

464 Establish confidence intervals for daily milk yield measures by robust bootstrap. P. M. Saama*¹ and I. L. Mao², ¹Michigan State University, East Lansing, MI, ²National Institute of Agricultural Science, Denmark.

Prior to statistical analyses of daily milk yield data, outliers due to equipment malfunction or confirmed milk recording errors should be removed. However, those outliers that are caused by health status, body condition, stress, energy balance, or BST would be valid data. Hence, using the central limit theorem (CLT) to establish confidence intervals (CI) for yield measures could be misleading. The "ordinary" bootstrap performs poorly in these situations. This study demonstrates the use of a robust bootstrap resampling algorithm to construct CI for daily milk yield. The double bootstrap algorithm advances the notion that CI can be constructed from a function of the sample and the mean whose distribution is independent of the mean, the sample, or any other unknown parameter using pivotal quantities. In the algorithm, the mean of the data is computed. Then the first stage bootstrap sample (F) of size n is obtained from the observed data, with replacement (WR). The difference between the mean of F and the mean of the observed data is divided by the SE of the mean (SEM) of F, is a pivotal quantity that provides a robust bootstrap-t distribution of the mean daily yield. Then, the second stage bootstrap sample (G) of size n is randomly drawn WR from F. The difference between the mean of this bootstrap sample and the mean of F is now divided by the SEM of G. The first and second stage bootstraps are repeated B and K times, respectively. The CI for the mean can be obtained from the percentiles of the bootstrap distributions. Daily milk records for 89 first lactation cows from a Michigan herd were used for demonstration with B=500 and K=500. The distribution was skewed to the right at peak and in late lactation. The 95% CI given by the CLT were widest. The ordinary bootstrap gave narrow CI while the bootstrap-t and double bootstrap methods gave relatively stable CIs. After computing 99% confidence intervals using this approach, data that do not fall within the limits of that interval could be removed prior to statistical analysis.

Key Words: Milk yield, Variation, Bootstrap

465 Determination of covariance functions for lactation traits on dairy cattle using random-coefficient regressions on B-splines. R.A.A. Torres Jr and Richard L. Quaas, *Animal Science Department - Cornell University*.

Covariance functions for dairy cattle have been specified either by a multi-trait analysis of records within an interval of the lactation followed by extrapolation or by direct modeling of observations throughout the lactation using random-coefficient regressions. Here we present an approach using regressions on B-splines that is an extension of the within interval multi-trait analysis where the intervals are specified by the knots. It allows local fitting behavior and simultaneous modeling of every day of the lactation. The approach was applied to 296,601 test day records from 36,520 cows for milk yield, 180,474 records from 27,320 cows for fat and protein yield and 135,336 records from 26,628 cow for somatic cell score coming from 13 large dairy herds from New York State during 1989 through 1997. A longitudinal model with cows as subjects was used together with other effects to adjust for environmental effects and a heteroskedastic independent residual. Inferences about the dispersion parameters were made from the samples of a Markov Chain Monte

Carlo procedure. For milk yield the 3 largest eigenvalues of the covariance function for the cow specific effect accounted for at least 85%, 11% and 2.5% of the total variance and the respective eigenfunctions were close to constant, linear and quadratic functions, with slight discrepancy at the extremes causing variance reduction. For fat and protein yield the first eigenvalue accounted for at least 94.88% and 96.20% of the total variance, respectively. This shows that a repeatability model with heterogeneous variance to account for smaller variation, mostly at the beginning of the lactation, should suffice for these traits. Somatic cell score had at least 5 eigenvalues accounting for more than 1% of the total variance. For this trait too, the first 3 eigenfunctions closely followed the constant, linear and quadratic functions.

Key Words: Covariance functions, Test-day model, B-splines

466 Comparison of random regression test-day models using Bayes factors. Pedro Lopez-Romero*¹, Romdhane Rekaya², Yu-Mei Chang², Daniel Gianola², and Maria J. Carabao¹, ¹Departamento de Mejora Genética y Biotecnología. INIA. Madrid-Spain, ²Department of Animal Sciences. University of Wisconsin. Madison, WI- USA.

