



Highlights

in Canadian Dairy Cattle Research

2010



Dairy Farmers
of Canada



Les Producteurs laitiers
du Canada

Reseau laitier canadien
CAN
Canadian Dairy Network



Agriculture and
Agri-Food Canada

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Canada has more than 16 main research institutions, with some 150 researchers doing scientific work connected with dairy production. This research yields a large amount of information essential to the growth and profitability of Canada's dairy industry. This information is disseminated in scientific journals that are often little known to and little used by dairy producers. The Dairy Farmers of Canada (DFC) and the Canadian Dairy Network (CDN) together asked, on behalf of Canadian dairy producers, that a document be developed to inventory the results of the research funded by all Canadian dairy industry partners. The purpose of this document would be to make the results published in the scientific journals accessible to as wide an audience as possible within the dairy industry.

First, we identified from last year's researchers list, the scientific articles published for the period of September 2008 to September 2009. Then we wrote a short abstract in non-technical language for each of the articles, which we grouped into various categories: animal welfare, environment, feeding, genetics, health, milk production and reproduction. Once the abstracts had been written, we contacted the corresponding author or a collaborator when the first author was unavailable to obtain their approval of the information. The necessary modifications were made.

This document is meant to showcase the results of research published by our Canadian researchers and to encourage Canadian industry stakeholders to consult the various scientific journals. With a view to proper interpretation of the results, each article includes a complete reference. Thus, you will be able to use the additional information to access the scientific articles for a better understanding of the research results. Copyright in the scientific articles cited in the document remains the property of the various scientific journals. The document has been revised by Réjean Bouchard, PhD, of the DFC; Brian Van Doormaal, of the CDN, and Jacques Surprenant, PhD, of Agriculture and Agri-Food Canada (AAFC).

Acknowledgements:

This document was made possible by funding from DFC, the CDN and AAFC. It required close collaboration and exceptional teamwork. I want to thank Réjean Bouchard, Brian Van Doormaal and Jacques Surprenant for their support. I also want to thank all the researchers who generously participated in revision of this document, Josée Toulouse, who inventoried the scientific articles, Steve Mason who wrote the abstracts and produced the document and Hélène Lavigne and Shelley Crabtree for validation of the document. Finally, all my thanks to Translation and Text Revision Services of AAFC for its excellent work.

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Animal Welfare





1

Behavior of Dairy Calves after a Low Dose of Bacterial Endotoxin

Journal of Animal Science, November 2008, Volume 86, Number 11, pages 2920-2927

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Changes in behaviour may provide early indicators of pathogen infection in calves. To determine which behavioural indicators might be relevant for this purpose, low doses (0.025 or 0.05 $\mu\text{g/kg}$ body weight) of bacterial endotoxin (lipopolysaccharide, LPS) were administered to calves at 3 or 20 weeks of age. Control calves received an injection of saline. Rectal temperatures of all calves increased in response to endotoxin irrespective of LPS dose or calf age. Behaviour was monitored for a 4 hour period while temperatures were elevated. LPS-treated calves ruminated for an average of 6.4 ± 3.7 minutes and ate hay for 23.1 ± 6.9 minutes in that 4 hour period versus 24.6 ± 6.6 minutes and 31.5 ± 7.5 minutes, respectively, for the same 2 measures in control calves. Self-grooming also decreased in treated calves while they spent more time lying and standing inactive. However, control and LPS-treated calves did not differ in terms of total time spent lying down. Milk and concentrate intakes were also similar for both treated and control calves.

2

Gait Assessment in Dairy Cattle

Animal, January 2009, Volume 3, Number 1, pages 87-95

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Gait assessment, also referred to as gait or locomotion scoring, is aimed at identifying animals that are lame, due to discomfort or pain caused by leg or hoof lesions. This paper reviews research on the development and use of gait assessment methods, including both subjective and automated approaches. Subjective methods are based on scoring components of cow posture and movement. The simplest of these uses a combination of back arch, abnormal gait and reluctance to bear weight on one or more limbs to assign a locomotion score in whole units from 1 to 5; 1 being normal, 5 severely lame. Other systems include observation of abduction (swinging out) and adduction (swinging in) of limbs, evenness of gait, and other abnormal behaviours observed in combination to assign scores. An alternative system, developed by the authors and their colleagues, scores 6 specific components (head bob, back arch, tracking-up, joint flexion, asymmetric gait and reluctance to bear weight) separately, followed by calculation of a combined value from these 6 independent scores. While these subjective methods, based on visual assessment, vary in their ability to detect lameness, their implementation requires no technical equipment and their accuracy can be improved through observer training. Several automated approaches to gait assessment are described, including computerized motion analysis techniques and the use of platforms mounted on load cells that can record forces exerted by each hoof. The authors also discuss how animal (e.g., conformation, size, udder fill) and environmental (e.g., flooring) factors can affect locomotion.



3

Allogrooming in Cattle: Relationships between Social Preferences, Feeding Displacements and Social Dominance

Applied Animal Behaviour Science, January 2009, Volume 116, Number 2-4, pages 141-149

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This study examined factors affecting both positive and negative social interactions among 6 separate groups of 8 cows each, housed in pens with access to free stalls and a common feeding space. Positive interactions observed included close spatial proximity between pairs of cows and mutual licking (allogrooming). Displacement of one cow by another from a position at the feed barrier or from a free stall was considered a negative interaction. Allogrooming was most commonly observed at the feed barrier after fresh feed delivery and between 12 and 2 AM. Dominance rank, evaluated by observing displacements at the feed barrier over a 3 day period, had no effect on allogrooming activity. When social competition was increased by halving the space available to access feed, allogrooming activity decreased, particularly among low dominance rank, first lactation animals. Since allogrooming contributes to coat hygiene and appears to be a behaviour indicating friendship between cows, the authors conclude that first lactations are most likely to be negatively affected by increasing competition for feed.



4

The Impact of Meloxicam on Postsurgical Stress Associated with Cautery Dehorning

Journal of Dairy Science, February 2009, Volume 92, Number 2, pages 540-547

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Cauterizing the horn buds (debudding) of young calves is a routine practice aimed at preventing the growth of horns which, in later life, are an injury hazard for both animals and stock handlers. The use of local anesthetic by way of cornual nerve block is a recommended practice. However, the effect of anesthetic typically lasts only about 2 hours while there is evidence that horn cauterization may cause pain for as long as 24 hours. Earlier research examined the use of non-steroidal anti-inflammatory drugs (NSAIDs) to prolong the period of pain reduction. Both phenylbutazone and ketoprofen had been tested for this purpose but neither of these drugs provided a sufficiently extended analgesic effect. The present study tested another NSAID, meloxicam, which has a much longer half-life in blood, to see if it might be effective in reducing stress over a longer time period. Sixty weaned Holstein heifer calves, ranging from 6 to 12 weeks of age, were initially sham debudded by applying an unheated electric cautery iron to establish baseline responses. On the following day, local anesthetic (lidocaine) was administered 10 minutes before debudding with a hot iron. Half of the calves also received a dose of meloxicam at this time; the other (control) half received a dose of carrier without the drug. At intervals for 24 hours after both sham and actual debudding, heart and respiratory rates were recorded and blood samples were taken for measurement of serum cortisol (stress hormone). Meloxicam calves had lower cortisol levels than controls until 6 hours after debudding but this difference was not seen at 24 hours. Increases in heart and respiratory rates were greater in the control group compared with the meloxicam group indicating that meloxicam was effective in reducing stress due to debudding.



5

Weight Distribution and Gait in Dairy Cattle are Affected by Milking and Late Pregnancy

Journal of Dairy Science, February 2009, Volume 92, Number 2, pages 581-588

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Several methods of scoring cow gait or locomotion have been proposed to identify cows that may be lame. These methods are both time-consuming and subjective as well as being affected by factors other than lameness. Automated methods of assessing locomotion, while potentially less time-consuming and subjective, are still subject to other influences that affect weight distribution among the cow's 4 limbs. The objective of this study was to assess the effects on automated weight distribution measurements of udder fill, pregnancy and standing with front legs elevated. With a full udder, approximately 49% of the cows' weight was borne on the hind legs; after milking, this decreased to 47%. A full udder also resulted in the back legs swinging out more as cows walk which has the effect of increasing subjective gait score. Pregnancy had no effect on weight distribution; both before and after calving, the hind limbs carried 48% of the cows' weight. Although back arch and weight shifting between right and left legs both increased after calving, gait score was not affected. Weight distribution between front and back limbs was not affected when front legs were elevated, as is the case when cows stand with front legs in a stall, rear legs in the alley.



6

Haptoglobin as an Early Indicator of Metritis

Journal of Dairy Science, February 2009, Volume 92, Number 2, pages 621-625

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Metritis is an infection of the uterus that commonly occurs within a few weeks after calving. Haptoglobin (Hp) is a protein that binds to free haemoglobin released from red blood cells, inhibiting bacterial growth by reducing iron availability. Although Hp is a common constituent in the blood of most mammals, it is virtually absent in the blood of healthy cattle, increasing in response to infection. Previous work showed an association between metritis and serum Hp levels but it was not clear from those results whether Hp increased before or after infection. This study was designed to define the time course of Hp elevation in relation to infection in the hope that elevated Hp levels might be used to identify cows at risk of developing metritis. Blood collected from cows at intervals between 20 days pre-calving and 21 days post calving was analysed for Hp concentration. After calving, cows were diagnosed as being healthy or having mild or severe metritis. Compared with the healthy cows, those with metritis had elevated serum Hp levels for the first 12 days after calving. While Hp peaked at 3 days in cows with mild metritis and at 6 days for cows with severe metritis, clinical signs did not appear, on average, until 8.6 days post-calving for the mild cases; 5.3 days for the severe cases. Cows with serum Hp levels above a threshold of 1 gram/litre at 3 days post-calving were 6.7 times more likely to develop metritis than cows with lower Hp levels suggesting that testing for blood Hp concentration at this time might assist in the early detection of metritis.



7

Using Behavior to Predict and Identify Ill Health in Animals

Journal of Animal Science, February 2009, Volume 87, Number 2, pages 770-777

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This paper reviews research that deals with evaluating animal health by observing behaviours that are indicative of current illness or risk of future illness. The authors advocate the use of objective measures of behaviour that are more sensitive to subtle changes as opposite to cursory, subjective assessment. For example, they cite research showing that, when dairy farmers rely on the presence of an obvious limp to diagnose lameness, only a small fraction of cows with leg injuries or hoof lesions are identified. A gait scoring system incorporating a number of defined criteria is much more efficient in identifying potential candidates for closer inspection. Automated feed intake recording systems are cited as examples where objective measures can aid in the prediction and diagnosis of illness. Cows diagnosed with metritis after calving had lower feed intakes pre-calving than healthy herd mates. When calves are fed liquid diets through an automated system, records of intake and feeder visits can identify sick calves and those at risk. Observation of behaviours that may not be considered abnormal may also be useful in assessing disease risk. For example, cows that spend extended lengths of time standing on wet concrete flooring place themselves at higher risk of developing hoof lesions. The authors advocate further research aimed at understanding normal behaviours, including interactions between animals, so that departures from those behaviours might be used as indicators or predictors of illness.



8

Feeding Method Affects the Feeding Behavior of Growing Dairy Heifers

Journal of Dairy Science, March 2009, Volume 92, Number 3, pages 1161-1168

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Feeding total mixed rations (TMRs) to dairy cattle at various stages of development has become common practice on most dairy farms. Adoption of TMR feeding was stimulated by the belief that simultaneous consumption of concentrates and forage would reduce the risk of acidosis resulting from the rapid consumption of concentrate alone. However, TMR feeding is less commonly applied to calves immediately after weaning. The objective of the present trial was to demonstrate the advantages of providing a TMR to animals at this stage of development. Prepubescent Holstein heifers were offered identical diets offered in each of 3 different formats: forage (chopped grass hay) and concentrate offered separately, concentrate top-dressed on forage, and forage and concentrate mixed together as a TMR. Feeding behaviour, feed intake and feed sorting were recorded during the period when calves were offered each diet. Providing concentrate either separately or as a top-dress resulted in rapid consumption of large meals of concentrate before hay. Provision of the diet in the form of a TMR resulted in a more even distribution of both hay and concentrate consumption through the day, although the heifers were able to perform a small amount of sorting of the TMR in favour of short particles, largely consisting of concentrate.



9

Effects of Local Anesthetic and a Nonsteroidal Antiinflammatory Drug on Pain Responses of Dairy Calves to Hot-iron Dehorning

Journal of Dairy Science, April 2009, Volume 92, Number 4, pages 1512-1519

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Because horned animals can pose a safety hazard to other animals and animal handlers, horn development is routinely arrested by cauterizing horn buds (debudding) in calves, typically between 2 and 6 weeks of age. Debudding may cause pain to the calf for up to 24 hours and, although it is generally recommended that a local anesthetic (LA) be used, the analgesic effect of LA persists for only about 2 hours. Therefore, several authors have examined the use of non-steroidal anti-inflammatory drugs (NSAIDs) to extend the length of analgesia. In the present study, physiological responses to hot iron debudding with and without administration of LA and the NSAID, Meloxicam, were monitored in 46 calves with an average age of 33 days. Baseline values were established by sham debudding, using an unheated cauterizing iron. After debudding with neither LA or NSAID, heart rate increased by an average 35 beats/minute in the first 5 min and remained elevated for 3 hours. When debudding was performed after only LA administration, heart rate increased and body temperature decreased between 2 and 3 hours later suggesting that the analgesic effect of the LA had worn off. The addition of NSAID administration before debudding eliminated indicators of pain after LA analgesia had waned indicating that the NSAID had prolonged the duration of analgesia.



