

## **COLLECTED ABSTRACTS**

### **Editorial: Donkey milk can improve our health, and that's why we should increase donkey milk production**

Iolanda Altomonte

**Italy**

As a member of the Journal of Dairy Research Editorial Board I would like to draw the attention of JDR readers to the donkey milk sector in Europe and particularly in Italy. Although many of the donkey breed populations in Europe have been decreasing, in the last few years in my country we have seen an increase in the numbers of some breeds reared for milk production (chiefly Ragusana and Amiata) and in the number of donkey dairy farms. In addition, several investigations support the suggestion that donkey's milk could be useful in babies suffering from cow milk protein allergy (CMPA: Martini et al., 2018a) and also later in life and in certain health conditions (e.g. elderly people, in cases of dyslipidaemias and obesity). Although there has been a rise in consumer interest about donkey milk, it is still a niche product, and its price is high (about 14 euros per liter for pasteurized milk). In my opinion we should try to increase the quantity of donkey milk on the market in order to reduce the price and promote the donkey milk sector.

Given the low udder capacity of jennies, the average milk yield is only about 1-1.5 l/d (Martini et al., 2018b). I believe that the best way forward would be to select the jennies in terms of milk yield and quality, similarly to what has been done for the other dairy species. Donkeys seem to have a wide individual variability in productive traits and this feature is an opportunity for genetic selection. The selection could be focused on creating two genetic lines in relation to the milk fat content. Donkey milk is low in fat (lower than 1%), making it useful in hypocaloric diets, whereas a genetic line producing higher fat milk would be more suitable in the diet of non-weaned babies. Furthermore, we need to investigate the nutritional needs of lactating donkeys, and take into account that foals are naturally milk-fed on donkey farms. We also need to find an optimal management for their separation and weaning in order to increase the amount of milk that is collected for sale. Future production systems must be able to combine profitability with the responsibility of protecting human and animal welfare, as well as the environment. Consumers in Italy often consider animal welfare as an indicator of healthier and tastier products, therefore, welfare aspects in dairy donkey farming are key for consumers looking for "healthy and natural food". Despite this, there are still only a few robust design studies on dairy donkey management and welfare. What do I expect for the near future? I look forward to seeing new investigations into dairy donkey genetic traits and farm management in order to increase donkey milk yield and improve milk production.

## Bioactive peptides from milk: animal determinants and their implications in human health

Einar Vargas-Bello-Perez, Roberto I. Márquez-Hernández and Lorenzo E. Hernández-Castellano  
Denmark and Mexico

### Abstract

Milk is an important protein source in human diets, providing around 32 g protein/L (for bovine milk, which constitutes some 85% of global consumption). The most abundant milk proteins are  $\alpha$ -lactalbumin,  $\beta$ -lactoglobulin,  $\alpha$ s-casein,  $\beta$ -casein, and  $\kappa$ -casein. Besides their nutritional value, milk proteins play a crucial role in the processing properties of milk, such as solubility, water bonding, heat stability, renneting and foaming, among others. In addition, and most importantly for this review, these proteins are the main source of bioactive components in milk. Due to the wide range of proposed beneficial effects on human health, milk proteins are considered as potential ingredients for the production of health-promoting functional foods. However, most of the evidence for bioactive effects comes from *in vitro* studies, and there is a need for further research to fully evaluate the true potential of milk-derived bioactive factors. Animal genetics and animal nutrition play an important role in the relative proportions of milk proteins and could be used to manipulate the concentration of specific bioactive peptides in milk from ruminants. Unfortunately, only a few studies in the literature have focused on changes in milk bioactive peptides associated to animal genetics and animal nutrition. The knowledge described in the present review may set the basis for further research and for the development of new dairy products with healthy and beneficial properties for humans.