Test-day milk yields (TD) from Spanish Holstein cows were analysed with a set of random regression models (RR), including Wilmlink (W) and Ali-Schaeffer (A) functions, and Legendre polynomials (L) of varying order on additive (3 and 5) and permanent (3,5 and 6) effects. Data were 47,982 completed first lactations. L were selected from a previous study, where a wider range of L models was evaluated using REML, assuming constant residual variance (CRV). Model performance had been assessed via goodness of fit, predictive ability, and behaviour of estimated daily variance and of correlations involving yields at different parts of lactation. These RR were revisited from a Bayesian perspective, allowing for heterogeneous residual variance (HRV) between 3 intervals. Gibbs Sampling was used to draw from marginal posterior distributions. The log-marginal likelihood (LML) was estimated for each model using the harmonic mean of likelihood values. Estimated LMLs can be used to compute the Bayes Factors (LBF). LBFs were greater than 150 in all cases, showing a very strong evidence in the Jeffreys's scale. The A model did not reach the convergence after 460,000 iterations. L models reached the convergence very fast since orthogonal polynomials lower the correlation between samples. The most plausible specification was an L model of 3rd order for additive effects, and of 6th order for permanent effects. Further analysis was done for 3 L models of order 3 for additive 3,5 and 6 for permanent effects, assuming CRV. A better performance than for its corresponding HRV counterpart was noted for the 5th and 6th order models for permanent effect.

MODEL	LML (x10 ⁹)	RV		
		5-75d	76-265d	266-335d
L(3,3)	-4.906	15.53	10.06	9.24
L(3,5)	-4.798	13.10	9.40	8.30
L(5,5)	-4.796	13.12	9.41	8.38
L(3,6)	-4.782	12.69	9.31	8.29
W	-5.663	21.94	17.11	28.07
L(3,3)	-4.912		10.96	
L(3,5)	-4.781		9.81	
L(3,6)	-4.770		9.58	

Key Words: Test day models, random regression, Bayes factor

ASAS/ADSA Food Safety: Microflora Surveillance

467 Effect of Shipping Stress in Beef Cattle on Prevalence Levels of Enterohemorrhagic E. coli and Salmonella spp. from the Feedlot to the Packing Plant. A.R. Barham¹, B.L. Barham*¹, A.K. Johnson¹, D.M. Allen², J.R. Blanton, Jr.¹, and M.F. Miller¹, ¹Texas Tech University, ²Excel Corporation.

Two hundred steers and heifers, from ten pens were used to determine prevalence of Enterohemorrhagic E. coli (EHEC) and Salmonella spp. (SAL) prior to and after shipping to a packing plant. Two samples were collected per animal: ventral midline hide swab and fecal sample, two weeks prior to transportation and at the packing plant. Samples were collected from all trucks before loading animals. EHEC and SAL tests were conducted following USDA & FSIS approved protocols.

Prevalence levels were generated using the frequency procedure in SAS (1995). Changes in prevalence levels were analyzed using the T-test procedure in SAS (1995). Overall prevalence of EHEC on hides and in feces at the feedlot were 18% and 9.5% respectively and 4.5% and 5.5% at the packing plant. Results indicated a numerical decrease in EHEC prevalence from feedlot to packing plant for hides and feces (P>.05). Overall prevalence of SAL on hides and in feces at the feedlot were 6% and 18% respectively, while prevalence at the packing plant was 87% and 43%. Data indicated an increase in SAL prevalence from feedlot to packing plant with the only significant increase seen on hides (P<.0001). Twenty percent of pens at the feedlot had positive EHEC feed samples while no feed samples were positive for SAL. Water samples taken at the feedlot indicated 10% of the pens were positive for both EHEC and SAL.

Seven percent of trucks were EHEC positive and 74.5% were SAL positive. Sex significantly affected EHEC status of fecal samples ($P<.05$). Moreover, there was a relationship between sex and SAL status of fecal samples ($P<.10$). Date slaughtered impacted the change of SAL status on hides ($P<.05$). Data indicate a relationship between date slaughtered and change of SAL status in fecal samples ($P<.06$). Available pen space per animal altered the EHEC and SAL in fecal samples ($P<.05$). Truck did not significantly impact EHEC or SAL status.

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

468 Prevalence, incidence, and duration of fecal shedding of *Escherichia coli* O157:H7 by feedlot cattle throughout the feeding period. S Younts^{*1}, D Smith¹, R Moxley¹, J Folmer¹, J Gray², S Hinkley¹, L Hungerford¹, M Khaita¹, and T Klopfenstein¹, ¹University of Nebraska-Lincoln, Lincoln, NE, ²USDA, ARS, ARRU, Athens, GA.

