion and killing of *Staphylococcus aureus* decreased ($P < 0.01$) over the 6 wk study. For wk 0, ingestion of *S. aureus* averaged 11.5 bacteria per neutrophil and for wk 6 averaged 8.4 bacteria per neutrophil. Killing for wk 0 averaged 34% of added bacteria compared to 23% for wk 6. Ingestion and killing also were lower ($P < 0.01$) for winter versus spring calves. Ingestion of *S. aureus* averaged 10.8 bacteria per neutrophil for winter calves and 9.1 bacteria per neutrophil for spring calves. Similarly, killing averaged 33% of added bacteria by neutrophils from winter calves compared to 26% killing of added bacteria by neutrophils from spring calves. In conclusion, nitrate-N at concentrations up to 40 ppm in milk replacer did not greatly affect growth or immune function of neonatal calves, however, neutrophil function decreased as calves aged and was lower in spring compared to winter.

Key Words: Nitrate, Calves, Immune function

1370 Brain cholinesterase activity in cattle exposed to coumaphos in Mexico. V. Pardio*[1], N. Ibarra[1], A. Velasquez[2], B. Nochebuena[1], E. De la Cruz[1], and J. Alfaro[1], [1]*Universidad Veracruzana, Veracruz, Veracruz/Mexico,* [2]*Instituto Mexicano del Seguro Social, Veracruz, Veracruz/Mexico.*

The purpose of the study was to determine Cholinesterase (ChEs) activity values from brain stem of cattle to evaluate the exposure to organophosphate pesticides, since the intensive use of these pesticides in Mexico represents a potential danger to livestock and to human health. Four groups of ten animals from different areas were sampled. Brain stems from mature male adults *Bos taurus x Bos indicus* 18 months of age were collected at the Federal Inspection slaughter in Veracruz, Mexico. Brain stem was removed according to official Mexican procedure and placed into cold buffered isotonic solution. Brain stem was sectioned into protuberance, medulla oblongata and the cervical region of spinal cord. ChE activity determination was according to Ellman method and AchE was assayed with iso-OMPA. Cholinesterase activities were expressed as UI/mL. Results were analyzed by ANOVA with Minitab 10.5. The mean AchE activity in medulla oblongata section of the animals dipped in coumaphos at the recommended dose every 21 days during 3 months was significantly lower than a group of animals not treated with any pesticide from the farm of the Faculty of Veterinary Medicine, but not statistically different from the other groups analyzed. The mean ChE and AchE activities in medulla oblongata and spinal cord of a group of animals dipped in coumaphos at the recommended dose every 21 days during 6 months were significantly lower than a group of animals not treated with any pesticide and than the group of animals dipped during 3 months. The mean AchE activities in medulla oblongata and spinal cord of the group of animals sprayed with coumaphos at the recommended dose twice a month during 6 months were significantly lower than the group of animals not treated with any pesticide and than the group dipped during 3 months. Among the three sections of tissue analyzed, medulla oblongata mean AchE activity was statistically higher than the AchE activities of the protuberance and spinal cord. Results indicate that medulla oblongata of cattle represents a useful area for measurement ChE activity for laboratory screening as an aid in diagnosing anticholinestersase insecticides exposure in cattle.

Key Words: Organophosphate pesticides, cholinesterase activity, brain stem

1371 Extension needs and approaches towards livestock health improvement in Bangladesh : Proshika experience. Nuru Miah, *Proshika Manobik Unnayan Kendra, Bangladesh.*

Livestock contributes 6.5% percent of the GDP and 13% of the agricultural GDP in Bangladesh. The livestock sector has been trapped in low productivity levels due to inadequate extension coverage of the Govt. sector. Proshika, one of the largest Non Govt. Development Organisations in Bangladesh has been operating livestock extension and support services in its working areas. It provides livestock extension services to cattle and poultry farmers through well trained resource persons. About 60 to 100 vaccinators, 10 to 15 paravets, 4 to 6 A.I. technicians, and 4-6 feed sellers in each upzilla are engaged in providing vaccination, deworming, treatment, artificial insemination services, and suppling quality feeds to rearers of cattle and poultry. Veterinary drug pharmacies are operated by advanced paravets. These extension agents are selected from proshika group members, trained for particular skills, provided equipment boxes with equipment's, and are allocated to working areas to maintain strong links to producers. Vaccines not produced locally are imported by Proshika and supplied to the beneficiaries. Fees are collected from cattle and poultry owners for their livelihood. These extension activities have reduced poultry mortality from 20 percent to 5 to 7 percent; cattle mortality has been recuced from 10 to 15 percent to 3-4 percent. These efforts have significantly increased the livestock and poultry population. Farmers undertaking different livestock rearing projects have increased their income up to 150 percent. The monthly net income of vaccinators, paravets and A.I. technicians varies from US Dollar 15 to 45 based on the activity. These extension approaches contribute towards the increased productivity of livestock resources, enhancement of gender equity and equality, and ensure participation of vulnerable women in productive enterprises.

Key Words: Paravet, Deworming, Extension

1372 Bedding material preferences of crossbred cattle. DilipKumar Garikipati*[1], Kailash M. M.[2], and Sarjan Rao Kapa[1], [1]*College of Veterinary Sciences,Tirupati,* [2]*Bangalore Agricultual University.*

Twenty crossbred HF cattle were examined for preferences in bedding materials. Two experiments were done. In experiment 1, crossbred cattle (n=10) were housed individually with access to 3 free stalls, each with different bedding material: Paddy straw, sawdust, or deep-bedded sand. After access to all 3 materials for 10 days, preference was determined by stall use and lying times, recorded for 24 h by videotape recorder. Each animal was then restricted to either sand or paddy straw for period of one week. Average lying time, and numbers of transitions between standing and lying, were significantly ($p<0.05$)less than when animals were restricted to sand or paddy straw. After the restriction phase, animals were again allowed access to all 3 bedding types and final preference was determined. Nine of 10 animals continued to choose sawdust. In experiment 2, 10 more animals were tested with sawdust, sand (both as described above), and paddy straw and waste paper materials. Initial & final preference tests as described above showed that 8 of 10 cows preferred sawdust. In the middle stage of the experiment all animals were restricted to each bedding material, in turn, for 5 days. Average lying time, time spent in the stall, and transition to lying were significantly ($P< 0.02$) lower for the sand - bedded stalls but there was no differences between other 2 surfaces. These results indicate that (1) cows prefer deep bedded sawdust, (2) that lying time, time spent in the stall, and number of lying transitions are affected when cows are provided with sand and paddy straw, but not with paddy straw covered with waste paper material.

Key Words: cow comfort, behavior, well-being

1373 Economic Efficacy of Treatment Protocols for Clinical Mastitis. E.H. Shim*, R.D. Shanks, and D.E. Morin, *University of Illinois, Urbana.*

The objective was to quantify the costs associated with two treatment protocols and the differences between the use of supportive therapy alone versus antibiotics in addition to supportive therapy. Between January 1994 and December 1995, 116,876 daily milk (DM) records on 676 lactations were taken at the University of Illinois Dairy Unit. Mastitis was diagnosed during 124 lactations with 25,047 DM records. Based on examination, each cow with clinical mastitis was assigned a severity score of 1 (least severe) to 3 (most severe) and randomly assigned to one of two treatment groups: N (supportive treatment only) and A (antibiotics in addition to supportive treatment). Extent of antibiotic and supportive treatment varied according to protocols. A treatment cost analysis was calculated for all lactations with at least one case of mastitis. Predicted and actual DM yields were estimated utilizing a random regression-test day model. The differences between the predicted and actual DM were summed for all 305 days of lactation to estimate milk loss for each mastitic lactation. Lactations that utilized antibiotics in addition to supportive therapy incurred $21 per lactation more in average treatment costs than lactations utilizing supportive therapy alone. Cows treated with supportive therapy alone lost 1,530 kg more marketable milk per lactation than cows treated with both antibiotics and supportive therapy. The addition of antibiotics to supportive therapy reduced the total economic loss by $382 per treated lactation. Based on smaller milk yield and economic losses, the efficacy of antibiotics

in addition to supportive therapy was greater than supportive therapy alone.

