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Biology of Lactation
in Farm Animals

ICFAE

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Farm Animal Endocrinology

Proceedings

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Introduction

The Workshop on “Biology of Lactation in Farm Animals” (BOLFA) was started in 1992 (first BOLFA was held in Madrid, Spain) and was maintained as a biannual conference as a satellite either of the American Society of Animal Science (ASAS) or of the European Federation of Animal Science (EAAP) conference until 2018. Traditionally, endocrinology topics have always been very present at the BOLFA. The International Congress on Farm Animal Endocrinology (ICFAE) was held for the first time in Budapest, Hungary, in 1995, and was continued as a triannual conference with a few intervals being more than three years. The endocrinology of lactation was always an important topic at the ICFAE. A merge of these two conferences was therefore a thought since longer time. In 2018 it was decided to change the intervals between BOLFA meetings also to triannual. The Covid pandemic in 2020 enforced the shift of the BOLFA into 2021. Thus, the first joint BOLFA and ICFAE conference was held in 2021 at Isle of Palms, South Carolina, USA, organized by ASAS. Because of the considerable overlap of topics of both meetings, this merge turned out as a successful way to bring scientists of both areas from around the world together for exchange of knowledge although beyond their individual own research topics. Thus, colleagues from both ASAS and EAAP decided that the triannual joint BOLFA/ICFAE conference will be maintained. In 2024 the conference is held in Bern, Switzerland, again jointly organized by ASAS and EAAP, as a satellite of the EAAP meeting in Florence, Italy.

As local organizers the newly elected head of Veterinary Physiology of the Vetsuisse Faculty, University of Bern, Prof. Josef J. Gross, and his just retired predecessor Prof. Rupert M. Bruckmaier are glad to welcome scientists from all over the world. The organization of the BOLFA/ICFAE is about to move on to the next generation of enthusiastic farm animal scientists to exchange knowledge on current topics related lactation and endocrinology.

The 2024 BOLFA/ICFAE will provide an invaluable opportunity for networking among highly respected and established as well as start of career scientists to create and discuss future research projects.

Scientists in the different fields of farm animal lactation and farm animal endocrinology will be presenting plenary lectures during the congress in Bern. Topics include effects of milk on human health, interactions between farming technology and animal physiology, endocrine changes from gestation to lactation, endocrine regulation of milk secretion, growth, and reproduction. A huge number of posters related to the main topics of the conference will provide the newest results in addition to the plenary talks.

Bern, August 2024

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We thank our sponsors for supporting the Workshop on “Biology of Lactation in Farm Animals” (BOLFA) and the International Congress on Farm Animal Endocrinology (ICFAE)!



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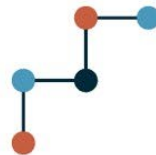


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Invited talks

I01

The convergent evolution of adult human milk consumption*Jay T. Stock¹ and Jonathan C. K. Wells²*¹Department of Anthropology, Western University, Ontario, Canada; ²University College London, United Kingdom
jay.stock@uwo.ca**Introduction**

There is widespread evidence for the importance of dairy products to the diet of ancient farming and herding populations throughout Eurasia and Africa. Cultural variation in consumption of primary products like milk which is high in lactose content, and secondary products such as cheese and yoghurt which contain lower amounts of lactose, emerged independently or spread to different regions throughout the past 12,000 years, driving geographic variation in the prevalence of lactase persistence in populations where raw milk consumption was common. This study reports the results of preliminary tests of the Lactase Growth Hypothesis, which suggests that milk consumption and the evolution of human lactase persistence promoted skeletal growth and increased survivorship in specific contexts where humans were depended upon milk consumption to mediate extreme nutritional stress in the past.

Materials and Methods

We compare geographic variation in the use of dairy products on human growth among populations: a) throughout Eurasia over the past 20,000 years (n=3507 individuals from n=366 archaeological sites), and b) using national level data on dairy consumption, lactase absorption prevalence, and average height using r societies globally, to test the relationships between milk consumption, lactase persistence, and human body size. We tested archaeological data for significant differences between time series cohorts using univariate ANOVA with Hochberg GT2 post hoc tests, and national level modern data using linear regression.

Results

Our results demonstrate that stature and body mass increases in Central and Northern Europe coincide with the timing of selective sweeps for lactase persistence, that milk consumption per capita correlates with the prevalence of lactase absorption in different regions, and that both of these variables correlate with adult statures.

Conclusions

Our analyses provide correlative support for the “Lactase Growth Hypothesis”, suggesting that milk is often an important fallback food that both drives natural selection in our species and influences variation in human body size.

I02

Innovation towards a dairy-based platform for effective, next-generation, prebiotics and probiotics*David A. Mills*

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Milk is well known to direct the enrichment of the neonatal gut microbiome—an assembled “organ” with important consequences on host health. A key driver of that guided enrichment are the glycans present in milk including free oligosaccharides, glycoproteins and glycolipids. In human infants, these glycans are responsible for enrichment of a beneficial bifidobacterial population. The basis for milk glycan consumption by bifidobacteria has been the subject of active research, mostly focused on the mechanisms by which free oligosaccharides enrich bifidobacteria in infants. Milk also contains an abundance of glycoconjugates presumed to participate in a similar enrichment of microbes in the gut, yet the molecular nature of this interaction is poorly defined. The dairy industry produces abundant glycoconjugate rich streams that may be useful in enriching bifidobacteria, or other mucin-consuming microbes, in a gut. Recently, we employed culturomics, genomics, metabolomics and transcriptomics approaches to characterize the growth of bifidobacteria on media containing whey protein phospholipid concentrate, a glycoconjugate-rich byproduct of cheesemaking. Different bifidobacterial species possess unique glycosidases and transport systems to efficiently utilize whey protein phospholipid concentrate and, surprisingly, such growth induces a “weaning” phenotype in a subset of bifidobacterial species. This suggests a co-evolutionary relationship between mammalian milk glycoconjugates, bifidobacteria and neonate hosts enabling an enrichment of a protective bifidobacterial-dominant community during nursing but also an unusual pre-programming of the gut microbiome for eventual plant fiber degradation during weaning. Characterization of this system will shed light on novel pre-, pro- and synbiotic tools to support and ensure a protective gut microbiota in a range of at risk populations.

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The importance of dairy foods for human health

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Consumer demand for milk and milk products is driven by nutrition as well as taste, affordability, and sustainability. Adequate dairy intake is promoted by most countries throughout the lifespan, primarily for bone health. Most countries recommend 2 to 3 servings daily for most age groups. This recommendation is because of the huge contribution of dairy to intake of essential nutrients. On a global scale, milk consumption is ranked very high compared to other food sources for bone building nutrients i.e. first for calcium, second for phosphorus and potassium, third for protein, and fourth for magnesium. Milk also ranked among the top three foods contributing to the global intake of essential amino acids such as lysine, threonine, methionine, and tryptophan. The dairy matrix includes the complex organizational structure of these essential nutrients and other constituents. The dairy matrix may promote health and lower the risk of many chronic diseases beyond the contribution of essential nutrients. Modeling shows that replacing dairy foods with non-dairy foods to meet nutrient intakes is not feasible. Dairy foods are sustainable as well as nutrient dense and are more affordable than plant-based beverage alternatives to milk.

Adequate dairy intake is promoted by most countries throughout the lifespan, primarily for bone health. Most countries recommend 2 to 3 servings daily for most age groups. This recommendation is because of the huge contribution of dairy to intake of essential nutrients. On a global scale, milk consumption is ranked very high compared to other food sources for bone building nutrients i.e. 1st for calcium, 2nd for phosphorus and potassium, third for protein, and fourth for magnesium (1). Milk also ranked among the top three foods contributing to the global intake of essential amino acids such as lysine, threonine, methionine, and tryptophan and for several B vitamins, especially vitamin B 12 which only comes from animal sources. Beyond the contribution of essential nutrients in dairy, the dairy matrix which includes the complex organizational structure of these nutrients and other constituents that may be health promoting has led to associations of dairy intake with lowering the risk of many chronic diseases (2). Modeling shows that replacing dairy foods with non-dairy foods to meet nutrient intakes is not feasible (3). A recent systematic review of dairy intake and bone health across the lifespan showed that evidence was strongest for middle age and older adults and evidence was weak for infants and toddlers, children aged 3 to < 10 years, and young adults (4). Large prospective studies show the cardiovascular benefits of diets high in dairy products (5).

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Milking research and practice: a 60-year learning curve

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Most young science graduates need one or more guiding stars to help kick-start their careers. My 60-year learning curve began in 1964 under the guidance of two leading NZ scientists: Doug Phillips and Wattie Whittlestone. Five years later, after losing our family home in a bushfire in 1969, I had the good fortune to study under the guidance of two other giants in the field of milking and mastitis, UK scientists, Frank Dodd and Cliff Thiel. I learned two critical lessons from that early access to such top-class mentors.

- 1) From Doug Phillips (a physicist and inventor): *the more sophisticated your measurement system or your modelling technique is, the more important it becomes to devise a simple check-test, just to make sure your results make sense physically and/or biologically.*
- 2) From my years working with Dodd and Thiel: *results obtained from research herd studies should be field tested before they can be recommended to commercial farmers.*

For the next three decades up to 2000, when the global milking research community was relatively small but surprisingly active, it was easy to make productive connections (and lasting friendships) with international colleagues. We continued to learn so much from one another.

My long association with Rupert Bruckmaier began in 1996, when I was asked to be an external examiner for his habilitation thesis. It was easy to write a glowing review for a thesis which met three key criteria, viz: interesting, original and of potential practical value. Since then, I've taken a 'fatherly interest' in his academic development and scientific contributions. As an aging 'academic father', I am proud to acknowledge Rupert's ongoing contributions to the science and practice of milking. His publications and presentations have helped to fill critical gaps in our understanding of good milking management in modern dairy herds. Two of his most valuable take-home messages for me were:

- *Because oxytocin has a very short half-life (only 2-3 min) in the blood stream, milk ejection stops soon after the particular tactile stimulus is terminated. In nature, this allows a milk ejection according to the needs of the suckled offspring.*
- *During machine milking, however, the goal is to empty the udder as completely as possible at each milking. Hence, a continuously elevated concentration of oxytocin is needed throughout the entire milking process. This is achieved by the stimulatory effect of pulsation (defined as cyclic liner movement).*

When Rupert presented those data at NMC (Bruckmaier, 2013), I was 74 years old. Another invaluable part of my 60-year learning curve!

One other life-long role-model for me is Leonardo da Vinci. His extraordinary creative genius was based on skills that all scientists, young and old, can keep trying to improve in ourselves. Leonardo's skills included: curiosity, careful observation, imagination and a willingness to question existing knowledge.

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Bimodal milk flow in dairy cows – diagnosis, risk factors, possible effects, and opportunities*Matthias Wieland*

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mjw248@cornell.edu**Introduction**

Milk flow curve dynamics offer valuable information that can improve milking and parlor efficiency, udder health, and animal well-being in dairy operations (Sandrucci et al., 2007). Among the many variables concerning milk flow curve dynamics, bimodality has been used extensively to assess the quality of premilking teat stimulation. A bimodal milk flow curve is defined by an increasing milk flow rate followed by a decreasing milk flow rate during the first 2 minutes of milking (Tančin et al., 2006). This decrease can present as a partial or complete cessation of milk flow and, more practically, may be identified as an ‘empty or dry claw’ shortly after milking unit attachment. Bimodality is due to removal of the cisternal milk fraction before the alveolar milk reaches the gland cistern and is a result of insufficient stimulation before milking (Bruckmaier and Blum, 1998); bimodality has been associated with decreased milking efficiency (Bruckmaier and Blum, 1996), reduced milk yield (Bruckmaier, 2005), and impaired udder health (Bruckmaier, 2005; Zeccconi et al., 2018). Traditionally, bimodality has been detected with a portable milk flow meter (i.e., the Lactocorder, WMB AG, Balgach, Switzerland) that measures continuous milk flow. Indeed, the Lactocorder is highly popular among veterinarians and milk quality consultants and has been considered the gold standard for the field assessment of bimodality in dairy cows. The goal of this paper is to review diagnostic tools to detect bimodality, risk factors for bimodality, its impact on milking performance, and opportunities to mitigate them.

Materials and Methods

A total of five field studies have been conducted to (1) assess if a vacuum recorder can be used to detect bimodality as determined with the Lactocorder, (2) investigate if electronic on-farm milk flow meters can be used to detect bimodality as determined with the Lactocorder, (3) investigate risk factors for bimodality, (4) study the influence of bimodality on milking performance, and (5) study the effect of supplemental stimulation on milking performance in dairy cows.

Results

Our results showed that both (1) the vacuum recording device and (2) the electronic on-farm milk flow meters can be used to detect bimodal milk flow curves in dairy cows, (3) cow characteristics such as parity, stage of lactation and health events are risk factors for bimodality, (4) bimodality negatively impacts milking performance, and (5) the supplemental stimulation system tested had no beneficial effects on milking performance.

Conclusions

Bimodality in dairy cows remains a fascinating topic warranting future research to optimize the milk harvesting process in dairy cows.

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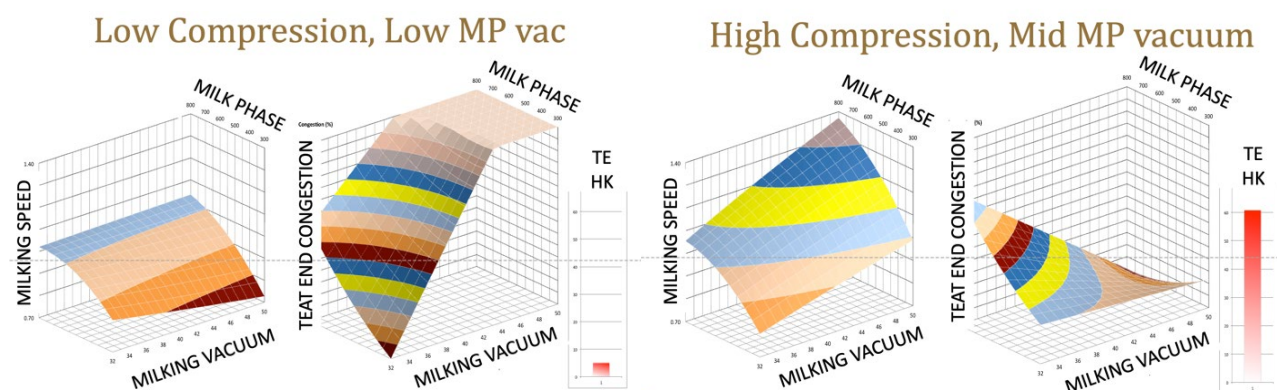
Effects of liner compression and vacuum on milking performance and teat condition

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This presentation will provide an overview of research conducted over the past three decades at the University of Wisconsin Milking Research and Instruction lab to provide better understanding of the biomechanics of machine milking. These studies have included methods of measuring liner compression and quantifying the interactive effects of liner fit, vacuum, pulsation, liner compression and teatcup removal on milking speed and teat condition. The complex interactions between factors affecting milking speed and teat condition are illustrated in the following 'liner maps'.



Implications of the interactive nature of milking biomechanics are that the effect of milking vacuum on milking speed and teat congestion is not the same for different pulsation settings (milk phase duration). Likewise, the effect of milking vacuum and pulsation are not the same for different liner compression. This illustrates the point that recommendations for vacuum level, pulsation settings, and teatcup removal settings cannot be made without knowing all of these as well as liner compression and liner fit. The milking speed for the low compression liner is comparable to the high compression liner at low milking vacuum level and across a wide range of milk phase duration due to the low level of mouthpiece chamber (MP) vacuum and resulting low teat barrel and teat end congestion. The low compression liner is not capable, however, of achieving the high milking speed of the high compression liner at high vacuum and milk phase duration. High liner compression is the primary contributor to teat end hyperkeratosis (TEHK) so that while the high compression liner can achieve higher milking speed and lower teat-end tissue congestion, the high compression liner will result in greater occurrence of rough teat ends. The fit of the liner to the teat as well as liner shape influence the MP vacuum, especially in the low flow period of milking. High MP vacuum results in congestion of the teat barrel, ringing of teats, congestion of teat end tissue and slower milking.

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Milking time testing as tool to detect udder health risk factors*Morten D. Rasmussen*

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The purpose of milking machines is to milk the animals quickly, gently and completely and ensure that milk intended for human consumption has the best possible milk quality. The ultimate test of milking machine installations is the performance of the dairy animals and the influence on milk yield, milk quality and udder health and by such the outcome of the interaction between the animal, the machine and the operator. Such effects may be evaluated indirectly through tests of the milking machine installation in between milking, during milking or by observations made during milking. This paper comprise knowledge of how vacuum measurements during milking may be interpreted and potentially influence udder health.

Materials and Methods

It is recommended to mount pressure transducers in the short pulse tube, short milk tube (SMT) or claw, and in the mouthpiece chamber (MPC) of one or two teats (for cows front and rear). Measurement of mouthpiece vacuum seems important in order to subdivide measurements of single animals into the attachment, main milking and overmilking period. Measurement in the short pulse tube is needed whenever data are analyzed in relation to the pulsation phases. Sampling rate is often set at 200 Hz and a response rate of at least 2.500 kPa/s.

Results and Conclusions

A liner slip is counted when vacuum in the claw or liner drops 8 kPa or more from the mean milking vacuum in less than 0.25 s. A vacuum drop from mean claw or liner vacuum to less than 4 kPa is counted as a unit fall-off. Liner slip is negatively correlated with udder health. The detachment phase is counted as too short if vacuum in the claw or liner decay at cluster removal in less than 0.5 s and is counted as too long if it last more than 5 s. A trapped vacuum in the MPC may create a reverse pressure gradient (RPG) across the teat canal and negatively influence udder health. MPC vacuum reflects the forces applied to the barrel of the teat during milking. Mean MPC vacuum higher than 25 kPa is logged for the individual milking or phase of milking. A MPC vacuum equal to liner or claw vacuum is counted as “no seal” and indicates no massage of the teat during milking, which influence blood circulation in the teat and the passive immune response negatively. A marked change in MPC vacuum and fluctuations indicates start of overmilking (no distinct threshold is given). The MPC vacuum typically follows the pulsation during the overmilking phase. A MPC vacuum higher than the liner or claw vacuum during the detachment phase is counted as “trapped MPC vacuum” and could potentially create an RPG across the teat canal

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Interactions between the effect of milking machine settings on milking duration and cow milk flow-rates*John Upton, Martin Browne, and Pablo Silva Bolona*

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Increasing the milk flow-rate switch point during milking has been shown to significantly reduce milking duration without negatively affecting milk yield or udder health, and it also improves cow comfort and teat health. Similarly, dynamic pulsation systems have been shown to increase peak milk flow-rates without compromising milk yield. However, studies to date have not documented the interactions between cow milk flow-rate and the effect of these milking technologies. Hence, the objective of this experiment was to investigate if high and low flow-rate cows respond differently to increased milk flow-rate switch point and dynamic pulsation settings.