10

Neck-rail Position in the Free Stall Affects Standing Behavior and Udder and Stall Cleanliness

Journal of Dairy Science, May 2009, Volume 92, Number 5, pages 1979-1985

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Ideally, a well-designed free stall will allow the cow to lie down comfortably without encountering obstructions or to stand with all 4 hooves in the stall. In either position, her tail should be positioned near or over the rear curb so that feces and urine are deposited in the alley rather than contaminating the stall. A critical component of stall design is the neck rail – a rail placed too far back will result in the cow ‘perching’ with only her 2 front hooves in the stall and her rear hooves in the alley, increasing her risk of lameness. If the rail is positioned too far forward, she may defecate and urinate in the stall and if it is too low, it may inhibit her ability to lie down. The present study was designed to evaluate the effects of neck rail placement on the standing, lying and excretion behaviour of Holstein cows ranging from 570 to 880 kg in weight. Neck rails were placed at 130, 145, 160, 175 or 190 cm from the rear curb, 125 cm above the bedded surface. Time spent standing with 4 hooves in the stall increased and perching decreased linearly as the neck rail was moved forward. The response was greatest for small cows, perhaps because, even at 190 cm from the curb, the neck rail inhibited large cows from fully standing in the stall. As expected, when neck rails were placed further forward, stalls were contaminated with more manure and cow hygiene declined. Lying time was not affected by rail position.

11

Cow Comfort in Tie-stalls: Increased Depth of Shavings or Straw Bedding Increases Lying Time

Journal of Dairy Science, June 2009, Volume 92, Number 6, pages 2684-2690

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Although many recent studies have evaluated bedding options for free stalls, few have done so for tie stalls in spite of the fact that almost half of North American dairy cows are housed in the latter. This paper reports on the results of 3 experiments that examined lying behaviour of cows associated with different amounts of shavings or straw used in tie stalls. Total daily lying time, number of lying bouts and the duration of each lying bout were recorded by data loggers attached to the hind limb of each cow. By means of a mercury switch, the data loggers determined once each minute whether the cow's limb was vertical or horizontal. In experiment 1, 4 levels of shavings were tested: 3, 9, 15 and 24 kg/stall. Cows spent approximately 3 more minutes per day lying down for each additional kg of shavings added to the stall. In experiment 2, where 4 levels of straw were tested (1, 3, 5 and 7 kg/stall), lying time increased by 12 minutes for each additional kg of bedding. When 4 lower levels of straw were tested in experiment 3, no differences in lying behaviour were observed between cows in stalls with 0.5, 1, 2 or 3 kg of straw/stall.



12

Feeding Behavior of Calves Fed Small or Large Amounts of Milk

Journal of Dairy Science, June 2009, Volume 92, Number 6, pages 2843-2852

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In 2 separate trials, calves were offered conventional or increased volumes of milk fed through an automatic milk feeding system. The objective was to test whether feeding calves larger than normal amounts of milk would increase calf growth and improve the efficiency of feeder use while having no negative effects on calf health. In the first trial, 2 groups of 25 calves were offered either 4 litres of milk replacer (4MR) or an unrestricted volume of milk replacer (ad lib: AL) per calf each day. Intake by the AL group increased in the first 2 weeks to a plateau averaging 14-16 litres per day that persisted until weaning at 6 weeks. In the second trial, 2 groups of 14 calves were offered either 4 or 12 litres of whole milk/calf/day (4WM or 12 WM). Milk intake by the 12WM group increased to a plateau that averaged 9-10 litres/day after 16 days. Although all groups were offered calf starter from day 1, AL and 12WM calves consumed insignificant amounts of starter before weaning while the 4MR and 4WM calves began consuming solid feed after 22 days on trial. The 4MR calves were consuming an average of 2.3 kg of starter per day at weaning; 4WM calves were consuming an average of about 0.6 kg/day. 4MR and 4WM calves visited the automatic feeders 2.3-2.5 times as often as AL and 12WM calves but 90% of these visits went unrewarded, limiting access to the feeders by other calves. Weight gain was higher for the AL calves in trial 1 while, in trial 2, the 12WM calves grew faster for the first 4 weeks but after that, gains were similar for both groups. Calf health was not affected by the volume of liquid feed offered in either trial.



13

The Stall-design Paradox: Neck Rails Increase Lameness but Improve Udder and Stall Hygiene

Journal of Dairy Science, July 2009, Volume 92, Number 7, pages 3074-3080

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The purpose of the neck rail, as a component of free stall design, is to allow the cow to stand with all 4 hooves in the stall and her hindquarters over the rear curb such that excreta are deposited in the alley rather than contaminating the stall. If the neck rail is positioned too far toward the curb, the cow may 'perch', with 2 hooves in the stall and 2 in the alley. Since the alley surface is typically hard, wet and contaminated with feces, it is possible that perching may compromise the integrity of the rear hooves. This study was designed to test that possibility. Cows were housed in pens with free stalls where neck rails were placed at 118 cm above the stall surface and either 130 or 190 cm from the rear curb. When provided with a stall having a neck rail only 130 cm from the curb, the average cow spent less time (1 versus 27 minutes/day) with all 4 hooves in the stall and more time (49 versus 33 minutes/day) with only 2 feet in the stall, compared with the average cow in a less restrictive stall. After 5 weeks, cows housed with the more restrictive stalls had an average gait score of 3.5, compared with 2.5 for cows in the less restrictive stalls, indicating an increase in lameness. Of 13 new cases of diagnosed lameness, 11 were in the group provided the more restrictive stalls. However, these stalls were less contaminated with manure and cows using them had cleaner udders, requiring less time to prepare them for milking. The authors conclude that improvements in stall and cow cleanliness came at the expense of cow comfort and hoof health.



14

Competition at the Feed Bunk Changes the Feeding, Standing, and Social Behavior of Transition Dairy Cows

Journal of Dairy Science, July 2009, Volume 92, Number 7, pages 3116-3123

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Reduced feed intake in the period between 3 weeks before and 3 weeks after calving (transition period) is believed to be a predisposing factor in the development of a number of post-calving health disorders. This study was designed to demonstrate the effects of increased competition for feed on the behaviour and feed intake of transition cows. To establish baseline intakes, cows were housed in small free stall pens accommodating 6 cows with individual computerized feed bins from 23 to 18 days before their expected calving dates. From 18 days until calving, they were housed in pens accommodating 9 cows with 6 feeders. Three cows in each of these pens had access to their own feeder while pairs of the remaining 6 cows had to share a feeder. Cows were removed from their pre-calving pen for a day to allow for calving in a separate maternity pen after which they were moved to a post-calving pen, again accommodating 9 cows with 6 feeders as before. Feed intake as well as standing and feeding behaviour were recorded for a week before and 2 weeks after calving. Cows that had to compete for access to a feeder displaced one another more often than cows that were assigned their own feeders. Competition did not affect feed intake or standing time of first lactation (primiparous) cows but pre-calving meal length increased. Multiparous cows forced to share a feeder spent less time eating at each visit to the feeder in the week before and the week after calving than did their pen mates that did not have to compete. Competitively-fed multiparous cows also spent more time standing without eating in the week after calving.



15

Effect of Dietary Dry Matter Concentration on the Sorting Behavior of Lactating Dairy Cows Fed a Total Mixed Ration

Journal of Dairy Science, July 2009, Volume 92, Number 7, pages 3292-3298

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When a total mixed ration (TMR) is thought to be too dry, it is common practice to add water with the aim of reducing ingredient separation. The objective of this study was to determine if adding water to a high-moisture TMR reduces sorting. Two diets containing the same ingredients but differing in dry matter (DM) content were offered to each of 2 groups of cows. Diet 1 (dry) was 57.6% DM; diet 2 (wet) was reduced to 47.9% DM by adding water. After 3 weeks on their respective diets, each group was then switched to the other diet for the same period. Dry matter intake, milk production and milk composition were monitored as well as particle size distributions in fresh feed and orts (unconsumed feed). Contrary to popular belief and the hypothesis of this study, cows sorted the wet diet more extensively than the dry diet. Sorting of the dry diet was limited to a tendency to refuse short particles, whereas the wet diet was sorted against long particles and in favour of short and fine particles. Water addition reduced DMI, neutral detergent fibre intake, and starch intake of cows on the wet diet. Neither milk production nor milk component yields were affected by treatment. These results suggest that water addition to high-moisture total mixed rations, containing primarily haylage and silage as forage sources, may not be an effective method to reduce sorting. Furthermore, water addition may negatively affect DMI and encourage sorting, resulting in the consumption of a ration with different nutrient composition than intended.



16

Does a Calf's Motivation to Ingest Colostrum Depend on Time Since Birth, Calf Vigor, or Provision of Heat?

Journal of Dairy Science, August 2009, Volume 92, Number 8, pages 3915-3921

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This study examined factors affecting calves' motivation to consume colostrum. Calf vigour was assessed by observation of behaviour in the first hour after birth and suckling events were recorded over the period in which the calf remained with its dam. Calves were removed from their dams and placed in individual pens at either 55-70 minutes or at 4-5 hours after birth. Half of the individual calf pens were equipped with heat lamps which were turned on immediately after placing the calf. After acclimating to their individual pens for one hour, each calf was offered an unlimited amount of thawed and heated colostrum in a teat-bottle. Colostrum was drawn from several common pools, frozen in individual portions before the beginning of the study. Vigour was again assessed while feeding. Interval from birth to first feeding had no effect on colostrum consumption; neither did the presence of a heat lamp in a calf's pen. Birth weight and calf vigour did account for higher rates of consumption by some calves. Duration of colostrum feeding was longer for calves that had suckled their dams and when room temperature at feeding time was higher.

17

Preference and Usage of Pasture versus Free-stall Housing by Lactating Dairy Cattle

Journal of Dairy Science, August 2009, Volume 92, Number 8, pages 3651-3658

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It is often assumed that pasture access is more favourable for cattle welfare than our typical total confinement dairy production facility. The objective of this study was to test that assumption by allowing cows to express their preferences for these two environments. Lactating cows were initially acclimated to both indoor and pasture environments by keeping them in the (free stall) barn between AM and PM milkings; moving them to pasture 2 hours after PM milking until they were brought in for the next AM milking. After a week of acclimation, half of the cows were restricted to barn access for 2 days, pasture access for the next 2 days, then offered the choice of barn or pasture for a subsequent 3 days. The sequence of restriction was reversed for the other half of the cows. These patterns were repeated 3 times to allow for changes in environmental and climatic conditions. When given a choice, cows spent an average of 13 hours/day on pasture, mainly at night. Pasture preference decreased during the day when temperature-humidity index rose and at night when it rained. Cows chose to spend more (average 69%) of their lying time on pasture rather than in their free stalls. When cows were provided access to both barn and pasture, a large proportion of their time spent in the barn was attributed to feeding, although they spent 1 hour per day less time eating and consumed 2.9 kg/day less dry matter than cows that were confined in the barn.



18

Competition for Feed Affects the Feeding Behavior of Growing Dairy Heifers

Journal of Dairy Science, August 2009, Volume 92, Number 8, pages 3922-3929

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This study was designed to test the effect of competition on the feeding behaviour of prepubertal dairy heifers. Thirty-six heifers averaging 7.5 months of age and 235 kg in weight were divided into 4 groups of 9 animals each. Total mixed rations (TMRs) were offered in computer monitored and controlled feed bins such that 3 heifers in each group had access to their own individual bins while the other 6 animals were required to share a bin with one other pen mate. Feeding behaviour, feed intake and feed sorting were monitored for the last 7 days of a 14 day trial period. Heifers required to share feed bins had 10% shorter total daily feeding times and consumed 9% fewer meals per day. However, the duration of their meals averaged 10% longer and their feed consumption during those meals was 13% greater. These animals also exhibited greater day-to-day variation in feeding time, meal duration, and meal size although their average daily dry matter intake was no different from the heifers that were not required to share feed bins. Feed sorting activity was also similar between treatments; all heifers selected against long particles in favour of medium and short particles.

19

Temperature Preferences and Feed Level of the Newborn Dairy Calf

Applied Animal Behaviour Science, August 2009, Volume 120, Number 1-2, pages 56-61

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The objective of this study was to examine whether the amount of milk offered to neonatal calves affected their preference for ambient temperature or their lying posture. Calves were housed for 3 days after birth in pens 1.96 metres wide by 3.81 metres long in which 2 heat lamps were placed in one end, establishing a temperature gradient from that end to the opposite (cold) end of the pen. Every 20 minutes for 24 hours, each calf's lying posture and position in the pen was recorded. A temperature logger, fitted on the back of each calf, recorded ambient temperature. Twelve calves were offered a daily allowance of milk equal to 8% of their body weight (BW); another group of 15 calves were offered milk at 30% of BW. Groups were balanced for sex, birth weight and coat colour. Feeding level did not affect either the ambient temperature chosen by calves or their lying posture. Calves in both milk allowance groups spent a little over 50% of their time lying in the heat lamp end of their pens. The authors recommend the use of heat lamps, based on the observation that all calves preferred to lie under them.



20

Laterality of Lying Behaviour in Dairy Cattle

Applied Animal Behaviour Science, September 2009, Volume 120, Number 3-4, pages 125-131

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This report summarizes data from 6 previous experiments where the lying and standing behaviour of cows was observed by continuous video recording. It examines factors that influence the side of the body on which cows lie down (laterality). Although a few groups of lactating cows housed in free stalls spent more time (56%) lying on their left sides, most, including cows on pasture, showed no preference for lying on either side. More often than not (64% of the time) cows switched sides in consecutive lying events, particularly if the previous event was either recent or relatively long (e.g., 80+ minutes). The probability of terminating a lying bout (by either standing or switching sides) also increased with the length of the previous bout. Switching sides was more common in mattress stalls with higher levels of sawdust bedding. Cows showed no preference for lying on either side after eating, irrespective of time spent eating or length of eating bouts before lying down. The slope of the lying surface also had no effect on laterality of lying.