	A	N
Average number of days with clinical mastitis	13	22
Average treatment cost	$49	$28
Average 305-day milk yield loss (kg)	182	1809
Milk loss due to unmarketable or unproduced milk (kg)	513	2043
Cost of lost milk*	$135	$538
Total cost (cost of lost milk and treatment)*	$184	$566

*based on the Feburary class 1 mover milk price of $0.2632 per kg

Key Words: Mastitis, Economics, Antibiotics

1374 Effect of Left Displacement of Abomasum (LDA) Corrected by Toggle Pin Suture on Performance of Holstein Dairy Cows. J.E.P. Santos[1], E. Raizman*[1], L.G. Corbelini[2], and R.A. Cerri[1], [1]*University of California, Davis,* [2]*Universidade Federal do Rio Grande do Sul.*

Objectives were to evaluate the effects of left displacement of abomasum (LDA) corrected by toggle pin suture on performance of lactating Holstein cows. Cows calving between June 1997 and February 2000 (374; 188 LDA and 186 controls) from a commercial dairy farm in central California were followed during the first 320 days in milk (DIM). Cows were diagnosed with LDA by the herd veterinarian and treated by the herd personnel by rolling the cow over her back and tacking the abomasum with toggles introduced after trocar punctures. Every LDA cow was matched with a herdmate that calved within 14 d of the affected cow's calving date, was in the same lactation and had a similar 305-d mature equivalent (ME) milk yield in the previous lactation (300 kg). Only cows that stayed in the herd for more than 60 DIM were included in the analyses. Continuous variables were analyzed by ANOVA and binomial data by Chi-square. Cows affected with LDA produced less milk (33.3 vs 31.6 kg/d; P< .01) and 3.5% FCM (33.2 vs 32.0 kg/d; P< .06), and had a lower 305-d ME milk yield (12,563 vs 12,073 kg; P< .05). However, milk fat yield (1.16 vs 1.13 kg/d; P< .22) and linear SCC (2.68 vs 2.86; P< .24) were similar, and milk fat content tended to be higher for cows with LDA compared with controls (3.51 vs 3.60; P< .08). Cows with LDA had a prolonged period from calving to first AI (77.9 vs 92.8; P< .001), but first AI conception rate, percentage of cows pregnant by 150 and 320 DIM were unaffected by LDA. Mean days open for pregnant and for all cows were unaffected by LDA, as well as the incidence of abortion and mastitis. Days in milk at the first clinical mastitis case tended to be lower for cows with LDA (140.7 vs 94.0; P< .12). Nevertheless, more LDA cows died (1.1 vs 8.0%; P< .001) or were sold (9.1 vs 27.7%; P< .001) by the end of the study. In addition, LDA cows left the herd (dead or sold) earlier in lactation (230.2 vs 102.1 DIM; P< .001). In conclusion, cows affected with LDA produced less milk, but most of the lactation losses were caused by increased death and culling early in lactation.

Key Words: Left displacement of abomasum, Dairy cows, Lactation

1375 Effect of disease on fertility traits in Swedish dairy cattle using survival analysis methodology. D.O. Maizon*[1,2] and P.A. Oltenacu[1], [1]*Department of Animal Science, Cornell University,*, [2]*Facultad de Ciencias Veterinarias, Universidad de Buenos Aires.*

Fertility traits are economically important in dairy cattle herds. In this study, the effect of disease on days from calving to conception was estimated. A sample of 20% of all cows that calved in Swedish farms in 1991 was used. The final data set was restricted to cows in their second or greater lactation with at least one service after calving, and consisted of 18,114 Ayrshire (SRB), and 12,530 Friesian (SLB) cows. Since days from calving to conception is a time-to-event variable, and about 26% are censored records in each breed, the analyses were performed using the Survival Kit software. A Weibull model was assumed, each breed was analyzed separately, and assessments were based on the likelihood ratio test. The models included the following confounders: season of calving, length of previous dry period, herd size, and average fat corrected milk of herd and cow, all treated as classificatory variables. The diseases studied were dystoccia (DYST), milk fever (MKFV), and retained placenta (REPL) as time-independent covariates, and cystic ovaries (CYST), metritis (METR), other reproductive diseases (OTRE), mastitis (MAST), ketosis (KETS), feet and leg disorders (FFET), and other diseases (OTHE) as time-dependent covariates. The diseases with negative and statistically significant ($p < 0.05$) effects on days from calving to conception were RTPL, CYST, and MAST in SRB cows, and DYST, OTRE, METR, MAST, and OTHE in SLB cows. The corresponding risk ratios, statistically different from one ($p < 0.05$), for these diseases were 0.785, 0.893, and 0.876 in SRB cows, and 0.638, 0.669, 0.742, 0.871, and 0.82 in SLB cows, respectively. These risk ratios indicate that these diseases decrease the probability of conception, and increase the time-to-conception.

Key Words: Dairy Cattle, Diseases and Fertility, Survival Analysis

1376 The diagnostic value of serum cholesterol in cows and newborn calves. R. Skrzypek*, *Agricultural University of Poznan, Poland.*

The study was conducted on 210 cow-calf pairs of crosses between native Black-and-White cattle and Holsteins. Cows were sampled 7-8 weeks before expected parturition, and 12-14 hours after parturition. Calves were sampled at the age of 24 hours, and 5 days after birth. There was found no significant relationship between the course of the parturition period (normal delivery, dystocia, placenta retention, and milk fever), and concentration of serum total cholesterol (TCh) in cows, as well as in their calves. In cows, serum TCh concentration tested before parturition was correlated negatively with the number of insemination services per confirmed pregnancy, and length of calving interval ($r \sim -0.2$; $P \leq 0.05$). Before parturition, cows with the shortest calving interval ($\leq$365 days) compared to cows with the longest calving interval (401$\leq$ days) had significantly higher serum TCh concentration by 0.20 mmol/L ($P \leq 0.05$). After parturition, the highest serum TCh concentration was found in the group of cows with medium length of calving interval (366-400 days). In calves that were afflicted with diarrhoea or died during the first month after birth, at both samplings it was found significantly lower serum TCh concentration (range from 0.10 mmol/L; $P \leq 0.05$ to 0.19 mmol/L; $P \leq 0.01$), as compared to control calves. It is concluded, that serum total cholesterol concentration is associated positively with reproductive performance of cows, as well as health and survival of the newborn calves.