The objective of this study was to describe patterns of fecal shedding of *E. coli* O157:H7 in cattle during the feeding period. One hundred steers were randomly assigned to 10 pens (10 animals each) upon arrival. Steers were fed a high concentrate finishing diet for 136 days starting in June. Once a week, fecal samples were collected from the rectum of each animal and cultured for *E. coli* O157:H7. New cases were those animals shedding the organism that had been culture negative the week before. Animals culture negative the prior week were considered the at-risk population. Duration of shedding was the number of consecutive weeks an individual was culture positive. *E. coli* O157:H7 was recovered from each animal at least once during the study. The percentage of pens with at least 1 steer shedding *E. coli* O157:H7 ranged from 10% (week 1) to 100% (weeks 10, 11, 13, 14, 15, 16, 18). The point-prevalence of cattle shedding the pathogen ranged from 1% (week 1) to 80% (week 10). The first 7 weeks of the feeding period were characterized by low incidence (≤ 0.1 new cases/animal-week) of shedding with short mean duration (≤ 2.5 weeks). Incidence increased dramatically in week 9 (0.5 new cases/animal-week), reached a maximum in week 14 (0.7 new cases/animal-week) and then gradually decreased. Mean duration of fecal shedding was longest mid-feeding, lasting 4.7 and 4.8 weeks for weeks 8 and 9 respectively. On the last sampling date, 30% of animals were culture positive, those positive had been shedding the organism a mean of 3.4 weeks, and at least one animal was shedding *E. coli* O157:H7 in 9 of the 10 pens. We concluded that prevalence of *E. coli* O157:H7 shedding within a group of feedlot cattle varied widely by time and space and that variability in prevalence was due to changes in both incidence and duration.

Key Words: Food safety, *E. coli* O157:H7, Feedlot cattle

469 Occurrence of verotoxin-producing *Escherichia coli* in beef and dairy heifers grazing the same pasture. B. H. Thran^{*} and H. S. Hussein, University of Nevada - Reno.

Verotoxin-producing *Escherichia coli* (VTEC) are foodborne pathogens that have been associated with human illness outbreaks due to consumption of contaminated beef or milk. Although there are over 60 VTEC serogroups that have been implicated in human illnesses, research in the U.S. has focused mainly on *E. coli* O157:H7. Therefore, the objective of this study was to assess the occurrence of VTEC in beef and dairy cattle under the same environment during a 1-year period. A herd of 23 yearling Angus heifers and another of 24 yearling Holstein heifers were allowed to graze an irrigated grass pasture with supplementation of alfalfa (*Medicago sativa*) hay only during winter. Each herd was sampled during four periods (spring [April], summer [July], fall [October], and winter [December]) of 1999 resulting in a total of 86 and 91 fecal samples (directly removed from the rectum) for the beef and dairy heifers, respectively. Using classic microbiological methods (based on sorbitol fermentation and 4-methylumbelliferyl- β -D-glucuronide [MUG] metabolism), 290 and 530 potential VTEC isolates were obtained from the beef and dairy heifer samples, respectively. Potential VTEC isolates were tested for verotoxigenicity and screened by the polymerase chain reaction for the presence or absence of the verotoxin genes (VT1, VT2, or both). The sequence and expression of the verotoxin genes were confirmed. A total of 22 VTEC isolates were detected in both herds. Serotyping of the VTEC isolates identified five isolates from the beef fecal samples as O26. Members of serogroups O6 ($n = 2$), O39 ($n = 1$), O157 ($n = 4$), O113 ($n = 1$) were isolated from dairy fecal samples. In

addition, nine VTEC isolates (one from beef and eight from dairy fecal samples) were unreactive with the 181 "O" and 52 "H" monovalent antisera used for serotyping. Based on detection of both O157 and non-O157 VTEC in our herds, it is clear that screening of cattle for VTEC should not be limited to O157. It is worth noting that members of the serogroups O6 and O26 were previously associated with human illness outbreaks in the U.S. and worldwide. Identification of VTEC-positive cattle prior to slaughter should help in reducing the risk of human foodborne illnesses and can be a critical step in any on-farm strategy to minimize the risk of food contamination with such pathogens.

