45 Histological examination of the organs of the rats administered varying levels of *Vernonia amygdalina* leaves. A. H. Ekeocha,* P. C. Ekeocha, and T. Fasola, *University of Ibadan, Ibadan, Oyo, Nigeria.*

Histology of the liver, kidney and pancreas of rats administered with *Vernonia amygdalina* was examined. Thirty male Albino rats were divided into 6 groups of 5 rats each. Four groups with basal blood glucose levels of 88.0 ± 0.16, 89.2 ± 0.23, 85.2 ± 0.27, and 85.8 ± 0.25 mg/dL were injected with 10% alloxan in saline to make them diabetic (277.6 ± 6.55, 284.8 ± 3.80, 256.4 ± 1.39 and 265.6 ± 4.41 mg/dL fasting blood glucose (FBG) respectively). The 4 diabetic groups were then treated with different doses (g/kg of BW) of an aqueous extract of dried *Vernonia amygdalina* Leaves herein referred to as VaL. A fifth group (nondiabetic) was treated with 400mg VaL /kg BW. VaL was administered twice daily for 2wks using an oral canula. The sixth group (nondiabetic) received no VaL as a positive control. Blood was collected from the tail to determine blood glucose level on a glucometer. The FBG levels of the 6 albino rat groups were recorded every 2 d for 2 wk. At the end of wk 2, the rats were slaughtered and their liver, kidney and pancreas were examined histologically to ascertain if VaL was toxic to the organs or not. Data were analyzed using ANOVA (SAS, 1999). The plant extract was observed to have hypo-glycaemic effect on each group of diabetic rats as it reduces their fasting blood glucose (FBG) levels from 277.6 mg/dL to 142.2mg/dL (Group 1) 284.8 mg/dL to 102.0 mg/dL (Group 2), 256.4 mg/dL to 86.3 mg/dL (Group 3) and 265.6mg/dl to 82.1 mg/dL (Group 4) over a period of 2 wk. The fifth group consisting of noninduced rats was administered with 400 mg/kg of *Vernonia amygdalina* leaves for a period of 2 wk and the FBG level (91.4 to 81.6 mg/dL) was compared with that of the control group (87.1 to 86.9 mg/dL). The last group i.e the sixth group of rats used as control were only fed with feeds and given clean water only. Their FBG (86.9 mg/dL) were also checked every 2 d and compared with the FBG of the other groups from groups 1–5 (142.2, 102.0, 86.3, 82.1 and 81.6 mg/dL). The rats were sacrificed and their organs (liver, kidney and pancreas) of the experimental animals were examined histologically to ascertain if 400 mg/kg of the plant extract was toxic to the organs. The results show that the plant extract had no adverse effect when administered on normal rats except for a marked congestion of the mesenteric blood vessel. The extract reduced the level of damage to the kidney, liver and pancreas when administered on diabetic rats, which is an indication that the plant extracts of *Vernonia amygdalina* leaves has antidiabetic properties that slowly heals partly damaged organs but not packed up organs.

Table 1. Fasting blood sugar (mg/dL) of normal and induced-diabetic albino rats administered with varying doses of Vernonia amygdalina leaf extracts

Day	G1	G2	G3	G4	G5	G6	SEM
2	277.6^{a}	284.8^{a}	256.4^{b}	265.6^{b}	91.4^{c}	87.1^{c}	8
4	237.4^{a}	243.5^{a}	203.2^{b}	201.8^{b}	85.2^{c}	87.5^{c}	6
6	231.2^{a}	226.6^{a}	151.6^{b}	143.2^{b}	84.6^{c}	88.3^{c}	9
8	217.1^{a}	200.0^{a}	106.0^{b}	94.0^{c}	83.5^{c}	87.9^{c}	9
10	196.0^{a}	176.0^{a}	89.0^{b}	87.3^{b}	82.2^{b}	89.4^{b}	7
12	163.4^{a}	132.0^{b}	87.3^{c}	84.8^{c}	81.8^{c}	86.2^{c}	7
14	142.2^{a}	102.0^{b}	86.3^{c}	82.1^{c}	81.6^{c}	86.9^{c}	6

$^{a–c}$Means in the same row with different superscripts differ significantly ($P < 0.05$).