Key Words: Cattle, Serum cholesterol, Diagnostic value

1377 Development of a DNA-based vaccine for the prevention of staphylococcal mastitis. E.W. Carter* and D.E. Kerr, *University of Vermont, Burlington VT.*

Staphylococcus aureus often results in a chronic infection of dairy cows. Current vaccine formulations are often ineffective in preventing infection. The objective of this study was to stimulate an immune response in dairy cows through injection of plasmid DNA containing a staphylococcal Protein A gene. To determine the most effective method of DNA-based vaccination of cows, intramuscular and intradermal vaccination sites were evaluated using a plasmid containing the jelly-fish Green Fluorescent Protein (GFP) gene. DNA was delivered by needle and syringe, or by high or low-pressure jet injection. For each injection site, 15 animals, six weeks prepartum, were assigned to receive DNA by one of the three delivery techniques. On each of three days, at two week intervals, each animal was given three injections (0.17 ml) containing a total of 0.5 mg of plasmid in 0.15 M saline. Serum samples were collected at two week intervals for anti-GFP antibody analysis by ELISA. Antibody titers (1/100 dilution) peaked approximately two weeks after the final injection. It was found that intradermal high-pressure jet-injection elicited the best response (3 of 4 animals responding). In contrast, needle and syringe delivery was ineffective while response to low pressure injection was intermediate. Similar results developed from intramuscular injection. A Protein A expression plasmid was constructed with an 827bp fragment of the Protein A gene inserted downstream of the CMV promoter region in the pcDNA3 vector. Protein A expression by eukaryotic cells was evaluated by transfection of COS-7 cells followed by Western blot analysis. The results confirmed the expression of the expected 32 kD Protein A. This plasmid is currently being evaluated as a vaccine in cows using high-pressure jet-injection. Specific humoral (ELISA) and cell mediated (lymphocyte proliferation assay) immune responses will be monitored. We have shown that an antibody response to a foreign antigen can be raised in dairy cows using a DNA-based vaccine. The needle and syringe delivery method was not as effective as jet injection

1378 Immunogenicity of a putative intranasal vaccine against bovine respiratory syncytial virus (BRSV)in calves. B. Earley*[1], O. Kavanagh[2], B. Adair[2], and R. Fallon[1], [1]*Teagasc, Grange Research Centre, Dunsany, Co. Meath, Ireland.*, [2]*Veterinary Science Division, Stormont, The Queen's University of Belfast, BT4 3SD, Northern Ireland.*

The objective of the study was to evaluate the effects of microencapsulated synthetic peptides of bovine respiratory syncytial virus (BRSV), following intranasal administration, on the cellular and humoral immune responses of calves. F peptide (F111-148)(0.5mg) and G peptide (G174-187)(0.5mg) representing known protective epitopes against BRSV were microencapsulated. Fifty-two Holstein/Friesian calves, approximately 56 days of age, were allocated randomly to one of the following 4 treatments (13 per treatment); 1). Soluble F-peptide (SF) and soluble G-peptide (SG) in phosphate buffer saline (PBS); 2). Microencapsulated poly(lactide-co-glycolide (PLG)-peptide-F and PLG-peptide-G); 3). Microencapsulated coupled to ovalbumin (OVA) (PLG OVA-peptide-G and PLG OVA-peptide-F); 4). Phosphate buffered saline control. Interferon (IFN) gamma production in response to concanavalin-A (Con-A) was determined in cultured lymphocytes on days 28 and 142 post-primary inoculation. Serum immunoglobulins (Ig)were measured quantitatively by single radial immunodiffusion on day 28 post-inoculation. The data were analysed using analysis of variance procedure and if a significant difference was observed, Duncan's multiple range F-test was applied to determine statistical differences between treatments. There was no difference ($P>0.05$) between treatments in response to Con-A induced IFN gamma-production from lymphocytes cultured on days 28 or 142 indicating that the vaccination had no effect on the cellular immune response. There was no significant difference in serum Ig concentrations between treatments ($P>0.05$). A sustained antigen-specific IgA response was induced in the calves nasal mucosa and lungs by all treatments ($P<0.05$) when compared with PBS control calves on day 28 for the SF and SG peptides. On Day 77 post-primary inoculation the antigen-specific IgA response to the F peptide subunit was maintained in all treatments compared with control while the response of the G peptide subunit ($P>0.05$) was not sustained. Non-live subunit vaccines encapsulated in biodegradable microparticles are a novel approach to achieving a protective immune response against specific pathogens in the hosts' respiratory tract.

Key Words: Bovine Respiratory Syncytial Virus, Microencapsulation, Immune Function

1379 Differential tyrosine phosphorylation on bovine PMN. Kynita Campbell[1], Max Paape[2], Mulumebet Worku*[1], and Yan Wang[2], [1]*North Carolina Agricultural and Technical State University,* [2]*USDA Beltsville, Maryland.*

Protein tyrosine phosphorylation is a key step in pathogen destruction by neutrophils (PMN) following ligand receptor interaction. Bovine blood PMN were isolated by differential centrifugation and hypotonic lysis of red blood cells. Viability was assessed by Trypan blue dye exclusion, purity by differential cell counts and concentration using a Coulter counter. Isolated PMN (10^7) were treated with Escherichia coli lipopolysaccharide (LPS)(1,000ng/ml, 100ng/ml), Dexamethasone (0.25mg/ml, 0.15mg/ml) and Sodium Butyrate (160μM, 80μM)or maintained in buffer (1 hour, 37°C). Cells were then lysed with Laemmli buffer, boiled, sonicated and analyzed by SDS-PAGE. Tyrosine phosphorylation was detected by western blotting with a mouse anti-phosphotyrosine monoclonal antibody and alkaline phosphatase conjugated goat anti-mouse antibody using BCIP-NBT substrate (Kirkegaard and Perry Laboratories). The number and intensity of phosphorylated protein bands after treatment were compared to untreated PMN. Lysates from untreated PMN contained six distinct bands. LPS and Dexamethasone treatments resulted in dephosphorylation of all bands. One unique band remained phosphorylated after treatment with Sodium butyrate. Dexamethasone (0.25mg/ml) resulted in the appearance of a unique band (64KDa). These proteins may play a significant role in modulation of bovine PMN function.

Key Words: Polymorphonuclear (PMN), Protein phosphorylation

1380 Bovine PMN release the COX-2 protein when stimulated with bacterial lipopolysaccharide. Jenora Waterman and Mulumebet Worku*, *North Carolina Agricultural and Technical State University.*

Neutrophils (PMN) are central to the inflammatory response of mammals. Cyclooxygenase-2 (COX-2) is one of two isoforms of the enzyme that catalyzes the production of prostaglandins from arachidonic acid. We show here that bovine PMN release the COX-2 enzyme when stimulated with *Escherichia coli* lipopolysaccharide (LPS). Bovine blood PMN from the caudal vein were isolated by differential centrifugation and hypotonic lysis of red blood cells. Viability was determined by Trypan blue dye exclusion, purity by differential cell counts and concentration using a hemocytometer. Isolated viable PMN (10^7) were incubated for 0.5, 1, 1.5, 2, 2.5, and 3 hours in the presence or absence of 10 ng/ml LPS. Cells were harvested, lysed and fractionated using 12% SDS-PAGE. COX-2 was identified by western blot analysis and enhanced chemiluminescence. COX-2 is expressed by bovine PMN. Expression of COX-2 can be modulated by treatment with LPS. Thus, bovine PMN may actively influence the eicosanoid composition during inflammation.