Materials and Methods

This study was carried out at the Teagasc Research Centre at Moorepark, Ireland. A 46 unit Dairymaster rotary milking system was used to milk the cows on the trial twice per day. Four treatments consisting of different milk flow-rate switch-points and pulsator settings were deployed: 1) S0.2, cluster was removed at 0.2 kg/min with static pulsator settings; 2) D0.2, cluster was removed at 0.2 kg/min with dynamic pulsator settings; 3) S0.8, cluster was removed at 0.8 kg/min with static pulsator settings, and 4) D0.8, cluster was removed at 0.8 kg/min with dynamic pulsator settings. The dynamic pulsator settings applied a shorter d-phase and longer b-phase above a milk flow-rate of 1.5 kg/min. Each group had 26 cows (13 low and 13 high average milk flow-rate, AMF), and transitioned through each treatment in a cross over design at two week intervals. All data were analysed in SAS 9.4 using mixed models.

Results

The effect of treatment on daily milking duration was significant ($P < 0.001$). The milking duration for S0.2 was 135 s (20%) longer than S0.8, while the difference between D0.2 and D0.8 was 147 s (22%). The low AMF cows had a 35% longer milking duration than the high AMF cows. There was also a significant effect of treatment on AMF ($P < 0.001$), where the AMF for S0.2 was 0.27 kg/min lower (24%) than S0.8, there was also a 24% difference in AMF between D0.2 and D0.8. We found a significant increase in peak milk flow-rate due to treatment for AM milkings but not PM milkings. There were significant interactions between treatment and AM milk yield, milking duration and AMF.

Conclusions

This study sheds new light on how cows of varying AMF respond to different milking management settings comprising increased milk flow-rate switch point and dynamic pulsation settings. Our study showed that low flow-rate cows have a greater reduction in yield and milking time compared with high flow-rate cows when they were milked on flow-rate switch point settings of 0.8 kg/min compared with 0.2 kg/min.

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Producing piglets in a free farrowing system*Alexander Grahofer*

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The birth process and colostrum quality are of crucial importance for the animal health and welfare of sows and their piglets in the free farrowing system. Dystocia often led to an increased mortality rate of piglets and to uterine and mammary inflammation in breeding sows. Since the prevalence of dystocia is up to 58% in free farrowing sows, new methods are needed to recognize birth complications as early as possible. Hence, non-invasive methods are needed to evaluate the hormone status of a sow. Therefore, the goal of one of our study was to evaluate the relation among colostrum traits and characteristics of the farrowing process and the reproductive performance of sows in a free farrowing system. In total, 61 crossbred sows were included in the study and after the birth of the first piglet, a colostrum sample (5-10 mL) was collected. Progesterone, serum albumin and alpha-lactalbumin levels of the colostrum samples were measured in the defatted colostrum samples. In addition, the course of the farrowing process and litter parameters were monitored. Overall, 921 piglets were born and the mean total farrowing duration (first piglet-last placenta) was 529 ± 276 min. The mean P4 level was 21.9 ± 10.9 ng/mL, the serum albumin was 7.4 ± 1.5 mg/mL and the alpha-lactalbumin concentration was 1.78 ± 1.23 µg/mL.

Colostrum P4 and the total farrowing duration were positively correlated (Spearman Rank correlation: $r = 0.26$; $p = 0.04$), as well as the birth duration of piglets (first-last piglet; 241 ± 129 min) (Spearman Rank correlation: $r = 0.30$; $p = 0.02$) and the placenta expulsion duration (first-last placenta; 353 ± 265) (Spearman Rank correlation: $r = 0.27$; $p = 0.03$). In addition, P4 positively correlated with serum albumin (Spearman Rank correlation: $r = 0.30$; $p = 0.01$) and alpha-lactalbumin (Spearman Rank correlation: $r = 0.41$; $p < 0.01$).

In a further study with 151 sows in the free farrowing system, the same sampling procedure was conducted. The Brix-values of colostrum samples were measured and relation among sow and piglets trait were evaluated.

Overall, the mean parity of the sows was 4.2 ± 2.4 and 17% of the sows received a birth induction with PGF2alpha. The mean Brix-values of the colostrum samples was 27.2 ± 3.0 %. The Brix-values are significantly increasing with the parity numbers. A significant difference between Parity 1 (26.1 ± 2.6 ; $p < 0.01$) and 2-3 (26.6 ± 2.7 $p < 0.01$) compared with ≥ 6 (28.2 ± 3.1) could be detected. Furthermore, a significant difference between the sow with and without birth induction and the brix-value of the colostrum could be detected. Sows with birth induction had lower Brix values (Mean: 26.0 ± 2.5 %) compared to sows without birth induction (Mean: 27.4 ± 3.0 %; $p < 0.01$).

These two studies evaluated the influence of specific colostrum traits on sow traits and the farrowing process in a free farrowing system. Especially, the progesterone level in the colostrum could be a reliable parameter to identify sows, which are at risk for a prolonged farrowing duration. Furthermore, it could be shown that birth induction of sows negatively influence the Brix-values of colostrum and therefore, this litters are at higher risk for insufficient colostrum uptake.

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Pros and cons of cow-calf-contact systems in dairy farming - can we use established indicators?

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Introduction Interest in cow-calf contact (CCC) systems has increased dramatically in recent years and a scientific opinion from EFSA (Nielsen et al., 2023) recommends their wider implementation as the system that best meets the needs of the animals. In addition to the high demands on animal observation, the change in management and the necessary structural adjustments, the effects of CCC on milk yield and milking are the biggest challenges for farmers. This is especially true in systems where calves are reared by their own mother. In addition to the large amounts of milk consumed by the calves, there is also the impairment of milk ejection, which can also lead to a depression of milk production until the end of lactation (Barth, 2020). However, this impairment does not occur equally in all animals, suggesting that other factors besides calf suckling must be involved. Since blood oxytocin measurements are difficult to obtain under practical conditions, other methods must be investigated to assess the degree of udder emptying. Evaluating fat content in the strippings could be one option (Jenni et al., 2024).

Materials and Methods Of a total of 50 German Holstein dairy cows, 34 (number of lactations: 2.5 ± 1.64 lactations) were kept with their calves and 16 (number of lactations: 1.9 ± 1.06) were kept without contact during the first 3 months of lactation. Thereafter, none of the cows were suckled. All animals were milked twice daily in a tandem milking parlor. Stripping samples were collected during one evening milking at three time points (8 ± 3.7 , 59 ± 2.1 , 108 ± 2.0 DIM) and analysed for fat content.

Linear mixed models were used to investigate the effect of suckling (yes/no) on the fat content in strip milk on the first and second sampling day. To take the repeated measurements into account udder quarter nested in cow was included as random effect. Furthermore, fat content was used to classify the status of udder emptying. According to Jenni et al. (2024) a fat content of strip milk lower than 4% was defined as incomplete milking.

Results There was a significant interaction between sampling day and the effect of suckling ($p=0.017$) causing a significant lower fat content in the strippings of suckled than not-suckled cows at the second sampling day (-3.59% , S.E.: 0.945 , $p<0.001$). By the third sample, there were no differences between treatments. The same result was obtained when considering the degree of udder emptying as a function of fat content: While 95% of the evaluable strippings of the non-nursing cows showed complete quarter emptying on the second sampling day, this was only the case for 72% of the cows with contact to their calf. However, there was a large variation among the cows in the contact group, which only decreased after the calves were weaned and separated (figure 1).

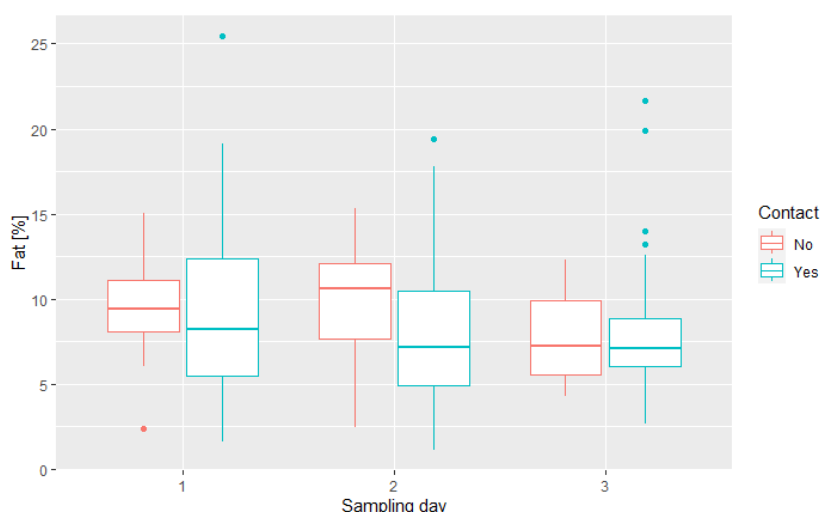


Figure 1: Fat content of strippings from cows that suckled their calf (contact = yes) or did not suckle their calf (contact = no) during the first three months of lactation (sampling day 1 and 2) and on day 3 when no cow was suckling a calf.

Conclusions Although the average fat content in the strippings of cows nursing their calf can still be very high, the influence of calf suckling on the degree of udder emptying was still evident.

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Placental contribution to the endocrinology of gestation and parturition*Karl Klisch*

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The placenta of mammals is probably the organ, which shows the greatest diversity on macroscopical, histological and molecular levels. This is the case despite seemingly uniform functions of the placenta, which are the transfer of nutrients, gases, and waste products. In addition to these functions, the placenta releases a variety of hormones into the organism of the pregnant female. In this review the production and release of placental hormones in domestic animals is viewed in context of the structural diversity of the organ.

Materials and Methods

Ultrastructural and histological studies of bovine, ovine, equine, camelid and porcine placenta are discussed in relation to other information about placental endocrine functions.

Results

Structural diversity of the different placentae is accompanied by great diversity of endocrine functions. Close to parturition the placenta has taken over a significant role in the production of gestagens in some species (sheep, horse), while in other species the corpus luteum remains the main source of gestagens (cow, pig). This is linked to differences in the induction of the prepartal decline of gestagens in these species (Schuler et al., 2018).

The epitheliochorial placenta of domestic animals is an evolutionary derived feature, which evolved from a more invasive endotheliochorial or hemochorial placenta type. In the equids and in ruminants mechanisms evolved, which allow the release of proteohormones, produced in fetal trophoblast cells, into the maternal organism. These mechanisms are the trophoblast girdle cells and endometrial cups in equids and the binucleate trophoblast cells, which fuse with maternal uterine epithelial cells, in ruminants. In equids eCG is transferred and in ruminants placental lactogen, prolactin-related protein(s) and pregnancy-associated glycoproteins are released into the mother. In addition to these proteins the binucleate trophoblast cells also release extracellular vesicles into the mother, which might have local or systemic functions in intercellular communication.

Conclusions

The great placental structural diversity is linked to diverse mechanisms for the release of endocrine signals from the fetus into the mother. This reflects the evolutionary dynamic in placental development.

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Hormonal control of the mammary blood-milk barrier*Kerst Stelwagen*

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The blood milk-barrier (BMB) forms at parturition when the gland switches from a non-lactating state to one of copious milk production and becomes leaky again when milk removal ceases and mammary involution is initiated. In this review the importance of the BMB in milk production and, in particular, its hormonal regulation is explored.

Results

Tight junctions (TJ) between adjacent mammary epithelial cells form a barrier to the two-directional paracellular movement of small molecules between the blood and milk and are therefore responsible for establishing and maintaining the BMB. They form part of the cell's junctional complex and consist of transmembrane proteins (occludin and claudins) that are linked via specific scaffolding proteins to the mammary cell's cytoskeleton. This means that when, during lactation, TJ become "leaky" the resulting perturbation of the cytoskeleton interferes with the cell's milk synthesis and secretory function. As such, TJ are involved in regulating and maintaining milk production. Mammary TJ are under hormonal control, with progesterone, glucocorticoids, prolactin, parathyroid hormone-related peptide (PTHrP) and serotonin (5-HT) being the key hormones. Progesterone prevents closure of TJ and the significant immediate prepartum drop in its systemic concentration is a prerequisite for TJ closure at that time. A simultaneous prepartum increase in the levels of glucocorticoids and prolactin is necessary for full TJ closure and initiation and subsequent maintenance of lactation. The role of prolactin appears to be predominantly a synergistic one, to maximize the effect of glucocorticoids on TJ closure. Both PTHrP and 5-HT are important hormones in maintaining extracellular calcium concentrations; the latter is a critical requirement for establishing and maintaining TJ closure and integrity. They appear to play opposing roles. Whereas PTHrP reduces TJ permeability, necessary for maintaining milk production, 5-HT has an opening effect on TJ. The latter effect may help to speed up mammary involution and help facilitate the movement of immune factors into the gland, to help prevent new intramammary infections. The effect of these hormones on mammary TJ seems to be mediated through upregulation of the expression of the TJ proteins occludin, claudin-1 to -4 and the scaffolding proteins in the case of TJ closure or downregulation of these protein in the case of TJ opening.

Conclusions

In summary, mammary TJ make up the BMB and play a role in establishing and maintaining milk production and are under hormonal control, with progesterone, glucocorticoids, PTHrP and 5-HT being key regulatory hormones, and prolactin likely playing a supporting role.

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The history of research into nutrient transfer from mother to neonate*Christopher H. Knight*

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In an era of accessible information, it would be nice to think that there was no need for this presentation. We know a very great deal about the processes of synthesis, secretion and transfer of milk. That knowledge has been built up gradually over a period of a hundred years, give or take, and many (perhaps most) of the fundamental discoveries are now more than fifty years old. So it was with considerable surprise and disappointment that I heard recently of advice given, by a neonatal paediatrician, to our breastfeeding niece. Her baby daughter had developed a degree of temporary lactose intolerance, and the advice was that she (our niece) should avoid dairy products, presumably in an effort to reduce the lactose content of her breastmilk. Was this sheer ignorance of the process by which lactose is synthesised exclusively in the mammary Golgi vesicle, or just simply a device to provide advice of some sort, irrespective of its accuracy? It caused me to think. As Editor of the Journal of Dairy Research, I frequently come across examples of lack of understanding of basic mammary physiology, the secretion of lactose as the major osmolar component being a prime example. So in the first part of the presentation I shall briefly recap some of the most important discoveries, and by doing so also hope to point out where knowledge actually is incomplete. For example, and expanding on the lactose story, we know how glucose is taken up across the basolateral membrane of the mammary cell, and we know that it then appears in the Golgi as one of the two precursors of lactose. But is it simply pure chance that glucose translocates to the Golgi, or are more subtle mechanisms involved? As lactose is synthesized and trapped within the vesicle, we know that water is drawn in through osmotic potential, but how does that water get through what is essentially an impermeable membrane? Since this is a meeting of Farm Animal scientists, I shall then try to place the historical knowledge into the context of current dairy animal research, and I shall argue that all of the myriad of technological advances that pervade modern science can only be effective when combined with a full understanding of the underlying physiology. And I shall take some liberties, and consider where we, as animal scientists, can perhaps have a significant impact by expanding our very significant knowledge into the area of human lactation. It concerns me greatly, for example, that millions of pounds and years of effort are committed to mastitis in dairy species, whilst human mastitis is largely ignored. And try as I might, I could not convince my last PhD student that she should acquire a better knowledge of the process of lactogenesis. She was a midwife, and her Thesis was concerned with the problem of early post-partum discharge and failure to establish breastfeeding. The human baby is probably uniquely adapted to “managing without” during the first 24h or so of life, born with copious fat reserves for energy and transplacentally-transferred passive immunity, but she was committed to breastfeeding needing to start within minutes or hours. There are many more examples of where our knowledge could be useful, and I shall try to elaborate on some of them.

Hormonal and nutritional regulations of lactation persistency in dairy ruminants

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Introduction

It has long been recognized that the lactation curve in dairy ruminants is shaped by the regulation of mammary tissue cell numbers throughout lactation^{1,2}. However, the precise hormonal and nutritional factors driving lactation persistency are not been fully elucidated. Therefore, a set of experiments was conducted to investigate the effects of hormonal and nutritional challenges on cell turnover in the mammary tissue.

Materials and Methods

Initially, experiments on dairy cows were conducted to assess the effects of hormonal challenges. The effect of inhibiting prolactin release was assessed by administering daily injections of a dopamine agonist (quinagolide) to cows for 8 weeks, compared with control group receiving no injections. Additionally, the effect of sex steroids was studied by performing either ovariectomy or sham surgery on dairy cows, followed by a monitoring period of 52 weeks. Furthermore, the effects of feeding level were investigated in dairy cows assigned to either a basal diet or a severe feed restriction from 2 wks before calving to wk 11 postpartum, or during a 4-wk moderate feed restriction (-20% DMI). The effects of different types of diets were investigated by supplementing 32 dairy cows with either by pass starch or amino acids (rumen protected lysine, methionine, and histidine) from 8 wks to 26 wks postpartum. Similarly, 2 levels of NEL (1.48 vs. 1.65 Mcal/ kg DM) through concentrates and amino acid supply (rumen protected methionine) were tested on 24 mid-lactating goats for 4 wks. Mammary tissue cell turnover was assessed through immunohistochemical analyses in all experiments, and the exfoliation of mammary epithelial cells (MEC) into milk was examined in some cases.

Results

The impacts on milk yield (MY) resulting from hormonal challenges (-14% during prolactin inhibition or +10% after ovariectomy) were associated with alterations in apoptosis/proliferation balance in the bovine mammary tissue and a decrease of MEC exfoliation (only for ovariectomy). The decreases in MY due to feed restriction (- 38 % and - 8% respectively with a severe and a moderate feed restriction) in cows were associated with either a higher level of apoptosis or an increase in MEC exfoliation with no effect on cell proliferation. The increase in MY (+ 11%) induced by starch supplementation in cows was accompanied by enhanced cell proliferation. AA supplementations did not affect milk yield but resulted in increased cell proliferation in both dairy cows and goats and reduced cell apoptosis in dairy goats.

Conclusions

Taken together, these experiments support the hypothesis that the regulation of lactation persistency in dairy cows and goats, subjected to hormonal or nutritional challenges, involves changes in the number of mammary cells, mediated by adjustments in the apoptosis/proliferation balance and the rate of mammary cell exfoliation into milk. AA supplementation can also affect cell turnover.

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Local and systemic control of milk secretion*P. Lacasse¹, S. Lanctôt², and A.-M. Deacon²*¹AAFC-Sherbrooke R&D Centre, QC, Canada; ²Université de Sherbrooke, QC, Canadapierre.lacasse@agr.gc.ca

Biological mechanisms exist to adjust milk output to the demand. Experiments where different milking frequencies were used within the mammary gland have shown that the increase in milk production is limited to the frequently milked half. Similarly, cessation of milking of one quarter will induce the involution process in that quarter only. These data indicate that milk secretion is regulated at the level of the mammary gland. However, systemic factors, such as pregnancy, hormones and photoperiod, also modulate milk secretion. Therefore, we have initiated a series of experiments to examine the linkage between local and systemic regulations.

