Breeding and Genetics: Dairy Cattle Breeding II– Applied molecular biology and genomics

322 Effects of genomic inbreeding on production, reproduction, and udder health in Holstein dairy cows. D. W. Bjelland*[1], K. A. Weigel[1], D. J. Nkrumah[2], and N. Vukasinovic[2], *[1]Uinversity of Wisconsin-Madison, Madison, [2]Pfizer Animal Genetics, Kalamazoo, MI.*

The objective of the present study was to assess the effects of genomic inbreeding on production, reproduction, and udder health in Holstein dairy cows. First and second lactation data were collected from 1,061 Holstein dairy cows, which were born between 2006 and 2008, from 5 commercial herds across the United States. All cows were genotyped for 54,609 single nucleotide polymorphism (SNP) markers. SNPs were edited based on call rate and allele frequency. The remaining SNPs were separated into SNPs that were or were not in Hardy-Weinberg (HW) equilibrium. The SNPs that were not in HW equilibrium were assumed to be under selection and were removed from the analysis of genomic inbreeding. Homozygosity of the remaining 43,398 SNPs that were in HW equilibrium was calculated and utilized as the measure of genomic inbreeding in this study. Average homozygosity was 66.7 ± 1.6%, with a minimum and maximum of 60.4 and 75.3% respectively, and cows with a greater percentage homozygous loci were assumed to be more highly inbred. Total lactation milk, fat, and protein yield decreased by 15.8, 1.4, and 0.6 kg, respectively, per 1% increase in homozygosity. Furthermore, days open increased by an average of 1.96 d per 1% increase in homozygosity, but somatic cell score did not change with an increase in homozygosity. The results presented herein suggest a possible relationship between increasing homozygosity and decreasing lactation performance and reproductive ability.

Key Words: genomics, inbreeding

323 Maternal grandsire confirmation and discovery in dairy cattle. G. R. Wiggans[1], T. A. Cooper*[1], P. M. VanRaden[1], J. R. O'Connell[2], and L. R. Bacheller[1], *[1]Animal Improvement Programs Laboratory, ARS, USDA, Beltsville, MD, [2]University of Maryland School of Medicine, Baltimore.*

Accurate pedigree information is essential for selecting dairy animals to improve economically important traits. Two methods of maternal grandsire (MGS) discovery were compared. The first compared one single nucleotide polymorphism (SNP) at a time using a genotype from one or both parents (SNP method). The second compared haplotypes of a potential MGS to the animal′s maternal haplotype, which included linkage information across all loci (HAP method). Modified pedigrees with 5% of true MGS replaced by a random genotyped bull from the same birth year and 5% set to missing were created to test the HAP method. To test the SNP method, the same group of animals (including multiple genotypes for some animals and excluding imputed dams) was used. Both methods ranked the most likely MGS. The SNP method can be performed as soon as a genotype is received because no imputation is required. The HAP method was expected to have greater accuracy because it includes low-density genotypes imputed to the 45,187 SNP used for genomic evaluation as well as additional imputed animals. Accuracy of predicting true MGS was evaluated using genotypes for 4,620 Holsteins, 659 Jerseys, and 160 Brown Swiss with confirmed genotyped sires for the SNP method and modified pedigrees for 4,134 Holsteins, 552 Jerseys, and 142 Brown Swiss for the HAP method. Accuracy of true MGS prediction for Holsteins, Jerseys, and Browns Swiss was 95, 91, and 94%, respectively, with the SNP method and 97, 95, and 97% with the HAP method. Lack of imputed SNP decreased accuracy of the SNP method for low-density genotyped animals (78%) compared with BovineSNP50 genotyped animals (97%). Predicted MGS accuracy with the HAP method was 98% for BovineSNP50 genotypes, 94% for Bovine3K genotypes, and 92% for imputed genotypes. When the HAP method was extended to great grandsires, accuracy of maternal great-grandsire confirmation and discovery was 92% for 652 Holsteins, 95% for 33 Jerseys, and 85% for 20 Brown Swiss. Because most dairy bulls have been genotyped, parentage and MGS analysis can accurately confirm, correct, or discover parents and more distant ancestors for most animals.