Key Words: Cyclooxygenase-2, PMN

1381 Modulation of apoptosis in bovine blood PMN by actinomycin-D, lipopolysaccharide, and sodium butyrate. P Matterson*, S Knight, and M Worku, *NC Agricultural and Technical State University.*

The longevity of neutrophils (PMN) is critical to cell function and resolution of inflammation. The development of methods to asses apoptosis in bovine PMN is essential to development of new therapies to control inflammations such as mastitis. Here we describe a modified assay to detect apoptosis in bovine PMN by comparing the effect of actinomycin-D (160μM), sodium butyrate (160μM), *E. Coli* lipopolysaccharide (LPS)(10ng/ml) treatments versus untreated isolate of PMN. Whole blood was collected from healthy, lactating Holstein cows (N=3) in 15ml vacutainer blood collection tubes pretreated with 250 IU of heparin sodium. The blood was pooled, diluted with 1X PBS, separated by gentle centrifugation and RBC were lysed with 0.83% ammonium chloride several times until a white pellet and clear supernatant was obtained. Viable, isolated PMN were verified by microscopic observation and counting, using Trypan Blue exclusion for viability (98.0%) and Wright stain differentials. Treated and control PMN were spotted onto poly-L-Lysine, subbed slides. After drying, slides were then assayed for apoptosis detection using Promega's Apoptosis Detection Kit which is based on the TUNEL method of labeling fragmented DNA of apoptotic cells with Fluorescein. The percentage of cells incorporating green fluorescence was evaluated microscopically. Actinomycin-D induced apoptosis (37%)while LPS only tended to induce apoptosis (19%). Sodium butyrate maintained (12%) PMN longevity compared to control cells (13%). Although cow to cow variation was observed, this method was effective in determining the effect of modulators of inflammation on bovine PMN.

Key Words: apoptosis, neutrophil, mastitis

1382 Techniques for RNA isolation and cDNA integrity in bovine blood PMN. S Knight*, M Worku, P Matterson, and S Dance, *NC Agricultural and Technical State University Greensboro, NC USA.*

Obtaining full-length quality cDNA is critical to experiments involving RNA analysis and library construction. This is even more so in neutrophils. Studies were conducted to evaluate two methods of RNA isolation and a cDNA integrity kit developed by Kirkegaard and Perry Laboratories, for determination of quality cDNA in the human, mouse, and rat systems. Bovine neutrophils (PMN) were isolated by differential centrifugation and hypotonic lysis of red blood cells. Viability was assessed by Trypan blue dye exclusion, concentration was determined by hemacytometer counts and purity by Wright's stained smears. Two methods of RNA isolation (Tri-Reagent and RNAzol) were compared and RNA quality determined by electrophoresis. To determine cDNA integrity, RNA was isolated from viable PMN using Tri-Reagent and a denaturing MOPS/formaldehyde agarose gradient gel. The RNA was reverse transcribed and the cDNA amplified by PCR using six primers specific for regions of the 3' and 5' ends of the housekeeping gene GAPDH, two

common ribosomal protein genes, L3 and S6, and the 3' and 5' regions of the clathrin gene. Tri-Reagent produced the purest RNA preparation with minimal DNA contamination. The cDNA kit may also be applicable for determination of bovine neutrophil cDNA integrity, and identity of conserved sequences. Acknowledgement to KPL for enabling us to serve as Beta tester.

Key Words: Neutrophil, cDNA, RNA

1383 Isolation of membrane protein associated with IgM binding from bovine neutrophils. A Johnston-Ward*, S Knight, and M Worku, *NC Agricultural and Technical State University Greensboro, NC USA*.

Isolation of membrane protein Fc receptors for IgM is essential to the determination of their role in bovine neutrophil (PMN) function for the formulation of possible therapeutics against bovine mastitis. Bovine PMN from two Holtstein-Friesians were isolated by hypotonic lysis of red blood cells and centrifugation. PMN were biotinylated and lysed. The lysates were incubated with purified bovine IgM, followed by goat anti-bovine IgM antibody, and immunoprecipitated with Protein A/G sepharose beads. Proteins were detected using western blotting and enhanced chemiluminescence(ECL)following binding of horseradish peroxidase conjugation Streptavidin. Two to three proteins ranging from 30-90kDa were shown using Coomassie Blue stain. After biotinylation, six proteins of 38-90 kDa were seen using ECL detection. Following immunoprecipitaion a unique protein at 28 kDa was also detected with ECL. Further analysis is needed to identify and characterize this protein and any other associated membrane proteins.

Key Words: biotinylation, IgM, Neutrophils

1384 Establishing and comparing profiles of antimicrobial resistance in *Staphylococcus aureus* isolates from selected organic and conventional dairy farms in Vermont. C. Nugent*, P. Murdough, W. Panky, and J. Barlow, *University of Vermont, Burlington, VT*.

Resistance to antimicrobial agents has become a great concern in human medicine, and has been linked to the use of antimicrobials in agriculture (Khachatourians, 1998). Antibiotics are used in agriculture both to increase growth rates in livestock as well as to treat and prevent disease. In the dairy industry, antibiotics are commonly used to combat bovine mastitis. The purpose of this study is to describe and compare the antimicrobial resistance patterns among bacterial isolates obtained from individual quarter milk samples of cows on selected dairy farms in Vermont. Bacterial isolates were collected over an 18 month period from 10 dairy farms, including 6 certified organic dairies where antibiotic use is avoided, and 4 conventional dairy farms that routinely use antibiotics for treatment and prevention of disease. In this preliminary study, the antimicrobial resistance profile of 180 *Staphylococcus aureus* isolates (54 from conventional farms and 126 from organic farms) was established by standard disk diffusion and minimum inhibitory concentration (MIC; Sensititre, Trek Diagnostics, WestLake, OH) methods. Sixteen antibiotics were evaluated. Increased proportions of isolates showing resistance to ampicillin, penicillin, pirlimycin, sulfamethizole, and tetracycline were observed from conventional dairy farms compared with organic farms. No differences in proportion of resistant isolates were observed for amoxicillin plus clavulonic acid, cephalethin, ceftiofur, enrofloxacin, erythromycin, florfenicol, gentamycin, lincomycin, neomycin, oxacillin, and penicillin plus novobiocin. Data on the prevalence of mastitis isolates (such as *Staphylococcus aureus* and Escherichia coli) displaying antimicrobial resistance may be used to evaluate the potential impact of current treatment practices on the development of antibiotic resistant pathogens on dairy farms.

Key Words: Antibiotic Resistance, Dairy Cattle, Mastitis

1385 Improved quantification of total lipids from liver samples. B. N. Ametaj*, Y. Lu, G. Bobe, J. W. Young, and D. C. Beitz, *Iowa State University, Ames, IA*.

Fatty liver is a major metabolic disease of dairy cows, and early diagnosis, as based upon total lipid percentage of the liver, is important for effective treatment or prevention. Previous methods for assaying total lipids in liver biopsy samples involved freezing in liquid nitrogen, pulverization of samples by percussion, extraction by shaking overnight in 2:1 chloroform and methanol (vol:vol), evaporation of solvent, and weighing of total lipids. Pulverization causes variable losses of liver sample, and utilization of liquid nitrogen is hazardous. We evaluated combinations of sonication, homogenization, and pulverization, followed by shaking for extraction of total lipids from liver samples. Results from samples obtained by biopsies of livers of dairy cows indicate that sonication is safer and faster than pulverization of liver samples. The sufficient amount of time for sonication (0, 10, 20, 30, 40, 50, and 60 sec) was 30 sec. Homogenization for 2 min and shaking overnight in chloroform:methanol (2:1 vol:vol) did not affect the amount of total lipids extracted ($P > 0.1$) compared with sonication and shaking for 2 h. From different amounts of liver sample (50, 100, 150, 200, and 250 mg), the amount of lipid extracted from at least 150 mg resulted in repeatable results (CV < 10%). Our data indicate that sonication for 30 sec, shaking for 2 h, sitting overnight at 4° C, and drying samples under air for 6 h is an efficient and reproducible method for quantification of total lipids from bovine liver. (Partly supported by funds from CSREES-USDA agreement 99-35004-8576)

Key Words: Total lipids, Liver, Bovine

1386 Effect of slow-release insulin on bovine hepatic lipidosis. A. Hayirli*, J. E. Kayhart, S. J. Bertics, and R. R. Grummer, *University of Wisconsin, Madison*.