Prolactin (PRL) is a galactopoietic hormone. However, PRL concentration is affected by the environment and changes throughout the year without associated changes in milk production, suggesting that the mammary gland's sensitivity to PRL is adaptable. Accordingly, we have conducted an experiment that shows that milk production response to the PRL secretagogue domperidone, was enhanced by previous reduction of PRL concentration with a low dose of quinagolide [1]. We have evaluated the impact of milking frequency (1x vs 3x) on PRL signaling using a split udder design. Milking 3x a day increased milk secretion and that increase was paralleled by increases of PRL receptors expression and STAT5 phosphorylation [2] supporting the hypothesis that the responsiveness of the mammary gland to PRL is modulated by milking frequency. Milking induces an important release of PRL in the female circulation and milk removal is not essential as a manual stimulation of the teats can induce it. To determine if the PRL release at milking alone contribute to the effect of milking frequency on milk secretion, two 5 min manual stimulations were performed between milkings. Despite the fact that the manual stimulations induced PRL releases similar to that induced by milking, there was no effect on milk production. This indicated that the systemic lactogenic stimulation by PRL is not sufficient to stimulate milk secretion. To determine if the accumulation of autocrine factor(s) in milk during milk stasis can induce mammary gland involution, we infused milk from a lactating quarter or mammary secretions from an un milked quarter for 7 days in other quarters and then subjected the infused quarters to a 7-day milk stasis before resuming milking for 7 days. The infusion of mammary secretions hastens the involution of the recipient quarter supporting the hypothesis that the accumulation of one or more autocrine factor(s) in milk triggers involution. The results also indicate that milk stasis leads to changes in the miRNA profile of the milk, but whether such changes are a cause or a consequence of the involution process remains to be established [3].

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Prolactin and heat stress: focus on domestic ruminants*I. J. Clarke¹, S. S. Chauhan¹, and F. R. Dunshea^{1,2}*

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Prolactin (PRL) is a somewhat enigmatic hormone, initially identified as a protein that was lactogenic in pigeons. For many years, the role of PRL in the development of the mammary glands and lactogenesis was studied without particular reference to its broader functions. It is now apparent that PRL is produced by many organs in the body and serves as many as 300 functions including a range of homeostatic and homeorhetic functions. In addition to the direct effects of PRL, PRL is potentially a candidate homoerotic hormone that might exert some of its effects through changing tissue responsiveness to other signals such as insulin. This article focuses on its role in the response to heat stress, especially in domestic ruminants. It has long been established that circulating PRL concentrations increase during heat stress, but it was unknown whether this is an effect of heat stress or whether this is an adaptive response. Recent data suggest that ruminant animals that are better adapted to heat stress have lower PRL concentrations under thermoneutral conditions but a greater increment in PRL in response to heat stress. Heat stress increases insulin sensitivity in ruminants and pigs, and this may be an adaptive response to heat stress. Increased insulin sensitivity would ensure that heat stressed animals would reduce adipose tissue lipid mobilisation and not use non-esterified fatty acids as a preferred energy substrate despite the reduction in feed intake that generally occurs during heat stress. Instead, glucose would become the preferred energy substrate for peripheral tissues. Heat stress causes a reduction in blood pCO₂, an increase in blood pO₂, and a resultant decrease in base excess and an increase in blood pH in sheep. These changes in blood gas parameters are most likely a result of increased respiration rate and CO₂ exhalation during HS. The use of lipids as a metabolic substrate during HS would result in a low respiratory quotient and, therefore, further exacerbate the decrease in the blood base excess resulting in respiratory alkalosis. An increase in insulin sensitivity would inhibit lipolysis and fat mobilization and increase blood flow to the skin. A mild elevation in PRL levels has beneficial effects on metabolic function and insulin sensitivity in humans and rodents, acting on the pancreas, liver, adipose tissue, and hypothalamus. Speculatively, the heightened insulin sensitivity observed during heat stress could be influenced by increased prolactin concentrations. Further evidence for the role of PRL in heat stress is the observations that cattle with a point mutation in the PRL receptor gene (SLICK gene) have improved thermotolerance, sweating ability and have altered coat characteristics.

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Exercise and lactation in horses: What do we know?*Sarah Reed*

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During lactation, mares are generally provided with opportunities to exercise voluntarily through turnout, with minimal or no forced exercise. The metabolic response to lactation is variable in mares, with some gaining or maintaining body weight, and others losing weight. This may be at least partially attributed to the decreased circulating concentrations of leptin post-partum which allow the mare to increase feed intake sufficient in most cases to maintain her body condition. Some studies indicate that obesity may decrease milk production in mares, although prolactin concentrations are not significantly altered. Milk composition may also be altered, with some studies demonstrating increased fat content with a greater inflammatory plasma fatty acid profile and decreased protein content in milk from obese mares compared with lean mares. However, exercise may mitigate some of the negative metabolic changes associated with obesity in lactating mares. Exercise may help obese lactating mares reach a healthier body condition and improve insulin sensitivity. Exercise may also counteract the metabolic instability associated with the transition period. In lactating women, aerobic exercise improves cardiovascular fitness, decreases body weight, and has no adverse effects on milk production, circulating prolactin concentrations, or milk composition. As obesity in mares becomes more common, it's important to identify safe strategies to improve health outcomes for both the mare and foal. While reducing body condition prior to gestation would be the most ideal scenario, mares that remain obese throughout gestation may benefit from light exercise during lactation.

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Nutritional and endocrine regulation of muscle growth in neonatal swine*T. A. Davis, A. Surawan, K. Beom Jang, and M. L. Fiorotto*

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Growth is dependent on a higher rate of protein synthesis than protein degradation. In young animals, the high rate of lean growth is largely attributable to the high rate of protein synthesis. The ingestion of food stimulates the synthesis of protein in skeletal muscle and this response is profound in early life. This feeding-induced stimulation of protein synthesis is crucial to support the rapid muscle growth during early postnatal life and the maintenance of body protein in adulthood.

Studies in the neonatal pig have shown that the sharp increase in muscle protein synthesis after eating is triggered by the rise in amino acids and insulin. Amino acids and insulin stimulate protein synthesis by activating independent intracellular signaling cascades that converge at mechanistic target of rapamycin complex 1 (mTORC1), leading to the activation of key regulators of mRNA translation. These include the phosphorylation of 4EBP1 and S6K1 and the formation of the active eIF4E-eIF4G complex that binds mRNA and initiates translation. Activation of the insulin receptor by insulin leads to the activation of the signaling pathway that involves IRS-1, PI3-kinase, Akt, TSC2, and Rheb, which enables the activation of mTORC1. This process is governed by positive and negative regulators. Amino acid signaling involves the interaction of the Rag proteins with mTORC1, which results in mTORC1 activation. The cyclic changes in the activation of these signaling proteins with meal feeding parallel the changes in protein synthesis in skeletal muscle. These responses are elevated in the neonatal pig born at term and decrease with age. Among the amino acids, leucine is the most potent agonist of mTORC1 activation.

In pigs born prematurely, the protein synthetic response in skeletal muscle to feeding is blunted. This reduced protein synthesis response in the preterm is due to a reduced activation of the insulin and amino acid signaling pathways that lead to mTORC1 and translation initiation factor activation. This reduced response to feeding is not simply due to differences in the profile of insulin and amino acids after a meal in the preterm compared to the term. When equivalent increases in insulin and amino acids in the preterm and term born pigs are provided by infusion, mTORC1 signaling and protein synthesis rates in muscle are blunted in the preterm. The attenuated response to feeding in the preterm is independent of birth weight. This anabolic resistance to feeding likely contributes to the extrauterine growth restriction, altered body composition, and reduced lean mass accretion following premature birth.

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Obesity in horses and donkeys - when do we talk about metabolic syndrome?

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Obesity and overconditioning are increasingly common problems among horses, ponies and donkeys. In a clinical context, the nutritional status is classified using a horse- or donkey specific body condition score (BCS) and cresty neck score (CNS).^{1,2} Owners commonly tend to underestimate the BCS of their equids.^{11,9,2} Depending on the person that assesses the BCS (owner or veterinarian) and on the geographical region and equine population, the prevalence of obesity varies. It is 21-45% in leisure horses,^{13,11} with significantly higher prevalences over 70% in certain subgroups such as Shetland ponies.¹¹ The owner-assessed prevalence of obesity in donkeys is 33%.^{3,10} Certain horse breeds, particularly pony breeds, as well as donkeys are evolutionarily adapted to endure in harsh environments with limited food availability. Accordingly, these types of equids have a high metabolic efficiency (“easy keepers”) and easily gain weight under our husbandry conditions with food that is too rich and easily available.^{12,6} This is aggravated by a lack of exercise.^{4,10} Obesity in equids is not a cosmetic problem, but an important risk factor for equine and asinine metabolic syndrome (EMS and AMS, respectively) and its dreaded consequences, endocrinopathic laminitis - in addition to the main factor, systemic insulin dysregulation (ID).^{7,4} ID can manifest as basal hyperinsulinemia, excessive or prolonged hyperinsulinemia in response to a carbohydrate challenge and tissue insulin resistance.⁴ The Equine Endocrinology Group provides recommendations for the diagnosis and management of EMS, which particularly apply to horses and ponies.⁵ Donkeys show a lower insulin response to oral carbohydrate challenge compared to horses and consequently, diagnostic criteria for horses will have to be adapted for donkeys.⁸ Therapeutic principles are based first and foremost on management changes (diet, exercise). Medical therapy, for example with sodium-glucose co-transporter 2 inhibitors, is reserved for more severe forms of the disease, where the patient suffers from laminitis and unresponsive ID.⁵ Cases with laminitis that do not respond to therapy often have to be euthanized for animal welfare reasons. Awareness of EMS and AMS should be promoted among veterinarians and owners.

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Local and systemic regulation of endocrine and innate immune system by sperm and embryo toward early pregnancy in cattle*Akio Miyamoto*

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Early embryonic losses arise from either a failure of the maternal immune system to recognize and accept developing embryos or disruptions in endocrine processes. Therefore, the establishment of pregnancy requires a precise regulation and cross-talk between maternal innate immunity and endocrine functions. Recent studies have shown that this process begins shortly after insemination in cattle. Upon sperm deposition into the uterus, there is a modest induction of inflammatory responses, characterized by the release of proinflammatory chemokines and the recruitment of polymorphonuclear cells into the uterine lumen. Furthermore, sperm interaction with the endometrial mucosa triggers transient inflammatory responses in circulating immune cells. This interaction between sperm and the uterine mucosa facilitates the efficient clearance of excess sperm and bacteria from the uterine environment, thereby creating favorable conditions for uterine receptivity to the impending embryo. Conversely, sperm binding to the oviductal epithelium prompts the release of anti-inflammatory molecules, notably prostaglandin E₂. In addition, the oviduct fluid protects sperm from phagocytosis by polymorphonuclear cells, thereby supporting sperm survival until fertilization. Once formed in the oviduct, the embryo communicates with the bovine oviduct through paracrine signaling, eliciting an anti-inflammatory immune response within the oviduct environment to facilitate early embryonic development and survival. Additionally, the oviduct epithelium stimulates the 16-cell stage bovine embryo, approximately on day 4 post-fertilization, to synthesize interferon tau (IFNT), a key pregnancy recognition signal in ruminants. As the developing embryos descend into the uterine horn, they initiate anti-inflammatory responses and express interferon-stimulated genes (ISGs) in the uterine epithelium and local immune cells, promoting embryo tolerance, primarily through the release of IFNT. Furthermore, the presence of day 7 embryos in the uterus induces anti-inflammatory immune responses and ISGs expression in circulating immune cells for embryo recognition. Moreover, IFNT plays a crucial role in regulating progesterone release by attenuating luteolytic pulses of uterine prostaglandin F_{2α} (PGF_{2α}) to maintain the corpus luteum (CL). Progesterone, in turn, supports the transport of embryos and uterine receptivity. Meanwhile, ovarian steroids play a pivotal role in regulating maternal innate immunity, crucial for fertilization, and embryo development within the female reproductive tract (FRT). Together, successful establishment of early pregnancy relies on the complex interactions between sperm/embryo and the maternal immune/endocrine systems. These interactions are crucial for triggering maternal recognition of pregnancy (MRP), facilitating embryo development and survival in cattle.

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Somatotropic axis and follicular dynamics*Matthew Lucy*

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The somatotropic axis [consisting of growth hormone (GH), the insulin-like growth factors 1 and 2 (IGF1 and IGF2), GH binding protein (GHBP), IGF binding proteins (IGFBP), and the cell-surface receptors for GH and the IGFs] has major effects on growth, lactation and reproduction in farm animals. The primary target tissues for GH are involved in growth and metabolism. The functionality of the somatotropic axis depends in part on the expression of liver GH receptor (GHR) that determines the amount of IGF1 that is released from liver in response to GH. The IGF1 acts as a pleiotropic growth factor and also serves as the endocrine negative feedback signal controlling pituitary GH secretion. Growth hormone and IGF1 undergo dynamic changes throughout the life cycle particularly when animals are either growing, early postpartum or lactating. In some farm species, including dairy cows there is a period of negative energy balance during the first four to eight weeks postpartum. During this period, the somatotropic axis becomes uncoupled and there is elevated GH and diminished IGF1 in the circulation. In other species (porcine; sows, for example) the somatotropic axis remains coupled early postpartum (during lactation) and both GH and IGF1 are elevated. Nonetheless, sows that become catabolic during lactation will have reduced IGF1 concentrations. Many of the mechanisms that control reproduction are linked directly to the nutritionally-controlled hormonal milieu of the animal. Cows in negative energy balance, for example, have lower blood concentrations of insulin and IGF1. Insulin and IGF1 concentrations, however, gradually increase postpartum as cows move from negative to positive energy balance. Insulin and IGF1 stimulate GnRH secretion from the hypothalamus and LH secretion from the pituitary. The hormonal control of GnRH and LH, therefore, arises from the metabolic and nutritional status of the animal via insulin and IGF1. The same metabolites and hormones that influence GnRH secretion and ultimately LH secretion may act directly on the ovary to influence the sensitivity of the ovary to LH and FSH. Thus, the effects of nutrition on reproduction are manifested at the ovary and at the pituitary and hypothalamus through metabolic hormones (GH, IGF1, and insulin) that are essential for nutrient partitioning. Cells within the reproductive tract can respond directly to GH but to a lesser degree than the primary target tissues of GH that include liver, muscle, and bone. The major impact that GH has on reproduction, therefore, may be secondary to its systemic effects on metabolism (including insulin sensitivity) or secondary to the capacity for GH to control IGF1 secretion. A threshold of free IGF1 protein in follicular fluid may be met by local ovarian (paracrine/autocrine) and endocrine sources of IGF1 and a reduction in IGFBP within the follicular fluid. Follicular development is increased when this threshold for IGF1 is met and there is sufficient stimulation from gonadotropins (LH and FSH). Understanding the mechanisms through which metabolic hormones including GH and IGF1 control ovarian function may lead to improved reproductive management because lactation and postpartum reproduction are closely tied.

Role of the innate immune system in the postpartal uterine disease complex in modern high yielding dairy cows

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Postpartum uterine diseases represent a significant challenge in modern high-yielding dairy cows, affecting their reproductive performance and therefore overall productivity. The innate immune system plays a critical role in the postpartum uterine disease complex by responding to microbial invasions that occur almost universally during parturition. In dairy cows, up to 100% experience bacterial contamination of the uterus immediately after calving, necessitating a robust immune response to prevent the progression to clinical or subclinical disease states.

The inflammatory response is a normal part of uterine involution; however, in some cows, this response becomes dysregulated, leading to persistent inflammation and subsequent uterine disease. While cows suffering from clinical metritis and clinical endometritis have been shown to harbor a significantly different uterine microbiome in comparison to healthy cows, this has not been the case for subclinical endometritis. The latter is characterized by an elevated presence of polymorphonuclear cells (PMNs) in the endometrium without overt clinical signs but leading to a significantly impaired fertility. This condition affects approximately 20-30% of postpartum cows and is diagnosed through cytological examination. The role of the innate immune system is pivotal in this context as it involves the initial detection and response to pathogens by PMNs, which are the first line of defense.

High-yielding dairy cows are particularly susceptible to postpartum uterine diseases due to metabolic and environmental stresses that may compromise their immune function. Especially the negative energy balance which is a hallmark of modern high-yielding dairy cows, has been shown to be associated with a higher prevalence of SCE, indicating a link between metabolic status and immune function.

Management practices that enhance the innate immune response during the peripartum period are therefore crucial for preventing postpartum uterine diseases. This includes nutritional strategies that minimize metabolic stress and support immune function, as well as timely and accurate diagnosis of all pathologic conditions.

In conclusion, the innate immune system is integral to managing the postpartum uterine disease complex in dairy cows. Effective modulation of this system through managerial and nutritional interventions can help mitigate the adverse effects of these diseases, thereby enhancing reproductive efficiency and overall herd productivity.

Poster presentations

S1_P01

Effect of feeding docosahexaenoic acid to sows on milk composition and microbiota of sows and pigletsT. Søndersby Bruun¹ and A. Varmløse Strathe²¹Livestock, SEGES Innovation; ²Department of Veterinary and Animal Sciences, University of Copenhagen, Denmark
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Growth and development of the piglets until weaning is highly dependent on the milk production of the sow and especially gut development and health is crucial for successful weaning and robustness of the piglets. The objective of the study was to test if addition of docosahexaenoic acid (DHA) in lactation diet of sows affects milk composition, microbiota of sows and piglets and piglet growth.

Materials and Methods

Sows (Landrace x Yorkshire) were fed a standard diet as control (Con group) diet or a standard diet plus 50 mg/kg feed DHA originating from a microalgae product (DHA group). Sows were fed the diets from farrowing in previous cycle and until next weaning. After farrowing litters (n=248) were standardized to 14 piglets. The litter was weighed at standardization and weaning.

Colostrum (n=19) and milk (n=33) samples were obtained by removal of piglets and injection with 2 mL Oxytocin at 12 hours, day 6 and 17 postpartum. The colostrum and milk were analyzed for dry matter, fat, protein, lactose, fatty acid composition and immunoglobulin A, G and M. Fecal samples were collected from sows and three piglets per litter at d 17 of lactation. The samples from the three piglets from one litter was pooled into one sample. Samples were collected in tubes with stabilizer (Canvax Biotech, Cordoba, Spain) and stored at -60°C until analysis. Milk and litter data was described in a mixed model with diet and parity as fixed effects and week batches of sows as random and analyzed using ANOVA in R. Fecal DNA was extracted using Power soil isolation kit according to the manufactory's instruction, and the Illumina sequencing was performed with a two-step PCR method amplifying the V3 hypervariable region of the 16S rRNA gene (He et al., 2022). The USEARCH pipeline and UNOISE3 were used to build zero radius Operational Taxonomic Units (zOTUs) as described by Edgar (2016) and Krych et al. (2018). Statistic differences of alpha and beta diversity were assessed by a student t-test and pairwise PERMANOVA, respectively.