Key Words: haplotype, maternal grandsire, SNP

324 Sequence analysis and methylation patterns of the bovine IWS1 gene localized to a region of BTA2 involved in postnatal growth. I. G. Imumorin*[1], M. De Donato[1,2], S. O. Peters[1,3], A. M. Corn[1], Y. Bing[1], H. E. Rudolfo[2,4], M. Al-Abri[1,4], and T. Hussain[1,5], *[1]Cornell University, Ithaca, NY, [2]Universidad de Oriente, Cumana, Venezuela, [3]Federal University of Agriculture, Abeokuta, Nigeria, [4]Sultan Qaboos University, Muscat, Oman, [5]University of Veterinary and Animal Sciences, Lahore, Pakistan.*

Our previous genome scan identified the proximal end of bovine chromosome 2 as containing a putative parent-of-origin effect quantitative trait locus (POE-QTL) affecting postnatal growth in cattle. We embarked on bioinformatics and experimental search for possible positional candidate genes in this region. A search of human and mouse genomes of known and putative imprinted genes identified the homolog of *Saccharomyces cerevisiae* serine protease inhibitor 1 (SPN1 also known as IWS1) in this region. This gene is part of a transcription factor complex that regulates transcription. We previously showed that this gene is expressed ubiquitously in 18 bovine tissues and has 99.9% homology with the predicted mRNA sequence in cattle. A comparison of this sequence with the predicted mRNA sequence in sheep and pig shows 97.9 and 92.9% homology, respectively. A phylogenetic analysis of the protein sequence predicted from these mRNAs and those of dog, panda, horse, human, marmoset, mouse, rat, elephant, opossum and platypus, using the lizard protein as an out group, shows high conservation among all the eutherian mammals but not as much with marsupials and monotremes. In this study we screened the coding region for mutations in 19 cattle breeds and found several SNPs. In addition, we carried out DNA methylation analysis in multiple bovine tissues and showed that this gene is differentially methylated. These further show that this gene may be of significance for the traits of interest mapped to bovine chromosome 2.

Key Words: SNP, cattle, IWS1 gene

325 Characterization of sequence diversity of IFNAA and INFB1 in Pakistani breeds of cattle. T. Hussain*[1,2], M. E. Babar[1], A. Nadeem[1], A. Ali[1], A. Wajid[1], M. Al Abri[2], M. De Donato[2,3], S. O. Peters[2], and I. G. Imumorin[2], *[1]Institute of Biochemistry and Biotechnology, University of Veterinary and Animal Sciences, Lahore, Pakistan, [2]Department of Animal Science, Cornell University, Ithaca, NY, [3]IIBCA, Universidad de Oriente, Cumana, Venezuela.*

Interferons (IFN) are glycoproteins made and released by host cells in response to the presence of pathogens to trigger the protective defenses

of the immune system. Among the type I IFNs, IFNA and INFB1 are very important to activate the initial immune response. The objective of this study was to characterize sequence polymorphisms in IFNA and INFB1 genes. We studied 46 samples from Pakistan: 4 Achai, 4 Bhagnari, 6 Cholistani, 4 Dhani, 4 Dajal, 4 Lohani, 4 Nari Master, 6 Red Sindhi, 6 Sahiwal and 4 Tharparker and compared them to sequences from 3 Hereford, 3 Angus and 8 Holstein catlle from the US and 4 Muturu, 11 White Fulani and 4 Sokoto Gudali from Nigeria. Sequences were analyzed by MEGA5 software and Panther to determine the phylogenetic and functional implications of the different variants found. We found 8 polymorphic sites in the amino acid sequence of IFNAA and 20 polymorphic sites in IFNB1. The phylogenetic analysis indicated significant amount of sequence variation and the divergence of Pakistani cattle breeds of these genes from other breeds of taurine cattle. Most of the amino acid changes were non-synonymous and produced changes in the protein structure. The phylogenetic analysis indicated significant divergence of Pakistani cattle breeds from other taurine breeds of cattle, and comparison were made with *Bubalus bubalis*, *Capra hircus* and *Ovis aries*. The functional analysis of the variants show amino acid changes that can affect the structure of the proteins potentially producing changes in the interaction between the other proteins involved in the immune response. This is the preliminary report on Interferon genes in Pakistani cattle breeds. This information is valuable for the association with the resistance or tolerance to infectious agents in cattle in this region.