The aim of this study was to determine if there is a dose of insulin that decreases concentrations of plasma NEFA and liver TG, without compromising plasma glucose concentration. Forty Holsteins were blocked according to calving date and assigned randomly to a single IM injection of one of four doses of slow-release insulin on d 3 postpartum. Cows were allowed ad libitum consumption of the same diet from 15 d prepartum to 6 d postpartum; on d 3 postpartum, they were fed hourly to minimize fluctuations in blood metabolites due to intake pattern. Blood samples were collected every hour from 0 to 24 h and every 6 h from 24 to 48 h following injection. Pre- and post-injection liver samples were taken on d 2 and 5 postpartum, respectively. Main and polynomial effects of insulin were tested by ANCOVA using a mixed model with repeated measures. One cow receiving .29 and two cows receiving .43 IU insulin/kg BW could not complete the trial due to severe hypoglycemia and their data were excluded from analyses. Both DMI ($P<.13$) and milk yield ($P<.15$) increased quadratically by insulin injection. Plasma glucose linearly decreased ($P<.0001$), serum insulin linearly increased ($P<.0001$), and plasma NEFA quadratically decreased ($P<.05$) for 24 h following injection. On d 4 postpartum, serum insulin concentration remained higher in insulin-injected cows than in saline-injected cows ($P<.08$) and the quadratic effect of insulin on plasma NEFA continued ($P<.02$). Percent change in liver TG decreased quadratically by insulin injection ($P<.08$). The low dose of slow-release insulin could be considered for prophylactic use against hepatic lipidosis.

	Dose[1]				
Response Variable	0	.14	.29	.43	SEM
DMI, kg/d (d 3-5)	15.1	16.6	15.6	14.3	.9
Milk yield, kg/d (d 3-5)	30.1	34.7	31.9	29.3	2.3
0-24 h Insulin, μU/ml	2.0	3.5	5.7	7.6	.7
0-24 h Glucose, mg/dl	47.5	42.3	35.9	36.7	1.7
0-24 h NEFA, μEq/ml	576.0	512.0	538.8	643.4	41.0
Liver TG, % DM (d 5)	9.9	8.1	11.5	11.1	1.4
Change inTG, % (d 2 vs. 5)	26.7	-10.2	12.3	42.9	18.1

[1]Insulin, IU/kg BW

Key Words: Insulin, Bovine, Hepatic lipidosis

1387 Utility of RAP-PCR to identify genes in bovine liver differentially expressed following in vivo endotoxin (LPS) challenge. E. E. Connor*, C. M. Ashwell, S. Kahl, and T. H. Elsasser, *USDA-ARS, Beltsville, MD*.

The liver is a target organ of cytokine action during an immune response and is the principal site of acute phase protein synthesis. In the present study, RNA arbitrarily primed polymerase chain reaction (RAP-PCR) was used to evaluate hepatic gene expression profiles of saline and low-level LPS-treated cattle to gain novel information on gene expression in inflammatory disease. A single bolus of either saline (n = 4) or LPS (E. coli 055:B5, 0.2 μg/kg, n = 6) was administered through a jugular

catheter on two occasions 5 d apart. Liver biopsy was performed on each animal 8 h after the second LPS challenge to obtain tissue for total RNA isolation and subsequent RAP-PCR analysis. RAP-PCR analysis identified six genes as differentially expressed by LPS challenge. Increases in expression of vascular cell adhesion molecule-1 ($P = 0.02$) and a bovine homolog of a novel human protein, hT41 ($P = 0.04$) were confirmed in LPS-treated livers. No differences in expression ($P > 0.05$) of the four other genes were detected by Northern analysis. Our results indicate that RAP-PCR is a useful tool for identifying novel genes expressed in cattle; however significant validation is required for accurate interpretation of results. Additional study is needed to determine the role of hT41 in the acute phase response in cattle.

Key Words: Endotoxin, RAP-PCR, Cattle

1388 Prepartum body condition score and liver glycogen concentration decrease circulating memory activity to viral antigens in periparturient dairy cows. D. C. Donovan*[1], A. R. Hippen[1], and D. J. Hurley[1], [1]*South Dakota State University, Brookings*.

The objective of the study was to correlate physiological effects associated with periparturient condition, and the related fatty liver and ketosis complex, with suppression of nonspecific responses and specific responses to viral antigens. Eight Holstein multiparous cows with a mean prepartum body condition score (**PBCS**) of 3.38 (range: 3.0-4.0) were utilized from a concurrent trial. Blood samples were taken weekly from 7 to 21d postpartum. T cell memory activity to bovine viral disease virus (**BVDV**), bovine respiratory syncytial virus (**BRSV**), and parainfluenza 3 (**PI3**) were measured in vitro. Staphyloccus aureus exotoxin B (**SEB**) was used as an internal standard of nonspecific lymphocyte proliferation. A negative correlation ($r = 0.42$, $P = 0.02$) was demonstrated between T cell proliferation induced by SEB in culture and PBCS. T cell memory response to BVD in culture was also strongly and negatively correlated ($r = 0.41$, $P < 0.04$) with PBCS. In contrast, T cell memory response to BRSV in culture was not correlated with PBCS, but strongly, negatively correlated ($r = 0.46$, $P = 0.07$) with liver glycogen concentration. The proliferative response to PI3 was not correlated with any of the physiological parameters measured. Nor were any of the responses correlated to liver lipid concentrations. This data suggests that gross physiological measures (prepartum body condition score) and specific indicators of physiological status (liver composition) are predictive of the capacity to mount specific and nonspecific immune responses. As such, they are indicative of increased susceptibility to infectious disease.

Key Words: Preparum body condition, T cell memory, Virus

1389 Impact of season and heat stress on SCC from infected and uninfected quarters. B. A. Broaddus*, R. J. Harmon, R. W. Scaletti, K. Akers, B. A. Smith, S. H. Hayes, and C. H. Hamilton, *University of Kentucky, Lexington, KY.*.