Results

The fatty acid composition was similar between groups except for a tendency (P=0.09) in colostrum for a higher concentration of DHA in the DHA group and a significant higher concentration of DHA in milk from DHA sows at day 17 (P<0.001). The colostrum and milk content of dry matter, protein, fat, lactose, IgA, IgM and IgG was similar in Con and DHA group (P>0.05). Litter weaning weight was unaffected by dietary treatment (P>0.05). The microbial alpha diversity were similar between sows fed control and DHA diets (P=0.485) and between piglets from sows fed control and DHA diets (P=0.963), respectively. Piglets from sows fed DHA increased the relative abundance of Bacteroidetes compared to piglets from sows fed the control diet, which was also consistent with the trend between sows fed control and DHA diets. Sows fed the DHA diet showed a higher abundance of the genera including Clostridiales, Bacteroides, but lower Veillonellaceae compared to the sow fed control diet (P<0.05). Piglets from sows fed the DHA diet increased the abundance of Ruminococcaceae, Bacteroides, and Bacteroidales but suppressed Streptococcus, S24-7, and Lactobacillus reuteri relative to piglets from control sows (P<0.05).

Conclusions

In conclusion, dietary inclusion of DHA in sow diets during gestation and lactation affected FA composition of sows milk and altered the abundance of microbial genera in sows and piglets.

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S2_P01

Management practices and farmer perceptions on milkability in dam-calf contact rearing in Switzerland*J. Rell, C. Nanchen, P. Savary, C. Buchli, and C. Rufener*

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Cow–calf contact (CCC) farming is a developing alternative in dairy management with diverse practical approaches. In dam-calf contact (DCC) rearing – a subtype of CCC rearing where cows nurse their own calf in addition to being milked – milkability is perceived as a challenge (Vaarst and Christiansen, 2023). We aimed to examine the current status quo regarding management and milking of DCC farms in Switzerland by means of phone interviews. Additionally, farmer perceptions on animal health and milkability were inquired.

Materials and Methods

Semistructured phone interviews were conducted with 17 DCC farmers on farm characteristics, management practices, dam-calf management and perceptions regarding milkability. A table summarizing the answers to all questions was created and descriptively analyzed.

Results

On 47% (8) of the farms whole-day contact is provided. Twelve 12% (2) operate with half-day contact and 41% (7) provide short-time contact before or after milking with widely differing housing forms for calves. Weaning occurred between two and 12 months of age with differing approaches for male and female calves. Most farms occasionally had calves with self-recovering diarrhea, though none experienced it regularly. Cow health was reported to be unproblematic on 71% (12) of farms. On average 20% of the cows were reported to have milkability problems. 65% (11) of the farms had taken measures to address poor milkability.

Conclusions

Our pilot study offers an initial insight into the developing DCC rearing system in Switzerland. Adaptation to farm-specific preconditions resulted in highly individual operational decisions leading to a diverse overall picture regarding DCC management. Animal numbers are mostly below Swiss average. Animal health is mainly good while milkability is an ongoing concern, though not the primary challenging.

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S2_P02

Effect of cow-calf contact on udder emptying in the robotic milking unit in dairy cows*S. Ferneborg, S. Almlöf, and S. Agenäs*

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Introduction

The interest in keeping dairy cows with their calves is increasing worldwide, with system development to follow. One of the major issues the system is facing is the loss of saleable milk, which is partly due to the milk intake by the calf, but may also be an effect of impaired milk ejection due to low udder emptying or lower sensitivity to the oxytocin released at machine milking (1). If milk removal is incomplete it may lead to a lower daily milk yield, due to feed-back inhibition of milk secretion (2). The aim with this study was to investigate if the residual milk volume after machine milking is higher when cows have access to their calf in a cow-calf contact system between milkings.

Materials and Methods

19 cows (6 Swedish Holstein, 13 Swedish Red) had access to their calves in a contact area built into the automatic milking system (3). They could access the contact area at any time for 85±16 days, before undergoing fenceline separation with part-time nursing access for 10 days and finally total separation after another 7 days without nursing access. Cows were milked in a DeLaval VMS Classic milking unit (DeLaval, Tumba, Sweden) and residual milk was quantified using i.m. injection of 7 IU oxytocin after milking at three occasions; during full contact (phase 1, 62±10 DIM), during fenceline (phase 2, 94±8 DIM) and two weeks after separation (phase 3, 108±8 DIM). Proportion of residual milk was calculated as amount of residual milk divided by total milk yield, and ln transformed before statistical analysis due to non-normal distribution. Data was analyzed using the mixed procedure in SAS Enterprise guide 8.3, with a model including the fixed effects of phase, lactation number, breed and strip milk fat content as well as the repeated effect of cow. The Tukey-Kramer adjustment was used to account for multiple comparisons. Values are means with corresponding standard errors unless otherwise specified.

Results

The proportion of residual milk obtained showed considerable variation in all three phases, and was on average 24±3.3% in phase 1, 18±3.3% in phase 2 and 11±2.6% in phase 3, with significant differences between phase 3 and phases 1 and 2 ($p=0.0484$ and $p=0.0219$, respectively). Fat content of the residual milk was 8.4±0.4% in phase 1, 9.1±0.4% in phase 2 and 9.3±0.3% in phase 3, but did not differ significantly between phases. A higher strip milk fat content was associated with a lower proportion of residual milk ($p<0.001$) and a higher fat content of the residual milk ($p<0.001$). Cows of Swedish Holstein had a lower proportion of residual milk compared to Swedish Red ($p=0.0297$).

Conclusions

Udder emptying was impaired during cow-calf contact and improved after separation. Strip milk fat content was associated with both proportion of residual milk and fat content of the residual milk. Persisting variation in udder emptying indicates that some cow-calf contact cows struggle with milk ejection also after separation from the calf.

*References**(1) Churakov et al 2023**(2) Penry et al. 2017**(3) Johansson et al. 2023*

S2_P03

Investigations into the effects of altering the c-phase of pulsation*D. J. Reinemann*

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Claims have been made that reducing the duration of the c-phase of pulsation will improve milking performance by improving cow comfort during milking.

Materials and Methods

These claims were investigated with a review of literature on the relationship between pulsation phase duration, liner wall movement, the point in the pulsation cycle where milk flow starts and stops, and the documented effects of altering the c-phase of pulsation.

Results

An often-misquoted paper published in 1983 in the Journal of Dairy Science reported that there was no effect of changing vacuum-decrease time (c-phase duration) on milking. A subsequent paper published in 2001 concluded that the duration of a and c phases had no effect on udder health or teat end condition and that an 8% increase in the percentage of the c phase resulted in a 8% increase in average milk flowrate (AMF) and a 5% increase in peak milk flowrate (PMF). This paper postulates that it is possible that short phases induced animal reactions because of faster movement of the liner. Similar results were reported in 2007 study in which a 6% increase in percentage of the c phase resulted in a 5% increase in AMF and 7% increase in PMF.

Investigations into liner wall movement and the point in the pulsation cycle at which milk flow starts and stops has provided an explanation for these results in that increasing the c-phase of pulsation also increases the true milk:rest ratio or the percentage of the pulsation cycle during which milk is flowing. Similar increase in milk flowrate can be obtained by simply adjusting the pulsation settings. The effects of increasing the duration of the c phase on milking speed are larger for lower overpressure liners.

Conclusions

Increased milking speed resulting from increasing c phase duration can be explained by changes in milk:rest ratio and there is no need to resort to unproven speculation regarding cow comfort for this effect.

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S2_P04

Improved machine milking efficiency without additional load on teat tissue through increased pulsation ratio in rear quarters*P. Fürst¹, M. Affentranger¹, K. Kakoulis², C. O. Paulrud², and R. Bruckmaier¹*¹Veterinary Physiology, Vetsuisse Faculty, University of Bern, Switzerland; ²DeLaval SA, Tumba, Swedenrupert.bruckmaier@unibe.ch**Introduction**

Dairy cows produce mostly more milk in rear than in front quarters. Thus, at conventional machine milking the milk flow ceases earlier in front than in rear quarters, resulting in milking on empty teats in front quarters. We tested the hypothesis that an increased pulsation ratio (PR) in rear teat cups reduces machine-on time and the time of milking on empty teats in front quarters without an increased impact on teat tissue thickness (TTT).

Materials and Methods

Ten Holstein cows were milked at 14-h (morning) and 10-h (afternoon) milking intervals. Each treatment was performed twice at both milking intervals. The system vacuum was set at 42 kPa. Front quarter PR was 65:35, whereas PR in rear quarters was 65:35, 70:30, 75:25, or 80:20. Whole udder milk flow was recorded, and the time of individual quarter milk flow (tQMF) was determined by characteristic changes of the quarter mouthpiece chamber vacuum. The TTT of all 4 teats was measured with a cutimeter at 5 min after cluster detachment. Statistical evaluations were performed by ANOVA and Tukey's t-test.

Results

Total milk yield as well as tQMF of front quarters did not differ among treatments and was 21.1 ± 0.3 and 15.6 ± 0.2 kg, and 4.9 ± 0.1 and 3.8 ± 0.1 min at 14-h and 10-h milking intervals, respectively. In contrast, tQMF in rear quarters and consequently the interval of first to last quarter milk flow cessation decreased gradually with increasing PR from 65:35 to 80:20 from 6.8 ± 0.5 to 6.0 ± 0.4 min ($P < 0.05$) and 5.3 ± 0.3 to 4.7 ± 0.3 min ($P < 0.05$), and from 2.7 ± 0.3 to 1.8 ± 0.2 min ($P < 0.05$) and 2.1 ± 0.2 to 1.6 ± 0.2 min ($P < 0.05$) at 14-h and 10-h milking intervals, resp.. Because of immediate cluster detachment at cessation of milk flow machine-on time was reduced from 7.6 ± 0.4 to 6.9 ± 0.4 min ($P < 0.05$) and from 6.2 ± 0.3 to 5.5 ± 0.3 min ($P < 0.05$) at 14-h and 10-h milking interval, respectively. Independent of PR the TTT after milking was increased compared to pre-milking values only in front quarters ($P < 0.05$), but not in rear quarters.

Conclusions

In conclusion, increased PR in rear quarters increases the milking performance, and reduces milking on empty teats without increased impact on teat tissue.

S3_P01

Possible factors associated with colostrum quality and excellent transfer of passive immunity in calves: the reality in different European farmsA. Fernandez-Novo¹, I. Kolkman², M. Driesse³, M. Yarnall⁴, M. Cerviño⁵, F.J. Dieguez⁶, and S. Astiz⁷¹Veterinary Medicine, Universidad Europea de Madrid, Spain; ²A7 Noord dierenartsen, The Netherlands; ³Boehringer Ingelheim Animal Health, The Netherlands; ⁴Boehringer Ingelheim Vetmedica GmbH, Germany; ⁵Boehringer Ingelheim Animal Health, Spain; ⁶Veterinary Faculty, Santiago de Compostela University, Spain; ⁷Animal Reproduction, National Institute of Agronomic Research (INIA-CSIC), Spainmatt.yarnall@boehringer-ingelheim.com

Introduction Due to the synepitheliochorial placenta of the bovine dam, neonatal calves must receive all of their passive immunity through colostrum. Achieving the highest levels of transfer of passive immunity (TPI) in calves is important for health, welfare and performance, beyond just survival, and the aim should be to achieve >40% of calves in this category. There are different risk factors associated, both at the cow and calf level, and this study sought to understand those associated with colostrum quality and excellence of TPI.

Materials and Methods We studied 1,041 calves from 108 farms advised by 27 vets from France (FR;292), Germany (DE;129), Great Britain (GB;231), Italy (IT;50), Spain (SP;219) and Netherlands (NL;120). Information on farm was: herd size (n=710), access to pasture at dry-off (n=322), anionic salts feeding (n=308), calf housing (n=567) and bedding (n=446). On dam/calving: parity (n=139), type of calving (n=840) and night-day birth (n=633). On calf-colostrum: calf breed (n=823), gender (m=906), birth weight (n=559), frozen/fresh colostrum (n=709), type of colostrum feeding (n=875), volume (n=842), quality (n=892), time from birth to colostrum intake (n=749) and age at blood sampling (n=824). Colostrum and plasma samples, within 11 days of life and at least 24 hours after colostrum intake, were assessed with refractometry. Brix values in serum were converted into total protein values (TP; in g/dl). TPI was divided into 3 categories: 1) "poor" with calf serum TP <5.3g/dl, 2) "fair" with TP 5.3-6.2g/dl and, 3) "excellent" with TP >6.2g/dl (Lombard et al., 2020). SPSS®v.25 (IBM, Armonk, NY, USA) and R Statistical Softwarev.4.3.1 (R Core Team, 100 Vienna, Austria) were used for statistical analysis (univariate analyses and mixed-effects regression models).

Results Median values were: administered colostrum volume, 3l (Interquartile interval or IQI:2l), quality, 24.4%Brix (IQI:6%), interval birth to administration, 2h (IQI:3h). Calves were blood sampled on the fourth day of life (IQI:2d) for assessment of passive transfer. The percentage of calves in each TPI category by countries was 26.6% calves in the Poor category (277/1,041; 95% CI: 4.0–44.0%); 38.1% in the Fair category (397/1,041; 95% CI:27.1–62.3%) and 35.3% of calves in the Excellent category (367/1,041; 95% CI:14.5–54.2%).

Colostrum quality was affected by access to pasture during the dry-off period, which was associated with lower quality colostrum (<21%; P=0.012, OR=0.283, CI 95%=0.105-0.761). This was also associated with type of calving, specifically intensively-assisted calving (P=0.042; OR=0.492; CI95%:0.98-0.244). Being on pasture during the dry-off period probably reflects the problem of an imbalanced nutrition (Mudler et al., 2017), and the level of calving assistance may result in lower quality colostrum similarly to that previously observed (Renaud et al., 2020). Although we demonstrated this negative effect of assisted calving on colostrum quality through the model analyses, we surprisingly observed the opposite effect in the univariate analyses on the TPI in calves, with assisted calvings showing ten percentage points more ETPI calves than the average. This may be explained by the fact that assisted calving results in a better observance of the mother and the calf, with a less delayed administration of colostrum. In fact, calves born after assisted calvings in our study were fed colostrum one hour after (median 1 h; IQI: 1.5 h) while those born after not observed calvings were fed later (median 2.5 h; IQI: 3 h).

Although the univariate analyses on TPI and on colostrum quality detected a significant effect of the most factors studied, according to the regression model, only calves that received more colostrum were more likely to be in better TPI categories (1.553 times/l higher odds of being in category "Excellent-TPI" compared to "Poor-TPI"; 1.381 times/l higher odds of being in "Excellent-TPI" compared to "Fair-TPI"). Likewise, higher colostrum Brix values were more likely to lead to better TPI (1.199 times/Brix percentage point higher odds of achieving an "Excellent-TPI" vs "Poor-TPI" and 1.094 times/Brix point higher odds for "Excellent-TPI" vs "Fair-TPI").

Conclusions Farms in European countries manage and administer colostrum adequately to calves on average. However, certain practices should be improved to achieve the goal of >40% of calves with excellent TPI, with the affecting factors being different among Europe. Intensively-assisted calving and access to pasture during the dry-off period decreased colostrum quality and management interventions should be incorporated when considering TPI in calves affected by these factors. Study funded in part by Boehringer Ingelheim.

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S3_P02

Serum osteocalcin and CTX-I concentrations in transition dairy cows.S. van der Drift¹, A. Koopmans¹, L. Kroon², R. Grotentraast¹, and M. Holstege¹¹Royal GD, Deventer, The Netherlands; ²Schothorst Feed Research, Lelystad, The Netherlandss.v.d.drift@gdanimalhealth.com**Introduction**

Bone metabolism biomarkers such as osteocalcin (OC; indicator bone formation) and CTX-I (degradation products of C-terminal telopeptides of Type I collagen; indicator bone resorption) can be used to monitor bone metabolism in transition cows. The objective of this study was to investigate serum concentrations of OC and CTX-I in transition cows at several time points relative to parturition.

Materials and Methods

The study was performed at the dairy farm of Schothorst Feed Research B.V (Lelystad, The Netherlands) in 2019 - 2020. A total of 44 cows of different parities were enrolled in the study. Animals were housed in a loose housing system. Serum samples were collected around 10 days before the expected calving date, at the day of calving and at day 2, 7, and 28 after parturition. OC and CTX-I concentrations in serum were analyzed using, respectively, a N-MID Osteocalcin and CTX-I test kit on an IDS-iSYS Multi-Discipline Automated System. Descriptive statistics were performed and differences in serum OC or CTX-I concentrations 1) between sampling days and 2) between parity groups within the same sampling day were investigated using a Kruskal-Wallis test followed by a Dunn's test (significance level $P < 0.05$; analyses performed in Stata version 17.0, 2021).

Results

Concentrations of OC and CTX-I, and their ratio in serum at different time points are shown in Table 1. Heifers had larger serum OC concentrations than cows from third or higher parity at all time points ($P < 0.05$). Compared with second parity cows, OC concentrations were larger in heifers at day 7 and 28 ($P < 0.05$). CTX-I concentrations were larger in heifers than in cows from third or higher parity from day -10 until day 7 ($P < 0.05$). At day 28, CTX-I concentrations in serum were similar for all parity groups.

Table 1: Concentrations of osteocalcin (OC), CTX-I, and their ratio (mean and standard deviation) in transition cows at different sample moments relative to parturition at the dairy farm of Schothorst Feed Research (The Netherlands).

Moment of sampling	No. of cows	Osteocalcin*		CTX-I*		OC/CTX-I-ratio*	
		Mean	SD	Mean	SD	Mean	SD
Day -10	35	8,2 ^a	7,1	0,38 ^a	0,48	47,3 ^a	33,1
Day 0	41	5,0 ^a	3,2	0,34 ^a	0,29	21,7 ^b	15,4
Day 2	41	5,0 ^a	2,8	0,50 ^a	0,31	11,3 ^c	4,7
Day 7	40	6,3 ^a	3,5	0,95 ^b	0,47	7,8 ^d	5,2
Day 28	41	9,8 ^b	5,3	1,46 ^b	0,77	7,8 ^d	3,9

*Results with different superscripts in the same column differ significantly from each other ($P < 0,05$).