21

The Welfare of Dairy Cattle—Key Concepts and the Role of Science

Journal of Dairy Science, September 2009, Volume 92, Number 9, pages 4101-4111

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This paper reviews research dealing with the ways in which current management systems affect dairy cattle welfare. The authors suggest that 3 questions should be asked when assessing animal welfare: is the animal functioning well in terms of its health and productivity; is the animal feeling well; and is the animal able to perform natural behaviours that are thought to be important to it? They then propose examples of the type of research studies that can be designed to answer these questions and suggest that management systems that address all 3 questions yield the best results for both animals and livestock managers. Examples given include feeding systems for calves that allow for natural behaviours such as social interaction and teat suckling, provide comfortable environments that promote good health and facilitate expression of genetic potential for growth. Other examples are given of research studies that apply to welfare-sensitive management practices for mature animals including the monitoring of social behaviours that impinge on the welfare of subordinate cattle or identify those that may be at risk of illness or injury. The authors argue that the traditional focus on animal health and productivity must be augmented with concerns about the animals' comfort, pain and pleasure and their ability to express natural behaviours.



22

Lying Behavior: Assessing within- and between Herd Variation in Free-stall-housed Dairy Cows

Journal of Dairy Science, September 2009, Volume 92, Number 9, pages 4412-4420

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A common objective when managing dairy cows in a free stall facility is to maximize the time that cows spend lying down in their stalls. Twelve hours of lying per day is often suggested as a target. In order to assess whether such a target is being achieved, simple methods such as the cow comfort index (CCI) and stall use index (SUI) have been proposed. The CCI is defined as the proportion of cows touching a stall that are lying down. SUI is the proportion of cows in the pen not feeding that are lying down in stalls. The objective of the present study was to develop a reliable automated method of assessing lying times and to use that method to evaluate the validity of CCI and SUI. Lying and standing posture of 2,033 cows on 43 farms (approximately 50 cows/farm) were monitored over 5 day intervals per cow using electronic data loggers attached to the cows' hind limbs. A mercury switch in the device activated a signal indicating whether the limb was vertical or horizontal. Results were compared with those derived from analyzing only a portion of the data to determine the minimum required to produce a comparable result. Recording 30 cows/farm over 3 days provided the best balance between accuracy and the volume of data required. Neither CCI nor SUI, calculated from one observation at 2 hours after afternoon milking, showed any association with actual lying times recorded with the data loggers.



23

Using Gait Score, Walking Speed, and Lying Behavior to Detect Hoof Lesions in Dairy Cows

Journal of Dairy Science, September 2009, Volume 92, Number 9, pages 4365-4374

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Gait scoring, also referred to as locomotion scoring, is often recommended as a way to identify cows that are lame. Earlier work by these authors and their colleagues established a gait scoring method based on the observation of specific gait characteristics, including head bob, back arch, joint flexion, step asymmetry and reluctance to bear weight. An overall numerical gait score (NGS) is determined from values assigned to each characteristic; higher scores (maximum NGS=5) indicate more severe lameness. The objective of the present study was to relate the presence of specific hoof lesions to changes in gait characteristics and NGS as well as walking speed and lying behaviour. In an initial trial, it was found that multiparous, lactating Holstein cows with sole ulcers scored higher (3.3 ± 0.2) than their herdmates with no ulcers (2.8 ± 0.2). Back arch, joint flexion, asymmetric steps, and reluctance to bear weight all increased in cows with ulcers. Ulcers did not affect walking speed but cows with ulcers spent more time lying down, attributable to longer lying bouts. In a second trial, gait scores of 47 cows were monitored from 4 weeks before until 24 weeks after calving. Cows that developed a sole ulcer had higher scores for back arch, joint flexion, asymmetric steps, reluctance to bear weight, and consequently NGS (3.1 ± 0.1 versus 2.35 ± 0.1) than cows that did not develop an ulcer. Observation of an increase in NGS predicted development of a sole ulcer 4 weeks before a confirmatory diagnosis. The authors conclude that, of the 7 gait characteristics evaluated, back arch, joint flexion, asymmetric steps and reluctance to bear weight were the best predictors of sole ulcers.



24

Automated Measurement of Changes in Feeding Behavior of Milk-fed Calves Associated with Illness

Journal of Dairy Science, September 2009, Volume 92, Number 9, pages 4549-4554

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Reduced feed intake is often one of the first signs that an animal is sick. The primary objective of this study was to determine whether individual intakes recorded by an automated milk feeder could be used as an indicator of illness in calves. A secondary objective was to determine whether intake responses to illness might be dependent on the calves' daily milk allowance. Data from 4 previous experiments involving group-housed dairy calves up to 21 days of age were examined to answer these questions. Low allowance calves were provided a maximum intake of 4 litres of milk per day. High allowance calves were offered a maximum of 12 litres/day in 3 of the experiments; in the fourth, their allowance was unlimited. Calf health was monitored regularly. Milk intakes of calves in the days after they were recognized as sick were compared with intakes of calves that remained healthy. Sick calves provided high intake allowances reduced their milk intakes by an average of 2.6 litres/day for 4 days. While they reduced their frequency of visits to the automated feeder by an average of 2.4 visits/day during the same 4 days, they increased the length of those visits by 1.7 minutes/visit. In contrast, no significant changes in milk intake or feeder visit frequency were observed for sick calves provided only 4 litres of milk/day although the duration of visits decreased by an average of 1.4 minutes per visit.

Environment





1

Comparing the Environmental Impacts of Pasture-based and Confinement-based Dairy Systems in Nova Scotia (Canada) Using Life Cycle Assessment

International Journal of Agricultural Sustainability, January 2009, Volume 7, Number 1, pages 19-41

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The objective of this study was to simulate the total environmental impacts of 2 alternative dairy production systems that are, or could be, employed by Nova Scotia dairy farmers. A theoretical pasture-based operation milked 40 cows, spent 5 months on pasture (with concentrate supplementation) and produced 29 kg of milk/cow/day. A year-round confinement scenario milked 70 cows producing 31 kg/cow/day. Using a complete life-cycle approach for both systems, all material and energy inputs and outputs required to produce 1,000 kg of milk were estimated. Environmental impacts examined included those on non-renewable resource use (abiotic depletion), global warming, ozone layer depletion, human toxicity, freshwater aquatic ecotoxicity, terrestrial ecotoxicity, photochemical oxidation, acidification, eutrophication, land use, and cumulative energy demand. Both production systems had similar impacts on these factors. The use of concentrate feeds, nitrogen fertilizers, transport fuels and electricity were major contributors to the environmental impacts of both systems. Results of modeling suggested that a 7 month grazing period would reduce impacts in several areas while maintaining the energy efficiency of the confinement system. The one area in which prolonged grazing would have a more negative environmental impact was land use.



2

Crushed Sunflower, Flax, or Canola Seeds in Lactating Dairy Cow Diets: Effects on Methane Production, Rumen Fermentation, and Milk Production

Journal of Dairy Science, May 2009, Volume 92, Number 5, pages 2118-2127

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Methane produced by rumen microbes in the anaerobic fermentation of feed represents a loss of from 2 to 12% of the lactating cow's gross energy intake. This enteric methane, which escapes from the rumen during eructation, contributes to the accumulation of greenhouse gases in the upper atmosphere which is responsible for global warming. The amount of enteric methane produced by the cow is dependent on both the level of feed intake and diet composition. The objective of the present study was to evaluate the effect of feeding various sources of lipid on methane production by lactating cows. Methane production was measured in closed environmental chambers. A control diet contained calcium salts of long chain fatty acids, a lipid source which largely escapes rumen fermentation, adding 3.1% lipid to total dietary dry matter (DM). Treatment diets contained one of 3 sources of crushed oilseeds: sunflower, flax or canola, increasing dietary lipid content by 4.2, 3.7 and 3.9% of DM, respectively. The addition of crushed oilseeds to the diets decreased daily methane production by an average of 13%, compared with the control diet. However, although DM intakes were slightly higher with oilseed addition, the inclusion of either sunflower or flax seed in the diets decreased the digestibility of DM intake by 16% and 19%, respectively. Neither milk volume, milk component yields nor milk production efficiency were affected by source of lipid.



3

Feeding Saponin-containing *Yucca schidigera* and *Quillaja saponaria* to Decrease Enteric Methane Production in Dairy Cows

Journal of Dairy Science, June 2009, Volume 92, Number 6, pages 2809-2821

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Saponins are a class of complex chemical compounds found in plants and marine organisms. Earlier studies demonstrated that these compounds could inhibit the proliferation of some rumen protozoa that support the growth of methane-producing rumen bacteria. Rumen methane production is an inefficient use of feed energy and contributes to the accumulation of greenhouse gases in the upper atmosphere. Therefore, this study was aimed at investigating the potential of saponins as feed additives that might reduce rumen methane production. In a preliminary trial, one of 2 different saponins was added at 15, 30 or 45 grams/kg of substrate dry matter (DM) to laboratory (*in vitro*) cultures inoculated with dairy cow rumen fluid. At all 3 concentrations, methane production was lower than in cultures with no added saponin. However, saponin addition also reduced DM digestibility. The *in vitro* trial was followed by a trial where lactating cows diets were supplemented with saponins at 10 grams/kg of DM. The lower concentration was chosen in an attempt to avoid the negative effect on digestibility seen in the *in vitro* experiment. DM intake was greater for cows fed saponins, compared with unsupplemented cows and there was no reduction in methane production, measured by 2 different methods. Saponin supplementation had no effects on milk volume or milk component yields. Milk production efficiency (milk kg/feed kg) declined due to higher DM intake. The authors conclude that saponin supplementation is not an effective strategy for reducing rumen methane production by lactating cows.



4

Potential use of *Acacia mearnsii* Condensed Tannins to Reduce Methane Emissions and Nitrogen Excretion from Grazing Dairy Cows

Canadian Journal of Animal Science, June 2009, Volume 89, Number 2, pages 241-251

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Many trials, conducted throughout the world, have examined or are examining potential strategies for reducing methane emissions from ruminant livestock. In previous experiments, researchers had demonstrated the potential of condensed tannins (CT) to reduce rumen methane production in cattle and sheep. In this study, a CT derived from the Black Wattle tree was tested as a dietary treatment aimed at mitigating methane production by lactating dairy cows grazing ryegrass pastures in south eastern Australia. Sixty cows, allocated to 3 equal groups, were drenched with water (controls) or with one of two doses of CT after each milking for 5 weeks. The low dose (163 grams/day) reduced methane emissions by 14% compared to controls; the high dose (326 grams/day for 17 days, 244 grams/day thereafter) reduced emissions by 29%. However, milk yield by cows on the low dose averaged 31.8 kg/day versus 33 kg/day for controls while those on the higher dose produced only 29.8 kg/cow accompanied by a 19% decline in fat yield and a 7% decline in protein yield. Following the 5 week grazing period, representative cows from each of the 3 groups were used to evaluate the effects of CT on digestive metabolism. Digestibility of dietary energy was significantly reduced by CT supplementation; from 76.9% for controls to 70.9% for the low dose and 66.0% for cows receiving the higher dose. Dry matter intake was also reduced at both CT doses while urinary nitrogen losses fell significantly, from 39% in controls to 26% and 22% in cows receiving low and high doses, respectively. Reduced urinary nitrogen excretion may be particularly relevant where nitrate leaching, ammonia volatilisation or nitrous oxide reductions are targeted.



5

Dietary Mitigation of Enteric Methane from Cattle

CAB Reviews: Perspectives in Agriculture, Veterinary Science... September 2009, Volume 4, Number 035, pages 1-18

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This paper reviews current knowledge about methane production by cattle including its contribution to global greenhouse gas production and strategies for its mitigation. Approximately 2 to 12% of the gross energy consumed by cattle is converted to methane and emitted to the atmosphere through eructation. The actual amount of methane produced depends on both feed intake and diet composition. It is estimated that domestic ruminants account for 11-17% of the methane that enters the atmosphere globally each year. A number of dietary strategies aimed at mitigating rumen methane production have either been proven effective or are currently being investigated. Increasing the proportion of grain in the diet as well as the inclusion of ionophores or lipids are known to be effective strategies. Supplementation with biologically active plant compounds such as condensed tannins, saponins and essential oils are currently receiving research attention as are rumen fermentation modifiers such as yeast and bacterial direct-fed microbials. Improvements in forage quality may also contribute to lower enteric methane production. The choice of strategies applied will largely depend on economic considerations for individual livestock producers. The authors propose that efforts to mitigate ruminant methane production should be evaluated on the basis of intensity – the amount of methane produced per unit of milk or meat produced – rather than simply on total global production. They also advocate the assessment of methane production in the context of the complete life cycle of the livestock production system rather than as an isolated part of that system.