Infection data were obtained monthly from June, 1999 to September, 2000 at the University of Kentucky dairy. The herd was comprised of Holsteins and Jerseys, averaging 120 lactating cows per month. Quarter foremilk samples were collected for bacteriological determination and somatic cell counts (SCC). The Livestock Stress Index (LSI) estimated heat stress by using the peak mean value for the seven days prior to sample date. LSI is calculated by combination of temperature and humidity. During the sample period two different summer conditions were observed; the first summer had 20 days of temperature in excess of 35° C, but the second summer had nine days at those temperatures. The first summer was during a drought with no more than three cm of rain, compared to the second where an excess of nine cm was reported. For uninfected quarters the geometric mean SCC was 29,000 cells/ml. For infected quarters the geometric mean SCC was 207,000 cells/ml. Coagulase-negative staphylococci (CNS) infections comprised 61 percent of the total infected quarters with a geometric mean SCC of 146,000 cells/ml. *Staphylococcus aureus* infected quarters had a geometric mean SCC of 621,000 cells/ml. The relationship of SCC of infected quarters to LSI differed when comparing the two different summers. There were no significant correlations between log SCC and LSI when looking at the total sample period. However, when evaluating October, 1999 through September, 2000, significant positive correlations were found for LSI and log SCC of uninfected quarters ($P < 0.0013$), infected quarters ($P < 0.0001$), major pathogens ($P < 0.0175$), minor pathogens ($P < 0.0004$), and CNS ($P < 0.0003$). All correlation coefficients were less than 0.12. The correlation of SCC and LSI for uninfected quarters was 0.049. The results suggest no marked changes in SCC were observed in uninfected quarters during hot summer weather. Hot summer weather may have a minor impact on SCC in infected quarters, but the effect is variable. Thus, infection status of the mammary gland, not heat stress, is the major factor determining SCC.

Key Words: Somatic Cell Count, Mastitis, Heat Stress

1390 Relationship of somatic cell score with fertility measures. R.H. Miller[1], J.S. Clay[2], and H.D. Norman*[1], [1]*Animal Improvement Programs Laboratory, Agricultural Research Service, USDA, Beltsville, MD,* [2]*Dairy Records Management Systems, Raleigh, NC.*

Dairy Herd Improvement data from 284,450 Holstein and Jersey cows in 37 states were examined to determine relationships of test-day somatic cell score (**SCS**), herd, calving year, parity, lactation stage, and calving ease score with fertility measures [rate of nonreturn to estrus by 70 d after first service, days to first service (**D1**), and days open (**DO**)]. Relationship of elevated SCS prior to first insemination with nonreturn rate was of primary interest, but factors other than SCS were examined to ensure that estimation of SCS effect was independent of other variables. Nonreturn rates differed greatly by month. Rates were highest during April and May and lowest during June (a difference of 8 to 9% for Holsteins and 12% for Jerseys). Nonreturn rate also was affected by parity (6 to 7% higher nonreturn rate for first parity than for sixth parity and later) , lactation stage at first service (increase in nonreturn rate of 8 to 13% from early to late lactation), and calving ease [a 7% decline in nonreturn rate from score 1 (no birth difficulty) to score 5 (extreme birth difficulty)]. For Holsteins, linear regression of nonreturn rate on preceding test-day SCS was significant (P 0.01), particularly for SCS recorded within 2 to 3 wk prior to insemination; however, regression coefficients were small (0.004 to 0.005). A similar relationship was not found for Jerseys. Quadratic regression of D1 on SCS was significant for Holsteins ($P < 0.05$) and Jerseys ($P < 0.01$) ; linear regression of DO on SCS was significant ($P < 0.01$) for Holsteins. The magnitude of SCS effect on fertility traits does not warrant postponement of first service for cows in estrus because of elevated SCS on previous test day. However, additional emphasis on mastitis control and, consequently, a slight increase in the economic weight for SCS in genetic selection may be justified.

Key Words: Fertility, Nonreturn rate, Somatic cell score

1391 Efficacy of a concentrated equine serum product to prevent failure of passive transfer of immunity in neonatal foals. C.J. Hammer*[1], J.A. Booth[1], L. Etzel[2], and H.D. Tyler[1], [1]*Iowa State University, Ames, IA USA,* [2]*Proliant, Ames, IA USA.*

Colostral antibodies are the most important factor providing immunological protection to the neonatal foal. Foals that fail to obtain adequate amounts of these antibodies, those classified with failure of passive transfer, are at an increased risk for developing infection and(or) death. The primary objective of this study was to determine if an orally administered concentrated equine serum product provided in the first hours of life could prevent failure of passive transfer in foals. To achieve this objective, ten foals of Quarter Horse breeding were utilized. Foals were alternately assigned to serve as a control or to receive the oral product. Treated foals were administered 250 ml of the oral serum product at 1 and 3 h of age via nasogastric intubation. These foals were muzzled to prevent nursing from their dam. Supplemental milk replacer (200 ml/feeding) was provided to the treated foals at 6 and 9 h of age. The dams of treated foals had their udder stripped at 1, 3, 6, and 9 h post parturition. The initial colostrum collected (200 ml) was fed back to the treated foals when the muzzle was removed at 12 h of age. Control foals were allowed to nurse from their dams ad libitum. Ten ml jugular blood samples were obtained from all foals (5 treated/5 control) at 1, 3, 5, 7, 9, 10, 11, 12, 24, and 48 h of age for determination of concentrations of plasma IgG. Plasma IgG concentrations were higher ($p<.05$) for treated foals compared to control foals at 5 h and 48 h of age. Plasma IgG concentrations were not different ($p>.10$) between treated and control foals at all other time periods measured. All treated foals had plasma IgG concentrations over 700 mg/dl by 10 h of age. These data suggest that administration of this concentrated oral plasma product is effective

in raising foal serum IgG concentrations and minimizing the incidence of failure of passive transfer.

Key Words: Immunoglobulin, Colostrum, Foal

1392 Heritability of Ascaridia galli egg output in laying hens. Matthias Gauly*[1], Christian Bauer[2], and Georg Erhardt[1], [1]*Institute of Animal Breeding and Genetics, University of Giessen,* [2]*Institute of Parasitology, University of Giessen, Germany.*

Twenty week-old Lohmann LSL (n = 46) and Lohmann Brown (n = 40) laying hens were orally infected with 250 embryonated Ascaridia galli eggs. The hens descended from 11 different sires. During a 12 month laying period the animals were kept in battery-cages in an environmentally controlled chicken house and faecal samples were collected separately from each bird once a month (samples 1 to 12) to determine faecal egg counts (FEC). A modified McMaster technique was used. Weights of the birds were also taken at monthly intervals. A group of 15 (LSL) and 20 (Brown) control animals, kept under the same conditions, were sampled in parallel. No anthelmintic treatments were given at any time. FEC were transformed as the decimal logarithm of n (number of eggs) + 25 to correct for heterogeneity of variance and to produce normally distributed data. Statistical analysis were performed with the Statistical Package for Social Science for PC (SPSS-PC Version 9.0). The model for estimating heritabilities of log-transformed data included the father as a random effect and age as a fixed effect. All infected animals excreted A. galli eggs. The percentage of positive samples (FEC $>$ 50) was 69.1 and 33.7. LSL hens had a significantly ($p < 0.01$) (2.38, SE: 0.04) higher mean log10 FEC compared to Lohmann Brown (1.89, SE: 0.03). There was no significant ($p > 0.05$) difference in laying performance between control and infected groups. The relative body weight development of the LSL chicks was significantly ($p < 0.05$) higher in the control group. The estimated heritabilities for mean log10 FEC were between 0.0 (all samples) and 0.10 (SE: 0.041) (samples 1-6) for Lohmann Brown and 0.13 (SE:0.039) (all samples) and 0.19 (SE:0.029) (samples 1-6) for Lohmann LSL. This opens a way of genetic selection for A. galli resistance in chickens, which will be of importance for birds kept in alternative and organic farming systems.