Key Words: Cattle, Foodborne pathogens, *Escherichia coli*

470 Salmonella isolation on 12 Midwest and Northeast dairy farms. L.D. Warnick^{*1}, J.B. Kaneene², P.L. Ruegg³, S.J. Wells⁴, M. Saeed², C. Fossler⁴, and L. Halbert², ¹Cornell University, Ithaca, NY, ²Michigan State University, East Lansing, MI, ³University of Wisconsin, Madison, WI, ⁴University of Minnesota, St. Paul, MN.

An on-going 3-year longitudinal study is investigating the occurrence, risk factors and antimicrobial resistance patterns of *Campylobacter jejuni* and *Salmonella* on 130 dairy farms. The objective of this part of the project was to compare salmonella isolation among sample sources on 12 dairy farms sampled weekly for 7-8 weeks. Three herds per state were enrolled from MI, MN, NY and WI based upon predefined herd-size criteria. Samples were obtained from target animal populations, bulk tank milk, milk filters, water and feed sources and environmental sites during weekly herd visits and were submitted to a central laboratory for isolation of *Salmonella* using standard laboratory procedures. All herds had *Salmonella* isolated from at least 1 sample. *Salmonella* was isolated from 372 (9.3%) of 4005 fecal samples collected from cattle. The percentage of positive cultures varied significantly among categories of cattle (chi-squared test $P<0.0001$) and for different environmental sample sources (chi-squared test $P=0.03$). *Salmonella* was isolated from 8.8% of preweaned calves, 8.0% of healthy lactating cows, 7.1% of designated cull cows, 19.8% of dry cows due to calve within 2 weeks, 13.5% of lactating cows within 2 weeks of calving and from 13.7% of sick cows. The age of preweaned calves and days in milk of healthy lactating cows were not different for cattle with positive cultures compared with those that were culture negative (Wilcoxon rank sum test $P\geq 0.2$). *Salmonella* was isolated from 92 (11.4%) of 807 samples collected from other sources. Samples with *Salmonella* (percent positive) were bulk tank milk (2.2%), milk filters (10.1%), water tanks or drinking cups (13.8%), feed bunks (13.2%), hide swabs from cows designated to be culled (12.1%), calf pens (9.0%), calving pens (13.5%), sick cow pens (24.0%), lagoon or manure piles (14.0%), and bird feces (8.7%). Sample source had a significant effect on detecting *Salmonella* in these infected dairy herds.

Key Words: Dairy Food Safety, Salmonella

471 Isolation of *Mycobacterium paratuberculosis* (*M.ptb*) from thin market cows at slaughter. C.A. Rossiter^{*1} and W.R. Henning², ¹Cornell University, Ithaca, NY, ²Pennsylvania State University, State College.

The study had two objectives: 1. assess the prevalence of Johne's disease in sound, thin (low body condition score, < 2.5 on dairy score 1-5, < 4 on beef score 1-9) dairy and beef cows at slaughter, at high risk of clinical infection; and 2. assess *M.ptb* dissemination to liver (L) and two lymph nodes, the superficial cervical (SC) and the popliteal (P), associated with muscle used in ground product. Cows ($n=539$) were sampled at 3 large slaughter plants: 189 dairy (primarily one No. East plant) and 350 beef (plants in So. and No. Central U.S.). The study population represented 30-50% of dairy and 10-15% of beef cows processed at the respective plants. On all 539 cows ileocecal lymph nodes (IC) and feces (FC) were cultured for *M.ptb* (Cornell Double Incubation) to diagnose infection. Culture result categories are Maximum (>299), Moderate (31-299) and Few (1-30) colony forming units (CFU)/0.1 gm. Liver, SC and P were frozen at -70C and later cultured from 135 cows: 25/25 with Maximum/moderate CFUs, 24/49 with Few CFUs, and 86/465 negatives. *M.ptb* was isolated from IC or FC from 65/189 (34%) dairy and 9/350 (3%) beef cows. Relative diagnostic sensitivity based on + IC or FC was twice + FC alone (14% vs. 8%). Culture of L, SC and P isolated *M.ptb* from 16/135 cows (15 dairy, 1 beef) and only from those with Maximum CFUs on both IC and FC indicating disseminated late-stage infection. All 16 were positive on L; 7/16 on SC or P (6 dairy, 1 beef). CFUs were higher in L (9 Moderate, 7 Few) than SC and P

(1 Maximum, 9 Few). All cows with disseminated infection were likely identified, thus *M.ptb* was cultured from L of 16/539 (3%) of sound, thin market cows: 15/189 dairy, 1/350 beef, and only from cows with disseminated infection. *M.ptb* was cultured from SC and P from 7/539 (1.3%) cows, 6 /189 dairy and 1/350 beef. This work suggests occurrence of *M.ptb* in SC and P in the total market cow population is very low. Prevalence and risk associated with disseminated *M.ptb* should be further characterized.