Feeding





1

Protein Value of a New Genotype Oat (CDC SO-I) for the NRC Dairy Model: Protein Degradation Balance and Kinetics, Protein Fractions and Total Metabolizable Protein Supply

Canadian Journal of Animal Science, September 2008, Volume 88, Number 3, pages 507-513

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The objective of this study was to characterize the nutrient value of a new oat variety for feeding to dairy cattle. The new variety, CDC SO I, developed at the University of Saskatchewan's Crop Development Centre, is distinguished from other oat varieties by its low lignin hull and high fat groat. The model defined in the US National Research Council's Nutrient Requirements of Dairy Cattle (NRC Dairy 2001) was used to predict the potential nutrient availability from the new variety compared with 2 conventional varieties, CDC Dancer and Derby. Based on results from laboratory analyses and rumen incubations of SO I, protein fractions defined in NRC Dairy 2001 were calculated, including rumen-synthesized microbial protein truly absorbed in the small intestine; rumen undegraded feed protein truly absorbed in the small intestine; endogenous protein in the digestive tract; total metabolizable protein supply in the small intestine (MP), and protein degraded balance (PDB). These calculations led to the conclusion that SO I would supply 9% more MP than CDC Dancer and 13% more than Derby. Although the PDB value for SO I was also greater than that for the other varieties, its negative value indicated that, fed alone, SO I would not provide enough energy to take advantage of its rumen degradable protein contribution for microbial protein synthesis.



2

Chemical Characteristics and *in situ* Ruminal Parameters of Barley for Cattle: Comparison of the Malting Cultivar AC Metcalfe and Five Feed Cultivars

Canadian Journal of Animal Science, October 2008, Volume 88, Number 4, pages 711-719

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This study compared chemical analysis values and rumen degradation parameters of 6 barley cultivars grown and fed to dairy cattle in western Canada. All cultivars were grown in one location for 3 years. Samples from each year's harvest were subjected to chemical analysis as well as *in situ* rumen degradation analysis, where small samples contained in sealed nylon bags were incubated in the rumens of cannulated, dry Holstein cows for varying lengths of time. Rate and extent of rumen degradation was calculated from disappearance of sample from the bags at successive time points. The malting barley cultivar, AC Metcalfe, contained a higher concentration of neutral detergent fibre and lower concentrations of non structural carbohydrates, starch, acid detergent fibre, total digestible nutrients, and fermentable cell wall carbohydrates compared with the average values of these components in the 5 feed cultivars examined. The rumen degradation analysis revealed that AC Metcalfe had a higher soluble dry matter fraction, lower crude protein and starch degradation rates, and a lower degradable starch concentration compared with the mean of the feed cultivars. The authors conclude that, although differences were found between AC Metcalfe and the feed barley cultivars, differences in performance of dairy cattle fed the different cultivars would be minor.

3

Relationships among Milk Urea-nitrogen, Dietary Parameters, and Fecal Nitrogen in Commercial Dairy Herds

Canadian Journal of Veterinary Research, October 2008, Volume 72, Number 5, pages 449-453

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When nutrients in diets fed to lactating dairy cows are not well balanced, excess nutrients may be excreted. This is particularly true when diets contain excessive amounts of protein relative to other nutrients. The critical element in protein is nitrogen (N) which, if available in excess of requirements, is incorporated into urea in the liver and excreted in urine and milk. Undigested protein and other metabolic byproducts are also excreted in feces. Although monitoring N excretion would facilitate evaluation of the efficiency of N use for milk production, testing feces and urine for N excretion is impractical. The objective of this study was to determine whether milk urea nitrogen (MUN) concentrations could be used to evaluate N intake and fecal N excretion. Eighty-three Prince Edward Island dairy herds were visited after each of 3 DHI test days. Diets were evaluated for nutritional parameters after collection and analysis of forage samples. Fecal samples, taken from 6 cows on each farm, were pooled and analyzed for N content. DHI milk samples were tested for MUN. Although dietary N concentration correlated well with fecal N concentration, herd average MUN did not.



4

The Relative Merit of Ruminal Undegradable Protein from Soybean Meal or Soluble Fiber from Beet Pulp to Improve Nitrogen Utilization in Dairy Cows

Journal of Dairy Science, October 2008, Volume 91, Number 10, pages 3947-3957

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There is growing interest in increasing the efficiency of nitrogen (N) utilization in lactating dairy cows in order to decrease the environmental burden of excreted N. On the basis of previous research, proposed strategies to achieve this objective include reducing the proportion of rumen degradable protein (RDP) and increasing the proportion of rumen fermentable fibre (RFF) in the diet. The objective of the present study was to evaluate the efficacy of these two strategies by measuring N balance in cows fed diets with varying levels of RDP and RFF. A reference (negative control; NC) diet, based on alfalfa silage and high moisture shelled corn (HMSC), contained no supplemental protein source. Solvent-extracted soybean meal (SSBM) was added to the NC diet to produce a high RDP diet (HiRDP). In a third diet (LoRDP), the SSBM was replaced with expeller soybean meal (ESBM) in which the protein is less rumen degradable. A fourth (HiRFF) diet contained both SSBM and beet pulp, a rich source of RFF. In all diets, supplements replaced portions of the HMSC in the NC diet. Although milk and milk protein yields were lower with the NC diet, it produced the highest nitrogen utilization efficiency (in terms of milk N per unit of N intake). ESBM supplementation reduced milk fat yield and urea N concentration but did not improve yields of either milk or milk protein. Milk production was reduced by the HiRFF diet due to a reduction in dry matter intake. None of the supplemented diets achieved the N utilization efficiency of the NC diet.



5

Alfalfa Cut at Sundown and Harvested as Baleage Improves Milk Yield of Late-Lactation Dairy Cows

Journal of Dairy Science, October 2008, Volume 91, Number 10, pages 3968-3982

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When alfalfa is ensiled, 44 to 87% of the crude protein in the crop is broken down into non-protein nitrogen (NPN). When the silage is fed, this NPN is rapidly degraded to ammonia in the rumen, much of which is not captured for microbial protein synthesis due to inadequate concomitant availability of readily-fermentable non-structural carbohydrate (NSC). The objective of this study was to examine the potential to increase the NSC in alfalfa silage by harvesting the crop late in the day, following the period of maximum photosynthesis. Late lactation cows were fed 100% forage diets consisting of alfalfa baleage harvested either at sundown after a sunny day (PM) or at sunup the following day (AM). Differences in NSC concentrations between AM and PM bales fed over the 10-day feeding trial ranged from -10 to 50 grams/kg of dry matter (PM minus AM). On average, PM bales contained 128 grams/kg of NSC versus 105 grams/kg in the AM bales. Cows fed PM baleage consumed 1 kg/day more dry matter, produced 1 kg/day more milk, 70 grams/day more milk fat and 40 grams/day more milk protein than those fed AM baleage. Nitrogen (N) balance measurements demonstrated that feeding PM baleage resulted in a N efficiency of 18.2 grams of milk N per 100 grams of N intake compared with a figure of 16.8 when AM baleage was fed.



6

Repeated Ruminal Acidosis Challenges in Lactating Dairy Cows at High and Low Risk for Developing Acidosis: Feed Sorting

Journal of Dairy Science, October 2008, Volume 91, Number 10, pages 3958-3967

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Several studies have demonstrated that lactating cows will preferentially consume the more palatable components of their total mixed rations (TMRs) while rejecting coarse forage particles. When cows are fed diets containing minimal levels of physically effective fibre (PEF), such sorting behaviour places them at greater risk of acidosis since it is those coarse particles that contributed the most to PEF. Conversely, other studies have shown that cows experiencing low ruminal pH may preferentially consume (sort in favour of) long forage particles. The objective of the present study was to evaluate feed sorting in cows at high or low risk of ruminal acidosis and to determine whether their sorting behaviour changed in response to an acidosis challenge. High risk (HR) cows were in early lactation, fed a TMR containing 45% forage. Low risk (LR) cows were in mid-lactation, fed a 60% forage diet. The acidosis challenge involved restricting TMR intake to 50% of normal, unrestricted (ad lib) intake for 1 day, followed by a meal of 4 kg of a blend of ground barley and wheat before being offered their TMR again. Before being challenged, both HR and LR cows sorted against the longest as well as the shortest TMR particles, and sorted in favour of medium length particles. HR cows sorted to a greater extent than LR cows which was associated with lower ruminal pH levels in HR cows. In response to the acidosis challenge, the HR cows altered their sorting behaviour in favour of consuming more long particles. The authors speculate that this change was a response to reduce the effect of the very low ruminal pH levels produced by the acidosis challenge.



7

The Effects of Histidine-Supplemented Drinking Water on the Performance of Lactating Dairy Cows

Journal of Dairy Science, October 2008, Volume 91, Number 10, pages 3998-4001

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Histidine (His) is one of the 20 amino acids considered essential in mammalian diets because they cannot be synthesized by mammalian tissues. Although it is commonly assumed that the cow derives her His requirement from microbial protein, a number of studies have demonstrated positive production effects when His supply to the small intestine is increased. In the present study, 2.5 grams per litre of supplemental His was added to the drinking water provided to early lactation cows, based on the rationale that the passage rate of liquids through the rumen far exceeds that of particulate matter. Rapid passage in the liquid phase may allow a proportion of the supplemental His to escape rumen degradation. Water intake by His-treated cows increased to 92.1 litres/day, compared to 85.1 litres/day by control (CTRL) cows whose water contained no added His. By the seventh day of His supplementation, His concentration in blood plasma was 50% higher than in the CTRL cows. It was estimated that 0.4% of the His consumed bypassed rumen degradation. Milk yield was 1.7 litres/day higher and milk lactose yield increased by 90 grams/day higher in the His-treated cows with insignificant changes observed in other milk components. The authors conclude that, for His supplementation through water to be practical, a higher proportion of added His would have to bypass the rumen.



8

In vitro Metabolism of Flax Lignans by Ruminal and Faecal Microbiota of Dairy Cows

Journal of Applied Microbiology, November 2008, Volume 105, Number 5, pages 1585-1594

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Lignans are naturally-occurring plant compounds that can have estrogenic activity. Flaxseed is a particularly rich source of the lignan, secoisolariciresinol diglucoside which, in monogastric animals is converted to several mammalian lignans that have been shown to have various health-promoting properties. Although little is known about the metabolism of plant lignans in ruminants, the authors of this study speculate that, beneficial lignans may be produced in dairy cows and secreted in milk, enhancing its nutritional value. Therefore, their objective was to evaluate the metabolism of flax lignans by bovine rumen and fecal microbes. Lignans extracted from either flax seeds or flax hulls were incubated in laboratory cultures inoculated with microbial extracts from rumen contents or feces of dry cows. Flax hulls incubated with fecal inocula produced the greatest quantities of the mammalian lignin enterodiol (ED). The production of enterolactone (EL) was greatest when rumen inocula were incubated with lignans from either flax seeds or hulls. ED and EL are 2 mammalian lignans found in previous research to have beneficial health properties for humans. The authors suggested that the results of the present study might lead to the targeted manipulation of ED and EL concentrations in milk.



9

Fecal and Urinary Lignans, Intrafollicular Estradiol, and Endometrial Receptors in Lactating Dairy Cows Fed Diets Supplemented with Hydrogenated Animal Fat, Flaxseed or Sunflower Seed

Journal of Reproduction and Development, December 2008, Volume 54, Number 6, pages 439-446

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Results of previous research by these and other authors suggest that feeding flaxseed to dairy cows in the early lactation breeding period may reduce early embryonic loss. The proposed mechanism of this effect involves the suppression of prostaglandin F₂-alpha (PGF₂α) synthesis by the alpha-linolenic acid present in flaxseed. Another possible mechanism involves the intestinal conversion of flaxseed lignans into compounds that inhibit estrogen (estradiol) synthesis by the dominant follicle in the early stages of conception. Elevated follicular estrogen secretion stimulates the synthesis of both PGF₂α and uterine oxytocin receptors which, when activated, causes embryonic death. The objective of this study was to investigate the possibility that intestinal conversion of the flaxseed lignan secoisolariciresinol diglycoside (SDG) into the mammalian lignans, enterolactone (EL) and enterodiols (ED) might result in reduced follicular estradiol secretion and uterine oxytocin receptor synthesis. Twenty-seven lactating, cycling Holstein cows were fed diets containing one of three sources of lipid, each providing 750 grams of supplemental lipid/cow/day: saturated fatty acids (SAT), flaxseed (FLX) or sunflower seed (SUN). Cows fed FLX had higher fecal concentrations of both SDG and ED and higher urinary ED concentrations than cows fed either SAT or SUN. However, no differences were detected in either follicular estradiol concentrations or in uterine concentrations of estradiol or oxytocin receptors.



10

Digestion, Ruminal Fermentation, Ciliate Protozoal Populations, and Milk Production from Dairy Cows Fed Cinnamaldehyde, Quebracho Condensed Tannin, or *Yucca schidigera* Saponin Extracts

Journal of Dairy Science, December 2008, Volume 91, Number 12, pages 4765-4777

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This study investigated the potential of 3 different types of plant extracts to alter rumen fermentation and milk production in lactating dairy cows. Cinnamaldehyde (CIN; 1 g/cow per day), condensed tannins from quebracho trees (QCT, 150 g/cow per day), or saponins from *Yucca schidigera* extract (YSE, 60 g/cow per day) were added to diets fed to ruminal cannulated cows. Although YSE supplementation reduced dry matter intake, neither CIN nor QCT had this effect. At the concentrations fed, none of the plant extracts had significant effects on any of the variables measured, including digestibility of feed components, degradation of feed samples incubated in the rumen in nylon bags, ruminal pH, volatile fatty acid production, rumen protozoal populations, nor milk or milk component yields. The authors conclude that administration of higher doses may have elicited more favourable results.