Key Words: *Vernonia amygdalina* leaves, organs, rat

46 Toxicological properties of liquid dishwashing detergent in Swiss albino mice. M. S. Gulay,* O. Yildiz Gulay, A. Ata, A. Demirtas, and S. Gungor, *Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Burdur, Turkiye.*

Detergent exposure can pose serious health risks to humans and animals. However, the effect of detergent exposure on animal health is yet to be studied. Thus, the aim of the current study was to investigate the toxicity of liquid dishwashing detergent on blood parameters and organ weights of male Swiss Albino mice. Fourty healthy male Swiss albino mice were randomly assigned to 5 groups of 8 animals each. Animals in TR1 served as control and received tap water while TR2, TR3, TR4 and TR5 received 0.1, 0.5, 1 and 5% v/v of the liquid detergent in tap water, respectively, as the only source of water. All treatments started right after weaning (3 wk of age) and continued for 60 d. Mice were kept in plastic cages under standard laboratory conditions. Food and water were provided ad libitum. At the end of the experiment, mice were kept under Sevorane anesthesia and blood samples were collected into Na-heparinized capillary tubes from the beating hearth. After blood collection, animals were sacrificed and internal organs were removed. Data were analyzed by one way ANOVA. Results of the current study indicate that water consumption among the groups did not differ ($P > 0.1$). There was no treatment effect on hematocrit, RBC, WBC, MCV, MCH, MCHC, total lymphocytes, monocytes, or basophils ($P > 0.1$). No effect of detergent treatment was detected on the weights of liver, kidney, spleen, lung, testis or brain ($P > 0.1$). However, total hemoglobin (TR1 = 15.2 ± 0.39, TR2 = 14.7 ± 0.35, TR3 = 14.4 ± 0.56, TR4 = 12.7 ± 0.54 and TR5 = 13.1 ± 0.40 g/dL; $P < 0.01$), plasma protein (TR1 = 6.88 ± 0.26, TR2 = 6.52 ± 0.13, TR3 = 6.71 ± 0.27, TR4 = 5.98 ± 0.11 and TR5 = 6.08 ± 0.31; g/dL $P < 0.05$), total neutrophils (TR1 = 0.83 ± 0.08, TR2 = 0.78 ± 0.13, TR3 = 0.91 ± 0.05, TR4 = 1.04 ± 0.10 and TR5 = 1.68 ± 0.41 x10^3; $P < 0.03$) and total eosinophils (TR1 = 0.071 ± 0.012, TR2 = 0.096 ± 0.024, TR3 = 0.082 ± 0.025, TR4 = 0.272 ± 0.092 and TR5 = 0.263 ± 0.083x10^3; $P < 0.03$) were affected adversely due to detergent treatment. In addition, serum alkaline phosphatase and aspartate aminotransferase levels were significantly elevated in TR4 and TR5 ($P < 0.05$). Thus, our results suggest that especially the higher doses used in the current study could be toxic and cause health risks to Swiss albino mice.

Key Words: detergent, toxicity, hematology

47 Isolation of lactobacillus strains with high adhesive ability to the intestinal epithelial cells. W. M. Zhang*[1], H. F. Wang[1,2], and J. X. Liu[1], *[1]Institute of Dairy Science, MOE Key Laboratory of Molecular Animal Nutrition, Zhejiang University, Hangzhou, China, [2]Department of Animal Science, College of Forestry and Biotechnology, Zhejiang A & F University, Hangzhou, China.*