Key Words: Johne's, paratuberculosis, food safety

472 Weekly shedding of *Campylobacter jejuni* on 12 Midwest and Northeast dairy farms. P.L. Ruegg^{*1}, J.B. Kaneene², L.D. Warnick³, S.J. Wells⁴, A.M. Saeed², C. Fossler⁴, and L. Halbert², ¹University of Wisconsin, ²Michigan State University, ³Cornell University, ⁴University of Minnesota.

Campylobacter jejuni has become the most common foodborne cause of diarrhea in humans. An on-going 3-year longitudinal study of 130 dairy herds is studying the occurrence, risk factors and antimicrobial resistance patterns of *C. jejuni* and *Salmonella* spp. obtained from cattle and environmental samples. The objective of this part of the study was to compare shedding of *C. jejuni* from animals located on 12 dairy farms that were sampled weekly for 4 weeks. Three herds per state were enrolled from MI, MN, NY and WI based upon predefined herd-size criteria. Fecal samples were obtained from target animal populations during weekly herd visits and were submitted to a central laboratory for *C. jejuni* isolation using standard laboratory procedures. *C. jejuni* was isolated from 154 of 2106 fecal samples (7.3%, .26 SD) and the prevalence of isolation was significantly different between farms ($F=2.25$, $p=.01$) ranging from 3.7 to 12.0%. *C. jejuni* was isolated from 6.25% (95% CI, 4.0-8.5) of preweaned calves, 7.13% (5.7-8.5) of healthy lactating cows, 12.8% (2.9-22.7) of designated cull cows, 8.4% (3.1-13.8) of close-up dry cows, 12.4% (6.6-18.2) of recently calved cows and 2.7% (.01-6.6) of sick cows. Calves that were culture positive for *C. jejuni* were 13.1 days older than calves that were culture negative ($p=.0002$). Week of sampling was not associated with prevalence of *C. jejuni* (chi-square = 4.4, $p = .22$) during the 4-week period.

Key Words: *Campylobacter jejuni*, Food Safety, Epidemiology

473 Multiple *Campylobacter coli* genotypes from sows and piglets in a commercial swine operation. M. E. Hume^{*}, R. E. Droleskey, and R. B. Harvey, USDA, ARS, SPARC, FFSRU.

Genotypes of *Campylobacter coli* isolates obtained from the feces of three sows, and rectal swabs from seventeen piglets from a farrow-to-finish swine operation were examined by pulsed field gel electrophoresis (PFGE). Five *C. coli* colonies were picked from a single culture plate for each sample following differential broth enrichment and growth on Campy-Cefex agar. Isolate genotypes were examined by PFGE following genomic DNA restriction endonuclease digestion with *Sma*I and *Sac*II. Twenty-three *Sma*I and thirteen *Sac*II genotypes were detected among ninety-nine *C. coli* isolates. One *Sma*I genotype was detected among isolates from each of two sows and each of seven piglets, two different genotypes were among isolates from one sow and each of seven piglets, and three different genotypes were detected in each of three piglets. Digestion with *Sac*II revealed one genotype among isolates from each of two sows and eight piglets, two genotypes among isolates from each of eight piglets, and three genotypes among isolates from each of two piglets. Although some piglets were related to sows in the study and some were littermates, there was no discernable pattern of shared genotypes among piglets and their respective sows or between related piglets. Data indicate that the pigs had been exposed to multiple *C. coli* genotypes, and that detection of co-colonizing genotypes is possible by PFGE.