11

Studies on the Production of Conjugated Linoleic Acid from Linoleic and Vaccenic Acids by Mixed Rumen Protozoa

Applied Microbiology and Biotechnology, December 2008, Volume 81, Number 3, pages 533-541

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Animal studies and human clinical trials have demonstrated beneficial health effects attributable to some of the conjugated linoleic acids (CLAs) found in ruminant meat and milk. CLAs and other intermediates, including vaccenic acid (VA: trans-11 C18:1), arise from the ruminal biohydrogenation and isomerisation of linoleic (LA: C18:2) and linolenic (LNA: C18:3) acids. The objective of this study was to characterize the roles of rumen protozoa (RP) in these transformations. RP were isolated from rumen contents collected from fistulated cows then resuspended in sterilized rumen fluid. These preparations, along with appropriate substrates, including LA and VA, were incubated anaerobically in laboratory fermentation flasks with (+AB) or without (-AB) added antibacterial agents. Neither +AB nor -AB incubations were able to convert VA to CLA through a desaturase pathway. Several different CLA isomers were produced from LA in the +AB incubation, the predominant one being rumenic acid (RA: cis-9, trans-11 C18:2). The rate of RA production was very high, 37% of the maximum concentration being produced within the first 6 minutes of adding LA substrate. Production of the trans-10, cis-12 C18:2 CLA isomers was only 10% that of RA. Although +AB incubations were unable to convert LA to VA or stearic acid (C18:0) these conversions did occur in the -AB incubations suggesting that rumen protozoa themselves were unable to biohydrogenate unsaturated fatty acids but that bacteria present in the -AB incubations were able to do so. The authors conclude that rumen protozoa, although incapable of desaturation or biohydrogenation, are able to perform fatty acid isomerisation reactions.



12

Long-Term Monensin Supplementation Does Not Significantly Affect the Quantity or Diversity of Methanogens in the Rumen of the Lactating Dairy Cow

Applied and Environmental Microbiology, January 2009, Volume 75, Number 2, pages 374-380

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There is a great deal of current interest in developing strategies to mitigate methane production from ruminant livestock, primarily because of its contribution to global greenhouse gas emissions. Methane is produced in the rumen by a group of archaea referred to as methanogens. An earlier study by these authors and their colleagues demonstrated a 7% reduction in ruminal methane production due to long-term monensin (MON) supplementation of lactating cow diets. The mechanism proposed to account for this effect involves inhibition of the activity of other rumen microbes that provide substrates to methanogens which, in turn, reduces methanogen proliferation. The objective of the present study was to confirm whether long-term MON supplementation exerted its effect on ruminal methane production through reduction of methanogen populations and/or the diversity of archaeal species comprising those populations. Rumen samples were withdrawn by stomach tube from lactating cows before and during supplementation of a total mixed ration (TMR) with 24 mg MON per kg of dietary dry matter over a 6-month period. Control cows were fed the same TMR without MON supplementation. Methanogen populations were estimated using a polymerase chain reaction technique which identifies target DNA sequences in archaeal genomes. Population diversity was evaluated by detecting fragments of a ribosomal RNA gene unique to each methanogen species. No differences were detected between MON supplemented and control cows in either the quantity or the diversity of rumen methanogens over the course of the trial.



13

Meta-analysis of the Effect of Oral Selenium Supplementation on Milk Selenium Concentration in Cattle

Journal of Dairy Science, January 2009, Volume 92, Number 1, pages 324-342

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This paper reviews and aggregates the results of 42 reports published between 1977 and 2007 where relationships between selenium (Se) intake and milk Se concentration were evaluated in dairy cows. Across all studies, oral Se selenium supplementation resulted in an average increase in milk Se concentration of 12.6 micrograms(μg)/litre, ranging from 7.9 to 17.4 μg /litre. Variation in responses was dependent upon geographic location, stage of lactation, Se dose and Se source. Among the studies conducted in the US, supplementation with Se yeast (typically 6 mg/head/day) raised milk Se concentration by an average of 29.2 μg /litre. It is suggested that the amino acid-bound Se found in Se yeast may be the preferred method of supplementation for enhancing milk Se concentration.

14

Timothy Hays Differing in Dietary Cation-Anion Difference Affect the Capability of Dairy Cows to Maintain their Calcium Homeostasis

Journal of Dairy Science, January 2009, Volume 92, Number 1, pages 238-246

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Hypocalcaemia is a problem encountered by high producing dairy cows at calving mainly because of the increased calcium (Ca) requirement for milk production. Lowering dietary cation-anion difference (DCAD) has been shown to decrease the risk of hypocalcaemia. Previous trials by these authors have demonstrated the use of low-DCAD hay for this purpose. The present trial was designed to establish the level of DCAD required to achieve metabolic effects considered indicative of successful treatment. Low-and medium-DCAD timothy hays were produced by fertilizing established stands with Ca chloride (CaCl_2); high-DCAD hay was grown on soil with no CaCl_2 fertilization. Diets fed to dry, pregnant cows containing one of each of the 3 hays at 71% of dietary dry matter (DM) had DCAD levels as follows: LOW – 0.7, MED – 7.3, HIGH – 14.4 mEq/100g DM. After 12 days of consuming these diets, cows were subjected to a challenge test, which induces artificial hypocalcaemia by infusion of EDTA solution into the blood circulation, to determine the time required for blood Ca concentration to decrease to 60%, and the time required to recover to 90% of pre-challenge concentrations. Although the first parameter was not affected by the hay consumed, recovery time was shorter for cows fed the LOW diet. Urine pH increased with increasing dietary DCAD. Blood pH in cows fed the LOW and MED diets was significantly lower than that in the cows fed the HIGH diet.



15

Empirical Prediction of Net Portal Appearance of Volatile Fatty Acids, Glucose, and their Secondary Metabolites (β -hydroxybutyrate, lactate) from Dietary Characteristics in Ruminants: A Meta-analysis Approach

Journal of Animal Science, January 2009, Volume 87, Number 1, pages 253-268

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Current diet formulation systems for ruminant animals, including dairy cattle, attempt to satisfy the animals' energy requirements by estimating the amount of metabolizable or net energy delivered by a blend of dietary ingredients. More recent work in this area is moving toward using chemical characteristics of the diet to predict the nature and amount of specific energy-yielding nutrients absorbed from the gut. The authors of this paper attempted to derive such prediction equations using data from previously published research. Within a limited range of dietary intakes and compositions, accurate equations were derived to predict volatile fatty acid (VFA) absorption from ruminally fermented organic matter (RfOM) intake and to predict glucose absorption from starch digested in the small intestine. An equation predicting beta-hydroxy butyrate (BHBA) absorption from RfOM was species-dependent; another predicting lactate absorption from starch digested in the rumen gave variable results. Both BHBA and lactate absorption rates were found to be closely related to VFA absorption rates; BHBA to acetate and butyrate; lactate to acetate.



16

The Effect of Short-term Hyperammonaemia on Milk Synthesis in Dairy Cows

Journal of Dairy Research, February 2009, Volume 76, Number 1, pages 49-58

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When lactating dairy cows are fed diets containing excess rumen degradable protein, relative to readily fermentable carbohydrate, high levels of ammonia (NH₃) are produced and absorbed into the bloodstream, requiring detoxification by the liver. In converting NH₃ to urea (CON₂H₄) the nitrogen (N) atom from a molecule of NH₃ is combined with an atom of N from the amino acid, aspartic acid. It has been suggested that, when excessive NH₃ is absorbed from the rumen, this process might consume amino acids which might otherwise be used for milk protein synthesis. The objective of this study was to examine this possibility by measuring metabolic and production responses to raising blood NH₃ blood concentrations in lactating cows. Cows were administered intravenous infusions of either ammonium acetate (AA; treatment) or sodium acetate (SA; control). While AA-infused cows produced 20% less milk volume, protein and lactose compared with SA-infused cows, no differences were observed in milk fat yield or in milk protein or lactose concentrations. Although blood levels of several important amino acids were decreased by AA infusion, measurement of other metabolic parameters failed to support the possibility that the observed reduction in milk yield was due to lower availability of amino acids. The authors speculate that a decrease in feed intake was the likely cause of the lower production.



17

Effects of Supplements of Folic Acid, Vitamin B12, and Rumen-protected Methionine on Whole Body Metabolism of Methionine and Glucose in Lactating Dairy Cows

Journal of Dairy Science, February 2009, Volume 92, Number 2, pages 677-689

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Methionine (Met) and lysine are usually identified as the 2 amino acids whose low levels of absorption in the small intestine limit milk production by high-producing cows. In addition to its requirement for protein synthesis, methionine plays important roles related to energy metabolism. The B-vitamins folic acid (FA) and vitamin B12 (B12) are important co-factors in both protein and energy metabolism, particularly in relation to the roles played by methionine. The objective of this research was to evaluate the effects of supplemental Met, FA and B12 on protein and energy metabolism, the latter in terms of the availability of glucose for milk lactose synthesis. Early lactation cows received supplemental rumen protected Met (RPMet) at either a level providing 76% of their Met requirement (LoMet) or at a level exceeding requirement (HiMet). Cows within these 2 Met treatment groups either received or did not receive weekly intramuscular injections of FA and B12. Vitamin injections increased blood and milk FA and B12 concentrations as well as milk volume, milk lactose, milk protein and milk total solids yields. Glucose synthesis and utilization increased in proportion to the increase in milk lactose yield. Vitamin supplementation increased the availability of Met for protein synthesis; in LoMet cows by increasing protein turnover and in HiMet cows by decreasing Met breakdown.



18

A Randomized Herd-level Field Study of Dietary Interactions with Monensin on Milk Fat Percentage in Dairy Cows

Journal of Dairy Science, February 2009, Volume 92, Number 2, pages 777-781

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This paper describes a large-scale field trial designed to evaluate the effect of feeding monensin on bulk tank milk fat concentration (BTMF%). Forty-seven Québec herds participated in the study. Of these, 42 were housed in tie-stalls; 29 were fed total mixed rations. Twenty-four herds were fed monensin during the first 12 weeks of a 7 month observation period; 23 herds were fed monensin during the last 12 weeks. Monensin was fed at a rate of 16 mg/kg of dietary dry matters. Responses were calculated from observations of BTMF% when monensin was either fed or not fed. Monensin feeding resulted in a statistically significant 0.12 percentage point decrease in BTMF%, an effect that was greater in herds feeding diets that were higher in non-fibre carbohydrates, lower in feed particles greater than 8 mm in length or not feeding dry hay as the first morning meal.



19

Effects of Supplementing Glycerol and Soybean Oil in Drinking Water on Feed and Water Intake, Energy Balance, and Production Performance of Periparturient Dairy Cows

Journal of Dairy Science, February 2009, Volume 92, Number 2, pages 698-707

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Many of the periparturient metabolic disorders experienced by dairy cows are thought to be triggered by negative energy balance resulting from reduced feed intake during the last week before calving. Energy intake after calving also lags behind the requirements for rapidly increasing milk production. To mitigate this negative energy balance, it is often recommended that the energy density of dietary dry matter (DM) be increased during the period of transition from gestation to lactation. This study evaluated the use of water as an alternative vehicle for delivering energy-yielding substrates in this period. Ninety cows, all fed the same late gestation (close-up) and early lactation diets, were randomly assigned to one of 3 treatment groups. A GLY group was offered drinking water containing 20 grams of glycerol per litre. An SBO group had soybean oil added to their water at 10 grams/litre. Water offered the control (CTRL) group contained no supplements. The trial began at 7 days prior to each cow's expected calving date and ended at 7 days post-partum. Both water treatments reduced TMR DM intake compared with CTRL across all 14 days. Intake of water containing SBO was lower than CTRL for all 14 days; intake of GLY-treated water was lower than CTRL prepartum but similar to CTRL post-partum. Energy intakes, energy balance, serum non-esterified fatty acid levels and serum glucose concentrations were similar among all cows for the duration of the trial. Serum betahydroxy butyrate concentrations were influenced by treatment and time relative to calving. Neither treatment affected calf birth weight, milk production or milk composition.



20

Responses in Mammary and Splanchnic Metabolism to Altered Lysine Supply in Dairy Cows

Animal, March 2009, Volume 3, Number 3, pages 360-371

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Lysine is generally recognized as one of the essential amino acids (EAA) whose inadequate supply to the small intestine most often limits milk production. However, experimental evidence has shown that the uptake of lysine by the mammary gland usually exceeds the amount secreted in milk. In contrast, it has been found that the mammary uptake of many non-essential amino acids (NEAA) is less than that secreted in milk and that lysine is consumed by the gland in the synthesis of those NEAA. This study was designed to evaluate whether the mammary gland has an absolute requirement for lysine to support NEAA synthesis or whether it can maintain NEAA synthesis in the face of a reduced supply of lysine. The effect of reducing lysine availability on lysine metabolism and milk protein and lysine secretion were secondary objectives. Cows were administered abomasal infusions of amino acid mixtures including (HiLys) or not including lysine (LoLys). For both treatments, lysine supply exceeded mammary uptake and mammary uptake exceeded lysine output in milk although the mammary uptake to output ratio decreased from 1.37 for HiLys to 1.12 for LoLys. This suggested that the mammary gland had some ability to reduce lysine use for NEAA synthesis but that there may be an absolute requirement for some proportion of the lysine taken up by the mammary gland to be used for this purpose.

21

Effects of Carvacrol and Cinnamaldehyde on Microbial Fermentation When Added to a Barley- or Corn-based Diet in a Continuous-culture System

Canadian Journal of Animal Science, March 2009, Volume 89, Number 1, pages 97-104

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Earlier research by these and other authors suggested that some plant extracts may have potential as modifiers of rumen fermentation. In particular, carvacrol (CAR), a derivative of oregano, and cinnamaldehyde (CIN), derived from cinnamon oil, have been shown to modify rumen microbial fermentation, improving the efficiency of nitrogen and energy metabolism. The present experiment examined the effects of these 2 compounds on microbial fermentation in laboratory (*in vitro*) fermenters inoculated with rumen extracts from lactating cows. Fermentation substrates included either barley or corn grain. Although grain source affected fermenter pH and crude protein degradation, neither CAR nor CIN addition affected any of the fermentation parameters monitored, including pH, volatile fatty acid production, ammonia nitrogen concentration, nutrient digestibility or efficiency of microbial protein synthesis.



