The objective of present new work was to know the effect of year period on mastitis prevalence and to characterize the routine procedures of milking in dairy herds of Culiacan, Sinaloa, Mexico. Total cows analyzed in the year were 976 with 3,904 quarters, in 8 representative herds randomly selected. The work was realized of September 2007 to June 2008, taking like representative year periods different environmental conditions, September month (it warms up and humid, 28.95 °C t and 78% RH); January (fresh, 19.05 °C t, 70% RH); and June (it warms up and dry, 29.65 °C, 60% RH). The health of each quarter was determined with the California Mastitis Test (CMT). The routine practices of milking in each herd were characterized taking 10 minutes from video with hidden camera, udder preparation, teats disinfection before and after milking, milking unit positioning, and over milking occurrence was analyzed. Traces result level was considered not affected, the proportion of negative quarters to CMT was 67.88% without differences (P>0.05) between year periods, corresponding 64.68, 69.26 and 69.69% to September, January and June, respectively. The proportion of positive quarters to CMT was 28.42% without differences (P>0.05) between year periods, corresponding 31.74, 26.89 and 26.63% to September, January and June, respectively. The fibrous quarters proportion was 3.70%. Udder preparation is not adapted one, teats disinfection before milking is not realized, in 50% herds use teat sealant, milking unit positioning is not advisable one, and the over milking is frequent. One concludes that the mastitis prevalence is elevated and is not affected by the year period, because the routine procedures of milking are not adapted.

Key Words: mastitis, prevalence, milking

T11 Effects of *Mangifera indica* peel extracts on *Staphylococcus aureus* mammary infections. S. Stella and D. Tedesco*, *University of Milan, VSA Dep.*, *Milan, Italy*.

In our previous trial, lower *Staphylococcus aureus* mammary infections was evidenced in milk obtained from dairy cow treated with Mangifera indica peel (personal communication). To verify this important effect, we performed an in vitro trial to evaluate the activity of Mangifera indica peel water and ethanol extracts on *Staphylococcus aureus* by agar gel dilution method. One g of each tested extract (water and ethanol) was solubilised in distilled water (10 mL) and sterilized by filtration. One mL aliquots of each solution were inoculated into fluid trypticase soy agar (100 mL) at 45°C. Then the inoculated media were plated into Petri dishes. *Staphylococcus aureus* suspensions (10^2 and 10^3 CFU/mL) were spread on the surface of the media and the plates were incubated aerobically at 37°C for 48 hours. After the incubation period, the number and the size of colonies in the extract-containing and extract-free plates were compared. *S. aureus* colonies were observed by optical microscope Olympus BX41 (12.5X); images were acquired by Image ProPlus software (Media Cybernetics Inc.). The diameter of at least 30 colonies for each medium was measured. The test also included plates containing only the culture medium and the culture medium plus ethanol, in order to obtain a control of the solvent antimicrobial effect. *S. aureus* strains were considered susceptible in case of complete growth inhibition (bactericidal effect) or when colonies were smaller than colonies in control plates (bacteriostatic effect). *S. aureus* strains were considered resistant when the number and size of colonies didn't change compared to the control plates Our results showed an efficient bacteriostatic action due to all Mangifera indica extracts tested: sizes of the tested colonies were smaller than in control (Table 1). The extract effects are following reported according to their efficacy: - Mangifera indica ethanol extract 1 mg/mL - Mangifera indica water extract (0.5 mg/mL) + ethanol extract (0.5 mg/mL) - Mangifera indica water extract 1 mg/mL. In conclusion treatment with this natural wastes extracts can reduce mammary infection.

Table 1. Bacteriostatic effect of Mangifera indica peel extracts on *S. aureus*

Blank (TSA)	Water extract	Ethanol extract	Mixed W/Et extract
2109,5 +/- 228,1	1549,2 +/- 189,2	1188,5 +/- 54,9	1477,0 +/-54,6

S. aureus colony size (µm, mean +/- st. dev.)

Key Words: *Staphylococcus aureus*, Mangifera indica peel extracts, mammary infections

T12 Effects of OmniGen-AF on mammary mucosal responses to an *Escherichia coli* challenge. Y.-Q. Wang*, A. Rowson, N. E. Forsberg, and S. B. Puntenney, *OmniGen Research*, *Corvallis, OR*.