Key Words: cattle, immune genes, SNP

326 Effect of GHR *Alu*I polymorphism on reproductive performance of Holstein cows. A. Schneider*[1], M. N. Corrêa[1], and W. R. Butler[2], *[1]Veterinary College, Federal University of Pelotas, Pelotas, RS, Brazil, [2]Department of Animal Science, Cornell University, Ithaca, NY.*

The recovery of the growth hormone (GH)/insulin-like growth factor (IGF-I) axis is implicated in an early return to ovulatory cycles and a shorter calving to conception interval in postpartum dairy cows. Based on this, the aim of this work was to determine effects on the reproductive performance of Holstein cows of a GH receptor (GHR) AluI polymorphism, a point mutation upstream from its mRNA start codon. Holstein cows (n = 80) were on the study until 210 days in milk (DIM). Blood samples were collected at 7 and 21 DIM for serum IGF-I determination. For GHR genotyping, DNA was extracted from blood and the presence of the alleles determined after digestion of the corresponding GHR gene region with the *Alu*I enzyme. Milk samples were collected 2 times/week for progesterone analysis. Progesterone higher than 1 ng/mL in 2 consecutive samples was considered indicative of ovulation. Cows were submitted to an OvSynch-TAI protocol at 55 DIM and repeated for cows diagnosed as not pregnant 30 and 60 days after AI. Data was analyzed with SAS for polynomial effects of the presence of the two GHR *Alu*I alleles. Among the cows, 40% had the *Alu*I(+/+) genotype, 50% had *Alu*I(–/+) and 10% were *Alu*I(–/–). Interval from calving to first ovulation was not different among these three genotypes ($P > 0.05$), but reduced for all cows carrying at least one *Alu*I(–) compared to *Alu*I(+/+) cows: 27 ± 3 vs. 33 ± 3 days, respectively ($P = 0.07$). There was a linear effect of the *Alu*I(–) allele on the calving to conception interval, being 80 ± 13, 94 ± 6 and 115 ± 8 days for the GHR *Alu*I(–/–), *Alu*I(–/+) and *Alu*I(+/+) cows, respectively ($P = 0.02$). For serum IGF-I concentration there was a quadratic effect of the *Alu*I(–) allele ($P = 0.04$): 101 ± 10, 60 ± 5 and 63 ± 5 ng/mL for GHR *Alu*I(–/–), *Alu*I(–/+) and *Alu*I(+/+) cows, respectively. In summary, the presence of the GHR *Alu*I(–) allele in Holstein cows was associated with increased serum IGF-I concentrations and fewer days to first postpartum ovulation that contributed to a shorter calving to conception interval.

Key Words: fertility, GHR, SNP

327 Genomic evaluation of rectal temperature in Holstein cattle. S. Dikmen*[1], J. B. Cole[2], D. J. Null[2], and P. J. Hansen[3], *[1]Department of Animal Science, Faculty of Veterinary Medicine, Uludag University, Bursa, Turkey, [2]Animal Improvement Programs Laboratory, ARS, USDA, Beltsville, MD, [3]Department of Animal Sciences, University of Florida, Gainesville.*