22

Selenium Uptake by Ruminal Microorganisms from Organic and Inorganic Sources in Dairy Cows

Canadian Journal of Animal Science, March 2009, Volume 89, Number 1, pages 105-110

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Selenium (Se) is an essential trace mineral which is often deficient in unsupplemented feeds. Current regulatory limits on Se supplementation in both US and Canada are 0.3 mg Se/kg of dietary dry matter (DM). However, currently available and widely used inorganic Se sources, including sodium selenite and selenate, are poorly absorbed by ruminants, probably due to ruminal conversion of these compounds to insoluble forms. The objective of this study was to compare the kinetics of Se uptake and loss by rumen microbes when Se was supplemented in either inorganic (selenite) or organic (Sel-Plex®) form. In the first of 2 trials, dry cows were fed a maintenance diet for a 14 day period after which Se supplements were administered directly into the rumen every 6 hours for 9 days. Rumen samples were withdrawn before each Se administration. After the final dose of Se was given on day 9, rumen samples were collected every hour for 24 hours to evaluate the rate (half-life) of Se loss from rumen microbes. When inorganic Se was used, rumen microbial Se concentration reached a plateau of about 100 µg/kg after 1 day of supplementation compared with a plateau of close to 400 µg/kg after 2 days when the organic source was used. The half-life of Se loss from rumen microbes was 16 hours after organic Se supplementation; 48 hours following inorganic supplementation. In the second trial, Se administration and sampling periods were shortened, based on data collected in trial 1. Again, uptake of organic Se was greater, reaching approximately 330 µg/kg, compared with 125 µg/kg for the inorganic source.



23

Supplementing Selenium Yeast to Diets with Adequate Concentrations of Selenium: Selenium Status, Thyroid Hormone Concentrations and Passive Transfer of Immunoglobulins in Dairy Cows and Calves

Canadian Journal of Animal Science, March 2009, Volume 89, Number 1, pages 111-122

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Current regulations in both the US and Canada limit the level of supplemental selenium (Se) permitted in dairy feeds to 0.3 mg/kg of dietary dry matter (DM). This is also the minimum level recommended in the US National Research Council's Nutrient Requirements of Dairy Cattle (2001). However, experimental evidence suggests that higher levels may be of benefit at some stages of the production and reproduction cycles. The objective of this study was to evaluate the potential beneficial effects of adding supplemental Se to dry and lactating cow diets already containing levels of Se considered adequate. Cows were fed basal diets containing 0.38-0.40 mg Se/kg DM from 150 days before their expected calving dates. At 60 days pre-calving, cows were allocated to one of two treatment groups. One group was offered dry and lactation diets where basal diets were supplemented with an additional 0.30 mg Se/kg DM in the form of Se selenite (SS); basal diets for the second group were supplemented with the same level of Se as selenized yeast (SY). Feeding of Se supplemented diets (containing 0.62-0.81 mg Se/kg DM) continued until 60 days post-calving. In whole blood drawn at intervals during the trial, average Se concentrations were 11% higher in cows fed SY compared with those fed SS. Colostrum and milk from cows fed SY also contained more Se, resulting in whole blood levels in their calves that were 19% higher. Neither Se source affected the cows' blood thyroid hormone concentrations or the passive transfer of immunoglobulins to calves.



24

Feeding High Proportions of Barley Grain in a Total Mixed Ration Perturbs Diurnal Patterns of Plasma Metabolites in Lactating Dairy Cows

Journal of Dairy Science, March 2009, Volume 92, Number 3, pages 1084-1091

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This study examined the effects of barley grain (BG) feeding level on blood metabolite concentrations. Cows in early- to late-lactation (60 to 140 days in milk) were fed total mixed rations (TMRs) containing 0, 15, 30 or 45% BG once per day at 8 AM for 21-day periods. Diets were identical except that, as BG inclusion level increased, barley silage decreased. Blood samples were collected periodically for 12 hours after feeding. Plasma glucose and lactic acid concentrations were highest in cows fed the most BG but BG feeding level did not have a significant effect on changes in concentrations of those metabolites across the 12 hour sampling period. Plasma concentrations of free fatty acids also rose with increasing dietary BG level reaching significant peaks at 2 hours after feeding before declining sharply to pre-feeding concentrations. Blood beta-hydroxybutyrate (BHBA) concentrations rose gradually, reaching plateau levels between 4 and 12 hours after feeding. BHBA plateaus were higher for the lower dietary BG feeding rates. Plasma cholesterol concentrations were also lower at the higher BG feeding rates with blood levels rising slightly after feeding before declining over the remainder of the sampling period. Interpretation of the relevance of the observed blood metabolite concentrations will require further research.



25

Alfalfa Cut at Sundown and Harvested as Baleage Increases Bacterial Protein Synthesis in Late-lactation Dairy Cows

Journal of Dairy Science, March 2009, Volume 92, Number 3, pages 1092-1107

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Grasses and legumes harvested late in the afternoon of a sunny day contain higher concentrations of water-soluble carbohydrates (WSC) than crops harvested earlier in the day, due to high photosynthetic activity during exposure to sunlight. In an earlier report on the present study, these authors found that alfalfa cut at sundown (PM) contained 22% more WSC than the same crop cut at sunup (AM). Alfalfa cut at sundown required 18 more hours to wilt than the AM-cut crop, likely resulting in greater breakdown of protein into more rumen degradable peptides, amino acids and non-protein nitrogen – together measured as total nitrogen (N). The present report evaluates the effects of these differences in AM and PM harvested alfalfa, preserved and stored as baleage, on nutrient metabolism and digestibility in the rumen and lower digestive tract. Late lactation cows fitted with rumen cannulae were fed either AM or PM baleage with no concentrate supplement. Although intakes were similar for both baleages, the proportion of N intake digested in the rumen was significantly higher in cows fed the PM baleage (PM: 79%; AM: 74%). The fraction of protein (non-ammonia nitrogen: NAN) associated with rumen bacteria flowing out of the rumen increased while the non-bacterial NAN fraction decreased. These observations point to greater capture of feed N by rumen bacteria, probably due to both the more degradable N in the PM baleage as well as its higher concentration of WSC which are readily fermentable in the rumen.



26

The Effect of Dietary Fiber Level on Milk Fat Concentration and Fatty Acid Profile of Cows Fed Diets Containing Low Levels of Polyunsaturated Fatty Acids

Journal of Dairy Science, March 2009, Volume 92, Number 3, pages 1108-1116

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Up until the late 1990s, it was commonly believed that milk fat depression (MFD) was caused by ruminal pH depression resulting from the feeding of diets containing insufficient effective fibre and excessive starch. However, a summary of research trials aimed at demonstrating this association concluded that ruminal pH accounted for only 39% of the variation in MFD observed. Subsequently, another theory explaining the role of ruminal pH in MFD emerged – the biohydrogenation theory. In this theory, low ruminal pH will only precipitate MFD when the diet contains an adequate level of polyunsaturated fatty acids (PUFAs). Low pH favours the conversion of these PUFAs to conjugated linoleic acids (CLAs) which, in very low concentrations, can inhibit fat synthesis by the mammary gland. The objective of the present study was to test this theory by comparing milk fat production in cows fed diets containing high (HF) or low (LF) concentrations of effective fibre along with similar low levels of PUFAs. Over a 4-week trial, cows fed the LF diet had significantly lower average ruminal pH and ruminal pH remained below 5.6 for an average 357 minutes/day, compared with 103 minutes/day for cows fed the HF diet. Diet had no effect on milk yield or milk component yields or concentrations. Minor differences were observed in the concentrations of specific milk fatty acids between the 2 diets although no differences were seen in concentrations of the CLAs thought to cause depressed mammary fat synthesis.



27

A Grain-based Subacute Ruminal Acidosis Challenge Causes Translocation of Lipopolysaccharide and Triggers Inflammation

Journal of Dairy Science, March 2009, Volume 92, Number 3, pages 1060-1070

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Lactating dairy cows are often fed substantial quantities of grain to increase dietary energy availability in support of high milk production. Most of the energy provided by grain is derived from starch which is rapidly fermented to volatile fatty acids by rumen microbes. In addition, because grain consumption does not provoke significant chewing and rumination, salivary buffer secretion is minimal. These two effects frequently lead to subacute ruminal acidosis (SARA), characterized by low rumen pH and the death of gram-negative bacteria, resulting in the release of lipopolysaccharide (LPS), a component of their outer cell membrane. The primary objective of this study was to determine the extent to which LPS released in the rumen crosses into the bloodstream. Cows were fed a total mixed ration (TMR) containing a 50:50 ratio of forage to concentrate over a 6 week period. In week 6, 21% of TMR dry matter (DM) was replaced with pellets containing equal proportions of ground wheat and barley to provoke SARA. The additional grain significantly reduced average daily ruminal pH, from 6.17 to 5.97, and increased the length of time each day during which rumen pH was below pH 5.6, from 118 to 279 minutes per day. DM intake, milk yield and milk fat concentration decreased significantly while milk protein concentration rose slightly. The grain challenge caused the free LPS concentration in rumen fluid to rise by 380% accompanied by a 10-fold increase in blood LPS concentration. Increases in the concentrations of plasma proteins associated with inflammation indicated that transfer of rumen-generated LPS caused an acute systemic inflammatory response.



28

Use of Real-time PCR to Predict Dry Matter Disappearance of Individual Feeds in a Total Mixed Ration

Animal Feed Science and Technology, March 2009, Volume 149, Number 3-4, pages 240-249

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The energy available to an animal fed any given diet is largely dependent upon the digestibility of dietary ingredients. When formulating diets for livestock, it is generally assumed that the components (fibre, protein, lipid) of each ingredient have constant digestibilities although this is, in fact, not true. Digestibilities are influenced by both animal factors and by other ingredients in the diet. This paper describes a novel method that has the potential to determine digestibilities of individual ingredients fed together in mixed diets. The proposed method involves identifying unique genetic markers in each ingredient and measuring the rate of degradation of those markers using an assay based on the polymerase chain reaction (PCR) technique. The assumption is made, based on previous research, that the rate of plant cell DNA degradation is proportional to the rate of degradation of other cellular components. To test the proposed method, samples of corn and alfalfa were individually incubated in rumen fluid maintained at pH 6.8 or 5.5. Disappearance of dry matter (DM) and DNA markers were measured for up to 48 hours. Relationships between DM and DNA disappearance rates for the separate ingredients were then used to predict DM disappearance rates in incubations containing corn and alfalfa in various proportions: 75:25, 50:50 and 25:75. When actual DM disappearance rates in these incubations were compared with rates predicted from DNA degradation estimated with PCR, correlations were very high. The PCR-based method also very accurately predicted DM degradation rate differences at pH 6.8 versus pH 5.5.



Chemical Characterization, Energy Values, Protein and Carbohydrate Fractions, Degradation Kinetics of Frost Damaged Wheat (with Severely Overall Weight Loss) in Ruminants

Animal Science Journal, April 2009, Volume 80, Number 2, pages 140-148

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Although barley is the predominant feed grain fed to dairy cattle in western Canada, in some years large quantities of wheat become available at very competitive prices due to damage to crops normally destined for human consumption. This study examined the nutritive value for cattle of wheat that had been damaged by frost. Samples of frozen wheat were compared with samples from crops that had not experienced damage, in terms of chemical analysis, calculated nutrient fractions and rumen degradability. The frozen samples examined had 24% lighter bushel weights (bulk densities) than standard samples of the same variety (AC Barrie). Frozen samples contained significantly lower levels of starch, total non-structural carbohydrates and non-protein N with significantly higher concentrations of crude fat, acid and neutral detergent fibre, lignin, as well as acid and neutral detergent insoluble crude protein (CP). Concentrations of other chemical components including CP, ash and total organic matter were similar to those in undamaged samples. Chemical analysis values were used to calculate nutrient subfractions defined in the Cornell Net Carbohydrate Protein System. Frozen wheat was lower in both intermediately degradable CP and carbohydrate (CHO) but higher in rapidly degradable and unavailable CP and CHO. Energy values, calculated using equations in the 2001 Nutrient Requirements of Dairy Cattle publication, indicated that frozen wheat would contribute less energy to dairy diets than would undamaged wheat. Rumen degradability values were calculated from *in situ* incubations in which ground samples of grain were suspended in the rumens of cannulated Holstein dry cows. Compared with undamaged grain, frozen wheat dry matter (DM) and CP solubility was lower while undegradable DM and CP were higher. Starch degradability values were similar for both frozen and undamaged samples.



30

Effects of Feed Naturally Contaminated with Fusarium Mycotoxins on Metabolism and Immunity of Dairy Cows

Journal of Dairy Science, April 2009, Volume 92, Number 4, pages 1585-1593

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The objective of this study was to evaluate production and immune response parameters of cows fed diets contaminated with deoxynivalenol (DON), a toxin produced by Fusarium mould. Mid-lactation dairy cows were offered one of two total mixed rations (TMRs) over a 9 week trial period. A control (CTRL) ration contained uncontaminated hay, haylage, corn silage, corn grain, wheat and supplement. In the DON ration, hay, corn grain and wheat were contaminated to the extent that the TMR contained 3.5 mg DON per kg of dry matter (DM). Among the parameters monitored, DM intake, body weight, milk yield and milk composition were similar in cows fed the 2 diets. Isolated neutrophils (white blood cells) from cows fed the DON ration had lower ability to engulf (phagocytize) experimental glass beads indicating depressed immune function. Serum sodium concentrations and antibody response to immunization were elevated in DON-fed cows.