This study was conducted to investigate the adhesive ability of lactobacillus and how the surface proteins of lactobacillus play a vital role in its adhering to the intestinal epithelia. Intestinal mucus and chyme were collected from a 20-d-old piglet. They were spread on de Man, Rogosa, Sharpe (MRS) agar plates for the selective culture. Agar plate was incubated at 37°C under anaerobic condition for 48 h, and the visible colonies were identified using microscope after stained by methylene blue. The selected colonies of lactobacillus were cultured anaerobically in MRS broth at 37°C. The partial 16s rRNA gene fragment of lactobacillus were then PCR amplified. The 16s rRNA sequences were deposited in GenBank, aligned with Clustal W. Phylogenetic analysis of

these strains was performed using MEGA 3.0. The S-layer proteins were isolated with 5 M LiCl, and then analyzed by SDS-PAGE. Eighty-five strains of lactobacillus were obtained and classified into 1 *Lactobacillus johnsonii*, 54 *L. salivarius*, and 30 *L. reuteri* strains. Depending on the phylogenetic analysis of the pictures of the colonies, 40 strains were selected to adhere to caco-2 cells as model of intestinal epithelial cells. Five of 40 strains were found to have higher adhesive ability (4.9–15.9 cfu/cell) than the others ($P < 0.05$), including 4 *L. reuteri* strains and 1 *L. salivarius* strain. They were named as *L. reuteri* ZJ616 (accession number JN981858), *L. reuteri* ZJ617 (JN981859), *L. reuteri* ZJ621 (JN981863), *L. reuteri* ZJ623 (JN981865) and *L. salivarius* ZJ614 (JN981856), respectively. These 5 strains ($P < 0.05$) inhibited the *Escherichia coli* K88 and *Salmonella enteritidis* 50335, with *L. reuteri* ZJ621 indicating the higher inhibition to *S. enteritidis* 50335 than the others ($P < 0.05$). The molecular masses of s-layer proteins from these strains ranged from 34 to 75 kDa and differed from each other ($P < 0.05$). The ability for the strains without s-layer protein to inhibit the *S. enteritidis* 50335 was reduced, indicating that the S-layer protein plays an important role in inhibiting pathogens.

Key Words: *Lactobacillus*, adhesive ability, surface proteins

48 Effect of mycotoxins on the intestine: Analysis of the interaction between fusariotoxins. B. Grenier*[1,3], A. P. Loureiro-Bracarense[2], G. D. Pacheco[1,2], J. Lucioli[2], A. M. Cossalter[1], W. D. Moll[3], G. Schatzmayr[3], and I. P. Oswald[1], *[1]Institut National de la Recherche Agronomique-ToxAlim, Immuno-Mycotoxicology, Toulouse, France, [2]Universidade Estadual de Londrina, Lab Patologia Animal, Londrina, Brazil, [3]Biomin Research Center, Tulln, Austria.*

Most fungi are able to produce several mycotoxins simultaneously in separate feedstuffs, and considering it is a common practice to use multiple grain sources in animal diets, the risk to be exposed to several mycotoxins at the same time increases. Interactions between concomitantly occurring mycotoxins can be antagonistic, additive, or synergistic. The data on the in vivo combined toxic effects of mycotoxins are somewhat limited. Similarly, data with regard to the impact of these metabolites on intestinal functions and integrity have only gained significant interest over the last decade. Intestinal cells are the first cells to be exposed to mycotoxins, and mycotoxins specifically target high proteins turnover- and activated-cells, which are predominant in gut epithelium. We thus investigated the effect of Fusarium toxins in the gastrointestinal tract of piglets fed deoxynivalenol (DON) and fumonisins (FUMO), alone or in combination at low doses. Piglets received separate diets for 5 weeks: a control diet; a diet contaminated with either DON (3 mg/kg) or FUMO (6 mg/kg); or both toxins. Chronic ingestion of these contaminated diets induced morphological and histological changes, as shown by the atrophy and fusion of villi, the decreased villi height and cell proliferation, and by the reduced number of immune cells. Cytokines analysis (TNF-α, IL-1β, IFN-γ, IL-6, and IL-10) revealed an upregulation of their expression. In addition, ingestion of contaminated diets reduced the expression of the adherent and tight junction proteins, E-cadherin and occludin, respectively. Regarding the association of both mycotoxins, several types of interactions were observed depending on the parameters and intestinal segments assessed. In conclusion, further investigations are required to evaluate the impact of mycotoxins in intestine, especially for the multi-contamination. So far, our findings along with the current data reveal that mycotoxins may affect nutrient absorption, increase the nutrient requirement, predispose animals to infections by enteric pathogens, and lead to chronic intestinal inflammations.