## Estimation of genetic variation for macro- and micro-environmental sensitivities of milk yield and composition in Holstein cows using double hierarchical generalized linear models

Jamshid Ehsaninia, Navid Ghavi Hossein-Zadeh and Abdol Ahad Shadparvar

Iran

### Abstract

The aim of this study was to estimate genetic parameters for environmental sensitivities in milk yield and composition of Iranian Holstein cows using the double hierarchical generalized linear model (DHGLM) method. Data set included test-day productive records of cows which were provided by the Animal Breeding Center and Promotion of Animal Products of Iran during 1983 to 2014. In the DHGLM method, a random regression model was fitted which included two parts of mean and residual variance. A random regression model (mean model) and a residual variance model were used to study the genetic variation of micro-environmental sensitivities. In order to consider macro-environmental sensitivities, DHGLM was extended using a reaction norm model, and a sire model was applied. Based on the mean model, additive genetic variances for the mean were 38.25 for milk yield, 0.23 for fat yield and 0.03 for protein yield in the first lactation, respectively. Based on the residual variance model, additive genetic variances for residual variance were 0.039 for milk yield, 0.030 for fat yield and 0.020 for protein yield in the first lactation, respectively. Estimates of genetic correlation between milk yield and macro- and micro-environmental sensitivities were 0.660 and 0.597 in the first lactation, respectively. The results of this study indicated that macro- and micro-environmental sensitivities were present for milk production traits of Iranian Holsteins. High genetic coefficient of variation for micro-environmental sensitivities indicated the possibility of reducing environmental variation and increase in uniformity via selection.

## Inclusion of grape marc in dairy cattle rations alters the bovine milk proteome

Richard A. Scuderi, David B. Ebenstein, Ying-Wai Lam, Jana Kraft and Sabrina L. Greenwood  
USA

### Abstract

Grape marc (GPM) is a viticulture by-product that is rich in secondary compounds, including condensed tannins (CT), and is used as a supplement in livestock feeding practices. The aim of this study was to determine whether feeding GPM to lactating dairy cows would alter the milk proteome through changes in nitrogen (N) partitioning. Ten lactating Holstein cows were fed a total mixed ration (TMR) top-dressed with either 1.5 kg dry matter (DM)/cow/day GPM (GPM group; n=5) or 2.0 kg DM/cow/day of a 50: 50 beet pulp: soy hulls mix (control group; n=5). Characterization of N partitioning and calculation of N partitioning was completed through analysis of plasma urea-N, urine, feces, and milk urea-N. Milk samples were collected for general composition analysis, HPLC quantification of the high abundance milk proteins (including casein isoforms,  $\alpha$ -lactalbumin, and  $\beta$ -lactoglobulin) and liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis of the low abundance protein enriched milk fraction. No differences in DMI, N parameters, or calculated N partitioning were observed across treatments. Dietary treatment did not affect milk yield, milk protein or fat content or yield, or the concentrations of high abundance milk proteins quantified by HPLC analysis. Of the 127 milk proteins that were identified by LC-MS/MS analysis, 16 were affected by treatment, including plasma proteins and proteins associated with the blood-milk barrier, suggesting changes in mammary passage. Immunomodulatory proteins, including butyrophilin subfamily 1 member 1A and serum amyloid A protein, were higher in milk from GPM-fed cows. Heightened abundance of bioactive proteins in milk caused by dietary-induced shifts in mammary passage could be a feasible method to enhance the healthfulness of milk for both the milk-fed calf and human consumer. Additionally, the proteome shifts observed in this trial could provide a starting point for the identification of biomarkers suitable for use as indicators of mammary function.

## Association between feed sorting and the prevalence of metabolic disorders in Hungarian large-scale dairy herds

Viktor Jurkovich, László Könyves and Mikolt Bakony

Hungary

### Abstract

This research communication describes the possible association between feed sorting and the risk of metabolic disorders in dairy cows. Feed sorting, that is selecting smaller size TMR particles over longer length fibers, can lead to imbalanced energy input. In addition, sorting can lead to lower nutritive value of leftover TMR. To detect a possible relationship between TMR sorting and the occurrence of metabolic disorders in large-scale herds, TMR separation and metabolic profile tests were performed in 22 Hungarian dairies. Feed sorting was defined as >5% alteration in the mass proportion of any of the TMR fractions between the time of feed distribution and 5-6 hours later. The prevalence of ketosis and subclinical acidosis differed between feed sorting and non-sorting groups. Inhomogenous TMR seems to be a predisposing factor for imbalanced energy status. TMR homogeneity measurements should be routinely included in herd health monitoring.