31

Increasing Physically Effective Fiber Content of Dairy Cow Diets through Forage Proportion versus Forage Chop Length: Chewing and Ruminal pH

Journal of Dairy Science, April 2009, Volume 92, Number 4, pages 1603-1615

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Physically effective fibre is a concept intended to account for the effectiveness of the structural carbohydrate fraction of a diet to promote chewing and salivation and thus, mitigate ruminal acidosis. To quantify the concept, these authors evaluate the particle length distribution of dietary forages using the Penn State Particle Separator (PSPS), originally a series of 2 sieves with a bottom pan, later modified to include an additional sieve. The proportion of particles retained on the sieves (the longer particles) is then multiplied by the diet's neutral detergent fibre (NDF) concentration to derive the physically effective NDF (peNDF) value. One strategy for increasing dietary peNDF is to increase forage particle length (FPL) by including forages harvested with longer chop lengths. Another strategy is to increase the proportion of forage in the diet, since forages contain higher levels of NDF relative to concentrates. The present study was designed to evaluate these two strategies in terms of their effects on chewing and ruminal pH. Alfalfa silages, harvested to yield longer or shorter FPL were included at either 35% or 60% of dry matter in mixed diets fed to lactating cows. Increasing either dietary forage content or FPL increased total daily chewing time and number of chews. Increasing dietary forage raised average ruminal pH by 0.4 units. Increasing FPL raised ruminal pH by 0.2 units on average in cows fed both high and low forage diets but in low forage diets the effect of longer FPL was not enough to prevent acidosis.



32

Effects of Feeding Fermenten on Ruminal Fermentation in Lactating Holstein Cows fed Two Dietary Sugar Concentrations

Journal of Dairy Science, April 2009, Volume 92, Number 4, pages 1725-1733

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Fermenten® is a fermentation byproduct containing a high proportion of amino acids and short peptides. In laboratory (*in vitro*) experiments, Fermenten® addition to rumen fermentation cultures has been shown to increase the efficiency of microbial protein production (eMPP), nutrient digestibility and the ratio of acetic to propionic acids. Increasing eMPP requires that more of the organic matter digested in the rumen is used to make microbial cells rather than being fermented to yield volatile fatty acids (VFA). Reduced VFA production should mitigate the pH depression that often accompanies high acid production rates. The earlier *in vitro* studies also showed that adding sugar to the incubations further enhanced the positive effects of Fermenten®. The present research was designed to determine whether the effects of Fermenten® and sugar observed in the *in vitro* studies could be replicated in a whole-animal (*in vivo*) study. Mid-lactation cows fitted with ruminal cannulae were fed one of 4 different diets (F+S+, F+S-, F-S+, F-S-) formulated with or without Fermenten® (F+ and F-) and with or without added sugar (S+ and S-). The only significant ruminal effect attributable to Fermenten® inclusion was an increase in ammonia concentration. Rumen pH was not affected. Added sugar tended to increase daily average ruminal pH and decrease the length of time that pH remained below 5.8. Neither Fermenten® nor sugar had any significant or practical effects on milk or milk component yields.



33

Influence of Methionine Supply on the Response of Lactational Performance of Dairy Cows to Supplementary Folic Acid and Vitamin B12

Journal of Dairy Science, April 2009, Volume 92, Number 4, pages 1685-1695

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Earlier research by these authors demonstrated that combinations of supplemental methionine (Met), folic acid (FA) and vitamin B12 (B12) could increase milk volume and milk component yields. The present study focuses on evaluating which of the 2 primary metabolic roles played by FA might account for these milk production responses. One of these roles is related to DNA synthesis; the other involves the synthesis of methyl groups that are essential in several metabolic processes. If the latter role is responsible for the effect of FA on production, then the provision of supplemental Met should reduce that effect since Met is a source of methyl groups. Cows were fed diets incorporating either deficient (LoMet) or adequate (HiMet) concentrations of Met, provided in the form of rumen protected Met (RPMet). Sub-groups of cows within these 2 Met treatment groups either received or did not receive weekly intramuscular injections of FA alone or in combination with B12 from 3 weeks pre-calving to 16 weeks post-calving. Administration of FA and B12 together resulted in a slight increase in milk production with no differences attributable to level of RPMet supplementation. The latter observation suggests that the role of FA in providing methyl groups was not the mechanism responsible for its effect on milk production.



34

Alfalfa Pellet-induced Subacute Ruminal Acidosis in Dairy Cows Increases Bacterial Endotoxin in the Rumen without Causing Inflammation

Journal of Dairy Science, April 2009, Volume 92, Number 4, pages 1712-1724

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Subacute ruminal acidosis (SARA) in dairy cows is caused by the rapid fermentation of feed ingredients, resulting in high rates of volatile fatty acid production in the absence of enough physically effective fibre (PEF) to adequately stimulate chewing and salivary buffer secretion. Low rumen pH leads to the death of gram-negative bacteria, resulting in the release of lipopolysaccharide (LPS), a component of the outer cell membrane of these bacteria. Although SARA is commonly associated with the excessive intake of starch, it can also be provoked by diets containing lower levels of starch with inadequate PEF. In this study, SARA was induced by replacing chopped alfalfa hay with finely ground alfalfa in pellet form. The objective was to determine whether this would result in elevated levels of LPS in rumen and blood and whether, as found in previous studies, high blood LPS would lead to a systemic inflammatory response. Lactating cows were offered a diet containing a 50:50 ratio of forage to concentrate over a 6 week period. In the first week, all forage was provided by chopped hay; in each of the subsequent 5 weeks, 8% of the hay portion of dietary dry matter (DM) was replaced by alfalfa pellets. Based on a criterion of rumen pH below 5.6 for more than 3 hours per day, SARA was induced when pellets comprised at least 16% of dietary DM. As pellet content was increased, DM intake increased, milk yield and milk fat concentration decreased and milk protein concentration increased. Although free rumen LPS concentration increased by 345%, in contrast to previous studies where SARA was induced by feeding excessive starch, blood levels of LPS and plasma proteins associated with inflammation were not affected in the present study.



35

Timothy Silage with Low Dietary Cation-Anion Difference Fed to Nonlactating Cows

Journal of Dairy Science, May 2009, Volume 92, Number 5, pages 2067-2077

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Dietary cation-anion difference (DCAD) is a measure of blood acidification potential. In the formulation of diets for pre-partum dairy cows, the application of DCAD is based on the idea that a more acidic diet will help prevent milk fever by stimulating the mobilization of calcium from bone as milk production rapidly rises after calving. Although several methods for calculating DCAD have been proposed, the most basic subtracts the sum of chloride and sulfur ion equivalents (anions) from those of sodium and potassium (cations). Strategies for reducing DCAD have focused on feeding either anionic salts or other feeds containing high chloride levels, but these treatments often reduce dry matter intake. A previous study by these authors demonstrated the use of low-DCAD timothy hay to achieve metabolic effects considered indicative of successful DCAD treatment. The present study examined the use of low-DCAD timothy silage (LDTS), comparing its effects with those of a commercial low-DCAD fermentation by-product (BioChlor®; LDBP) and those of a control with normal-DCAD silage. LDTS was grown on a field with a soil potassium (K) content of 101 kg/ha also fertilized with 140 kg of chloride/ha; high-DCAD silage was produced on a field with a soil K content of 289 kg/ha. Both fields received 80 kg of nitrogen/ha. Both LDTS and LDBP tended to decreased dry matter intake compared to CTRL, suggesting the need for further research on the interaction between low-DCAD forage and conservation methods. Also, LDTS and LDBP diets reduced blood and urine pH as well as blood bicarbonate when compared with CTRL, indicating that LDTS was effective in reducing dietary DCAD.



36

Unprocessed Whole Flaxseed is as Effective as Dry-rolled Flaxseed at Increasing α -Linolenic Acid Concentration in Milk of Dairy Cows

Livestock Science, May 2009, Volume 122, Number 1, pages 73-76

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Flaxseed contains a high concentration of α -linolenic acid (α LA), an omega-3 fatty acid that has several beneficial properties. Evidence from previous trials suggests that α LA supplementation via flaxseed feeding may reduce embryonic loss in early lactation dairy cows. Increasing the concentration of α LA in milk is expected to confer health benefits to consumers. The objective of this study was to determine whether feeding dry-rolled flaxseed would produce higher levels of α LA in milk compared with feeding whole, unprocessed seed. After consuming a total mixed ration (TMR) containing sunflower seed for 14 days, 10 mid- first lactation cows were divided into 2 groups that were fed TMRs in which 10% of dry matter was either dry-rolled (RF) or whole flaxseed (WF). Digestibility of ether extract (total lipid) in the WF diet was significantly lower than that in the RF diet (48.6 versus 62.4%, respectively) and more α LA (and some intact seed) was excreted in the feces of cows on the WF diet (259 vs. 129 grams α LA/day). However, cows on both diets had similar milk α LA concentrations (0.83% for WF; 0.86% for RF) suggesting that the amounts of α LA absorbed from the gut were similar. These milk α LA concentrations were 3 times those found in milk when the cows were fed the TMR containing sunflower seed. The authors speculate that, offsetting the lower digestibility of lipid in the WF diet, α LA in the RF diet may have been subject to greater losses through biohydrogenation in the rumen.



37

Effects of Glycerol on Lactation Performance, Energy Balance and Metabolites in Early Lactation Holstein Dairy Cows

Animal Feed Science and Technology, May 2009, Volume 151, Number 1-2, pages 12-20

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Increasing production of biodiesel from oilseeds has resulted in the increased availability of glycerol (GLYC), a by-product of the process. Previous studies have demonstrated that GLYC can serve as a precursor for glucose production in transition cows, reducing the negative energy balance often experienced by cows in early lactation. This trial evaluated effects on production and metabolism when diets fed to early lactation cows were supplemented with GLYC. Thirty-six multiparous cows between 4 and 63 days in milk (DIM) were fed total mixed rations (TMRs) supplemented with 0 (CONT), 100 (LG), 200 (MG) or 300 (HG) grams of GLYC per cow per day. Level of GLYC supplementation had no significant effects on TMR intake, milk volume yield or milk component yields. Energy balance improved in proportion to GLYC intake, particularly during the first 17 and last 20 days of the 63 day trial. As a result, GLYC supplemented cows also tended to lose less body weight during those periods. Plasma glucose concentrations increased while plasma levels of non-esterified fatty acids, beta-hydroxybutyrate and urine ketones decreased in proportion to GLYC intake. These effects were indicative of increased energy availability and reduced reliance on body fat reserves to satisfy requirements.



38

Milk Concentrations of the Mammalian Lignans Enterolactone and Enterodiols, Milk Production, and Whole Tract Digestibility of Dairy Cows Fed Diets Containing Different Concentrations of Flaxseed Meal

Animal Feed Science and Technology, June 2009, Volume 152, Number 1-2, pages 103-111

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Flaxseed is a rich source of secoisolariciresinol diglucoside (SDG), one of a number of estrogenic plant lignans. In monogastric animals, such plant lignans are converted by colonic bacteria into mammalian lignans, including enterodiol (ED) and enterolactone (EL), which have been shown to have positive human health benefits. Previous studies in ruminant animals have demonstrated that SDG can be converted to EL and ED by rumen microbes with EL being the main product found in rumen fluid, blood plasma and milk. Increased concentrations of EL in milk may enhance its nutraceutical value. In addition, it has been shown that EL increases the proliferation of cultured mammary epithelial cells, suggesting that it may promote mammary development and lactation. The present study was designed to assess the effect of increasing levels of SDG intake on milk EL and ED concentrations. Since the highest concentration of SDG is found in flaxseed's fibre fraction, flaxseed meal (FM) was the ingredient used to increase dietary SDG intake. Diets containing 0, 5, 10 or 15% FM, on a dry matter basis, were fed to Holstein cows between weeks 33 and 37 of lactation. While the concentration of EL in milk increased in direct proportion to dietary FM level, ED was not detected. Feed intake, milk yield and milk composition were similar in cows fed the 4 diets.

39

Using a Complex Non-TDN Based Model (the DVE/OEB System) to Predict Microbial Protein Synthesis, Endogenous Protein, Degradation Balance, and Total Truly Absorbed Protein Supply of Different Varieties of Cereal Oats for Ruminants

Animal Science Journal, June 2009, Volume 80, Number 3, pages 273-279

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A new variety of oats, CDC SO I, recently developed at the University of Saskatchewan's Crop Development Centre, is distinguished from other oat varieties by its low lignin hull and high fat groat. A previous study compared the potential metabolizable protein contribution of SO I with those of 2 conventional varieties, CDC Dancer and Derby, using the evaluation system described in the US National Research Council's Nutrient Requirements of Dairy Cattle (NRC Dairy 2001). The present paper describes a similar comparison using the DVE/OEB model, a protein evaluation system used more widely outside of North America. Use of the DVE/OEB model yields estimates for truly absorbed rumen synthesized microbial protein (TAMP), truly absorbed rumen undegraded feed protein (TAUP) and total truly absorbed protein (TTAP) in the small intestine; endogenous protein (EP) in the digestive tract; and protein degraded balance (PDB). SO I had TAMP and EP levels that were similar to the other varieties. The TAUP value for SO I was higher than that for CDC Dancer but similar to Derby. Although TTAP was similar for the 3 varieties, PDB was positive and significantly higher in the SO I variety compared with the negative values calculated for CDC Dancer and Derby. The latter indicates a more favourable balance of rumen degradable crude protein and rumen fermentable energy in SO I.