Key Words: mycotoxin, intestine, co-contamination

49 Dietary supplementation of young broiler chickens with capsicum and turmeric oleoresin increases resistance to necrotic enteritis. S.-H. Lee*[1], H. Lillehoj[1], S.-I. Jang[1], D.-K. Kim[1], M.-S. Park[1], E. Lillehoj[2], and D. Bravo[3], *[1]Animal and Natural Resources Institute, ARS-USDA, Beltsville, MD, [2]University of Maryland, School of Medicine, Baltimore, [3]Pancosma S.A., Geneva, Switzerland.*

The *Clostridium*-related poultry disease necrotic enteritis (NE) causes substantial economic losses on a global scale. In this study, a mixture of 2 plant-derived phytonutrients, capsicum oleoresin and turmeric oleoresin (XT), was evaluated for its effects on local and systemic immune responses using a co-infection model of experimental NE. Chickens were fed from hatch with a diet supplemented with XT, or with a non-supplemented control diet, and orally challenged with virulent *Eimeria maxima* oocyts at 18 d and *Clostridium perfringens* at 20 d of age. Parameters of protective immunity were (a) body weight gain, (b) gut lesions, (c) serum levels of *C. perfringens* α-toxin and NetB toxin, and (d) intestinal levels of proinflammatory cytokine/chemokine gene transcripts. Chickens fed the XT-supplemented diet had increased body weight gains and reduced gut lesion scores compared with birds given the non-supplemented diet. The XT-fed group also displayed decreased serum α-toxin and NetB toxin levels and reduced intestinal IL-8 and LITAF mRNA levels, but increased TNFSF15 mRNA levels, compared with non-supplemented controls. In conclusion, this study documents the molecular and cellular immune changes following phytonutrient dietary supplementation that are relevant to protective immunity against avian NE.

Key Words: phytonutrients, necrotic enteritis, protective immunity

50 The identification of candidate genes for BSE and the application to chronic wasting disease in mule deer. J. Thomson*[1], V. Bowles[1], U. Basu[1], Y. Meng[1], P. Stothard[1], and S. Moore[2], *[1]University of Alberta, Edmonton, AB, Canada, [2]University of Queensland, Brisbane, Qld, Australia.*

Previous work in our lab identified 64 regions throughout the bovine genome associated ($P < 0.05$) with classical BSE in European cattle. Bioinformatic analysis identified 89 candidate genes within the regions. Data from 10 different tissue mRNA sequence libraries and 2 whole genome sequences, created using next generation sequencing technology, was interrogated to identify structural variation in these candidate genes. Single nucleotide polymorphisms were identified in 87 of the 89 candidate genes and the identified polymorphisms were tested in 729 animals from 2 populations (193 controls and 225 case samples from paternal half sib families, and 14 control and 397 case samples from a second population). There were 31 markers identified as associated with BSE susceptibility ($P < 0.05$ and MAF >0.01) including 7 marker clusters on chromosomes 2, 10, 14, 17, and 20. The functions of the genes associated with significant markers include prion protein binding and proteasome structure and function as well as neurodegenerative disease in humans. A de novo assembly of the mule deer genome has been constructed and the candidate genes are being annotated to provide research targets in the gene pathways underlying prion disease progression, which will enhance our understanding of the disease.