Conclusions

Bone metabolism biomarkers OC and CTX-I in transition cows show specific concentration patterns around parturition. Parity-specific reference intervals are necessary for a correct interpretation of OC and CTX-I concentrations in transition cows

S3_P03

Characterization of prepartum serum and mammary secretion immunoglobulin G concentration and its association with colostrum immunoglobulin G yield in Holstein cattle*A. J. Fischer-Tlustos, K. Klein, C. McQuaig, J. Petrou, J. P. Cant, and M. A. Steele*

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amandaf@uoguelph.ca**Introduction**

The importance of adequate colostrum immunoglobulin G (IgG) quality and mass for neonatal development and health is well-established; yet, factors controlling the prepartum transfer of IgG from dam serum to prepartum mammary secretions, and ultimately colostrum, remain to be fully elucidated. Thus, the objective of this study was to characterize IgG concentrations in prepartum serum and mammary secretions, and to determine potential associations with colostrum IgG mass.

Materials and Methods

From d -58 ± 2.9 prior to calving, parity 2 (P2, n = 11) and 3 (P3, n = 10) Holstein cows were dried off and fed a controlled energy diet (NEL = 1.48 Mcal/kg DM) until calving. Serum was collected weekly from wk-9 to d-9, followed by every other day until calving, as well as at calving. Prepartum mammary secretions were collected at d-14 and d-7, followed by every other day until calving by stripping a maximum of 15 mL of secretion/d. Colostrum was collected within 1.4 ± 0.76 h of calving and yield was recorded. Total IgG content was determined by radial immunodiffusion. Data were analyzed using PROC GLIMMIX of SAS considering the fixed effects of parity and time and the random effect of cow.

Results

Serum IgG concentration was highest ($P < 0.01$) from wk-9 to wk-2, and was 24.4, 27.6, and 27.6% higher than d-2, -1, and calving, respectively. Secretion IgG was highest ($P < 0.01$) at d-7 (267.8 ± 21.48 g/L) and was greater than d-2 (158.8 ± 25.6 g/L), d-1 (157.6 ± 22.0 g/L) and colostrum (89.9 ± 16.45 g/L). Despite a lack of difference ($P = 0.29$) in serum IgG between parities, P3 cows had 1.5x greater ($P = 0.006$) secretion IgG than P2 cows from d-14 to d-1. Correlation analyses demonstrated that colostrum yield was correlated ($P = 0.02$, $r = 0.73$) with secretion IgG concentration at d-14 but not at any other timepoints. In addition, the magnitude of decline in serum IgG from wk-3 to calving was correlated ($P = 0.02$, $r = 0.65$) with secretion IgG concentrations at d-5.

Conclusions

The results demonstrate that P3 cows may have a greater capacity for IgG transport than P2 cows and that IgG concentration gradually declines from d-7 to calving, likely due to dilution. Furthermore, correlation analyses suggest that a larger decline in serum IgG from wk-3 to calving may be important in determination of secretion IgG content and that early establishment of high levels of prepartum mammary IgG may be an important factor dictating colostrum IgG yield at calving.

S3_P04

Colostrum intake of piglets in relation to birth order

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Introduction

Colostrum production of sows is estimated by assessing weight gain of piglets over a 24-hour period after start of parturition (Theil, 2017). During this timeframe, the composition of colostrum undergoes gradual changes (Quesnel & Farmer, 2019). In this study, we aim to estimate colostrum intake of piglets within this 24-hour period by repeatedly measuring the weights of the first, middle, and last-born piglets.

Materials and Methods

Twenty-eight sows (TN70, parity 2-5) and their litters were used. The body weight of the first, middle, and last-born piglets were assessed at birth and 8, 12, 16, 20 and 24 hours after the birth of the first piglet. Colostrum intake (eCI) was estimated per piglet based on piglet weight gain (pWG) in the time period 0-12, 12-24 and 0-24 hours, according to Krogh et al. (2015). pWG and eCI were analyzed using a linear mixed model (R v4.2.1), with birth order and time period and their interactions as fixed effects, and sow as a random factor.

Results

During the first 24 hours, average and SEM of pWG and eCI per litter were 78 ± 7 and 396 ± 12 g/piglet. Sow colostrum production (Σ eCI) averaged 7.2 ± 0.2 kg and farrowing duration averaged 298 ± 29 minutes. pWG and eCI were higher for the first piglet during the period 0-12 and 0-24, compared to the middle and last-born piglet. eCI was higher for middle-born piglet in the period 0-12 hours, compared to the last-born piglet (<0.001). During the 12-24 hours period pWG and eCI were similar for all piglets.

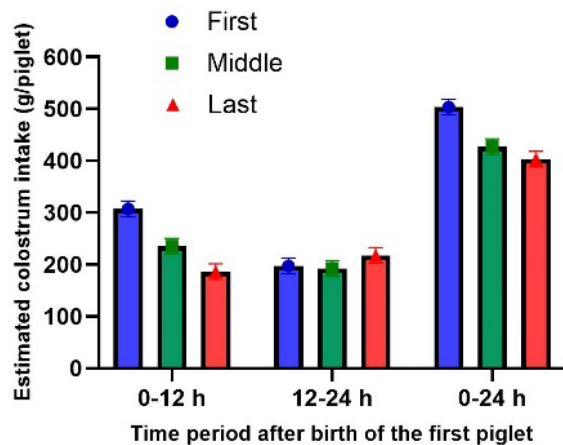


Figure 1. Estimated colostrum intake of piglets by birth order and time period.

Conclusions

Piglets contribute differently to the estimated colostrum production of sows, partly related to the time of birth. The first-born piglets have a higher colostrum intake during the first 12 hour period after start of parturition, a period with higher colostrum quality. This indicates that first-born piglets may have an advantage in terms of immunity and robustness later in life.

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S4_P01

Study of the molecular mechanisms involved in the induction of milk lipolysis in dairy cows subjected to feed restriction

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Introduction

Milk lipolysis is defined as the hydrolysis of triglycerides, which are the main component of milk fat. Milk lipolysis leads to the release of free fatty acids, responsible for rancid flavor. It is induced in cows subjected to feed restriction, but molecular mechanisms involved in this induction are not well documented. The objective of our study was to identify genes involved in the induction of lipolysis during feed restriction.

Materials and Methods

Holstein dairy cows were fed a restricted diet (i.e. 65% of their ad libitum DM intake (DMI)) to induce spontaneous lipolysis in milk. Two groups of 7 cows each received a control diet (100% of ad libitum DMI) or a restricted diet over 2 periods according to a 2 x 2 crossover design. The restricted diet was fed for five days. Milk samples were collected to prepare mammary epithelial cells (MEC) using an immunomagnetic purification method. Mammary biopsies were also collected at each period. Total RNAs were extracted for both milk purified MEC and mammary biopsies using TRIzol. Bovine 8 x 60K single color RNA microarrays were hybridized with RNA samples collected from mammary biopsies. Expression data were normalized with 75th percentile scaling and analyzed using the R software. Statistical analyses were performed using moderated paired t-tests with a Benjamini-Hochberg correction for multiple testing, and a significance threshold defined using adjusted p-values ≤ 0.10 and absolute fold changes threshold ≥ 1.5 . A subset of genes of interest were also studied by RT-qPCR from mammary biopsy and milk purified MEC samples.

Results

As expected, feed restriction increased milk spontaneous lipolysis and this was associated with an increase in lipoprotein lipase enzyme activity. Transcriptomic analyses from mammary biopsy samples revealed 85 DEGs between the restricted and control diets ($P < 0.1$; 61 upregulated and 24 down regulated with feed restriction) including upregulation of the R1OK2 gene, which codes for a kinase involved in the 40S ribosome maturation ($P < 0.05$). TopGO functional enrichment analysis showed that DEG were mainly involved in biosynthetic process, transcription and cellular assembly without any clear connection with milk lipolysis. Microarray results were confirmed by qPCR with observed trends suggesting downregulations of LPIN-1 and COL3A1 and upregulation of HPSA8 genes. LPL mRNA analyzed by qPCR was down-regulated in mammary biopsy samples and did not vary in milk purified MEC. This suggests that the downregulation of LPL gene in the mammary tissue probably occurs in cells other than mammary epithelial cells.

Conclusions

Taken together, these results suggest that lipolysis is not under the control of transcriptomic regulations and the downregulation of LPL with feed restriction could be a retrocontrol to counteract the higher LPL activity under feed restriction.

S4_P02

The effect of dietary energy source on lactating sow energy metabolism and nitrogen retention*N. Gregory and L.-A. Huber*

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Adequate energy intake is crucial for lactating sows to optimize milk yield and piglet growth and to minimize sow body condition loss. The objective of the study was to assess the impact of dietary energy source on energy metabolism and nitrogen utilization in lactating primiparous sows.

Materials and Methods

Immediately after farrowing, twenty-eight primiparous sows (Yorkshire × Landrace and Yorkshire) were assigned to one of two isocaloric diets with similar standardized ileal digestible (SID) lysine contents (2,567 kcal/kg net energy and 0.76% SID lysine): [1] a diet that provided 87.5% of calories from carbohydrates and 2.5% from fat (CARB) or [2] a diet that provided 3.8× more calories from fat at the expense of carbohydrates (FAT). Litters were standardized to 12 ± 1 piglets within 24 hr of birth and were not provided creep feed; weaning occurred on lactation day 21. On days 4 and 14 of lactation, a jugular catheter was placed in a subset of sows ($n \sim 18$) to perform a frequently sampled intravenous glucose tolerance test (FSIGTT) on days 5 and 15 and an oral fat tolerance test (OFTT) on days 6 and 16 of lactation. Area under the curve (AUC) was calculated for glucose and insulin for the FSIGTT and triglycerides (TG) for the OFTT. Two nitrogen balances using total urine collection and fecal grab sampling were conducted between days 7 and 11 (NB1) and days 17 and 21 (NB2) of lactation from a subset of sows ($n \sim 22$). An ANOVA analysis was performed with the fixed effects of dietary treatment, day of lactation, and the interaction between dietary treatment and day of lactation; the interaction was not significant, thus, only main effects were presented.

Results

No dietary treatment differences were observed for sow average daily feed intake (5.5 ± 0.3 kg), body weight change (-20.6 ± 3.4 kg), backfat loss (-3.4 ± 0.5 mm), milk production (9.0 ± 0.7 kg/day) or piglet average daily gain (194 ± 9 g) over the 21-day lactation period. The AUC for glucose and insulin was not different between dietary treatments but decreased between days 5 and 15 (367 vs. 217 ± 81 mM min and 15 vs. 10 ± 3 nM min for glucose and insulin, respectively; Day $P < 0.01$). FAT-fed sows had lower overall AUC for TG compared to CARB-fed sows (1.2 vs. 1.7 ± 0.4 mM hour; Treatment; $P < 0.05$) with no difference between days. Nitrogen intake, total nitrogen excretion, and nitrogen retention did not differ between dietary treatments but increased from NB1 to NB2 (Day; $P < 0.05$).

Conclusions

The apparent clearance rate of glucose and nitrogen retention increased later in lactation, regardless of energy source, suggesting that lactating primiparous sows are less sensitive to insulin in early lactation. FAT-fed sows appeared to clear more TG throughout lactation, suggesting an increased ability to utilize energy from ketogenic sources. Therefore, lactating sows are capable of adapting to dietary energy source with no apparent impact on nitrogen utilization or performance

S4_P03

Mastitis induces proteomic cargo changes in a subpopulation of CD81 extracellular vesicles in milk

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Introduction

Mammary gland cells release extracellular vesicles (EVs) in milk, and changes in their content occur during pathological conditions such as mastitis (Saenz-de-Juano et al., 2022). EVs are a heterogeneous family of lipid bilayer-bound vehicles that shield their cargo from enzymatic degradation in body fluids, facilitating the transportation of proteins, nucleic acids, or lipids to target cells over distances. The tetraspanins (e.g CD9, CD63 and CD81) are EV surface proteins. Their primary functions include EV formation, release, and uptake (Giovanazzi et al., 2023). Common milk EVs isolation techniques do not allow the purification of EV subpopulations based on their surface tetraspanins. In this study, we used a novel portable microstructured electrochemical device (PMED; Krivitsky et al., 2023) to isolate specific CD81-milk EVs and to assess the different proteomic cargo in quarters affected by mastitis.

Materials and Methods

Milk samples were manually obtained from 3 dairy cows exhibiting symptoms of mastitis in single quarters. From each cow, two quarters were selected and classified as either inflamed or healthy based on the somatic cell count (SCC). The SCC of inflamed quarter milk ranged between 434'000 and 4'493'000 cells/ml, while the SCC of healthy quarters ranged between 21'000 and 53'000 cells/ml. Before EVs isolation, milk fat, milk cells and caseins were removed by differential centrifugation, acid precipitation and filtration. Then, EVs were extracted from skimmed milk using a novel PMED to isolate CD81-EVs. Nano tracking analysis (NTA) was employed to quantify the extracted EVs, and a proteomic analysis of EV cargo was conducted using bottom-up proteomics coupled with Data-Independent Acquisition (DIA).

Results

The novel PMED EV isolation method was successfully implemented. The results showed an increase in CD81-EVs yield that correlated with the milk SCC. The proteomic analysis identified 1332 unique proteins in CD81-milk EVs. Comparing the proteome content from high and low SCC quarters EV revealed significant alterations in the protein cargo related to inflammatory responses, complement cascade activation, and plasma lipoprotein regulation, confirming the involvement of milk EVs in mastitis pathology.

Conclusions

The utilization of PMED represents a promising advancement for studying milk EVs, particularly in discerning specific subpopulations such as CD81-positive EVs. The observed alterations in protein cargo between high and low SCC quarters underscore the potential diagnostic and prognostic utility of milk EVs in mastitis management.

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S5_P01

Litter independent effects of colostrum intake on piglet growth and survival*P. Langendijk and H. van Hees*Trouw Nutrition Research and Development/Wageningen University, The Netherlands
pieter.langendijk@trouwnutrition.com**Introduction**

Intake of colostrum has been reported by various authors to be important for survival and development of suckling piglets. However, the effects of colostrum are often confounded with birth weight and dam effects (Devillers et al., 2011). For example, heavy birth weights are associated with both a high colostrum intake and high gain to weaning, and it can be assumed that sows that produce more colostrum inherently produce more milk throughout the rest of lactation. This paper describes relationships between colostrum intake and neonatal growth and survival, corrected for birth weight and litter effects.

Materials and Methods

Data were available from 154 litters and 2436 piglets, and included birth weight, weaning weight (at 3.5 wks), colostrum intake, and neonatal survival. Litter effects were accounted for by expressing individual weaning weight relative to litter average, and the resulting weight was then analysed in a linear model with relative colostrum intake (g per kg birth weight) as a class variable and birth weight as a covariate.

Results

Pre-wean mortality was strongly related to colostrum intake, and increased from around 3 % in piglets with >250 g intake, to 9 % when intake was 200 to 250 g, 23 % at 150 to 200 g, and 75 % at <150 g. Weaning weight was strongly related to colostrum intake and birth weight ($R^2 = 0.40$), and corrected for litter effects and birth weight, weaning weights increased from 6.1 kg in piglets with lowest intake to 7.0 kg in piglets with highest colostrum intake ($P < 0.01$; Table 1).

Table 1. Weaning weight, corrected for litter effects and birth weight, in relation to colostrum intake.

	Colostrum intake, g/kg birth weight						
	<150	150-200	200-250	250-300	300-350	350-400	>400
N	224	129	317	507	562	309	120
Weaning weight, kg	6.1 ± 0.2	6.0 ± 0.1	6.3 ± 0.1	6.4 ± 0.1	6.8 ± 0.1	6.7 ± 0.1	7.0 ± 0.1

Conclusions

Independent of birth weight and litter effects, variation in colostrum intake contributes considerably to piglet growth and survival, and is associated with 1 kg variation in weaning weight. These data re-emphasize the essential role of colostrum intake by the neonate piglet.

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S5_P02

Consequences of extended suckling in cow-calf contact systems for growth and fat deposition of calves*C. L. van Zyl^{1,2}, E. A. M. Bokkers², H. K. Eriksson³, B. Kemp¹, S. Agenäs^{3,4}, and A. T. M. van Knegsel¹*¹Adaptation Physiology, WUR, The Netherlands; ²Animal Production Systems, WUR, The Netherlands; ³Applied Animal Science and Welfare, SLU, Sweden; ⁴Beijer Laboratory for Animal Science, SLU, Swedencoenraad.vanzyl@wur.nl**Introduction**

In cow-calf contact (CCC) systems, calves have maternal contact and can suckle until a later age than in conventional dairy production systems, where cow and calf are separated shortly after birth. Pre-weaning growth of calves with prolonged CCC is mostly greater than conventionally reared calves. However, post-weaning growth generally decreases after prolonged CCC, possibly due to the weaning and separation stress experienced. We aimed to determine the consequences of prolonged CCC followed by gradual fence-line weaning at 12 wk on growth and fat deposition of calves until a month after weaning.

Materials and Methods

Calves either had CCC (CCC, n = 19) or were group-housed and conventionally reared with no contact with cows (NC, n = 22). From separation at 12 h after birth onwards, NC calves received 4 L of whole milk twice daily from teat buckets. Unrestricted contact between CCC cows and calves was allowed until the start of gradual weaning and separation in the 10th week. During weaning, physical CCC was possible, but suckling was restricted via a fence-line. CCC calves were weaned in week 12 and separated from cows in week 13. NC calves were gradually weaned over the same period and at the same age. Calves were weighed at 9 moments, and backfat thickness (BFT) at 9 moments, via ultrasonography. Body weight and BFT of calves were analyzed separately via linear mixed models with repeated measures. Average daily gain (ADG) of all calves during the pre-weaning period, 1 wk post-wean, 1 wk post-separation and during the total post-weaning period were analyzed via linear mixed models. Blood of all calves was sampled for analysis of glucose, insulin, insulin-like growth factor 1 and non-esterified fatty acid concentrations. Values represent LSM and SEM.

Results

CCC calves were consistently heavier than NC calves. Average daily gain of FC calves was greater than NC calves pre-weaning (1.37 ± 0.04 vs. 1.03 ± 0.04 kg/d; $P < 0.01$), but lower during the weaning period (0.59 ± 0.11 vs. 1.05 ± 0.12 kg/d; $P < 0.01$). During the wk after separation, ADG of CCC calves was lower than NC calves (1.00 ± 0.10 vs. 1.42 ± 0.11 kg/d; $P < 0.01$). Backfat thickness of CCC calves was greater than NC calves from start of weaning until 4 wk post-weaning (4 wk post-weaning: 0.73 ± 0.03 vs. 0.61 ± 0.03 cm, $P < 0.01$).

Conclusions

The higher ADG of the CCC calves preweaning was likely due to higher milk intake. Decreased ADG shortly after weaning could be due to weaning and separation stress, as well as CCC calves not being as adapted to solid feeds pre-separation as NC calves. Backfat thickness of FC was greater than NC calves. Additional studies are necessary to assess the long-term effects of higher ADG and back fat thickness in calves raised in CCC systems, particularly regarding their metabolic status and reproductive performance both during adolescence and adulthood.