Heat stress negatively affects the production, fertility, and health of dairy cattle. Rectal temperature (RT) has unfavorable genetic correlations with production, longevity, economic merit, and somatic cell score in Holstein cows. The objectives of the current study were to perform a genome-wide association study (GWAS) for rectal temperature in dairy cows under heat stress conditions, and to determine with what genes single nucleotide polymorphisms (SNP) of large effect are associated. Rectal temperature was measured between 1500 and 1700 h in 5,590 lactating Holstein cows sired by 3,322 bulls during the summer in north central Florida. Rectal temperature averaged 38.8°C ± 0.57°C, and ranged from 37.0°C to 41.6°C. The model included fixed effects of parity; random effects of herd-year, animal, and permanent environment; and regressions on temperature-humidity index and test-day milk yield. The pedigree file included 886 sires with Illumina BovineSNP50 BeadChip (Illumina, Inc., San Diego, CA) genotypes. After edits for call rates <0.90, minor allele frequencies <0.05, and Mendelian conflicts, 30,0018 markers remained. Genotypes for 9 animals were dropped due to low call rates. (Co)variance components and breeding values were calculated using the AIREMLF90 and BLUPF90 software packages from the University of Georgia (Athens). The GWAS was performed with a one-step procedure as implemented in the POSTGSF90 software. The heritability and repeatability of RT were 0.06 and 0.22, which is lower than recent estimates, possibly because most sires had only a few daughters with records and pedigree ties were limited. The 20 SNP with the largest solutions were examined to determine if they were located in or near genes that could account for heat stress effects. Five SNP were located in introns: *C1H21orf59* on *Bos taurus* autosome (BTA) 1, *INADL* on BTA3, *ZNF335* on BT13, *VPS13* on BTA16, and *FTO* on BTA18. The third-ranked SNP was located in an exon of *CCT6B* on BTA12. These results will help identify genes involved in physiological responses to heat stress. Additional phenotypes are needed to improve estimates SNP effects.

Key Words: rectal temperature, genomic selection, genetic evaluation

328 Feasibility of genomic prediction of fatty acids composition in milk of dairy cattle from Luxembourg using single-step procedure. P. Faux*[1], V. M.-R. Arnould[1,2], H. Soyeurt[1,3], and N. Gengler[1,3], *[1]Animal Science Unit, Gembloux Agro-Bio Tech, University of Liege, Gembloux, Belgium, [2]CONVIS s.c., Ettelbruck, Luxembourg, [3]National Fund for Scientific Research (FNRS), Brussels, Belgium.*

Milk composition in fatty acids (FA) portrays a class of novel traits of interest for both human health and animal robustness. With the exception of Wallonia, Luxembourg is currently the only place in the world where, using mid-infrared spectrometry, milk composition in 29 FA is routinely recorded for dairy cows. Since 2007, spectral data has been recorded so far on 87,368 cows from 690 different herds, by 2 main control methods (T-method: one sample of only one milking, morning or evening, and S-method: proportionate sample of all daily milkings).

Additionally, milk, fat and protein yields are available since 1990. The availability of FA allows many options for management use and animal breeding but requires advanced modeling (e.g., adapted to the testing methods). In the context of animal breeding, genomic selection has been widely developed in dairy cattle, where single-step approach (ssGBLUP) is particularly well suited for small-sized populations, as the dairy cattle population of Luxembourg (365,892 animals currently in pedigree) and is completely integrated into mixed modeling of phenotypic data. The objectives of this study were: (1) to assess the potential benefits of a single-step genomic evaluation on milk FA composition in a small-sized population and in particular (2) to quantify the impact of genomic information on reliability (REL) of estimated breeding values (EBV) of FA in Luxembourg. In a preliminary study for a single FA, oleic acid (C18:1 *cis* 9) genetic evaluations were performed on 47,613 milk records; collected by S-method, from 8,000 cows in first parity with a random regression test-day model using second order Legendre polynomials. For this sample, molecular data was simulated for 422 AI sires, ancestors of recorded cows. Prediction error variances (PEV) were used to compute REL and effective daughter contributions (EDC). First results showed a low increase in REL and EDC. Extension of this research to all sampling methods and research on the optimum structure of the reference population (bulls, cows) will be done to fit the Luxembourg-specific situation.