Key Words: bovine spongiform, chronic wasting disease, genomics

51 Phosphorus utilization in broilers fed soybean and benniseed-based diets with and without microbial phytase supplementation. O. Adebiyi, A. Ologhobo, A. Omojola, O. Olusola, W. Muhammed, and M. Olumide*, *University of Ibadan, Ibadan, Nigeria.*

The effects of microbial phytase (Natuphos 500) supplementation of corn- soybean meal and benniseed meal based diets were studied with One hundred and sixty-eight (168) day- old Arbor Acre broiler chicken on the parameters of growth performance, hematology, carcass characteristics and phosphorus and calcium retention in tibia bone for 8 week. The chickens were within a weight range of 40–43 g at day old and allotted in a completely randomized design (CRD) to 6 dietary treatments with 7 broiler chickens per replicate having 4 replicates per treatment. Three levels of phytase (0, 300 and 600 FTU/kg diet) were added to the diets based on soybean meal and benniseed meal respectively. The diets contained 2972.00/ 3299.90 ME kcal/kg DM energy, 23.26/23.10% protein, 1.67/1.72% calcium and 0.85/ 0.87% phosphorus in the soybean meal and benniseed meal based diets respectively at the starter phase. At the finisher phase, the diets contained 3061.20/ 3363.44 ME kcal/kg DM energy, 21.02/ 21.09% protein, 1.52/ 1.57% calcium and 0.75/ 0.77% phosphorus in the soybean meal and benniseed meal based diets respectively. Body weight gains of 48.60 g/day and feed intake of 101.98 g/day were significantly ($P < 0.05$) increased by 300 FTU/kg phytase supplementation in benniseed meal diets when compared with the group without phytase supplementation (control group). Significant ($P < 0.05$) increases were also obtained for percentage retentions of nitrogen (N) 67.22, phosphorus (P) 45.58, ash 74.85, ether extract (EE) 65.43 and crude fiber (CF) 22 in the benniseed diet supplemented with 300FTU phytase. Phosphorus and calcium retentions in the tibia bone were not significantly ($P < 0.05$) affected by supplementation of soybean meal and benniseed meal based diets with microbial phytase supplementation. Likewise, the treatments also failed to induce any statistical effects on hematological parameters of broilers including the PCV, WBC, Hb and RBC respectively. There were however, significant ($P < 0.05$) differences in the magnitude of response of birds to the diets supplemented with phytase. Birds fed benniseed meal with 300 FTU/kg microbial phytase supplementation had the highest feed intake g/day (101.98), weight gain g/day (48.60) and phosphorus retention in tibia bone (24.29).

Key Words: phytase, phosphorus, tibia bone

52 Effects of tropical legume supplementation on parasite burden and health parameters in goats. M. A. Zarate*[1], J. C. Hamie[1], J. J. Romero[1], E. N. Muniz[2], Y. J. Jang[3], K. G. Arriola[1], O. C. Queiroz[1], and A. T. Adesogan[1], *[1]University of Florida, Gainesville, [2]Empresa Brasileira de Pesquisa Agropecuária, EMBRAPA, Aracajú, Sergipe, Brazil, [3]Gyeongsang National University, Jinju, South Korea.*

The objective of the experiment was to evaluate the effects of supplementing bahiagrass hay (BG; *Paspalum notatum*) with perennial peanut (PP; *Arachis glabrata*), soybean (SB; *Glycine max*), cowpea (CP; *Vigna unguiculata*) and lespedeza (LES; *Lespedeza cuneata*) on infection by *Haemonchus contortus* and *Eimeria* sp. in goats. Forty naturally infected male Kiko × Spanish, 8 mo old, kids (23.8 ± 5.2 kg) were blocked by weight, placed in 3 pens per treatment (each with 2 or 3 kids), and fed diets containing BG hay alone or BG hay supplemented with PP, SB, CP and LES at 50% of the diet. Body weight (BW) was measured on 2 consecutive days at the beginning and end of the 4 wk trial. On d 0 and every 7 d thereafter, blood packed cell volume (PCV), fecal egg counts (FEC) and BW were recorded. Data was analyzed as a randomized block design. The model included the effects of treatment, week and their interaction. The PDIFF statement of SAS with Tukey adjustment was used to evaluate means. Feeding PP, SB or LES hay ($P < 0.05$) reduced gastrointestinal nematode FEC compared with BG alone, but LES had the least values ($P < 0.01$). Goats fed LES had lower ($P < 0.01$) Eimeria FEC compared with BG alone and similar tendencies were evident for goats fed PP and SB ($P = 0.08$, $P = 0.06$). Legume supplementation increased PCV ($P < 0.05$) compared with feeding BG alone. Treatments did not affect BW or ADG. We conclude that feeding LES resulted in the greatest reduction in total FEC and it also increased PCV. Feeding PP and SB reduced total FEC to a lesser extent and also increased PCV but feeding cowpea only increased PCV.