Key Words: *Campylobacter*, Swine, Pulsed Field Gel Electrophoresis

474 Chlorate supplementation in drinking water reduces *E. coli* O157:H7 populations in cattle prior to harvest. T. R. Callaway^{*1}, R. C. Anderson¹, T. J. Anderson¹, T. L. Poole¹, and D. J. Nisbet¹, ¹Agricultural Research Service/USDA-Southern Plains Agricultural Research Center.

Cattle are a natural reservoir of *E. coli* O157:H7. Therefore, strategies that reduce *E. coli* O157:H7 in cattle will reduce human exposures to this pathogen. Nitrate reductase converts nitrate to nitrite, but also co-metabolically reduces chlorate to chlorite, which is cytotoxic to bacteria. Nitrate reductase-positive bacteria (e.g., *E. coli*) exposed to chlorate die. As a result of this bactericidal effect, it was suggested that chlorate be used as a strategy to reduce *E. coli* O157:H7 in cattle prior to harvest. This study was designed to determine the effect of chlorate treatment on experimentally inoculated *E. coli* O157:H7 populations in cattle. Cattle ($n=8$) were fed a feedlot-style high grain ration, and were experimentally infected with 3 strains of *E. coli* O157:H7 identified via different antibiotic resistance markers. Fecal and ruminal counts of each O157:H7 strain, as well as generic *E. coli* and total coliforms were determined prior to chlorate treatment. Cattle were given access to drinking water supplemented with 2.5 mM KNO_3 and 100 mM NaCl (Controls) or 2.5 mM KNO_3 and 100 mM $NaClO_3$ (Treatment). Sodium chlorate treatment reduced the population of each O157:H7 strain approximately 2 logs (from 10^4 to 10^2) in the rumen and from 10^6 to 10^3 in the feces. Chlorate treatment reduced total coliforms and generic *E. coli* from 10^6 to 10^4 in the rumen and by 3 logs throughout the rest of the gastrointestinal tract (ileum, cecum, colon and rectum). Strains of *E. coli* O157:H7 were reduced throughout the intestinal tract. Chlorate treatment did not alter total culturable anaerobic bacterial counts. Therefore, it appears that chlorate supplementation is a potential strategy to reduce *E. coli* O157:H7 populations in cattle prior to harvest.

Key Words: *E. coli* O157:H8, Pre-harvest intervention, Sodium chlorate supplementation

475 Integron gene sequences within poultry farms and processing plants. M.T. Roe^{*1}, A. Byrd², D. Smith³, and S. D. Pillai¹, ¹Texas A&M University, College Station, TX, ²United States Dept. of Agriculture, College Station, TX, ³Gainesville College, Gainesville, GA.

The dissemination of multiple antibiotic resistant bacteria is a major issue in the poultry industry. It has been shown that the integron gene sequence plays an important role in the transfer of antibiotic resistance cassettes between pathogens. The objective of this study was to examine the occurrence of integron gene sequences within poultry production and processing. Microbial community DNA from chicken carcass wash samples at key points within poultry farm and processing plants were PCR analyzed utilizing primers specific for the 5' and 3' conserved segments of the integron gene sequence to determine the presence of integrons in sample DNA. Out of 96 total samples, 51% of the samples were positive for the integron sequence. Seventy-one % of on farm samples were integron positive, while, 66%, 46%, and 42% were positive at the post-feather removal, pre-chiller tank immersion, and post-chiller tank immersion location of a processing plant respectively. When two independent chiller immersion tanks were sampled at four discrete points over a 48-hour period, integron sequences were repeatedly detectable. These results indicate that integron sequences are widely distributed within poultry production and processing. Thus, there are multiple locations at which lateral gene transfer of antibiotic resistance gene cassettes can potentially occur.

Key Words: Antibiotic Resistance, Integron Gene Sequence, Poultry Processing

476 Detection of transgenic DNA in bovine milk: Results for cows receiving a TMR containing maize grain modified for insect protection (MON810). R.H. Phipps^{*1}, D.E. Beaver¹, and A.P. Tingey², ¹The University of Reading, Reading, UK., ²Reading Scientific Services Ltd, Reading, UK. .