Key Words: Genetic resistance, Ascaridia galli , Chicken-Nematoda

1393 Antibiotic effects of Tylosin in the large intestine of swine fed sub-therapeutic concentrations of Tylan. M.D. Howard*[1], J.A Zahn[1], and D.L. Harris[2], [1]*National Swine Research Information Center, USDA-ARS,* [2]*Iowa State University.*

The objectives of this study were to: 1) measure the concentration of tylosin and bioactive tylosin metabolites in the large intestine of pigs fed a typical corn-soy finishing diet, containing a sub-therapeutic level of tylosin (20 g/907 kg of feed); 2) determine the in vitro minimum inhibitory concentration (MIC) for *Micrococcus luteus* and *Brachyspira hyodysenteriae*. Large intestinal digesta of three pigs (107.0 kg 2.0) were solvent-extracted and antibacterial compounds were isolated by coupled preparative reverse-phase liquid chromatography (P-RPLC) followed by a plate diffusion assay with *M luteus* as the indicator microorganism. Four major bioactive fractions were purified to homogeneity by a second P-RPLC step and structures of the compounds were assigned using electrospray-ionization tandem mass spectrometry (MS-MS). The identity, concentration, and yield of bioactive compounds purified from the digesta were: Tylosin A (112.2 8.2 mg/kg, yield = 74%); tylosin D (241.6 19.3 mg/kg, yield = 81%); demycarose-tylosin A (36.0 2.3 mg/kg, yield = 78%); and demycarose-tylosin D (21.0 1.3 mg/kg, yield = 79%). The MIC of purified tylosin A for *M. luteus* was found to be 4.3 1.3 mg/kg, and the MIC for *B hyodysenteriae* was found to be 1.1 0.4 mg/kg. The MIC of the four bioactive compounds were found to be statistically ($a = 0.05$) identical in MIC assays performed using *M luteus*. Therefore, the normalized concentration of all bioactive forms of tylosin was used to estimate potential sub-therapeutic effects of tylosin on susceptible bacteria in the gut of swine. The total concentration (0.52 mg/kg) of all bioactive forms of tylosin was found to be in the MIC range of *B hyodysenteriae* (1.0 0.6 mg/kg). This result suggests that sub-therapeutic levels of tylosin may influence microbial community structure in the gut of swine through direct antibiotic effects. Additionally, results of this study show, for the first time, the identity and concentration of tylosin metabolites in the gut of swine that exhibit antibacterial activity.

Key Words: Tylosin, growth promotion, antibiotic resistance

1394 Adhesion of *Actinobacillus pleuropneumoniae* to swine soluble fibronectin. R.C. Hamer*[12], I. Enriquez[2], D. Godinez[2], R.Z. Martinez[2], P. Talamas[2], S. Vaca[3], and M. de la Garza[2], [1]*FMVZ- Universidad Autonoma de Sinaloa. Culiacan, Sinaloa Mexico.,* [2]*CINVESTAV-IPN. Zacatenco. Mex. D.F. Mexico.,* [3]*ENEP- Iztacala. Universidad Nacional Autonoma de Mexico. Mexico.*.

Actinobacillus pleuropneumoniae (Ap) is an important cause of swine respiratory disease. Adhesion to the swine respiratory tract is an initial step in infection. Fibronectin is one of the most abundant extracellular matrix proteins in lung connective tissue. The objective of this work was to show the interaction of Ap with swine soluble fibronectin. Ap cells were seen adhered to fibronectin, using negative staining by transmission electron microscopy. This interaction was observed as protein nets with bacteria adhered. Adhesion kinetic studies of Ap to swine fibronectin films was performed in microtitration plates, adhesion was observed for the first 5 min of incubation and it was maintained for 3 hours. Heat killing of bacteria inhibited adhesion almost completely; heparin or swine serum from convalescent pigs diminished adhesion in less proportion. Also, some carbohydrates and metaperiodate had an important effect on adhesion. Immunofluorescence using anti-swine fibronectin antibodies revealed bacteria adhered to fibronectin when both were in contact. These experiments revealed bacterial-protein interactions, and a possible important role of fibronectin in bacterial recognition of swine lung, colonization, and subsequent infection. Project supported by CONACyT-Mexico grant no. 28755B.

Key Words: Pneumonia, Fibronectin, Swine

1395 Testing for the Presence of Enterotoxigenic *Escherichia coli* Infections Causing Diarrhea in Swine Using PCR and ELISA Techniques. S. Cole*[1] and R. R. Marquardt[1], [1]*University of Manitoba* .

Five strains of Enterotoxigenic *Escherichia coli* (ETEC) K88 (F4), K99 (F5), 987P (F6), F41, and F18 (F107) have been shown to cause diarrheal disease in young pigs. It is important to identify specific strains of *E. coli* by their colonization factor genes if they are to be treated with therapeutic antibodies as each antibody is specific for a given strain. Among different procedures only genetic and immunological techniques are specific. The objectives of this study were to adapt two types of assays to detect the presence of these five strains of ETEC in swine, the polymerase chain reaction (PCR) and the enzyme linked immunosorbent assay (ELISA). The polymerase chain reaction is a genetic assay applied to the testing of fecal samples from animals with diarrhea where *E. coli* is the suspected cause. PCR has been called the DNA photocopier that replicates many copies of a target DNA strand which can be used for identification of the organism. The ELISA technique can be used to test for the presence of anti-*E. coli* colonization factor antibodies in the blood of swine. The antibodies that result from an infection will persist for a matter of weeks in the animal. In this way even if no animals are sick at the time of investigation, the disease exposure of healthy animals could indicate the presence of a pathogen that has been a problem and may be again. Preliminary refinement and testing of these methods at the University of Manitoba have proven successful by indicating the presence of specific pathogenic *E. coli* strains in the feces of pigs specifically inoculated. In addition the ELISA method too has proven the ability to detect anti-*E. coli* colonization factor antibodies in the blood of pigs. The assays have proven to be fast, specific, sensitive and accurate for the application of therapeutic antibodies in an ETEC control program.

Key Words: Enterotoxigenic E. coli, PCR, ELISA

1396 Differential effect of dexamethasone on lymphocyte proliferation and immunoglobin production *in vitro*. M.R. Rogers*[1], S.C. Lozano[1], K.M. Kammlah[1], T.H. Welsh, Jr.[2], and J.C. Laurenz[1], [1]*Texas A&M University-Kingsville,* [2]*Texas A&M University-College Station*.

The present study investigated the effect of the synthetic glucocorticoid, dexamethasone (DEX), on concanavalin (ConA)-induced lymphoproliferation and immunoglobin M (IgM) production by pig lymphocytes *in vitro*. Blood was obtained from male, crossbred pigs (n=3-6 pigs/experiment; 40-45 days of age) and lymphocytes obtained by density gradient centrifugation over Lymphoprep (Nycomed, Oslo, Norway). Lymphocytes were plated in 96-well plates at 1 X 10^5 cells/well in DME/F12 containing 10% fetal bovine serum, 2 mM L-glutamine, 10 uM 2-mercaptoethanol, ConA (0 to 10 ug/ml) and/or DEX (0 to 10^{-6} M). Cultures were incubated for 96 h and lymphoproliferation determined using the CellTiter proliferation assay (Promega, Madison, WI). In replicate cultures, supernatants were removed and IgM production determined using an ELISA specific for pig IgM. As expected, ConA induced a dose-dependent increase ($P < .01$) in lymphoproliferation and IgM production with maximal effects occurring at .6 and 1.25 ug/ml, respectively. Although not effecting basal lymphoproliferation, DEX dose-dependently inhibited ($P < .01$) ConA-induced (0.3 and 1.25 ug/ml) lymphoproliferation with maximal effects occurring at 1 x 10^{-8} M. However, the suppressive effects of DEX (1 x 10^{-8} M) on lymphoproliferation could be overcome ($P < .05$) with the addition of higher concentrations of ConA (5 to 10 ug/ml). Similarly, when cells were stimulated with low concentrations of ConA (.3 ug/ml), DEX dose-dependently inhibited ($P < 0.01$) IgM production with maximal effects occurring at 10^{-8} M. In contrast, in cultures treated with 1.25 ug/ml ConA, low concentrations of DEX (10^{-10} to 10^{-9} M) actually augmented ($P < .05$) IgM production. Furthermore, DEX (10^{-8} M) induced additional 1.7 to 1.9 fold increases ($P < .05$) in IgM production by lymphocyte cultures treated with higher concentrations of ConA (2.5 to 10 ug/ml, respectively). Collectively, these results demonstrate that glucocorticoids have primarily a suppressive effect on lymphocyte proliferation, but can augment immunoglobin production depending on both the degree of lymphocyte stimulation and the concentration of glucocorticoid.