Key Words: genomic selection, fatty acids, small population

329 Microsatellite markers based genetic evaluation of Pakistani cattle breeds. M. E. Babar*[1], T. Hussain[1,2], A. Nadeem[1], A. Ali[1], A. Wajid[1], S. A. Shah[1], K. Abbas[1], A. Azam[1], Z. Ahmad[1], M. De Donato[1,3], S. O. Peters[1], and I. G. Imumorin[1], *[1]Institute of Biochemistry and Biotechnology, University of Veterinary and Animal Sciences, Lahore, Pakistan, [2]Dept. Animal Science, Cornell University, Ithaca, NY, [3]IIBCA, Universidad de Oriente, Cumana, Venezuela.*

Animal Genetic Resource of Pakistan is very diverse. There are 15 distinct breeds of cattle in Pakistan all belonging to zebu (humped type) cattle (*Bos indicus*) for which very little information on genetic architecture is available. Microsatellite markers are being widely used for breed characterization in animals. In the present study 345 individuals of 11 breeds (10 Pakistani cattle breeds and exotic Holstein Friesian breed) were genotyped using 22 labeled microsatellite markers to assess genetic variation and relationships among them. All markers were polymorphic and observed number of alleles ranged from 8 (TGLA122) to 18 (ILSTS029, BM6526) with mean value 13.54 ± 2.80 per locus. Average values of observed and expected heterozygosity were calculated as 0.462 ± 0.155 and 0.823 ± 0.06. Mean values of Fis, Fit, Fst and gene flow were 0.328, 0.432, 0.155 and 1.362 respectively. The average PIC value was 0.81 showing suitability of these markers for forensic analyses. Nei's genetic distance estimates indicated relatively close genetic identity between Tharparker and Red-Sindhi breeds of Sindh Province of Pakistan while Tharparker and Dajal breeds were found most distinct. The UPGMA-based phylogenetic tree constructed from the genetic distances also indicated that the cattle breeds of Pakistan can be classified into distinct genetic groups based on these markers. This is the first comprehensive report on molecular characterization of Pakistani cattle breeds using microsatellite markers. This study can be helpful for making breed conservation strategies of cattle in Pakistan in future.

Key Words: microsatellite markers, heterozygosity, Pakistani cattle breeds

330 Effects of β-casein, κ-casein and β-lactoglobulin gene allelic variants on milk production and protein composition traits of Brown Swiss cows. C. Ribeca,* A. Cecchinato, M. Penasa, V. Bonfatti, F. Tiezzi, P. Carnier, and G. Bittante, *Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), Legnaro, Padova, Italy.*

Milk protein composition influences many aspects of the dairy industry as well as the nutritional value and the technological properties of milk. The aim of this study was to investigate the effect of β-casein (*CSN2*), κ-casein (*CSN3*) and β-lactoglobulin (*BLG*) gene allelic variants on milk production and the relative concentration of the major milk proteins: α-lactalbumin (α-LA), β-lactoglobulin (β-LG), α_{S1}-casein (α_{S1}-CN), α_{S2}-casein (α_{S2}-CN), β-casein (β-CN) and κ-casein (κ-CN) of individual milk of Brown Swiss cows. A total of 1,271 cows distributed in 85 herds were milk sampled once from January 2010 to February 2011. Individual samples were collected during the evening milking and detailed milk protein composition was analyzed using reversed-phase high performance liquid chromatography (RP-HPLC). Genotypes of cows for *CSN2*, *CSN3* and *BLG*, were also derived by RP-HPLC. The allele frequencies for *CSN3* (A: 0.232, B: 0.768) and *BLG* (A: 0.331, B: 0.669) were unbalanced in favor of the B variant. All genes were in Hardy-Weinberg equilibrium. An association study for*CSN2*, *CSN3* and *BLG* allelic variants was performed using a linear model. The model included effects of days in milk, parity, *CSN2*, *CSN3*and *BLG* genotypes, and the random effect of the sire of the cow. Effects of genotypes on milk yield were weak. The *CSN2* genotype influenced the percentage of α_{S1}-CN, α_{S2}-CN and β-CN, calculated on total casein, the CN number, calculated on total protein κ-CN percentage, and total protein content. The *CSN3* genotypes affected the percentage of α_{S1}-CN, α_{S2}-CN, and β-LG, the latter calculated on total whey protein, percentage of β-CN, CN number, κ-CN percentage, and total protein content. The BLG influenced the percentage of β-LG, protein content, total whey protein, CN number, and percentage of β-LG. All these associations were statistically significant ($P < 0.05$). Our findings could be used to identify animals that produce milk with desired composition or desired processing and manufacturing properties.