## Assessment of on-farm welfare for dairy cattle in southern Spain and its effects on reproductive parameters

Laura Molina, Estrella Agüera, Francisco Maroto-Molina and Carlos Carmelo Pérez-Marín

Spain

### Abstract

In this Research Communication we analyse the animal welfare status of dairy farms located in southern Spain and test the hypothesis that monitoring of wellbeing could increase the profitability of dairy herds by improving indices of reproduction. Twenty dairy farms were visited and a total of 1650 cows were assessed using the Welfare Quality<sup>®</sup> (WQ) protocol to determine their welfare status. These farms were selected as representatives of the main types of dairy farms found in the south of Spain. No farms attained a welfare status of “excellent”, but all obtained an adequate score for most parameters. Feeding assessment showed relatively low variability among farms, whereas housing and health assessments exhibited high variability. Significant correlations were found between a number of welfare parameter pairings: between percentage of collisions and time needed to lie down; between cleanliness of water points and cleanliness of various animal parts; between farms with access to an outdoor loafing area and an inadequate body condition score and with animal cleanliness; between the frequency of animals lying partly or completely outside of the lying area and the percentage of integument alterations and finally between the presence of respiratory problems and farm hygiene parameters. Furthermore, significant correlations between welfare parameters, reproductive indices and milk production were found. The percentage of cows exhibiting an inadequate body condition score and farms where cows took longer to lie down were correlated with the calving-first insemination interval. Animals showing a higher incidence of coughing and hampered respiration presented lower heat detection rates and milk production and finally farms with dirtier animals had lower milk production. This study is the first step towards including welfare in the recording of routine data in dairy cattle farms in southern Spain.

## Berberine inhibits lipopolysaccharide-induced expression of inflammatory cytokines by suppressing TLR4-mediated NF- $\kappa$ B and MAPK signaling pathways in rumen epithelial cells of Holstein calves

Chenxu Zhao, Yazhou Wang, Xue Yuan, Guoquan Sun, Bingyu Shen, Feng Xu, Guyue Fan, Meiyu Jin, Xinwei Li and Guowen Liu

China

### Abstract

Subacute ruminal acidosis (SARA) can increase the level of inflammation and induce rumenitis in dairy cows. Berberine (BBR) is the major active component of *Rhizoma Coptidis*, which is a type of Chinese anti-inflammatory drug for gastrointestinal diseases. The purpose of this study was to investigate the anti-inflammatory effects of BBR on lipopolysaccharide (LPS)-stimulated rumen epithelial cells (REC) and the underlying molecular mechanisms. REC were cultured and stimulated with LPS in the presence or absence of different concentrations of BBR. The results showed that cell viability was not affected by BBR. Moreover, BBR markedly decreased the concentrations and mRNA expression of pro-inflammatory cytokines, including tumor necrosis factor- $\alpha$ , interleukin-1 $\beta$ , and interleukin-6 in the LPS-treated REC in a dose-dependent manner. Importantly, Western

blotting analysis showed that BBR significantly suppressed the protein expression of toll-like receptor 4 (TLR4) and myeloid differentiation primary response protein (MyD88) and the phosphorylation of nuclear factor- $\kappa$ B (NF- $\kappa$ B), inhibitory kappa B (I $\kappa$ B $\alpha$ ), p38 mitogen-activated protein kinase (MAPK), and c-Jun N-terminal kinase (JNK) in LPS-treated REC. Furthermore, the results of immunocytofluorescence showed that BBR significantly inhibited the nuclear translocation of NF- $\kappa$ B p65 induced by LPS treatment. In conclusion, the protective effects of BBR on LPS-induced inflammatory responses in REC may be due to its ability to suppress the TLR4-mediated NF- $\kappa$ B and MAPK signaling pathways. These findings suggest that BBR can be used as an anti-inflammatory drug to treat inflammation induced by SARA.

### **Exogenous phospholipase A2 affects inflammatory gene expression in primary bovine mammary epithelial cells**

Jacqueline P. Kurz, Mark P. Richards, Matthew Garcia and Zhongde Wang  
**USA**

#### **Abstract**

This Research Communication addresses the hypothesis that exogenously administered phospholipase A2 (PLA2) affects the inflammatory responses of bovine mammary epithelial cells (bMEC) *in vitro* with the aim of providing preliminary justification of investigation into the uses of exogenously administered PLA2 to manage or treat bovine mastitis. Primary bMEC lines from 11 lactating Holstein dairy cows were established and the expression of 14 pro-inflammatory genes compared under unchallenged and lipopolysaccharide (LPS)-challenged conditions, with and without concurrent treatment with bovine pancreatic PLA2G1B, a secreted form of PLA2. No differences in the expression of these genes were noted between PLA2-treated and untreated bMEC under unchallenged conditions. Following LPS challenge, untreated bMEC exhibited significant downregulation of *CXCL8*, *IL1B*, *CCL20*, and *CXCL1*. In contrast, PLA2-treated bMEC exhibited significant downregulation of *IL1B* and *CCL20* only. These findings indicate that exogenous PLA2 affects the expression of some pro-inflammatory factors in immune-stimulated bMEC, but does not influence the constitutive expression of these factors. Further investigation of the influence of exogenous PLA2 in the bovine mammary gland is justified.