Key Words: Pigs, Glucocorticoid, Lymphocyte

1397 Effect of oral administration of dehydroepiandosterone-sulfate (DHEAS) on pig lymphocyte function *in vitro*. S.C. Lozano*[1], T.H. Welsh, Jr.[2], and J.C. Laurenz[1], [1]*Texas A&M University-Kingsville,* [2]*Texas A&M University-College Station*.

The present study investigated the effect of chronic, oral administration of DHEAS on lymphoproliferation and immunoglobin (IgM) production by isolated lymphocytes *in vitro*. Crossbred, female pigs (n = 12; initial weight 9.3 .5 kg) were assigned by weight to one of two treatments (n = 6 pigs/treatment) and were fed either 0 or 1 mg DHEAS/kg body weight twice daily for 100 days. At the end of the treatment period, blood was obtained via jugular venipuncture and lymphocytes isolated by density gradient centrifugation over Lymphoprep (Nycomed, Oslo, Norway). Lymphocytes were plated in 96-well plates at 1 x 10^5 cells/well in DME/F12 containing 10% FBS, 2mM glutamine, 10 uM 2-merceptoethanol, ConA (0 to 2.5 ug/ml), or ConA (.3 or 1.25 ug/ml) in the presence of DEX (0 to 10^6 M). Cultures were incubated for 96 h and lymphoproliferation determined using the Celltiter proliferation assay (Promega, Madison, WI). In replicate cultures, supernatants were removed and immunoglobin M (IgM) production determined using an ELISA specific for pig IgM. As expected, ConA induced dose-dependent increases ($P < .01$) in lymphoproliferation and IgM production with initial increases apparent at .3 ug/ml and maximal effects occurring at 1.25ug/ml ConA. Although not effecting ($P > .05$) the response of lymphocytes to low concentrations of ConA (< .3 ug/ml), feeding of DHEAS to pigs increased ($P < .05$) the extent of lymphoproliferation and tended to increase ($P = .06$) IgM production by lymphocytes at higher concentrations of ConA (.6 to 2.5 ug/ml). In both treatment groups, DEX dose-dependently inhibited ($P < .01$) ConA-induced (0.3 and 1.25 ug/ml) lymphoproliferation with maximal effects occurring at 10^{-8} M. Similarly, when cells were stimulated with low concentrations of ConA (.3 ug/ml), DEX dose-dependently ($P < .01$) inhibited IgM production with maximal effects occurring at 10^{-8} M. In contrast, in cultures treated with 1.25 ug/ml ConA, DEX (10^{-10} to 10^{-8} M) augmented ($P < .05$) IgM production and the effect of glucocorticoid treatment was increased ($P < .01$) in pigs fed DHEAS. Collectively, these results indicate that oral administration of DHEAS can enhance the responsiveness of lymphocytes to antigenic challenge and suggest that DHEAS may be beneficial to enhance immune function in domestic livestock species.

Key Words: pigs, dehydroepiandosterone, glucocorticoid

ASAS/ADSA Breeding and Genetics: Gene Mapping, QTL, and Statistical Methods

1398 A novel and highly effective method to generate transgenic mice and chickens: linker-based sperm-mediated gene transfer. Jin Qian*[1], Yi-Hsin Liu[2], Mason Jiang[3], Tsehay Mekonnen[1], and Ken Wang[1], [1]*BioAgri Corp.,* [2]*Center for Craniofacial Molecular Biology, USC,* [3]*Dept. of Anesthesiology, UCLA*.

Genetic modification of domestic animal traits can be used to improve productivity and quality or to produce bioreactors for modern medicine. DNA microinjection, the current method to produce transgenic livestock, is time consuming and requires extensive training and special equipment. More importantly, except in mice, microinjection has reported only limited success in larger or higher species. Sperm-mediated gene transfer has been recognized as a potentially powerful alternative method, but has been questioned by many laboratories around the world since the original results were difficult to duplicate. We have developed a linker (mAb C) to bind with sperm and DNA. Using flow cytometry, it has shown cross-reactivity with sperm cells from all tested species including mouse, pig, cow, sheep, goat, chicken, and human. mAb C has been characterized as a basic protein and has been shown to bind DNA through ionic interaction. We report here the use of this novel linker with the sperm-mediated gene transfer method to successfully generate transgenic mice and chickens. Sperm from FVB/N mice were treated first with mAb C and then combined with a linearized DNA fragment, pGL3-control (Promega). After *in vitro* fertilization, fertilized eggs at the two-cell stage were implanted into pseudopregnant mice. 12 offspring were born and used to mate with wild type mice. 2 (16.7%) transmitted transgenic mouse lines (F1) were identified by Southern blot. Furthermore, this novel technology was also tested in the chicken through artificial insemination. The transgene was detected in 10 out of 88 (11.4%) chicken embryos by PCR. Combined with our high success rate in making transgenic pigs (58.3%) through surgical oviduct fertilization, our data strongly suggests that this novel linker-based sperm-mediated gene transfer method is both universal and effective and could be of great value to modern agriculture and medicine.

Key Words: Linker-based sperm-mediated gene transfer, Transgenic chicken, Transgenic mice

1399 Generation of transgenic pigs by sperm-mediated gene transfer using a linker protein (mAb C). Keejong Chang[2,3], Jin Qian[1], Mason Jiang[4], Ming-Che Wu[5], Chidar Chen[2], Hin-Lung Lo[3], Meng-Chun Hu[2], Wen-Wen Lin[2], Iris Ho[2], and Ken Wang*[1], [1]*BioAgri Corp.,* [2]*BioAgri Corp., Taiwan Division,* [3]*Dept. of Chemistry, Soochow University,* [4]*Dept. of Physiology, Taiwan Livestock Research Center,* [5]*Dept. of Anesthesiology, UCLA*.

Sperm-mediated gene transfer (SMGT) has been recognized as a potentially powerful method to make transgenic animals for many years. The current method of gene transfer, microinjection, used widely in transgenic mouse production, has had only limited success in producing transgenic animals from larger or higher species. We report here a sperm-mediated gene transfer method that uses a linker protein to vastly improve the efficiency of large transgenic animal production. A monoclonal antibody (mAb C) that could be used as a linker was identified after screening hybridomas immunized with mouse sperm cells. mAb C is reactive to a surface antigen on mouse sperm cells and is also cross-reactive with sperm cells from many different species such as pig, cow, sheep, goat, chicken, and human. mAb C has been characterized