Previous studies (Wang et al., 2007, 2009) have shown that feeding OmniGen-AF to animals increases blood-borne markers of immunity. The goal of this study was to examine effects of feeding OmniGen-AF on the mammary mucosal response during a pathogen challenge. A murine model of bovine mastitis was used. Twenty-four timed pregnant CD1 mice were assigned to three treatments: 1) negative control, 2) positive control and 3) OmniGen-AF-fed. Negative control animals were fed a control diet. At d10 of lactation, they were anesthetized and both L4 and R4 mammary glands infused with sterile PBS. Positive control animals were fed the same diet but infused with 50 colony forming units (CFU) of a bovine clinical isolate of Escherichia coli. The final group of animals was fed OmniGen-AF (0.5% w/w) for 14 d prior to the infusion of 50 CFU of E. coli into the L4 and R4 mammary glands. Infection was allowed to progress for 24 h after which animals were euthanized (ca. d10 of lactation) and whole mammary samples were recovered and RNA isolated using Trizol reagent. Concentrations of mRNAs encoding myeloperoxidase (MPO), major histocompatibility complex 2 class II (MHC), macrophage inflammatory protein (MIP) and beta-actin were assessed using either Sybr green or TaqMan-based quantitative PCR assays. Beta-actin was used as a housekeeping gene to standardize mRNA concentrations. Mammary concentrations of MPO and MHC mRNAs were elevated significantly (P<0.05) in OmniGen-AF-fed animals. MIP mRNA was unaffected by treatment (P>0.05). Enhanced mammary MPO mRNA implies that neutrophil infiltration into infected tissue was increased. An increase in mammary MHC mRNA implies that antigen presentation was enhanced by provision of OmniGen-AF in the diet. Wada et al (2008) reported that OmniGen-AF reduced incidence of mastitis in dairy cattle. Possible mechanisms may include enhanced neutrophil infiltration into infected mammary tissue and enhanced antigen presentation in mammary antigen presenting cells.

Key Words: OmniGen-AF, mastitis, mucosal immunity

T13 Decision-making for early postpartum subclinical mastitis. V. E. Cabrera*, J. Pantoja, P. Ruegg, and G. Shook, *University of Wisconsin*, *Madison*.

A decision tree model was developed to study the economic outcomes of testing and treating early postpartum cows for subclinical mastitis. The model evaluates sequential decisions that determine economic outcomes based on a 305-d lactation. Logistic regression models were used to predict positive and negative results of 2 diagnostic tests: quarter

somatic cell count (SCC) or California Mastitis Test (CMT). The tests were used to detect intramammary infection (IMI) for different DIM (2 to 8), parity statuses (heifer or cow), and a defined SCC threshold. Producer decisions for each cow included (1) test or no test, (2) if test is pursued, what type of test (CMT or SCC), and (3) a final decision: cull, segregate, administer antibiotics, or take no action. Each intermediate or final node of the model was associated with an economic outcome that the decision tree used to find the economically optimal pathway. The cost of subclinical mastitis was assessed as the aggregation of five factors: (1) milk loss, (2) milk premium loss, (3) premature culling, (4) clinical flare-ups, and (5) transmission to herd mates. These costs were a function of the lactation curve, milk price, defined SCC threshold, livestock prices, and a defined prevalence of contagious mastitis pathogens. Preliminary results indicate, in general, the selection of CMT and no action for negative cows. Seems that the administration of antibiotics could be a feasible option for positive cows, especially when a cow is in first parity (increased rate of cure), milk from a treated cow is used for heifer feeding, and the prevalence of contagious pathogens is high. The cost of mastitis under an optimal policy would vary between $142 to $225 per cow per 305-d lactation, and depend strongly on mastitis prevalence, SCC threshold, milk price, milk production level of cow, and parity.

Key Words: decision tree, mastitis cost, mastitis economic impact

T14 Effects of CpG ODN adjuvant on the immune responses elicited by a quadrovalent mastitis vaccine in dairy cows. S.-C. Lee[1] and J.-W. Lee*[2], [1]*Graduate Institute of Animal Vaccine Technology, National Pingtung University of Science and Technology*, *Neipu, Pingtung, Taiwan*, [2]*Department of Tropical Agriculture and International Cooperation, National Pingtung University of Science and Technology*, *Neipu, Pingtung, Taiwan*.