### **Septin6 Regulates Cell Growth and Casein Synthesis in Dairy Cow Mammary Epithelial Cells via mTORC1 Pathway**

Bin Li, Zhuzha Basang, Lijun Hu, Liu Liu and Nan Jiang  
**China**

#### **Abstract**

This research paper addresses the hypothesis that Septin6 is a key regulatory factor influencing amino acid (AA)-mediated cell growth and casein synthesis in dairy cow mammary epithelial cells (DCMECs). DCMECs were treated with absence of AA (AA-), restricted concentrations of AA (AAr) or normal concentrations of AA (AA+) for 24h. Cell growth, expression of CSN2 and Septin6 were increased in response to AA supply. Overexpressing or inhibiting Septin6 demonstrated that cell growth, expression of CSN2, mTOR, p-mTOR, S6K1 and p-S6K1 were up-regulated by Septin6. Furthermore, overexpressing or inhibiting mTOR demonstrated that the increase in cell growth

and expression of CSN2 in response to Septin6 overexpression were inhibited by mTOR inhibition, and vice versa. Our hypothesis was supported; we were able to show that Septin6 is an important positive factor for cell growth and casein synthesis, it up-regulates AA-mediated cell growth and casein synthesis through activating mTORC1 pathway in DCMECs.

### **The effect of 17 $\beta$ -estradiol on lactose in plasma and urine in dairy cows in late lactation**

Sigrid Agenäs, Idamaria Lundström and Kjell Holtenius

**Sweden**

#### **Abstract**

The aim of this study was to investigate the effect of 17 $\beta$ -estradiol on mammary tight junctions in cows in late lactation. The experiment included five non-pregnant cows around day 290 in lactation. The cows received injections of 17 $\beta$ -estradiol for six days. The effect of exogenous 17 $\beta$ -estradiol on milk yield, milk composition and lactose in plasma and lactose in urine was investigated before, during and after the treatment. Milk yield decreased after 17 $\beta$ -estradiol injections and lactose in plasma and urine increased, showing an effect on the integrity of the mammary tight junctions. However, there was a delay between hormone injections and the decrease in milk yield and opening of tight junctions, indicating that other factors are involved. A high correlation between lactose in urine and blood plasma was found. More than 30% of the total lactose production was lost in urine after 17 $\beta$ -estradiol treatment.

### **Treatment with etamsylate reduces haemolactia in lactating dairy cows.**

Lorenzo Fraile, Antonio Arcas, Luis M. Jiménez, Josep Mallo and Ramon Armengol

**Spain**

#### **Abstract**

This Research Communication describes the efficacy of etamsylate to reduce haemolactia in dairy cows. A dairy cow with haemolactia produces milk that is reddish or pinkish due to the presence of blood. Haemolactia causes economic loss because bloody milk is rejected by the industry and the consumers. A total of 58 dairy cows with haemolactia were included in the study and randomly divided into treated (n=31) and control (n=27) groups. Treatment consisted of three consecutive daily doses of etamsylate at 15 mg/kg, delivered intramuscularly. Milk production was recorded daily for 7 days, whether or not blood was detected in milk. The mean number of days with the presence of blood in milk in the treatment group was significantly lower (3.4 d) than in the control group (4.9 d). Treatment with etamsylate did not significantly affect milk yield. In conclusion, treatment with etamsylate reduces the number of days blood is observed in milk and it does not have any negative effect on milk production.