Table 1. Effects of legume supplementation on gastrointestinal nematode and Eimeria fecal egg counts (NFEC, EFEC), Hematocrit (HEM), initial and final body weight (IBW, FBW), and average daily gain (ADG)

	Bahiagrass	Peanut	Soybean	Cowpea	Lespedeza	SEM
NFEC, epg	2773[a]	2072[bc]	1914[b]	2641[ac]	1147[d]	161
EFEC, epg	2809[a]	2162[ab]	2197[ab]	2700[a]	1246[b]	284
HEM, %	21.5[a]	29.6[b]	30.1[bc]	26.4[bc]	27.5[c]	1.3
IBW, kg	24.9	25.1	23.5	24.4	24.7	3.3
FBW, kg	25.0	25.6	24.5	24.1	26.2	3.8
ADG, g/d	4.4	15.4	35.2	−9.2	51.6	22.2

[a–d]Means within a row with different superscripts differ by $P < 0.05$.

Key Words: goat, parasite, legume

53 Carboxymethylation and antioxidant activity of exopolysaccharides. M. Huang,* T. F. Zhu, Z. Q. Lu, G. X. Wu, and Y. Z. Wang, *National Engineering Laboratory of Bio-Feed Safety and Pollution Prevention and Key Laboratory of Animal Nutrition and Feed Science of Ministry of Agriculture, Institute of Feed Science, Zhejiang University, Hangzhou, Zhejiang, China.*

Polysaccharides have been demonstrated to play an important antioxidant role as free radical scavengers, and chemical modification is one of the methods to improve their bioactivities. In our previous study, exopolysaccharides (EPS) produced by *Enterobacter cloacae* Z0206 were able to improve antioxidant function in rats, tilapia and Broilers. However, modification of EPS is necessary to improve their bioactivities. Therefore, to validate the possibility of enhancing the antioxidant activities of EPS through the carboxymethylated modification and screen out the optimum reaction conditions, carboxymethylated derivatives were prepared in aqueous alkaline medium using monochloroacetic acid (MCA) as etherifying agent according to the orthogonal test. Nine modification conditions were designed to study the effect of 3 parameters such as the reaction time, reaction temperature and ratio of NaOH to MCA on reaction yield and degree of substitution (DS), 9 carboxymethylated derivatives with different DS were obtained. The data were analyzed by general linear model ANOVA, differences between groups were considered statistically significant at the 5% ($P < 0.05$) level.The antioxidant activities were evaluated in vitro, by scavenging abilities on superoxide radical and hydroxyl radical. The results indicated that the extent of the impact of variables on DS followed the order: molar ratio of NaOH to MCA > reaction time > reaction temperature. With the increase of the reaction time and reaction temperature from 50°C to 60°C, DS of derivatives increased gradually. However, the DS decreased when the temperature up to 70°C. The carboxymethylated derivatives showed noticeable antioxidant activities compared with EPS, and carboxymethylated derivative with DS of 0.86 showed highest antioxidant activities. The optimum carboxymethylated conditions of EPS were the reaction time of 4 h, the reaction temperature of 60°C and the ratio of

NaOH to MCA of 2:1. This study provided a theoretical basis for the exploration of a potential antioxidant in feed additives or food industry.