S5_P03

Serum osteocalcin and ALP concentrations in replacement heifers

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Serum concentrations of (bone-specific) alkaline phosphatase (ALP) and bone biomarkers such as osteocalcin (OC) can be used to monitor bone metabolism in animals. The objective of this study was to investigate serum concentrations of OC and ALP in 2- to 4-month-old and 12- to 14-month-old replacement heifers and to calculate reference intervals for these age groups.

Materials and Methods

Serum samples were collected on 52 Dutch dairy farms from replacement heifers of two to four months of age (n=126) and 12 to 14 months of age (n=131). OC concentrations in serum were analyzed using a N-MID Osteocalcin test kit on an IDS-iSYS Multi-Discipline Automated System (IDS). ALP concentrations in serum were analyzed with an enzymatic method on a UniCel DxC 600 Synchron Clinical System (Beckman Coulter). Descriptive statistics were performed and differences in serum parameters between age groups were investigated with a Wilcoxon Rank Sum Test. Associations between serum parameters were described for each age group with a Spearman rank correlation coefficient. All analyses were performed in Statistix Version 8.0 using a significance level of $P < 0.05$. Age-specific reference intervals were calculated using Reference Value Advisor (Geffre et al., 2011).

Results

Concentrations of OC and ALP are shown in Table 1. Serum OC and ALP concentrations were larger in younger than in older replacement heifers ($P < 0.001$). Correlation coefficients for OC and ALP concentrations in younger and older replacement heifers were $r_s = 0.32$ and $r_s = 0.30$, respectively ($P < 0.001$).

Table 1: Descriptive statistics and reference intervals for concentrations of alkaline phosphatase (ALP, in U/L) and osteocalcin (OC, in ng/mL) in replacement heifers of different age groups from 52 Dutch dairy farms.

Age group	Parameter	N	Mean	SD	Median	IQR	Reference interval
2 to 4 months	ALP	126	242	78	237	186 - 289	133 - 328
	OC	126	47.9	16.3	45.8	38.4 – 57.0	32.1 – 71.3
12 to 14 months	ALP	131	165	56	157	125 – 192	102 – 216
	OC	131	32.2	10.7	30.3	24.3 – 37.8	16.5 – 46.1

*SD = standard deviation; IQR = interquartile range

Conclusions

Serum ALP and OC concentrations differ in replacement heifers from different age groups. Age-specific reference values are needed for correct interpretation of serum ALP and OC concentrations.

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S5_P04

Effect of energy content of high-protein milk replacer on dry matter intake and growth performance of Holstein calves in summer and winter

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Introduction

Young calves are particularly sensitive to thermal stress, growth performance decreases when exposed to either heat or cold conditions. The energy requirements for maintenance are increased outside the thermoneutral zone [1]. However, the precise energy composition of milk replacers (MR) in relation to varying environmental temperatures remains unclear. Therefore, the objective of this study was to evaluate the effects of energy content of high-protein MR on dry matter intake (DMI) and growth performance in Holstein calves subjected to heat or cold stress.

Materials and Methods

Eighty Holstein heifer calves born in summer (average temperature: 20 °C) and winter (4.7 °C) were fed one of the three MR: (1) containing 29.1% CP, 20.5% fat and 4.68 Mcal/kg of metabolizable energy (ME) (n = 26), (2) 28.8% CP, 26.3% fat and 4.98 Mcal/kg of ME (n = 26), (3) 28.9% CP, 31.7% fat and 5.26 Mcal/kg of ME (n = 28), on a DM basis. The treatment MR were offered at 600 g/d (powder basis) from 7 to 13 d, 800 g/d from 14 to 20 d, 1,200 g/d from 21 to 41 d, 800 g/d from 42 to 48 d, and 600 g/d from 49 to 55 d, and then weaned at 56 d of age. Data were collected until 91 d of age. All the calves were fed calf starter and chopped hay ad libitum from 7 d of age. Data were analyzed by Fit Model procedure of JMP® 17 pro (SAS Institute Inc., Cary, NC).

Results

During the early stages (7-20 days), MR treatment had no significant impact on DMI, yet MR intake was lower and solid feed intake was higher during winter compared to summer (P < 0.05). However, growth performance remained unaffected by both MR treatment and season. From days 21 to 42, hay intake decreased as energy content of MR increased (P < 0.05), while calf starter intake was higher during winter (P < 0.05). Nevertheless, growth performance remained unaffected by both MR treatment and season. Subsequently (days 43-55), MR intake increased while calf starter intake decreased as energy content of MR increased (P < 0.05). Additionally, MR intake was lower and solid feed intake was higher during winter (P < 0.05). Notably, average daily gain (ADG) was higher in winter (P < 0.05). After weaning (56-91 days), DMI was unaffected by MR treatment but was higher during winter (P < 0.05). Growth performance remained unaffected by MR treatment, yet ADG and skeletal development were notably higher during winter (P < 0.05).

Conclusions

These results suggested that higher energy MR formulations enhance MR intake while reducing solid feed intake, with no discernible impact on growth performance. During winter, calves exhibit increased reliance on solid feed to satisfy the increase in maintenance energy requirements, accelerating rumen development and promoting post-weaning growth performance.

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S5_P05

Effects of calving interval and periconception conditions of dairy cows on development and metabolism of their offspring in early lifeY. Wang¹, A. Ipema¹, R. Goselink², E. Burgers^{1,2}, J. Gross³, R. Bruckmaier³, B. Kemp¹, and A. van Knegsel¹¹Adaptation Physiology Group, WUR, The Netherlands; ²Wageningen Livestock Research, WUR, The Netherlands;³Veterinary Physiology, Vetsuisse Faculty, University of Bern, Switzerlandyapin.wang@wur.nl**Introduction**

Extending the voluntary waiting period (VWP) for insemination in dairy cows is of interest to reduce the frequency of calving events and inseminate at a moment with better cow conditions around conception. Little is known about the calves in early life born from dams with an extended VWP, and maternal conditions around conception on offspring in early life. The objective of the current study was to identify the effect of extending dam's VWP, and periconception conditions on body condition and metabolic status of their offspring from birth until their first calving.

Materials and Methods

Holstein Friesian dairy cows (N = 154) were blocked according to parity, milk yield, and somatic cell count (SCC), and randomly assigned to a VWP of 50, 125, or 200 d. Heifer calves (N = 62) from cows with different VWP were monitored from birth until their first calving. Not all dams were successfully inseminated soon after the planned VWP, resulting in differences between the intended VWP and the actual calving interval (Clnt). Calves were regrouped according to their dam's actual Clnt (Clnt_1: 324 - 408 d; Clnt_2: 409 - 468 d; Clnt_3: 469 - 586 d). Body weight was recorded for dams and offspring. Dam's blood was collected every 2 weeks from 4 week before to 4 weeks after conception. Offspring's blood was collected every 2 weeks from birth to 11 weeks old, and then every 4 months until calving. Data analyses were performed using a mixed model, with repeated measurements and fixed affects for dam's Clnt, parity, offspring's age and their two-way interactions. To analyse effects of periconception conditions, those were included as a covariable in the model, separately.

Results

From birth to weaning, the calves born to dams in Clnt_1 had a higher plasma non-esterified fatty acids (NEFA) concentration than Clnt_3 calves (0.34 vs. 0.26 mmol/L). For primiparous dams, calves born to dams with a shorter Clnt (Clnt_1) had greater IgG against keyhole limpet hemocyanin (KLH) than Clnt_3 (6.02 vs. 4.60) before weaning. After weaning till calving, Clnt_1-calves from primiparous dams tended to have greater plasma NEFA concentration than Clnt_2-calves. From birth to weaning, dam's milk fat content around conception was positively related to offspring's insulin and glucose concentrations. During weaning to calving, dam's SCC and body weight around conception was positively related to offspring's IgG and IgM against KLH in plasma. Dam's insulin-like growth factor_1 was positively related to offspring's body weight, insulin and IGF_1 from birth to weaning.

Conclusion

From birth to weaning, a longer Clnt in dams can result in a lower natural antibodies, although effects were not present in all Clnt categories and parity groups. The periconception conditions of dams did not largely influence the offspring till weaning. After weaning, dam's Clnt had limited effects on offspring, but other periconception conditions like body weight and milk performance had stronger relationship with offspring's metabolism than before weaning.

S5_P06

Effects of calving interval and periconception conditions of dairy cows on milk performance and metabolism of their offspring in later lifeY. Wang¹, A. Ipema¹, R. Goselink², E. Burgers^{1,2}, J. Gross³, R. Bruckmaier³, B. Kemp¹, and A. van Knegsel¹¹Adaptation Physiology Group, WUR, The Netherlands; ²Wageningen Livestock Research, WUR, The Netherlands;³Veterinary Physiology, Vetsuisse Faculty, University of Bern, Switzerlandyapin.wang@wur.nl**Introduction**

Extending the voluntary waiting period (VWP) for insemination in dairy cows is of interest to reduce the frequency of calving events and inseminate at a moment with better cow conditions around conception. Little is known about the calves in later life born from dams with an extended VWP, and maternal conditions around conception on offspring in later life. The objective of the current study was to identify the effect of extending dam's VWP, and periconception conditions on body condition, metabolic status and milk production of their offspring during the first 100 days in milk (DIM) of the offspring's first lactation.

Materials and Methods

Holstein Friesian dairy cows (N = 154) were blocked according to parity, milk yield, and somatic cell count (SCC), and randomly assigned to a VWP of 50, 125, or 200 d. Heifer calves (N = 62) from those cows were monitored from their first calving until 100 DIM. Not all dams were successfully inseminated soon after the planned VWP, resulting in differences between the intended VWP and the actual calving interval (Clnt). Calves were regrouped according to their dam's actual Clnt (Clnt_1: 324 - 408 d; Clnt_2: 409 - 468 d; Clnt_3: 469 - 586 d). Body weight and milk yield were recorded daily. Dam's blood was collected every 2 weeks from 4 week before to 4 weeks after conception. Offspring's blood was collected weekly during 8 weeks after calving, and subsequently every 2 weeks until 100 DIM. Data analyses were performed using a mixed model, with repeated measurements and fixed affects for dam's Clnt, parity, offspring's age and their two-way interactions. To analyse effects of periconception conditions, those were included as a covariable in the model, separately.

Results

In offspring of primiparous dams, a longer Clnt of the dams resulted in greater body weight (Clnt_3), greater plasma glucose (Clnt_2), and lower milk yield (Clnt_2) during the first 100 DIM, compared with shorter Clnt of the dams (Clnt_1). In offspring of multiparous dams, a longer Clnt of the dams resulted in lower milk yield (Clnt_3) than a shorter Clnt (Clnt_1), and lower plasma insulin-like growth factor-1 concentration (Clnt_2 and Clnt_3) than Clnt_1. Dam's milk fat around conception was positively related to offspring's milk fat and SCC. Dam's milk lactose was negatively related to offspring's milk protein and lactose. Besides, dam's fat and protein corrected milk yield (FPCM) was negatively related to offspring's milk fat, lactose and SCC. Dam's body condition score (BCS) was positively related to offspring's IgG and IgM against keyhole limpet hemocyanin, and negatively to offspring's plasma glucose.

Conclusions

A longer Clnt in dams can result in a greater body weight, lower milk yield during the first 100 DIM, although effects were not present in all Clnt categories and parity groups. Besides calving interval, other periconception conditions like parity, milk fat, milk lactose and FPCM were related to offspring milk performance. Dam's parity and BCS were related to offspring's metabolism.

S5_P07

Metabolic and endocrine responses to different carbohydrates in milk fed calves*G. Bierlein, R. M. Bruckmaier, and J. J. Gross*

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georg.bierlein@unibe.ch**Introduction**

The carbohydrate lactose represents one of the major nutritional factors in milk fed calves. Since the synthesis of this disaccharide in the mammary gland is complex and requires energy, the question arises how lactose could endure in evolution. In addition, lactase is necessary to cleave lactose in the small intestine into its absorbable monosaccharides glucose and galactose. We investigated the endocrine response to different carbohydrates in formula as a substitute for lactose in milk fed calves.

Materials and Methods

Male and female calves ($n = 38$; age: 16.1 ± 0.4 days; body weight: 53.7 ± 1.0 kg) were assigned to 5 different experimental diets (4 kg of formula per meal, 2 meals/day) fed for 3 consecutive days. The composition of the formula was similar to whole milk (WM; 4% fat, 3.4% protein, 4.7% carbohydrates) and contained the following carbohydrates added to a formula, which was based on reconstituted protein extract with added milk fat: lactose (LAC); glucose (GLU), galactose (GAL), glucose + galactose (G/G) and xylose (XYL). On the last day of the treatment blood samples were taken from the jugular vein right before the first feeding (T1), two (T2) and seven (T3) hours later. Concentrations of glucose, triglycerides (TG), insulin, glucagon, and insulin-like growth-factor-1 (IGF-1) were determined in plasma. Data were logarithmized and evaluated using a mixed model (SAS, version 9.4). Fixed effects were treatment and timepoint of sampling and the individual calf as repeated subject.

Results

Plasma glucose concentration increased from T1 to T2 by tendency in G/G ($P = 0.09$) and was more elevated in GLU at T3 than at T1 ($P < 0.05$) and increased by trend in GAL from T2 to T3 ($P = 0.06$). At T1 plasma glucose concentration in XYL was lower than in LAC, GAL, and G/G ($P < 0.05$). At T2 the blood glucose concentration tended to be lower in XYL compared to GLU ($P = 0.10$) and was lower in LAC and G/G ($P < 0.05$). Triglyceride concentration increased from T1 to T2 in all treatments ($P < 0.05$) and remained elevated at T3 compared to T1 ($P < 0.05$). XYL had greater triglyceride concentrations at T1 in comparison with the other treatments ($P < 0.05$). Basal insulin concentration was lower in GLU ($P = 0.07$) and XYL ($P < 0.05$) than in the other treatments and remained lower in XYL at T2 (LAC: $P = 0.09$; GLU, GAL, G/G: $P < 0.05$). Insulin concentration increased from T1 to T2 in GLU and G/G ($P < 0.05$), while glucagon concentration increased from T1 to T2 in GAL and XYL ($P < 0.05$) and by trend in GLU ($P = 0.07$). In all treatments glucagon concentrations were elevated at T3 compared to T1 (G/G: $P = 0.07$; LAC, GLU, GAL, XYL: $P < 0.05$), and even more elevated in XYL ($P < 0.05$). At T1 concentration of IGF-1 was greater in GAL than in GLU and G/G ($P = 0.09$), while in XYL IGF-1 was constantly lower compared to the other treatments ($P < 0.05$).

Conclusions

In conclusion, we observed differences in the metabolic and endocrine response towards the different carbohydrates in formula. Despite a similar gross nutrient composition of the experimental diets the endocrine response following feeding differed between the carbohydrate sources. In calves receiving XYL, no postprandial insulin increase was detected. The increase of plasma glucagon concentration in XYL after feeding confirmed that xylose is absorbed from the intestine but cannot be used in intermediary metabolism.

S6_P01

Effect of feeding glycemic diets to sows in the weaning-to-estrus interval on development of follicles and embryos and piglet birth weight*M. F. Fenner¹, E. Østrup¹, T. Sønderby Bruun², and A. Varmiløse Strathe¹*¹Department of Veterinary and Animal Sciences, University of Copenhagen, Denmark; ²Livestock, SEGES Innovation, Denmarkavst@sund.ku.dk**Introduction**

Increased litter size in hyper-prolific sows leads to more low-birthweight or intra-uterine restricted (IUGR) piglets at birth and a higher within-litter variation in birth weight. The objective of the study was to test if addition of glucose or fructose to the sows' diet in the weaning-to-estrus interval would have an effect on 1) blood hormones and follicle number and size at ovulation, 2) embryo number and size eight days post mating, and 3) individual piglet birth weight.

Materials and Methods

Sows (Landrace x Yorkshire) in all three experiments were fed either a 1) 4.0 kg control diet, 2) 4.0 kg control diet + 400 g glucose, and 3) 4.0 kg control diet + 400 g fructose during the weaning-to-estrus interval. In experiment 1, 36 sows were included. Blood samples were taken at weaning (T0) and 3 days post-weaning (T1) from the jugular vein. Whole-blood was analyzed for glucose and serum for insulin and IGF-1 concentrations, respectively. The day after showing the first signs of standing heat, the sows were euthanized and the ovaries were removed and fixed in formalin for MRI scans, where number, size, and distribution between ovaries of follicles were measured. In experiment 2, 45 sows were included and slaughtered 8 days post-mating, where ovaries were removed, and embryos were flushed from the uterine horns. Ovaries were MRI scanned to detect number and size of corpora lutea and flushed embryos were staged using stereomicroscopy. In experiment 3, 290 sows were included. At the time of farrowing both litter size and individual birth weight were registered. Data were fitted using a mixed-effects model in ANOVA using the nlme package in R. Models included batch as a random effect, diet was included as fixed effect and parity as covariate.

Results

Experiment 1: The blood glucose concentrations were similar in all groups at T0 and T1 ($P > 0.05$). Systemically measured insulin and IGF-1 increased from T0 to T1 in both glucose and fructose groups, but not significantly ($P > 0.05$). The total number of follicles and distribution of follicle size was also similar between groups ($P > 0.05$). However, in the glucose and fructose groups there was a more even distribution of follicles between the two ovaries ($P = 0.20$) compared to control group ($P = 0.07$). Experiment 2: The total number of corpora lutea did not differ between groups. In individual sows the number of corpora lutea was similar between ovaries in the glucose group, whereas it was different in the control and fructose groups ($P < 0.001$). The mean corpus luteum volume was similar ($P > 0.05$), but in control and fructose groups there was a tendency for larger volume on the ovaries with lower number of corpora lutea ($P < 0.10$). Mean number of flushed embryos were similar between groups, but there was a larger variation in developmental stage in fructose and control sows compared to glucose. Experiment 3: Litter weight, average birth weight and within-litter variation in birth weight were similar between groups ($P > 0.05$). Sows in the glucose group had more total born piglets (23.7) compared to sows in fructose (22.3) and control (22.8) groups (SEM=0.38; $P < 0.05$).

Conclusions

The addition of glucose in the weaning-to-estrus interval ensured a more even distribution of follicles and corpora lutea between uterine horns, which could lead to increased embryo survival and explain the higher number of total born piglets.

S6_P02

Acute endotoxemia inhibits luteinizing hormone and decreases hypothalamic kisspeptin in adult cowsA. Renwick¹, B. Whitlock¹, C. Nestor², A. Esteller-Vico¹, and L. Amelse¹¹University of Tennessee College of Veterinary Medicine, USA; ²North Carolina State University, USAarenwick@vols.utk.edu**Introduction**

Inflammatory diseases such as metritis, mastitis, and subacute rumen acidosis are commonly associated with infertility in livestock. Lipopolysaccharide (LPS; endotoxin), a component of the cell wall of Gram-negative bacteria, is associated with inflammation during infections and non-infectious diseases and is a cause of impaired reproduction. Acute LPS administration suppresses kisspeptin expression in the arcuate nucleus female [2] rats. In ewes, a relatively low acute dose of endotoxin inhibits the LH surge and suppresses kisspeptin expression in the preoptic area and arcuate nucleus [1]. However, there is limited data in other domestic, non-seasonal breeding, livestock species.