## Effect of automatic cluster remover settings on milkability, milk quality and milking irregularities of crossbred cows

Ahmad Fahim, Madan Lal Kamboj, Ajayvir Singh Sirohi, Mukesh Bhakat and Tushar Kumar Mohanty

India

### Abstract

Automatic cluster remover (ACR) settings regulate the end of milking by detaching the clusters based on milk flow dropping below a preset level, which needs to be standardised for different breeds of dairy animals based on their production. A study was conducted to find out the best ACR setting for milking Indian crossbred cows based on milkability, milking irregularities and milk quality. Fifty six crossbred dairy cows in lactations 1 to 4 were categorised into three groups based on the level of production; low (N=16; <12 kg/day), medium (N=32; 12-18 kg/day) and high (N=08; >18 kg/day). The ACR settings tested were 0.1, 0.2, 0.3 and 0.4 kg/min, keeping the vacuum level and pulsation settings constant. The ACR settings significantly ( $P<0.01$ ) affected the milk yield at all levels of production with a significant effect ( $P<0.01$ ) on machine-on time at 0.4 kg/min. The yield during the first two minutes of milking, average flow and peak flow rates were not affected at any level of production. The average electrical conductivity in milk was significantly ( $P<0.01$ ) lower for the low and medium yield cows without affecting the mean somatic cell count. At 0.4 kg/min, more cluster reattachments were needed because of significant amount of milk remaining in the udders post-cluster removal.

## Casein composition and differential translational efficiency of casein transcripts in donkey's milk

Gianfranco Cosenza, Rosalba Mauriello, Giuseppina Garro, Barbara Auzino, Marco Iannaccone, Angela Costanzo, Lina Chianese and Alfredo Pauciullo

Italy

### Abstract

The amount of the four caseins ( $\alpha_{s1}$ ,  $\alpha_{s2}$ ,  $\beta$ -CN and  $\kappa$ -CN) in donkey milk was evaluated by Urea-PAGE analysis at pH 8.6, followed by immuno-detection with polyclonal antibodies, coupled to densitometric analysis. The results showed the percentage of each casein in decreasing order:  $\beta$ -CN (54.28) >  $\alpha_{s1}$  (35.59) >  $\alpha_{s2}$  (7.19) >  $\kappa$ -CN (2.79). The mRNA quantification of donkey casein transcripts, carried out by RT-qPCR, showed that the average percentage of corresponding gene transcripts (*CSN2*, *CSN1S1*, *CSN1S2 I* and *CSN3*) was 70.85, 6.28, 14.23 and 8.65, respectively. The observed translation efficiency, assessed as percentage of single milk casein fraction out of single percentage of transcript, was 0.76, 5.66, 0.50 and 0.32, respectively. The analysis of the sequences flanking the start codon, the codon usage frequencies and the coding sequence length might explain, at least in part, the differential transcriptional and translational rate observed among the casein transcripts.



## Milk $\beta$ -hydroxybutyrate concentration measured by Fourier-transform infrared and flow-injection analyses from samples taken at different times relative to milking

Mélissa Duplessis, Débora E Santschi, Sabrina Plante, Camille Bergeron, Daniel M Lefebvre, Jean Durocher and Roger I Cue

**Canada**

### Abstract

Analysis of milk BHB concentration by Fourier-transform infrared (FTIR) spectrometry more frequently than regular milk testing could help dairy producers in decision making, particularly if it would be possible to use small hand-stripped samples (hereinafter simply called samples) taken between dairy herd improvement (DHI) test-samples analysed using DHI algorithms. The aim of this Research Communication was to evaluate milk BHB concentration and the prevalence of elevated milk BHB concentration analysed by FTIR spectrometry compared with flow-injection analysis (SKALAR) from samples taken at different times relative to the milking. A total of 293 early-lactation cows in 44 commercial dairy herds were involved in the study. Herds were visited once during the morning milking when a routine DHI test-sample was obtained using in-line milk samplers. Additional milk samples were taken by hand stripping as follows: 1) Just before connecting the milking machine; 2) immediately after removing the milking machine; 3) 3 hours after milking and 4) 6 hours after milking. Milk samples were analysed for BHB concentration by FTIR and SKALAR, the latter being the reference method. Milk BHB concentration from samples taken before milking was different between FTIR and SKALAR whereas no difference was noted for other sampling times, although milk BHB concentration rose as time after milking increased. Except for DHI test-samples for which prevalence was not different between analysis methods, prevalence of elevated milk BHB concentration ( $\geq 0.15$  mmol/L) was greater for FTIR analysis. However, no difference in prevalence was observed between SKALAR and FTIR when using a threshold of  $\geq 0.20$  mmol/L. In summary, hand-stripped milk samples taken any time after removing the milking machine until 6 hours after the milking can be recommended for FTIR analysis of elevated milk BHB concentration prevalence provided a threshold of 0.20 mmol/L is used.