Staphylococcus aureus (*S. aureus*) and *Escherichia coli* (*E. coli*) remain to be common pathogens inducing bovine mastitis worldwide. Prevention of mastitis by using vaccines has not been very successful. Accumulated lines of evidence indicated that the efficacy of a vaccine can be enhanced by using bacterial DNA, or synthetic CpG oligodeoxynucleotides (ODNs), as the adjuvant. In the present study, a quadrovalent mastitis vaccine, containing formalin inactivated three strains of *S. aurues* (T5, T8, and smith compact) and *E. coli* J5, was formulated with or without a sequence of CpG ODNs that has been shown to be immunostimulatory to bovine cells. Eighteen healthy dairy cows were randomly assigned to three groups and received (1) the control (Freund's incomplete adjuvant, FIA, alone, n=6), (2) Vaccine + FIA (n=6), and (3) Vaccine + FIA + CpG (n=6). Serum antibodies specific to the four strains of bacteria and the expression of cytokines, including interferon-gamma (IFN-γ) and IL-4, in peripheral blood mononuclear cells (PBMC) in response to killed bacteria were analyzed by real-time PCR. In comparison with the control, titers of serum antibody specific to the three *S. aureus* strains were significantly ($p < 0.05$) increased. Addition of CpG ODNs into the vaccine did not enhance the production of antibodies. However, PBMC from cows immunized with CpG ODNs as the adjuvant had a significantly increased expression of IFN-γ (11 v.s. 4 folds) and decreased expression of IL-4 (2 v.s 10 folds) at the transcriptional level. Results indicated that inclusion of CpG ODNs as the adjuvant in an inactivated mastitis vaccine can enhance Th1 type immune responses, which might be beneficial to the elimination of bacteria by phagocytes.

Key Words: vaccine, CpG, mastitis

T15 Intramammary glucocorticoid treatment during LPS-induced mastitis. O. Wellnitz, M. Saudenowa, and R. M. Bruckmaier*, *University of Bern, Vetsuisse Faculty, Veterinary Physiology*, *Bern, Switzerland*.

Therapeutically used glucocorticoids have dose-dependent effects on the immune system. Glucocorticoids such as prednisolone (Pred) are traditionally added to antibiotic intramammary injectors aiming to support the cure of the inflamed mammary gland. The goal of the study was to evaluate the effects of Pred at the dosage commonly used in intramammary injectors on the immune system of the mammary gland and to evaluate the influence of Pred on the mammary immune response to E. coli lipopolysaccharide (LPS) stimulation. Five healthy lactating dairy cows with quarter somatic cell counts (SCC) below 120×10^3 cells/mL were intramammarily infused with either Pred (10 mg), LPS (100 μg), Pred+LPS, or saline solution (9 g/l) in one out of four quarters, respectively. Udders were completely emptied by machine milking every 12 h. SCC of each quarter, tumor necrosis factor alpha (TNF) in milk, and lactate dehydrogenase (LDH) in milk were measured at 0, 3, 6, 9, 12, 24, and 36 h. mRNA expression of TNF, interleukin (IL)-1beta, IL-8, IL-10, and lactoferrin (LF) were measured in milk cells at 0, 12, 24, and 36 h using qRT-PCR. Differences between treatments were considered significant if $P<0.05$. SCC increased in LPS stimulated quarters independent of Pred within 6 h until the end of the experiment. TNF milk concentrations increased immediately after LPS stimulation independent of Pred lasting until the 12 h milking. Milk LDH was elevated at the 9 h sample in the LPS quarters and at the 12 to 36 h samples in the LPS+Pred quarters. SCC, TNF, and LDH remained unchanged in the control quarters and in the Pred treated quarters. mRNA expression of TNF, IL-1beta, IL-8, IL-10, and LF increased in LPS treated quarters independent of the presence of Pred. No changes in mRNA expression of these factors in milk cells were observed in controls and Pred treated quarters. In conclusion, stimulation of udder quarters with LPS had a pronounced effect on the mammary immune response. The investigated parameters responded to LPS as typically expected. Based on the measured parameters no immune modulating effects of Pred were observed in healthy udder quarters despite a slightly delayed LDH response.

Key Words: prednisolone, mammary immunity, cow

Breeding and Genetics: Dairy Cattle Breeding II and Rabbit Breeding

T16 Ketosis – Managable by breeding strategies? F. Rehbock[1], G. Freyer[2], F. Klug[3], and N. Vukasinovic*[4], [1]*Landesforschungsanstalt für Landwirtschaft und Fischerei M-V, Institut für Tierproduktion*, *Dummerstorf, Germany*, [2]*FBN, Unit Genetics and Biometry*, *Dummerstorf, Germany*, [3]*Alexandrastr. 4*, *Graal-Müritz, Germany*, [4]*Newsham Choice Genetics, STL Research Center*, *Chesterfield, MO*.

Improving health and fertility is an economic prerequisite for increasing longevity and life performance in dairy cows. According to the literature, ketosis has been reported to be the cause for 9 to 26% reduction