Materials and Methods

Fifteen Angus cows (multiparous, 2.5 to 4 years old) were utilized in two experiments. In Experiment 1, cows (~2.5 years old; 560.5 ± 19.2 kg BW) were randomly assigned to two groups: control (CON1; n=3) and endotoxin (acute endotoxin; 400ENDO; n=3). The CON1 group received 2 mL saline intravenously (IV) and 400ENDO received 400 ng of LPS/kg of BW IV. On the experiment day, the cows underwent serial blood collection with a sample every 12 minutes for six hours (1-hour pre-LPS/saline treatment and 5 hours post-LPS/saline treatment). In Experiment 2, cows (~4 years old; 598.6 ± 17.2 kg BW) were randomly assigned to two groups: control (saline; CON2; n=4) and endotoxin (acute endotoxin; 600ENDO; n=5). Cows in CON2 received 2 mL saline IV while those in 600ENDO received 600 ng of LPS/kg of BW IV. On the day of the experiment, blood samples were collected hourly for 5 hours followed by hypothalamic tissue collection. Tissue was analyzed using immunohistochemistry to evaluate kisspeptin expression.

Results

Cortisol concentration was increased two hours after LPS treatment in 400ENDO (9.0 ± 1.8 ug/mL) and 600ENDO (7.2 ± 0.9 ug/mL) compared to CON1 (0.4 ± 0.1 ug/mL) and CON2 (1.3 ± 0.3 ug/mL), respectively. Concentration of IL-6 was increased in 400ENDO compared to CON1 and 600ENDO compared to CON2 beginning 2 hours after treatment administration through end of experiments. LH pulse frequency was decreased in 400ENDO (0.5 ± 0.1 pulse/hr) compared to CON1 (1.1 ± 0.1 pulse/hr) and LH concentration was decreased in 600ENDO (1.9 ± 0.1 ng/mL) following LPS compared to CON2 (2.4 ± 0.1 ng/mL). There were fewer kisspeptin immunopositive cells in the preoptic area of 600ENDO (36.3 ± 8.3 cells) compared to CON2 (73.4 ± 9.3 cells) and in the arcuate nucleus of 600ENDO (62.3 ± 6.2 cells) compared to CON2 (87.3 ± 8.0 cells).

Conclusions

Inflammation associated with LPS induced endotoxemia suppresses the reproductive neuroendocrine axis.

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S7_P01

Somatic cell count in milk of goats under Slovakian dairy practice

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Introduction

Mastitis in goats is responsible for a decrease in milk yield, change in content of protein, and a decrease of lactose and fat content (Novac & Andrei, 2020). The intramammary infection is the main factor causing an increase of somatic cell count (SCC) in milk and thus SCC considered as a key standard for mastitis detection. However, it is not so easy to interpret high SCC to detect mastitis in goats as it is in cows (Tvarožková et al., 2023). The aim of this study was to evaluate the SCC in milk of dairy goats under Slovakian dairy practice and to assess the effects of breeds and farms.

Materials and Methods

The study was performed at six dairy farms with differed breed of goats (Anglo-Nubian(AN); Alpine (AL); White Shorthair (WSH)). Milk samples were collected during late spring and summer at the morning milking over years 2021-2023. In total 1523 samples were collected. SCC was determined using a Fossomatic 90 instrument (Foss Electric, Hillerod, Denmark). According to individual SCC in milk of goats, samples were divided into four SCC classes (<500x10³, 501 – 1000x10³, 1001 – 2000x10³, > 2 001x10³ cells.ml⁻¹).

Results

From all analyzed samples there were 20.49%, 17.79%, 28.82%, 32.91% samples in above mentioned SCC groups, respectively. The effects of the years, farms and breeds on milk samples distribution in SCC groups are presented in Table 1. The frequency of distribution of milk samples are differed among the involved farms and even among the study years. Moreover, the percentage of milk samples in last SCC group increased over the study year (20.42%, 31.07% and 42,36%, in 2021, 2022 and 2023, respectively).

Table 1. The frequency of milk samples distribution in SCC classes at farm and breed levels within year

Farms (breed)	SCC classes in 2021, x.10 ³ .ml ⁻¹				SCC classes in 2022, x.10 ³ .ml ⁻¹				SCC classes in 2023, x.10 ³ .ml ⁻¹			
	< 500	501-1000	1001-2000	> 2001	< 500	501-1000	1001-2000	> 2001	< 500	501-1000	1001-2000	> 2001
Farm A (AN), %	42.31	28.85	21.15	7.69	20.00	34.29	22.86	22.86	35.42	12.50	16.67	35.42
Farm B (AL), %	43.78	28.98	13.04	14.49	41.67	22.22	16.67	19.44	18.03	21.31	22.95	37.70
Farm C (AN), %	43.33	21.69	23.33	23.33	65.52	17.24	10.34	6.90	36.00	16.00	24.00	24.00
Farm D (AN), %	19.28	21.69	19.28	39.76	34.92	7.94	28.57	28.57	21.00	34.00	26.00	19.00
Farm E (AL), %	38.46	18.18	27.27	16.08	30.26	39.47	11.84	18.42				
Farm F (WSh), %					9.84	14.75	36.75	39.34	0.54	7.34	40.49	51.63

Conclusions

High differences in milk samples distribution in SCC classes point out the importance of SCC in detection of health problems of the mammary gland in dairy goats. Although it is not so easy to use SCC as indicator of udder health, the regular SCC analysis should be involved into the management of dairy goats contributing to maintain good udder health. (Projects: VEGA 1/0597/22; APVV – 21 – 0134)

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S7_P02

Subclinical mastitis and antimicrobial susceptibility of *Staphylococcus* spp. isolated from two Slovakian goat farms

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Introduction

Many studies have reported a steady increase in the incidence of subclinical mastitis caused by coagulase-negative staphylococci (CNS) along with an increase of antibiotic resistance of bacterial pathogens in small ruminants (Lysitsas et al., 2023). The importance of CNS is currently highly controversial, but these microorganisms can persist in the udder and adversely affect milk yield and increasing somatic cell counts (Supré et al., 2011). Currently, traditional mastitis control programs are not applied in Slovak dairy goat farms. The aim of the study was to evaluate somatic cell count (SCC), the prevalence of bacterial pathogens and to determine their susceptibility to antibiotics at two Slovakian dairy goat farms.

Materials and Methods

At total, 90 half udder milk samples from two dairy herds (farm1= 34 goats, farm2= 11 goats) in Slovakia were aseptically collected during lactation for SCC evaluation, identified of pathogens and antimicrobial susceptibility testing. SCC were determined using a Somacount 150 (Bentley Czech, USA). According to individual SCC in milk of goats, samples were divided into four SCC groups: SCC1 < 500; SCC2 ≥ 500 < 1000; SCC3 ≥ 1000 < 2000; SCC4 ≥ 2000, all × 10³cells.ml⁻¹. Milk samples (10 µl) were cultivated on blood-agar plates and evaluated after 24-48 hours. Pure bacterial colonies were classified by MALDI-TOF MS (Bruker Daltonics, Bremen, Germany). Antimicrobial susceptibility tested according to the CLSI standard (2018). Test discs (Bio-Rad, Marnes-la-Coquette-France) were used to test the susceptibility of *Staphylococcus* spp. to 8 types of antibiotics. The diameters of the inhibition zones were evaluated (susceptible, intermediate, resistant) according to CLSI breakpoints.

Results

From all analyzed samples 10% of samples (farm1 5.6% and farm2 4.4%) were in SCC3 and 14.4% were in SCC4 (farm1 3.3% and farm2 11.1%), respectively. Our results showed higher prevalence of bacterial pathogens in milk samples in the farm2 77.3% (n= 17/22) compared with farm1 17.64% (n=12/68). The most common identified bacterial pathogens were CNS, especially *Staphylococcus* (*S.*) *epidermidis* (n=9) with *S. simulans* (n=6) and *S. caprae* (n=5). *S. aureus* was identified only in one sample and one sample were considered as contaminated. No evidence of high resistance of CNS was observed among the tested antibiotics. In total of nine *S. epidermidis* isolates, only one were resistant to tetracycline. In *S. aureus* we found 100% resistance to neomycin and ceftiofur

Conclusions

Although no clear evidence of high bacterial resistance to antibiotics was recorded in this study, monitoring of pathogen prevalence and antibiotic resistance should be routinely carried out to ensure the best treatment choices for mastitis in goats to guarantee the safety of goat products.

(Projects: VEGA 1/0597/22; APVV – 21 – 0134)

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S7_P03

The effect of entry order into milking parlour and stadium of lactation on milk yield and milk composition

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lucia.macuhova@nppc.sk**Introduction**

One of the factors affecting milk production in dairy animals is the order of entry into the parlor and the stage of lactation. A change in the order of entry of the animal into the parlor may be associated with a change in the health status of dairy animal or altered milking conditions (Tancin et al., 2001; Polikarpus et al., 2015). The aim of this study was to investigate whether the order of entry into the parlor and stadium of lactation has an effect on the milk yield and milk composition.

Materials and Methods

95, 91, 94, 96, and 94 animals of the Slovak dairy sheep breed were included in 1st, 2nd, 3rd, 4th, and 5th months of measurement from March to July in the study, resp. The ewes were milked in a one-platform milking parlour with 20 stalls and one milking unit per 2 milking places. According to the order of entry of ewes into the parlor (5 batches in total), animals were divided into three milking groups (1st group - animals from the first batch, 2nd group - animals from the second and third batch, 3rd group - animals from the fourth and fifth batch). Milk composition was determined (fat, protein, lactose, dry matter, and non-fat dry matter; (%)) using a MilkoScan FT120. Somatic cell count was measured with a Somacount 150. Project: APVV-21-0134.

Results

The effect of lactation stadium was significant on the milk yield as well as on its composition. The effect of milking group is shown in Table 1.

Table 1. Parameters of milk yield and milk composition according to ewes milking group (parlor entry)

Parameters	Milking group			P-value
	1 st	2 nd	3 rd	
Milk yield, mL	689.09±31.84	669.15±27.89	666.36±28.75	0.6354
Fat, %	5.92±0.13 ^a	6.39±0.09 ^b	6.70±0.10 ^c	<0.0001
Protein, %	4.91±0.06 ^a	5.15±0.05 ^b	5.29±0.05 ^c	<0.0001
Lactose, %	4.70±0.03	4.91±0.02	4.89±0.02	0.6354
Solids-not-fat, %	10.41±0.07 ^a	10.88±0.06 ^b	11.03±0.06 ^b	<0.0001
Log SCC	1.86±0.06	1.88±0.05	1.92±0.05	0.6317

^{a,b,c} means in the same line without a common superscript letter were significantly different (P < 0.05)

Conclusions

The stadium of lactation had an effect on milk yield and milk composition. The order of entry into the parlor affected only the milk composition, except lactose.

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S7_P04

Inventory of abdominal adipose depots in Dutch dairy cows at slaughter

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Introduction

In dairy cows, abdominal fat deposition is not always accompanied by a high body condition score. As a result, health problems related to (excessive) internal fat deposition in cows are probably underestimated in practice. To assess the prevalence of abdominal fattening in Dutch dairy cows and to investigate the association with external fat deposition, an inventory in a slaughterhouse was carried out.

Materials and Methods

During two inventory moments at a large slaughterhouse in the Netherlands in 2023, fat depositions in the abdomen (omentum) and on the outside of the carcass were scored in 400 Holstein Friesian dairy cows. The omental fat score (OFS) was based on a 5-point scale as described by Van Eetvelde et al. (2011). Carcass fat scores (CFS) were based on the SEUROP classification system (5-point scale) used in the slaughterhouse. Descriptive statistics were performed and associations between OFC and CFS categories were analyzed with median tests (Stata version 17.0, 2021).

Results

25,3% of the cows had an OFS ≥ 4 (95% CI: 21,1-29,8%). In 12,8% of these cows (95% CI: 9.6-16.4%), the CFS was ≤ 3. In 9.8% of the cows, a high OFS (≥ 4) was accompanied by a CFS ≥ 4 (95% CI: 7.0-13.1). Cows with a CFS of 4 always had considerable omental fat deposition (≥ 3).

Table 1. Percentage of cows within a specific omental fat score category per carcass fat score category in 400 dairy cows presented at a Dutch slaughter house.

		Carcass fat score			
		1	2	3	4
Omental fat score	1	1.9%	1.0%	0.0%	0.0%
	1.5	5.1%	1.0%	0.0%	0.0%
	2	22.4%	6.0%	0.0%	0.0%
	2.5	12.8%	8.0%	3.5%	0.0%
	3	34.6%	27.0%	19.8%	10.3%
	3.5	16.0%	35.0%	43.0%	22.4%
	4	5.8%	16.0%	23.3%	37.9%
	4.5	0.6%	5.0%	8.1%	13.8%
	5	0.6%	1.0%	2.3%	15.5%

Conclusions

Based on our data, fattening of dairy cows seems to start in the abdomen and is not always accompanied by a high carcass fat score. Thus, cows with normal body condition scores in practice can have abdominal fat deposition and be at risk for health problems related to (abdominal) fat mobilization.

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S7_P05

Improving IgG determination in goat colostrum using colour through Artificial intelligence

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Introduction

Immunoglobulin G colostrum estimation is crucial in ruminant dairy farms due to agammaglobulinemic or hypogammaglobulinemic status of the ruminant's newborn. Some methods are used at farm, such as colostrometer or refractometer. Colostrum colour was proposed to estimate IgG in goat colostrum by Argüello et al. (2005). Since 2005, artificial intelligence has advanced exponentially. Algorithmic advancements, the exponential increases in computing power and storage and an explosion of data, as highlighted in the McKinsey AI guide (Chui & McCarthy, 2020), have evolved synergistically. This evolution has facilitated access to computational and statistical tools, opening a new realm of possibilities for the scientific community. The objective of the present study was improving the IgG estimation through colour in goat colostrum using Machine learning and Deep learning methodologies.

Materials and Methods

The data set from Argüello et al. (2005) was used. IgG (using ELISA) and CIE colour (L, Cr and Hue) were measured in 813 goat colostrum samples. The data set was randomly divided in two subsets, one for training the models (650 registers) and one for testing the models (163 registers). The models have been generated using Python version 3.11.7. Techniques based on regression have been employed, specifically decision trees in Machine learning, utilizing the Sklearn package, and neural networks in Deep learning through the TensorFlow and Keras packages. Subsequently, in both techniques, the regression obtained has been factored into two values, HIGH in the case of IgG value greater than 20 mg/ml, or LOW otherwise, to generate evaluation metrics under the same conditions as those conducted in the original study.

Results

In the original 2005 study, an Accuracy of 0.87, a Sensitivity of 0.93, a Specificity of 0.71, and a Negative Predictive Value of 0.78 were obtained. Using the neural network-generated model, these values were 0.96, 0.94, 0.97, and 0.97, respectively. Using tree regression, resulting in an Accuracy of 0.98, Sensitivity of 0.97, Specificity of 1, and Negative Predictive Value of 0.94.

Conclusions

The utilization of Machine Learning or Deep Learning techniques represents a significant improvement compared to classical statistical methods on the same sample data, obtaining regression data for IgG that are nearly equivalent to those obtained with laboratory techniques.

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S7_P06

Differing planes of nutrition alter serum amino acid composition in nonpregnant, multiparous beef cows on day 3 of the estrous cycle

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Introduction

Prior to placentation, embryo survival and growth rely on endometrial-derived histotroph to supply nutrients, hormones, growth factors, cytokines, adhesion proteins, and enzymes. Amino acid composition is altered in fetal fluids from nutrient restricted beef cows during early and late gestation (Crouse et al., 2019; Swanson et al., 2022). To maintain a 12-month calving interval, cows need to re-breed during the post-partum period, when they are often in negative energy balance. We hypothesized that differing planes of nutrition would alter performance characteristics, and serum and histotroph nutrient composition in beef cows on day 3 of the estrous cycle.

Materials and Methods

Nonpregnant, multiparous beef cows (n = 18) were stratified by body weight to differing planes of nutrition: maintain body weight (no change in body weight, n = 9; CON) or moderate body weight loss (-0.7 kg/d, n = 9, NEG) for 62 days. Baseline (day 0) and ending (day 62) body weights, carcass ultrasonography, and jugular venous blood was collected. Cows were subjected to the 7-day CO-synch+CIDR estrous synchronization protocol, and slaughtered 3 days after estrus (day 62) for collection of carcass characteristics and uterine histotroph ipsilateral and contralateral to the corpus luteum (CL). Amino acid concentrations were determined by Ultra Performance Liquid Chromatograph. Glucose, non-esterified fatty acids (NEFA), and blood urea nitrogen (BUN) were determined by colorimetric assays. Data were analyzed using the PROC GLM procedure of SAS 9.4 for fixed effects of plane of nutrition. The threshold for significance is $P \leq 0.05$.

Results

There were no differences ($P > 0.05$) in cow age, initial body condition score (BCS), or initial or final body weight between treatment groups. Final BCS, average daily gain, average daily feed intake, and feed efficiency were decreased ($P < 0.05$) in NEG compared with CON cows. There were no differences in carcass ultrasound or characteristics, except dressing percentage was increased ($P = 0.02$) in NEG compared with CON cows. Serum NEFA were increased ($P < 0.01$) in NEG compared with CON cows. There were no differences ($P > 0.05$) in serum BUN, glucose, essential, nonessential, or total amino acid concentrations between treatments. Serum histidine concentration was greater ($P = 0.04$) in NEG vs. CON cows. Serum alanine, isoleucine, and tryptophan concentrations were decreased ($P = 0.02$) in NEG vs. CON cows. There were no differences ($P > 0.05$) in glucose concentrations between histotroph ipsilateral or contralateral to the CL. Amino acid concentrations in histotroph ipsilateral and contralateral to the CL were previously reported (Neville et al., 2023), and interestingly most differences between NEG vs. CON cows were in histotroph contralateral to the CL.

Conclusions

Nutrients are vital for conceptus growth and development, and perturbations in maternal nutrient intake may negatively impact reproductive function, specifically in the uterus as it prepares for the embryo. Furthermore, reductions in tryptophan warrant further investigation due to its impact as an essential amino acid and notable bioactivity.

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S7_P07

Variation of somatic cell count in Slovak dairy sheep

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Introduction

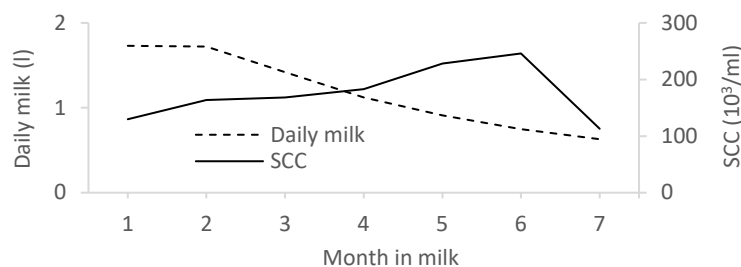
Somatic cell count (SCC) has negative effects on milk traits in sheep. Apart from intramammary infection, SCC is affected by physiological (non-pathological and/or environmental) factors i.e. parity, stage of lactation, season (Paape et al., 2001; Smistad et al., 2021). The objective of this study was to investigate associations between SCC and these factors.