## Persistence of coagulase negative staphylococcal intramammary infections in dairy goats

Véronique Bernier Gosselin, Simon Dufour, Pamela R. F. Adkins and John R. Middleton

**USA and Canada**

### Abstract

The objectives of the research described here were to describe the persistence of intramammary infections (**IMI**) caused by coagulase negative staphylococci (**CNS**) in goats using strain-typing, and to evaluate the relationship between species-specific CNS IMI and somatic cell score (**SCS**) at the udder-half level. Udder-half milk samples were collected from all 909 lactating goats (1817 halves; 1 blind half) in a single herd. Milk samples were cultured on Columbia blood agar, and 220 goats with at least one half yielding a single colony type CNS were enrolled for two additional half-level samplings at approximately 1-month intervals. Isolates were identified to the species level by matrix-assisted laser desorption-ionisation time-of-flight mass spectrometry or PCR amplification and partial sequencing of *tuf* or *rpoB*. An IMI was defined as persistent when  $\geq 1$  follow-up sample yielded the same species and strain as on Day 0 based on pulsed-field gel electrophoresis. A



generalised mixed linear model was used to evaluate the odds of persistence as a function of CNS species. A mixed linear model was used to evaluate the relationship between IMI status on a given day and SCS. Among 192 IMI, 69.8% were persistent based on species and strain-type. *Staphylococcus simulans* IMI had higher odds of persistence than *Staphylococcus arlettae* IMI. In primiparous goats, *Staphylococcus epidermidis* IMI was associated with higher SCS than *S. arlettae*, *Staphylococcus xylosus*, and 'other CNS' IMI. The differences detected in the present study between CNS species, with regard to persistence of IMI and association with SCS, highlight the need to study CNS at the species and strain level to understand the pathogenicity and epidemiology of CNS in goats.

### **Milk cathelicidin and somatic cell counts in dairy goats along the course of lactation**

Vittorio Tedde, Valerio Bronzo, Giulia Maria Grazia Puggioni, Claudia Pollera, Antonio Casula, Giulio Curone, Paolo Moroni, Sergio Uzzau and Maria Filippa Addis

**Italy**

#### **Abstract**

This research communication reports the evaluation of cathelicidin in dairy goat milk for its relationship with the somatic cell count (**SCC**) and microbial culture results. Considering the limited performances of SCC for mastitis monitoring in goats, there is interest in evaluating alternative diagnostic tools. Cathelicidin is an antimicrobial protein involved in innate immunity of the mammary gland. In this work, half-udder milk was sampled bimonthly from a herd of 37 Alpine goats along an entire lactation and tested with the cathelicidin ELISA together with SCC and bacterial culture. Cathelicidin and SCC showed a strong correlation ( $r = 0.72$ ;  $n = 360$  milk samples). This was highest in mid-lactation ( $r = 0.83$ ) and lowest in late lactation ( $r = 0.61$ ), and was higher in primiparous ( $0.80$ ,  $n = 130$ ) than in multiparous goats ( $0.71$ ,  $n = 230$ ). Both markers increased with stage of lactation, but cathelicidin increased significantly less than SCC. In addition, peak level in late lactation was lower for cathelicidin (5.05-fold increase) than for SCC (7.64-fold increase). Twenty-one (5.8%) samples were positive to bacteriological culture, 20 for coagulase-negative staphylococci and one for *Streptococcus* spp.; 18 of them were positive to the cathelicidin ELISA (85.71% sensitivity). Sensitivity of SCC > 500,000 and of SCC > 1,000,000 cells/mL was lower (71.43% and 23.81%, respectively). Therefore, the high correlation of cathelicidin with SCC during the entire lactation, along with its lower increase in late lactation and good sensitivity in detecting intramammary infection (**IMI**), indicate a potential for monitoring subclinical mastitis in dairy goats. However, based on this preliminary assessment, specificity should be improved (40.41% for cathelicidin vs 54.57% and 67.85% for SCC > 500,000 and > 1,000,000 cells/mL, respectively). Therefore, the application of cathelicidin for detecting goat IMI will require further investigation and optimization, especially concerning the definition of diagnostic thresholds.