Key Words: polysaccharides, carboxymethylation, antioxidant activity

54 Risk factors for switch in status from *Mycobacterium avium* ssp. *paratuberculosis* test positive to negative; data from the national Johne's disease control demonstration program. A. Kenyon*[1], S. Aly[1], and I. Gardner[2], [1]*Veterinary Medicine Teaching and Research Center, School of Veterinary Medicine, University of California-Davis, Tulare,* [2]*Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California-Davis, Davis.*

The objective of this study was to identify the risk of a switch in status of cows tested by *Mycobacterium avium* ssp. *paratuberculosis* (MAP) antigen-based tests (Agtest) and antibody-based tests (Abtest). A switch in status was recorded when a cow that previously tested positive subsequently tests negative. Data from 65 demonstration herds nationwide were used to study the rate of switch in status from a positive to a negative test result. Study herds' dairy herd improvement records for the respective testing period was downloaded and verified for correctness before importing into the relational database. The data set was comprised of 144,150 dairy herd information association (DHIA) records and 245,063 Agtest and Abtest results including Biocor, Kinetics ELISA, HerdChek (Idexx), liquid culture Trek ESP, Bactec, culture on Herrold's egg yolk medium, MGIT and PCR. Matched DHIA records and results yielded 194,535 records that represent longitudinal data for all the cows tested as part of the national Johne's disease demonstration program and their DHIA records over spanning the time period from 1999 to 2010. In 11 years, a total of 48,434 cows were tested for MAP using at least a single test and a total of 4839 (10%) cows switched status at least once in their lifetime using any of the study tests. In comparison to first parity, as a cow's parity increased to 2, 3, and 4, its odds of switching serostatus using increased by 60, 30 and 50% (P value < 0.01). For every 100 d in milk increase in a cow's lactation, its odds of switching serostatus increased by 7% (P value 0.01). Cows were more likely to switch in status as they aged which could also be explained by repeated testing. In addition, cows were also more likely to switch in status as they progressed in their lactation regardless of parity.

Key Words: switch test results, Johne's, risk

55 Expressing an antimicrobial peptide cathelicidin-BF by fusion with SUMO in *Bacillus subtilis*. C. Luan,* Y. G. Xie, H. W. Zhang, and Y. Z. Wang, *Institute of Feed Science, Zhejiang University, National Engineering Laboratory of Biological Feed Safety and Pollution Prevention and Control, Key Laboratory of Animal Nutrition & Feed Science, Ministry of Agriculture, Hangzhou, Zhejiang Province, People's Republic of China.*

Cathelicidin-BF, an antimicrobial peptide purified from the snake venoms of *Bungarus fasciatus*, is an excellent alternative tool for clinical or agricultural antibiotics. The cathelicidin-BF efficiently killed bacteria and some fungal species, especially active against gram-negative bacteria. No hemolytic and cytotoxic activity was observed at the dose of up to 256 μg/mL. Most antimicrobial peptides were expressed in *Escherichia coli*. In this work, we developed a highly efficient expression system in *Bacillus subtilis* to allow for potential application of cathelicidin-BF. The cathelicidin-BF gene was fused with a small ubiquitin-like modifier (SUMO) gene and ligated into a *Escherichia coli*/*Bacillus subtilis* shuttle vector pHT43, generating pHT43-SUMO-CBF, which was subsequently transformed into *B. subtilis* strain WB800N. The fusion protein SUMO-CBF was secreted into the culture medium, and 60mg of recombinant fusion protein was purified from 1 L of culture supernatant by Ni-NTA chromatography. After the SUMO-CBF fusion protein was cleaved by the SUMO protease 1 at 4°C overnight, the cleaved sample was reapplied to a Ni-NTA resin. Finally, peptide yields of 8mg/l recombinant cathelicidin-BF were achieved. The recombinant cathelicidin-BF had similar antimicrobial activity to the synthetic cathelicidin-BF. These results may lead to a cost-effective way for the mass production of cathelicidin-BF in *B. subtilis*.

Key Words: antimicrobial peptide, *Bacillus subtilis*, expression