Materials and Methods

Data (1,974 records) from routine milk recording of 487 ewes (single farm, tested in 2022) were analyzed. Mixed model with fixed factors (parity, month in milk, month of recording) and random factors (ewe, error) was considered. Ewes were on the first to third parity, lambed in autumn and winter seasons and tested four times per lactation on average (from 2 to 7 test-day records available). Somatic cell score (SCS) was investigated; inverted exponential transformation of SCS back to SCC was calculated.

Results

The median of SCC was 130 ths., the mode of SCC was 40 ths. per ml of milk. Up to 64% of records had SCC ≤ 200 ths., 12% had SCC >200 ths. ≤ 400 ths., 5.5% had SCC >400 ths. ≤ 600 ths. and SCC >600 ths. ≤ 1 mil., respectively; 13% had SCC >1 mil. SCC per ml. The dependence of SCC on month in milk is given in the figure below. For comparison, daily milk estimates were added.



An important proportion of SCC variation was associated with month of recording (57% of variance explained by the model). Two peaks of SCC (April and July) with a drop in August were found. The drop was probably caused by selection of ewes toward the end of lactation; two peaks could reflect the fact that ewes lambed in autumn (mainly in November) and in winter (mainly in February), respectively.

Conclusions

The study indicated that clinical mastitis probably occurred in analyzed ewes and SCC could contribute to their identification. Monitoring of SCC is optional in Slovakia and no thresholds of SCC have been determined until now.

The support of the project APVV-21-134 is gratefully acknowledged.

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S7_P08

Pathogens of mastitis in suspected mastitis in dairy cows in Slovakia*D. Tančinová, K. Tvarožková, B. Gancárová, M. Árvayová, L. Černek, and V. Tančin*

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Introduction

Bovine mastitis is the most widespread and important disease of the mammary gland worldwide. The health of the mammary gland is critical for dairy industry because this disease has negative impact on milk yield and quality, animal welfare, farm economy and food safety (Smistad et al., 2023). It is estimated that more than 40% of cows worldwide suffer from different types of mastitis. Last study in Slovakia showed, that mastitis is most commonly caused by the pathogens such as *Staphylococcus* spp., *Streptococcus* (Str.) *agalactiae*, *Str. dysgalactiae*, *Str. uberis* and various Gram-negative bacteria (Holko et al., 2019). The aim of our study was to determine the prevalence of mastitis pathogens in dairy cows with suspected mastitis.

Materials and Methods

The milk samples were taken by veterinarians from dairy cows suspected of mastitis. The samples came from 38 herds in 2023 (383 samples). The milk samples (10 µl) were incubated aerobically on blood agar plates (MkB Test a.s. Rosina. SR) at 37°C for 24 h. All grown colonies were identified using MALDI-TOF MS (Bruker Daltonics. Bremen. Germany). Milk samples were considered positive if at least one colony-forming unit (CFU) of contagious pathogens (*S. aureus*, *Str. agalactiae*) was detected. For other pathogens. positive classification required at least five CFU.

Results

In our study the most common mastitis pathogens were coagulase negative staphylococci (CNS) (42.82%). The second frequent identified pathogen was *Escherichia* (*E.*) *coli* (19.32%), followed by *Str. uberis* (9.40%), *Staphylococcus* (*S.*) *aureus* (9.40%), *Enterococcus* spp. (4.44%), *Klebsiella pneumoniae* (4.18%), *Serratia marcescens* and *Corynebacterium bovis* (both 2.35%), and *Str. agalactiae* (1.31%). Also, other pathogens were isolated with less than 1% (*Pasteurella* spp., *Streptococcus dysgalactiae*, *Pseudomonas aeruginosa*, *Trueperella pyogenes*). Major infection pathogens *S. aureus* was still often detected as compared to other infection one *Str. agalactiae*.

Conclusions

In our study, the most common mastitis pathogens were CNS, followed by *E. coli* and *Str. uberis*. *S. aureus* was still often detected. Identification of mastitis pathogens is necessary not only for the application of subsequent therapy but also for the application of appropriate anti-mastitis measures for further prevention in dairy farms.

The support of the project APVV-21-134 is gratefully acknowledged.

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S7_P09

The effect of somatic cell counts and month on the milk yield and its composition during year in dairy sheep

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Introduction

The French Lacaune is used in pure breeding or crossbreeding in order to improve the milk yield of domestic sheep populations (Antunović et al., 2024). Tančin et al. (2017) found out, that Lacaune had higher percentage of milk samples with somatic cell counts (SCC) over 1000×10^3 cells mL^{-1} as compared to other breeds or crossbreds and high percentage with SCC below 400×10^3 cells mL^{-1} which may contribute to the development of legislative limits for sheep milk. The aim of the work was to evaluate the effect of SCC and month on milk yield and its composition during year in dairy sheep.

Materials and Methods

The study was performed at sheep dairy farm with Lacaune with year-round production. Milk samples were collected monthly during year 2022 at morning or evening milking. In total 3131 samples were collected. We monitored the milk yield and its composition (DairySpec FT, Bentley Instruments, Inc., USA). SCC was done by Somacount FC, Bentley Instruments, Inc., USA. According to individual SCC in milk of ewes, samples were divided into five SCC groups ($\leq 200 \times 10^3$, 201 – 400×10^3 , 401 – 600×10^3 , 601 – 1000×10^3 , $> 1001 \times 10^3$ cells. mL^{-1}).

Results

The effect of the month on milk composition are presented in Table 1. We founded lowest milk yield in November and highest in January (920 versus 2003 mL). From all analyzed samples there were 66.48%, 10.53%, 4.83%, 5.29% and 12.87% samples in above mentioned SCC groups, respectively (Table 2). The highest milk yield was found in SCC group $\leq 200 \times 10^3$ and the lowest in $> 1001 \times 10^3$ cells. mL^{-1} .

Table 1. The influence of the month on the milk yield and composition of sheep milk

Month	January	February	March	April	May	June	July	August	September	October	November	December
n	265	266	266	500	273	264	266	155	189	180	176	327
Milk Yield, ml	2003±720	1830±680	1557±470	1583±610	1684±510	1547±450	1182±370	1154±460	1257±480	1288±580	920±470	1505±720
Fat, %	6.13±1.31	6.35±1.51	6.70±1.33	6.53±1.52	6.26±1.16	6.02±1.10	5.90±1.10	5.50±1.39	6.01±1.47	6.29±1.57	8.16±1.42	7.82±1.52
Protein, %	6.47±0.60	6.40±0.61	6.14±0.56	5.77±0.70	5.55±0.41	5.80±0.43	6.34±0.55	5.20±0.71	6.38±0.82	6.53±0.80	7.08±0.71	6.56±0.74
Lactose, %	4.72±0.32	4.65±0.26	4.64±0.24	4.66±0.27	4.78±0.26	4.70±0.30	4.60±0.28	4.79±0.49	4.85±0.50	4.66±0.36	4.45±0.38	4.61±0.38

Table 2. The influence of the somatic cells count (SCC) on the milk yield and composition of sheep milk

SCC groups, $\times 10^3$	≤ 200	201-400	401-600	601-1000	> 1001
n	1307	207	95	104	253
Milk Yield, ml	1645±660	1540±661	1587±674	1527±674	1373±607
Fat, %	6.39±1.56	6.35±1.65	6.48±1.45	6.76±1.45	6.83±1.56
Protein, %	6.03±0.80	6.17±0.77	6.31±0.67	6.24±0.67	6.24±0.80
Lactose, %	4.75±0.29	4.71±0.30	4.67±0.26	4.65±0.26	4.41±0.54

Conclusions

77.01% of the tested samples were below 400×10^3 cells mL^{-1} and only 12.87% over $> 1001 \times 10^3$ cells. mL^{-1} indicating a good status of udder health in studied dairy sheep farm.

The support of the project APVV-21-0134 is gratefully acknowledged

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S7_P10

Somatic cell count before drying off and after calving in antibiotic treated cows by drying off

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juliana.macuhova@lfl.bayern.de**Introduction**

Antibiotics are used to dry off cows to prevent or treat mastitis (Holko et al., 2019). The aim of this study was to evaluate the association between somatic cell count (SCC) before drying off and after calving in antibiotic treated cows before drying off.

Materials and Methods

From the years 2021 to 2023, milk samples were taken at the last milk yield testing before drying off and at the first milk yield testing after calving from 1530 cows during one and in 228 during two lactations on one farm. Milk samples were categorized into 6 SCC groups, and the samples of two lowest and two highest SCC groups before drying off were evaluated (Table 1). SCC was determined using a Fossomatic 90 instrument (Foss Electric, Hillerod, Denmark). Projekt: APVV-18-0121

Results

The count of milk samples according to SCC group (SCC in thousands per ml milk) before drying off and after calving is shown in Table 1. A conducted χ^2 test showed a significant association between the counts of milk samples in SCC groups before drying off and after calving ($p < 0.05$). After calving, the count of milk samples mainly changed in SCC group $> 100 \leq 200$ (722 before drying off vs. 198 after calving); and thereby, the count of milk samples mostly increased in SCC group ≤ 100 . Also, the count of milk samples after calving in SCC groups > 600 decreased (286 before drying off vs. 170 after calving).

Table 1. The count of milk samples observed (expected) according to SCC group (SCC in thousands per ml milk) before drying off and after calving.

SCC group before drying off	SCC group after calving						Total
	≤ 100	$> 100 \leq 200$	$> 200 \leq 400$	$> 400 \leq 600$	$> 600 \leq 1000$	> 1000	
≤ 100	764 (710)	78 (98)	63 (64)	19 (23)	16 (25)	38 (59)	978
$> 100 \leq 200$	509 (524)	88 (72)	43 (47)	19 (17)	17 (19)	46 (43)	722
$> 600 \leq 1000$	76 (97)	13 (13)	13 (9)	7 (3)	12 (3)	12 (8)	133
> 1000	92 (111)	19 (15)	11(10)	2 (4)	6 (4)	23 (9)	153
Total	1441	198	130	47	51	119	1986

Conclusions

The observed reduction in the count of milk samples in both SCC groups with SCC > 600 in thousands per ml milk (and even in the SCC group $> 100 \leq 200$) after calving compared to before drying off supports the positive effect of treatment with antibiotics before drying off. The increase in SCC after calving in some cows (even of cows of group SCC ≤ 100 before drying off) compared to before drying off, could point out on worsening mammary gland health after calving (or already in the time between last milk yield testing and drying off, i.e. even before drying off).

References

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S7_P11

Udder health status, occurrence and antibiotic resistance of pathogens from ewe's milk from one dairy farm in Slovakia*K. Tvarožková, V. Tančin, B. Gacárová, L. Mačuhová, M. Uhrinčať, and M. Vršková*

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Introduction

Mastitis is the health problem of the mammary gland in dairy animals. The disease is associated with economic losses due to a negative impact on milk yield and quality, welfare and treatment costs (Aref et al. 2018). Somatic cells count (SCC) is used to diagnose subclinical mastitis but there is still discussion about the legislative limits for SCC in ewes (Tvarožková et al. 2019). The aim of our study was to describe the frequency of distribution of ewes in SCC groups on the basis SCC per whole lactation and at the end of lactation to assess the occurrence of pathogens in milk samples at the half udder level from ewes with high SCC during whole lactation.

Materials and Methods

A total, 468 udder milk samples from dairy ewe's farm in Slovakia were collected during whole lactation (monthly) for evaluation of SCC. SCC were determined using a Somacount 150 (Bentley Czech, USA). On the basis of SCC from milk recording the ewes were divided into the five SCC groups: G1 = SCC <200 × 10³, G2 = SCC ≥200 <400 × 10³, G3 = SCC ≥400 <600 × 10³, G4 = SCC ≥600 <1000 × 10³ and G5 = SCC ≥1000 × 10³ cells/ml and to evaluate the distribution of ewes into SCC groups. At the end of lactation at drying time the ewes with high SCC during whole lactation (≥1000 × 10³ cells/ml) to detect mastitis pathogens in milk at the half udder level were sampled. Milk samples (10 µl) were cultivated on blood-agar plates and evaluated after 24-48 hours. Bacterial colonies were classified by MALDI-TOF MS (Bruker Daltonics, Bremen, Germany). Antibiotic resistance was tested according to the CLSI (2018). The identified pathogens were tested to 8 selected antibiotics. The diameters of the inhibition zones were evaluated (susceptible, intermediate, resistant) according to CLSI breakpoints (CLSI,2018).

Results

The main results of this study was excellent and surprisingly good udder health based on SCC in milk samples. There were 87.0%, 3.8%, 1.7%, 2.1% and only 5.3% samples throughout lactation in SCC group G1, G2, G3, G4 and G5 respectively. In ewes with high SCC throughout whole lactation the following pathogens at the end of lactation were detected: *Staphylococcus (S.) simulans*, *S. caprae*, *S. piscifermentans* and *S. carnosus*. The most effective antibiotics were sulfamethoxazole/trimethoprim, tetracycline, lincomycin and neomycin.

Conclusions

The regular milk recording and monitoring SCC are important tools to ensure good udder health and to identify ewes for antibiotic treatment or culling at the end of lactation. Results could be used to possible discussion about limits for SCC in raw ewe's milk.

(Projects: VEGA 1/0597/22; APVV – 21 – 0134; GA FAPZ 06/2023)

References

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S7_P12

Virtual fencing for managing lactating Holstein Friesian cows and its effect on animal welfare*P. Fuchs^{1,2}, M. K. Schneider^{2,3}, C. M. Pauler^{2,3}, A. Confessore⁴, C. Umstätter⁵, and M. Probo²*

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Introduction

Virtual Fencing (VF) is based on animal tracking and boundaries can be adapted to local conditions and animal needs. The use of VF in dairy farming could be beneficial as cows require high quality forage to meet their demand. This leads to more frequent paddock changes in pasture-based systems. However, the principle of VF is based on the animal's associative learning of an audio tone (AT; rising pitch) through electric pulses (EP; 0.2 J for 1 s), which might raise concerns from an animal welfare perspective.

Materials and Methods

Two separate experiments (E1 & E2) were conducted to investigate the adaptation process of 20 lactating Holstein-Friesian cows to a VF system during half-day grazing and its impact on animal welfare. In both experiments, the cows were naive to VF. In E1, the herd was divided into four stratified groups. Two groups were introduced to VF and two groups were managed with electric fencing (EF). Each group grazed simultaneously in separate paddocks for four consecutive periods in a rotational grazing system. To assess the effects on animal welfare, cow activity, feed intake, body weight, milk yield and cortisol were analyzed. In E2, the herd was divided into two groups of five younger cows (mean \pm SD: 2.8 \pm 0.3 and 2.8 \pm 0.3 yrs, 1st lactation) and two groups of five older cows each (mean \pm SD: 7.0 \pm 1.4 and 8.0 \pm 3.0 yrs, \geq 4 lactations) to analyze whether age affects cow activity, milk yield and hair cortisol under VF management. Each group grazed simultaneously in separate paddocks for five consecutive periods in a strip-grazing system.

Results

In both trials, the VF system effectively kept the cows within their assigned grazing area. The cows learned to adapt to the VF system and the repetitive situation of a new virtual boundary in both a rotational and a strip grazing management system. In E1, each cow received a mean (\pm SD) number of 1.9 \pm 3.3 ATs and 0.1 \pm 0.7 EPs per day. The number of ATs and EPs decreased over time ($P < 0.001$) with the highest learning curve during the first three days after virtual fence activation. This finding was also evident in E2, with younger and older cows adapting equally fast to VF. Finally, milk yield, milk and hair cortisol, feed intake, body weight as well as activity and lying behavior were not affected by the use of the VF system.

Conclusions

The VF technology can be a helpful tool for managing dairy herds in pasture-based systems. Dairy cows, regardless of age, adapted rapidly to the VF system without compromising their welfare according to the indicators measured and during the periods studied.

S7_P13

Transcriptome based assignment of bovine milk somatic cell typesM. Zorc¹, J. Levantic², S. Džeroski², and P. Dovč¹¹University of Ljubljana, Biotechnical Faculty, Slovenia; ²Institut Josef Stefan, Ljubljana, Slovenia
peter.dovc@bf.uni-lj.si**Introduction**

The mammary gland is a highly specialized regenerative organ and one of the few tissues that undergoes most of its development after birth. The cyclical phases of growth, differentiation, lactation and involution are controlled by hormones and growth factors. The presence of somatic cells in milk is also a consequence of the complex function of the mammary gland and the intense secretion of milk, which differs considerably between species. The most important fractions of somatic cells in milk are epithelial cells, lymphocytes, polymorphonuclear neutrophils (PMN) and macrophages. The majority of epithelial cells present in milk are viable and have the characteristics of fully differentiated alveolar cells. The somatic cell count and differential somatic cell count are often used as markers of udder health. In cattle and sheep, the epithelial cell fraction accounts for only a relatively small proportion of somatic cells in milk, whereas in goat and pig milk, epithelial cells are the predominant cell type in milk. In this study, we demonstrate the use of transcriptomic profiles for identification of cell types in bovine somatic milk cells.

Materials and Methods

In our experiment, we performed single-cell RNA sequencing of somatic milk cells from HF cows. Using 10X genomics chemistry and NovaSeq 6000 (Illumina) sequencing, we obtained 361 million reads and 257 million reads in two biological replicates. Sequencing saturation was determined using Cell Ranger, and the data were further processed using R (version 4.3.2) and the R package Seurat v5. We performed anchor-based integration analysis to analyse all cells in both samples simultaneously. Machine learning approach was applied for interpretation of data.

Results

After normalization and integration of the data, cell clustering revealed 21 distinct cell populations based on their gene expression profiles. We identified classical and intermediate monocytes, naïve, effector and memory CD4+ T cells, naïve and effector CD4+ T cells, immune cells with ISG expression, neutrophils, macrophages, mast cells, platelets, naïve B cells, progenitor cells, dendritic cells, luminal, ductal and alveolar cells. To facilitate the interpretation of our data, we applied various machine learning approaches to address the lack of comprehensive cell type-specific profiles for the bovine model.

Conclusions

Our single-cell RNA sequencing analysis of bovine milk has revealed a cellular landscape of bovine somatic cells highlighting a rich diversity of cell types critical for lactation, immune response and tissue homeostasis. The identification of a substantially higher number of cell types in the somatic cell fraction of milk compared to traditional expectations opens a new horizon for a more complex interpretation of biological processes in the mammary gland.

References

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