Key Words: milk protein composition, protein variant, dairy cow

331 Associations between single nucleotide polymorphisms in multiple candidate genes on milk yield, composition, coagulation properties and individual cheese yield in Brown Swiss cows. A. Cecchinato,* C. Ribeca, M. Penasa, C. Cipolat Gotet, M. De Marchi, A. Maurmayr, and G. Bittante, *Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, Legnaro, Padova, Italy.*

The fraction of milk used for cheese making is growing worldwide. Although genetic variation for traits related to cheese-making exists, their inclusion as breeding goals in conventional selection programs is hampered by phenotyping costs. A possible solution can be found in the identification of candidate genes that affect milk quality, composition and technological traits and that can be integrated in gene-assisted selection programs. The aim of this study was to investigate the association between 46 single nucleotide polymorphisms (SNP), in 33 candidate genes, and the aforementioned traits in individual milk samples from Italian Brown Swiss cows. A total of 1,271 cows were sampled once in 85 herds. Milk and blood samples were collected during the evening milking concurrently with the monthly test-day milk recording. Individual milks were used for measuring: milk quality traits (i.e., protein, casein and fat percentage), milk coagulation properties and individual cheese yield. Genotyping was performed by using a custom VeraCode

GoldenGate approach. A mixed linear model, considering effects of herd, days in milk, parity, SNP genotype and the random effect of the sire of the cow, was used for the association analysis. Each SNP was analyzed separately. Results showed that 16 out of the 46 SNPs were significantly associated with at least one of the traits. Within these SNPs, the ATP-binding cassette sub-family G member 2 (*ABCG2*) and α_{S1}-casein (*CSN1S1*) genes were found to be associated with technological traits considered. Although further research is needed to validate the SNPs in other populations and breeds, the association between these markers and milk technological traits could be exploited in gene-assisted selection programs for genetic improvement purposes.

Key Words: milk coagulation property, individual cheese yield, candidate gene

332 Sire and vaccine treatment effects on immune response to BVDV 1b challenge. E. D. Downey*[1], X. Fang[1], C. A. Runyan[1], J. E. Sawyer[2], T. B. Hairgrove[3], J. F. Ridpath[4], C. A. Gill[1], and A. D. Herring[1], *[1]Texas A&M University, College Station, [2]Texas AgriLife Research, College Station, [3]Texas AgriLife Extension, College Station, [4]National Animal Disease Center, USDA-ARS, Ames, IA.*