## Comparison of an on-farm point-of-care diagnostic with conventional culture in analysing bovine mastitis samples

Geoff Jones, Olaf Bork, Scott A. Ferguson and Andrew Bates

**New Zealand**

### Abstract

The performance of a new point-of-care diagnostic (Mastatest), an on-farm test designed to identify bacteria and provide antibiotic sensitivity testing information from milk samples, was compared with standard microbiological culture methods. A total of 292 milk samples from clinical mastitis cases in dairy cows on New Zealand dairy farms were examined, and latent class analysis was used to estimate the performance characteristics of both tests. Two hundred and fifty-six samples (87.7%) demonstrated bacterial infection in standard culture, and 269 (92.1%) using the point-of-care diagnostic. The most common bacterial species detected was *Streptococcus uberis*, found in 195 samples (66.8%) using standard culture and 190 samples (65.1%) using the point-of-care diagnostic. Latent class analysis found no significant differences in test characteristics between the point-of-care diagnostic and standard culture. The estimated sensitivity and specificity of the point-of-care diagnostic against all targets combined were 94.6% and 72.1% respectively; the corresponding estimates for standard culture were 90.5% and 73.9%. Comparison of antibiotic susceptibility testing using the point-of-care diagnostic and the reference method showed similar trends and, in some cases, identical MIC<sub>50</sub> and MIC<sub>90</sub> values, with at most one antibiotic dilution difference.

## Herd factors influencing free fatty acid concentrations in bulk tank milk

Lars Wiking, Martin Bjerring, Mette Marie Løkke, P. Løvendahl and T. Kristensen

**Denmark**

### Abstract

Free fatty acid (FFA) concentrations can be elevated in raw milk due to improper handling and management at the dairy farm, and high concentrations of FFA can lead to off flavours in milk. This study aimed to describe how the herd production system, milking system, feeding and technological factors impact on FFA concentrations in bulk tank milk. FFA concentrations in bulk milk samples from 259 organic and 3326 conventional herds were analyzed by FT-IR during one year. The FFA content was significantly lower in bulk milk from organic than conventional herds. This was most evident during the summer half-year when the organic cows graze pasture. Bulk milk from automatic milking systems (AMS) and tie-stalls contained greater concentrations of FFA than any other milking parlor systems. In AMS, high milking frequency was found to be the most significant contributor to elevated FFA content in milk. Moreover, a strong interaction was found between milking interval and production system (organic vs. conventional). The technical factors, pre-cooling, onset time for cooling after milk inlet, contact between milk and agitation also impacted on the FFA concentration, whereas other technical factors including centrifugal pump type, length and height of pumping line and type of AMS manufacturer were found to be without significant effect on FFA. Feeding variables, based on feeding plans and evaluation, only explained a small part of the variation in bulk milk FFA. Overall, this study demonstrated that AMS compared to other milking system contributes significantly to increased FFA concentration in bulk tank milk, and within AMS high milking frequency contributes to increased FFA concentration.

### **Analysis of fatty acid profile in milk fat of Wallachian sheep during lactation**

Martin Ptáček, Michal Milerski, Jaromír Ducháček, Jitka Schmidová, Vladimír Tančín, Michal Uhrinčať, Luděk Stádník and Tereza Michlová

**Czech Republic and Slovak Republic**

#### **Abstract**

In this Research Communication we evaluate the fatty acid (FA) profile of Wallachian sheep milk. The study was performed on 38 ewes in Beskydy Mountains. Samples were collected 4 times during the lactation, at monthly intervals. FA profile as well as groups of saturated, monounsaturated (MUFA), and polyunsaturated (PUFA) FAs were investigated. Considerable increase over the lactation was detected for lauric, myristic, myristoleic and palmitic acids, while stearic acid showed the opposed tendency. Variability, supported by significant differences among particular days of milk collection, was demonstrated for oleic acid; its highest distribution occurred at the beginning and at the end of the trial. The highest distribution of CLA was at the second sample day. Milk of Wallachian sheep naturally grazed at permanent pasture areas showed higher content of PUFA and MUFA in contrast with intensive or semi-intensive sheep breeds reported in the literature.