The aim of the study was to determine the presence or absence in bovine milk of transgenic DNA as a result of animals consuming maize grain genetically modified (GM) for insect resistance by the Bt gene (event MON810). Ten Holstein/Friesian cows (mean days in milk 207, milk yield 25.3 kg/d, DM intake 20.8 kg/d and live-weight 639 kg) received a

TMR, in which the forage component was grass and maize silage (non-GM) in a 1:3 DM ratio and formed 55% of the TMR DM. In study weeks 1-3 the TMR DM also contained non-GM supplements of 18.5% cracked wheat, 26.1% rapeseed meal and 0.4% minerals. In weeks 4-12 ground maize grain (MON810) replaced cracked wheat. Milk and TMR samples were taken weekly prior to and after the introduction of the GM diet. In milk samples spiked with Bt DNA, PCR analyses established a minimum detection level (MDL) of transgenic DNA, of 7.5 g/l of milk. Subsequent semi quantitative PCR analyses were carried out in duplicate on TMR and milk samples using three primer sets to establish the presence of GM DNA. Two of the maize primer sets recognise different regions of the Cauliflower Mosaic Virus (CaMV) 35S promoter and the third covers the integration site of the MON810 inserted DNA. The PCR analysis was capable of detecting GM DNA fragments greater than or equal to 200 base pairs (bp) in length. The feed and milk samples analysed at week 3 when cows received a TMR containing no GM feed ingredients were negative. The TMR feed samples at weeks 4 and 12 were positive for MON810 maize DNA, but all milk samples were negative. In conclusion the results show that GM DNA could not be detected in milk (MDL 7.5 g/l of milk) from cows receiving 18.5% of their diet DM as insect protected (MON810) maize grain. The study was funded by the Milk Development Council and the Dairy Industry Federation.

Key Words: Dairy cows, GM feed ingredients, Transgenic DNA

477 Assessment of novel feeds in animal nutrition. Karen Aulrich* and Gerhard Flachowsky, *Institute of Animal Nutrition, Federal Agricultural Research Centre.*

Apart from feed safety assessment including safety for consumers, animals and environment, nutritional assessment of feeds produced using recombinant DNA techniques (Genetically Modified Organism, GMO) is necessary. In 1997 we started a program to assess GMO's of the so-called first generation (feed plants with changed tolerance or resistance and with minor changes in content of valuable and desirable ingredients) including Bt-corn, Pat-corn, Pat-sugar beets and Gt-soybeans. Apart from main nutrients (crude protein, ether extract, crude fiber, crude ash), amino acids, fatty acids, minerals, fiber components and some mycotoxins were determined in seeds of corn and soybeans, sugar beets and silages from corn and sugar beet leaves. Digestion and feeding experiments were carried out with broilers (Bt-corn), layers (Bt-corn, Pat-corn), pigs (Bt-corn, Pat-corn, Pat-sugar beets, soybeans), sheep (Bt-corn silage, Pat-corn silage, Pat-sugar beet silage), growing bulls (Bt-corn silage) and fistulated cows (Bt-corn silage). Digestibility of nutrients, performance of animals and fate of DNA were investigated. Up to now, we did not find significant differences in nutritional assessment and food quality between feeds from isogenic and transgenic plants of the first generation, except a lower mycotoxin-content (deoxynivalenol and zearalenon) in Bt-corn. The so-called substantial equivalence could be demonstrated. Fragments of plant DNA could be detected in some animal tissues (e.g. muscle from chicken), but fragments of transgenic

DNA were not found. It may be assumed that the transfer of DNA fragments in the body is a process, which takes place constantly and consequently, but it is not a specific problem of genetic engineering. However, the subject should be pursued further. From the point of view of animal nutrition, further studies seem to be necessary on the following subjects: - Nutritional assessment of GMO's with substantial changes in composition (GMO's of second generation) - Influence of GMO's upon animal health and product quality.

Key Words: Novel feeds, Animal nutrition, Genetically modified organism

478 Differences in Transfer of Nicarbazine, Meticlorpindol and Ivermectin from Feed to Milk. C.A. Kan*¹, C.A.J. Hajee², J.A. van Rhijn², A. Klop¹, T. Zuidema², B.J.A. Berendsen², and H.J. Keukens², ¹ID TNO Animal Nutrition, P.O. Box 65, 8200 AB Lelystad, The Netherlands, ²RIKILT, P.O. Box 230, 6700 AE Wageningen, The Netherlands.