Yearling Angus-Nelore F_2 and F_3 steers born in spring of 2009 and 2010 (n = 182) were evaluated for immune response to vaccination and viral challenge. Calves were stratified by composition (F_2 or F_3) and sire across 3 vaccination treatment groups of non-vaccinated (NON, n = 61), 2-injection killed vaccine (n = 60), or single-injection modified live vaccine (MLV, n = 61). All calves were determined to be free of bovine viral diarrhea virus (BVDV) persistent infection and challenged with BVDV 1b (d 0); serum was collected on day of vaccination(s) and d 0, 14, 28, and 42 evaluated for neutralizing antibodies against IBR, and BVDV types 1a, 1b, and 2. Titers (reciprocal base 2 log of the highest neutralizing dilution) were analyzed as repeated measures with mixed models; fixed effects included vaccine treatment, day, vaccine treatment by day interaction, year, and sire nested within composition. No differences existed between F_2 and F_3 steers. Large ranges in animistic titer values among steers were observed, particularly for BVDV1b on d 14 among killed (0 to 12) and MLV (0 to 10) and d 28 for NON steers (3 to 9). Sire nested within composition affected ($P = 0.003$) IBR and approached significance ($P = 0.09$) for BVDV1b. LS means for IBR titers ranged from 0 to 2.2 for F_2 sires and 0.12 to 2.2 for F_3 sires. There was vaccine treatment by day interaction ($P < 0.001$) for IBR, and BVDV types 1a, 1b, and 2. Calves vaccinated with killed vaccine had higher ($P < 0.05$) titers at all post-challenge times compared with MLV or NON calves. NON calves had the lowest animistic titers from d 14 to 42 and appeared to reach peak titer at d 42 for all BVDV types (3.9, 7.5, and 2.9 for 1a, 1b and 2, respectively). MLV steers also had peak BVDV titers at d 42 (4.9, 7.4, and 3.0 for types 1a, 1b and 2, respectively). The killed treatment appeared to have peak BVDV titers on d 14 (9.2, 11.2, and 9.2 for types 1a, 1b and 2, respectively). These data indicate that variation among families in antibody response to vaccination and viral challenge can exist and that response across pathogens may not be uniform across families.

Key Words: *Bos indicus* crosses, BVDV challenge, vaccine response

333 Genome-wide DNA methylation fluctuation in mastitis mice infected by *Staph. aureus*. Y. Yu,* Y. Wei, L. Fan, Y. He, and Y. Wang, *China Agricultural University, China.*

Introduction and objectives: *Staph. aureus* is one of the most crucial causes of mastitis in dairy cattle worldwide. It can survive and reproduce in keratin and phagocyte that normally reject the growth of bacteria. What′s more serious is that *Staph. aureus* can generate β-lactamase which can inactivate antibiotics, resulting in great trouble on clinical therapy. Considering mastitis was corporately affected by bacteria, genetic and epigenetics, in addition, DNA methylation is a key epigenetic marker, which play an important role in the regulation of gene expression in eukaryotes, this study mainly aimed to exploring the DNA methylation changes in mastitis mice infected by *Staph. aureus*. Materials and methods: The *Staph. aureus* mastitis model of dairy cow was established with CD-1 mice by our lab (Fan et al. 2012). A total of 6 *Staph. aureus* mastitis mice and 6 control mice (CD-1) were randomly selected at 12~13 weeks old. We detected the cytosine methylation pattern of the mice genome of breast tissue at 24h post infection. The technique of fluorescent methylation-sensitive amplified polymorphism (F-MSAP) was used, which is a modification of the amplified fragment length polymorphism (AFLP) method to detect DNA methylation on genome-wide (Xu et al. 2005). A total of 7 primer pairs were designed for F-MSAP analysis. Results: The fragments range from 50 to 600bp were count to analyze the DNA methylation levels qualitatively between the 2 groups. Our results showed that a total of 147 bands were detected, and *Staph. aureus* infected group (51%) had decreased incidence of DNA methylation than the controls (55%) although the chi-squared test revealed no significant differences between the 2 groups. DNA methylation fluctuation on specific genes were significantly different between the 2 groups, which depends on the variances of 32 bands. Conclusions: DNA methylation modification is varied after *Staph. aureus* infection in the mastitis mice model. The demethylated and upmethylated region/gene and the role of DNA methylation regulation induced by *Staph. aureus* in dairy cows are warranted to study in further work.

Key Words: DNA methylation, *Staph. aureus* mastitis, F-MSAP