### **A rapid HPLC method for the determination of lactoferrin in milk of various species**

Efstathia Tsakali, Arhontoula Chatzilazarou, Dimitra Houhoula, Spiridon Koulouris, John Tsaknis and Jan Van Impe

**Greece and Belgium**

**Abstract** This Research Communication describes the adaptation and testing of an RP-HPLC method, previously tested for the determination of lactoferrin (LF) in whey, for its applicability to determine milk lactoferrin content. Milk samples of various species, namely, ovine, caprine, bovine, donkey and human milk, were tested. The advantage of this RP-HPLC method includes speed and convenience, as it does not include extensive pretreatment or separation steps. A simple pre-treatment step was added in order to remove fat and proteins of the casein family and the samples were tested. The results varied in terms of elution of the LF peak both between the milk of the different species as well as from the initial application on whey. The peak resolution was satisfactory in the cases of ovine, bovine and donkey milk samples while for caprine and human milk an interference with other peaks was observed. Nevertheless, quantification of LF was found possible for all samples. This new application of the modified method will allow the determination of LF in milk samples of the tested species either for everyday analysis or as a useful qualitative screening for presence or absence of LF.

### **Microbiological quality of milk from farms to milk powder manufacture: an industrial case study**

Lizandra F. Paludetti, Alan L. Kelly, Bernadette O'Brien, Kieran Jordan and David Gleeson

**Ireland**

#### **Abstract**

The experiments reported in this research paper aimed to track the microbiological load of milk throughout a low-heat skim milk powder (SMP) manufacturing process, from farm bulk tanks to final powder, during mid- and late-lactation (spring and winter, respectively). In the milk powder

processing plant studied, low-heat SMP was produced using only the milk supplied by the farms involved in this study. Samples of milk were collected from farm bulk tanks (mid-lactation: 67 farms; late-lactation: 150 farms), collection tankers (CTs), whole milk silo (WMS), skim milk silo (SMS), cream silo (CS) and final SMP. During mid-lactation, the raw milk produced on-farm and transported by the CTs had better microbiological quality than the late-lactation raw milk (e.g., total bacterial count (TBC):  $3.60 \pm 0.55$  and  $4.37 \pm 0.62 \log_{10}$  cfu/ mL, respectively). After pasteurisation, reductions in TBC, psychrotrophic (PBC) and proteolytic (PROT) bacterial counts were of lower magnitude in late-lactation than in mid-lactation milk, while thermoduric (LPC – laboratory pasteurisation count) and thermophilic (THERM) bacterial counts were not reduced in both periods. The microbiological quality of the SMP produced was better when using mid-lactation than late-lactation milk (e.g., TBC:  $2.36 \pm 0.09$  and  $3.55 \pm 0.13$  cfu/ g, respectively), as mid-lactation raw milk had better quality than late-lactation milk. The bacterial counts of some CTs and of the WMS samples were higher than the upper confidence limit predicted using the bacterial counts measured in the farm milk samples, indicating that the transport conditions or cleaning protocols could have influenced the microbiological load. Therefore, during the different production seasons, appropriate cow management and hygiene practices (on-farm and within the factory) are necessary to control the numbers of different bacterial groups in milk, as those can influence the effectiveness of thermal treatments and consequently affect final product quality.

### **Influence of milk pH on the chemical, physical and sensory properties of a milk-based alcoholic beverage**

Rodrigo A. Ibáñez, Stefanie Vyhmeister, María F. Muñoz, Natalia Brossard, Fernando Osorio, Fernando N. Salazar, and Einar Vargas-Bello-Pérez

**Chile and Denmark**

#### **Abstract**

The research reported in this Research Communication evaluates the effect of milk acidification on the physicochemical and sensory properties of *Licor de Oro* (or Gold Liqueur; *LO*), a traditional alcoholic beverage produced in *Chiloé* island, Chile, which is made by mixing milk acidified with lemon juice and alcohol at a ratio of 1.0:1.0, along with sugar and other spices. The mixture is stored for a couple of weeks and then filtered to obtain a product with a yellowish-transparent appearance, sweetness and acidic taste, milky and alcoholic notes. The lack of information regarding *LO* processing, mainly in the amount of acid added to the mixture, leads to products of highly variable quality. Thus, the objective of this study was to evaluate the effect of milk acidification on the physicochemical and sensory properties of *LO*. Raw milk was acidified using citric acid to six different pH values: 6.7 (control), 6.0, 5.3, 4.6, 3.9 and 3.2. These milk treatments were then used to make *LO*. A decrease of milk pH led to *LO* with higher levels of sensorial and titratable acidity. *LO* obtained at pH 6.7 and 6.0 had higher levels of total protein than other treatments, leading to excessive turbidity. In contrast, treatments made at  $\text{pH} \leq 5.3$  had a typical transparent appearance of *LO*. These results suggest that a minimum level of milk acidification is required to obtain *LO* with desired appearance and composition.