Cross contamination during feed production may result in contamination of feeds with feed additives or veterinary drugs. This may lead to contamination of milk, but the extent to which residues might occur was not known. We carried out carry-over experiments with nicarbazine, meticlorpindol and ivermectin. 6 Groups of 4 cows each (both high [> 35 kg/day] and low [< 20 kg/day] producing ones) received concentrate artificially contaminated at three different levels. The high-producing cows received 12 kg and the low producing ones 2 kg concentrate per cow per day. The contaminated feed was fed for three weeks. During exposure and at least 7 days post-exposure, milk samples were collected and mixed samples per group were analysed. Two cows (one high and one low producing one) served as controls. Milk samples were analysed by validated HPLC methods with UV or fluorescence detection. The LOQ in milk for nicarbazine and meticlorpindol was 25 ng/g and for ivermectin 0.1 ng/ml. Feed levels were in the 1-12.5 mg/kg range for nicarbazine, in the 2.5-25 mg/kg range for meticlorpindol and in the 0.3-3 mg/kg range for ivermectin. Nicarbazine (measured as DNC) could not be detected in any of the milk samples. Very low levels could be detected in body fat samples of some animals slaughtered after about 8 days withdrawal. Meticlorpindol was found in milk at levels between 5 and 50 ng/g during feeding the contaminated feed only. The levels scattered, but generally a dose-response relationship between levels in feed and in milk could be established. Ivermectin was found in milk throughout the whole exposure period and up to 10 days post-exposure. Levels up to 7 ng/g were found and good dose-response relationships between feed and milk level could be established. These data show that absorption from feed and excretion into milk of these three compounds in dairy cows differed considerably. No general pattern could be deduced from these data and no general conclusion on risks of residues in milk due to cross contamination in feed mills could be drawn.

Key Words: Nicarbazine in milk, Meticlorpindol in milk, Ivermectin in milk

ASAS/ADSA Physiology: General Physiology

479 Lutalyse alters the immune response in sows after intrauterine inoculation with bacteria. M. C. Wulster-Radcliffe*¹, R. C. Seals², and G. S. Lewis¹, ¹USDA-ARS United States Sheep Experiment Station, ²University of Virginia.

During luteolysis, increases in PGF_{2α} and decreases in progesterone lead to clearance of uterine infections. Thus, we conducted an experiment to determine whether Lutalyse, a PGF_{2α} analogue, alters the uterine immune response to bacterial challenge in the absence of luteolysis and the concomitant decrease in progesterone. Sows (n = 6/group) were assigned to treatments in a 2 × 2 factorial array; bacterial challenge and Lutalyse were main effects. Vena caval blood was collected daily on d 7 through 11 of the estrous cycle. On d 7, uteri were inoculated with either PBS or 70 × 10⁷ cfu of *Escherichia coli* and 150 × 10⁷ cfu of *Arcanobacterium pyogenes* in PBS (10 mL). On d 9, saline (2 mL) or Lutalyse (10 mg) was injected i.m. On d 11, uteri were collected. Sediment (packed-cell volume; PCV) and ability to culture *E. coli* and *A. pyogenes* from uterine flushings were used to diagnose infections. Differential white blood cell counts and basal and mitogen-stimulated lymphocyte proliferation were used to evaluate immune function. All bacteria-treated sows developed uterine infections. Sows treated with bacteria and Lu-

talyse had less severe infections than sows treated with bacteria and saline (PCV = 25 vs 67%; P < 0.01). No PBS-treated sows developed infections (PCV < 5%). Neither Lutalyse nor bacteria affected progesterone (64 ng/ mL) or estradiol-17β (< 1 ng/mL), indicating that luteolysis did not occur. Basal (10.2 vs 2.3 pmol) and lipopolysaccharide-stimulated incorporation (6.8 vs 2.9 pmol) of [³H]thymidine into newly formed lymphocytes was greater (P < 0.01) for Lutalyse-treated than for saline-treated sows. Lutalyse, compared with saline, increased vena caval PGF_{2α} (0.44 vs 0.28 ng/mL; P < 0.05), and Lutalyse increased neutrophils (65 vs 84/100 WBC; P < 0.01) and decreased lymphocytes (28 vs 16/100 WBC; P < 0.01). Thus, exogenous PGF_{2α} can initiate clearance of uterine infections without inducing luteolysis and decreasing progesterone and/or increasing estradiol-17β concentrations; effects of PGF_{2α} seem somewhat independent of changes in progesterone and estradiol-17β.

Key Words: Infection, Sow, Lutalyse