40

Effect of Dietary Forage to Concentrate Ratio on Volatile Fatty Acid Absorption and the Expression of Genes Related to Volatile Fatty Acid Absorption and Metabolism in Ruminal Tissue

Journal of Dairy Science, June 2009, Volume 92, Number 6, pages 2767-2781

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Lactating dairy cows fed high concentrate diets are subject to ruminal acidosis when the rate of accumulation of fermentation acids (volatile fatty acids, VFA) exceeds the rumen's buffering capacity and/or the rate at which acids are removed by absorption through the wall of the rumen. The results of previous studies suggested that the absorption rates of VFA increase when higher concentrate diets are fed. The objective of the present study was to evaluate the effects of dietary concentrate level on VFA absorption rates and to relate these rates to the expression of genes that code for proteins involved in transport and absorption of VFA across the rumen wall. Cows were offered diets containing either 8% (LC) or 64% (HC) concentrate on a dry matter (DM) basis for a period of 34 days. Data and samples were collected over the last 6 days. DM intakes were similar for both diets. Cows fed the HC diet had lower average rumen pH than the cows fed LC (6.03 vs. 6.48) and their rumen pH remained below 5.8 for a higher proportion of the day (376 vs. 10 minutes/day). Although total ruminal VFA concentration was significantly higher in the HC-fed cows, neither the rate of VFA absorption from the rumen nor the expression of genes coding for enzymes involved in VFA absorption were affected by dietary concentrate level.



41

Effects of a Propionic Acid-based Additive on Short-term Ensiling Characteristics of Whole Plant Maize and on Dairy Cow Performance

Animal Feed Science and Technology, June 2009, Volume 152, Number 1-2, pages 21-32

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It is normally recommended that ensiled forages be allowed a period of 6 to 8 weeks to achieve a low and stable pH. However, it is sometimes necessary to start feeding the forage before that stable state has been reached. Failure to achieve a low pH means that the forage may be subject to deterioration due to the growth of aerobic organisms upon exposure to air. The objective of this study was to test the efficacy of a silage additive in reducing the aerobic spoilage of corn silage that was exposed to air before the ensiling process was complete. The liquid additive was a blend of 70% propionic acid and 30% ammonium hydroxide applied at a rate of 5 litres per ton of wet, chopped forage. Additive-treated and untreated forages were ensiled in silage bags. Bags were opened the day after ensiling and were sampled daily for the next 30 days for analysis of microbial growth and chemical composition. Each of the 2 forages was fed to a group of 15 cows beginning at 2 days after ensiling. The additive significantly reduced yeast and mould growth on the forage between day 5 and day 14 post-ensiling. For the first 10 days post-ensiling aerobic stability (defined as the time required to raise silage temperature by 2°C) was also significantly improved by the additive. pH and organic acid concentrations were similar between the 2 forages. There were no significant differences in either dry matter intakes (average 23 kg/d) or milk yields (average 29 kg/d) between groups of cows fed treated or untreated forages.



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Developmental Changes in Clearance of Intravenous Doses of Glucose, Acetate and β -hydroxybutyrate from Plasma of Calves

Livestock Science, June 2009, Volume 122, Number 2-3, pages 177-185

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In the first few months of life, the calf's digestive system evolves from one that digests nutrients directly from milk to one that derives nutrients from rumen fermentation. This process involves both structural development of the rumen and metabolic changes which allow the calf to reduce its reliance on glucose while increasing its utilization of volatile fatty acids. This study characterized some of the changes that occur during this process. Calves were pail-fed milk replacer until weaning at 39 days of age and were offered unlimited access to one of 4 starter feeds from one week of age until 54 days of age. Starters were based on a commercial product containing a protein pellet, an extruded supplement, steam-flaked corn and rolled barley (starter 1). In starter 2, 10% alfalfa meal replaced a portion of the corn and barley; starter 3 contained 20% alfalfa meal. In starter 4, cracked corn replaced the processed grains. Calves offered the starters containing alfalfa consumed more starter and grew faster than those fed starters 1 or 4; at 54 days of age they averaged 9.5 kg heavier. Starter 3 also promoted improved rumen papillae development as well as resulting in a higher rumen pH and acetate:butyrate ratio at day 54. Glucose metabolism increased significantly up to 39 days of age and glucose remained the primary energy source until the end of the trial. Blood concentrations of β -hydroxybutyrate and acetate increased and their utilization rates also increased up to 39 days of age.



43

The Effect of Selenium Sources and Supplementation on Neutrophil Functions in Dairy Cows

Animal, July 2009, Volume 3, Number 7, pages 1037-1043

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Neutrophils are the most abundant of the white blood cells found in mammals and are an important component of the animal's response to infection. When an infection occurs, neutrophils migrate to the site of infection through the bloodstream and the intercellular spaces within tissues, following chemical signals secreted by immune cells at the infection site. On reaching the site, neutrophils engulf (phagocytize) the infectious organisms, isolating them in intracellular phagosomes into which reactive oxygen molecules (ROM) are released, destroying the infectious organism by oxidation. The synthesis of these ROM involves a rapid uptake of oxygen by the neutrophils, referred to as a 'respiratory burst'. Selenium (Se) plays important roles in a number of immune functions involving neutrophils, including the synthesis of ROM. The objective of the present study was to assess the effects on neutrophil function of feeding 2 different levels of dietary Se provided in either organic or inorganic form to dairy cows from 4 weeks before until 4 weeks after calving. Five groups, each containing 5 cows, were offered diets containing either no supplemental Se (control), 0.3 or 0.5 mg/kg Se as sodium selenite or the same concentrations of Se as an organic complex. Se supplementation had no effect on neutrophil phagocytosis but organic Se at both concentrations significantly increased the neutrophil respiratory burst. Programmed neutrophil cell death was lowest in cows fed the control or organic Se supplemented diets. Although cow plasma Se concentrations were not affected by dietary treatment, calf plasma Se concentrations were higher when cows received Se supplementation at the 0.5 mg/kg level from either source.



44

Differences in Splanchnic Metabolism between Late Gestation and Early Lactation Dairy Cows

Journal of Dairy Science, July 2009, Volume 92, Number 7, pages 3233-3243

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As the cow transitions from gestation to lactation, her nutrient requirements change significantly. After calving, the rapid rise in milk production places a high demand on her metabolism to provide for the synthesis of milk components. In particular, the synthesis of milk lactose requires her liver to produce large amounts of glucose (gluconeogenesis) from substrates including lactate, propionate and amino acids (AA). But AA are also required to satisfy requirements for milk protein synthesis. The objective of this study was to evaluate the flow of metabolites as they pass from the gut into the portal blood system and through the liver in late gestation and early lactation cows. As milk production increased after calving, both glucose absorption from the gut and gluconeogenesis by the liver increased. Lactate was found to be the main substrate for this increased gluconeogenesis rather AA, as has been suggested by other investigators. For most AA, extraction by the liver declined as lactation is initiated, suggesting that AA use for milk production was of higher priority than gluconeogenesis.



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Effects of Glucose, Propionic Acid, and Nonessential Amino Acids on Glucose Metabolism and Milk Yield in Holstein Dairy Cows

Journal of Dairy Science, July 2009, Volume 92, Number 7, pages 3244-3257

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The high-producing dairy cows required large quantities of glucose (GLC) to support the synthesis of milk lactose. Because she absorbs relatively little GLC from her diet, most of this requirement must be met through hepatic GLC synthesis (gluconeogenesis: GNG). The primary substrates for GNG are propionate (PROP), amino acid (AA) and lactate. The objectives of this study were to determine whether increased GLC availability would increase milk lactose synthesis and to quantify the potential contributions of intestinally-absorbed GLC, PROP and AA to whole body GLC supply. Mid-lactation Holstein cows fitted with ruminal and duodenal cannulae received iso-energy infusions (digestible energy) of GLU or AA into the duodenum or PROP into the rumen in supplement to a grass silage based diet. In control (CTRL), cows received duodenal infusions of water. Arterial GLC entry rate that mainly corresponding to GNG plus glycogenolysis with the grass silage diet was measured by using the dilution of a stable isotope of GLC infused into a jugular vein. GLC entry rate increased 2170, 3220, 2590 and 2490 g/d while milk lactose yields averaged 1180 g/d, 1220 g/d, 1230 g/d and 1125 g/d for CTRL, GLC, PROP and AA infusions, respectively. Although infusion of GNG substrates increased GNG, as indicated by GLC entry rate that were higher for infusions relative to CTRL. However increases in GNG did not result in higher milk lactose production. The authors suggest that this may have been due to a shift of GLC utilization away from lactose synthesis toward other pathways: either an increase of GLC utilization in other tissues than mammary gland mammary or an increase in GLC oxidation within the mammary gland.



46

Heat-induced Protein Structure and Subfractions in Relation to Protein Degradation Kinetics and Intestinal Availability in Dairy Cattle

Journal of Dairy Science, July 2009, Volume 92, Number 7, pages 3319-3330

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Ingredients used in diets fed to lactating cows may have been subject to heating. Byproducts such as distillers' grains are often heated in the process of removing moisture. Other ingredients may have been heated in an attempt to reduce the degradability of their protein. However, studies of the effects of heating on protein metabolism and animal performance have yielded conflicting results. The objective of the present study was to examine the effects of heating on the physical structure of flaxseed protein using synchrotron-based Fourier transform infrared microspectroscopy (FTIRM). Samples were also subjected to chemical analyses and *in situ* rumen incubations. Intact flaxseeds were heated at 120°C for 20, 40 or 60 minutes (treatments T1, T2 and T3, respectively). Compared with unheated control seeds, the FTIRM analysis revealed that T2 and T3 treatments altered the 3-dimensional structure of protein, increasing the ratio of α -helix to β -sheet structures. Heating also decreased the proportion of soluble CP and increased non-protein nitrogen, as well as neutral and acid detergent insoluble nitrogen resulting in a calculated decrease in overall protein degradability. Degradation characteristics of samples suspended in the rumens of cannulated Holstein dry cows indicated reductions in rumen-degradable protein and dry matter for all treatments. A laboratory procedure used to estimate intestinal digestibility revealed no changes in the digestibility of rumen undegradable protein due to heating.



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Increasing Dietary Sugar Concentration May Improve Dry Matter Intake, Ruminal Fermentation, and Productivity of Dairy Cows in the Postpartum Phase of the Transition Period

Journal of Dairy Science, July 2009, Volume 92, Number 7, pages 3341-3353

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Previous research by these authors and others suggested that replacing dietary starch with sugar in lactation diets might increase feed intake without depressing ruminal pH as might be expected with a more rapidly fermentable substrate. The objective of the present study was to determine whether adding sucrose to diets fed to fresh cows might mitigate the negative energy balance often experienced in early lactation. Immediately after calving, cows were offered diets containing either low (LS: 4.7%) or high (HS: 8.4%) sugar concentrations. At the higher concentration, sucrose replaced cracked corn. Compared with cows fed the LS diet, those fed the HS diet had higher dry matter (DM) intakes (18.3 vs. 17.2 kg/day) and higher average ruminal pH (6.21 vs. 6.06) although there was no difference in the proportion of time during which pH was below 5.8. Digestibilities of DM and nutrient fractions, including starch and neutral detergent fibre, were similar on both diets as were ruminal volatile fatty acid (VFA) concentrations and proportions of individual VFAs. Cows fed the HS diet had lower plasma glucose concentrations but higher concentrations of betahydroxy butyrate and non-esterified fatty acids. Plasma insulin concentrations were similar in cows on both diets. Neither milk yield nor milk composition was affected by dietary sugar concentration.

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Assessment of the Potential of Cinnamaldehyde, Condensed Tannins, and Saponins to Modify Milk Fatty Acid Composition of Dairy Cows

Journal of Dairy Science, July 2009, Volume 92, Number 7, pages 3392-3396

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This study investigated the potential of 3 different types of plant extracts to alter milk fatty acid composition when added to the diets of lactating dairy cows. Milk fatty acid profiles from cows fed total mixed rations supplemented with cinnamaldehyde (CIN; 1 g/cow per day), condensed tannins from quebracho trees (QCT, 150 g/cow per day), or saponins from *Yucca schidigera* extract (YSE, 60 g/cow per day) were compared with unsupplemented control cows. Supplemental CIN or QCT had no effect on milk fatty acid profiles while YSE decreased the relative proportions of saturated 6- and 8-carbon fatty acids (C6:0 and C8:0) as well as that of vaccenic acid (trans-11 C18:1).



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Fourier Transform Infrared Microspectroscopic Analysis of the Effects of Cereal Type and Variety within a Type of Grain on Structural Makeup in Relation to Rumen Degradation Kinetics

Journal of Agricultural and Food Chemistry, July 2009, Volume 57, Number 15, pages 6871-6878

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Starch, a non-structural carbohydrate contained within the endosperm of cereal grains, is the primary source of energy for high-producing dairy cows. The rate of degradation of that starch by rumen microbes is influenced by a matrix of protein that surrounds the starch granules, inhibiting microbial access. It is commonly recognized that this contributes to the slower ruminal degradation of corn starch relative to barley and wheat – the starch granules in corn grain are surrounded by a more robust protein matrix. Slower degradation is more favourable to digestive health and efficiency since rapid degradation of large quantities of starch can lead to ruminal acidosis. This study utilized Fourier transform infrared (IR) microspectroscopy analysis to predict rumen degradation kinetics by examining structural features of cereal grains. Rumen degradation kinetics were evaluated by incubating sealed nylon bags containing ground grain samples in the rumens of cannulated heifers. The IR analysis technique used the Canadian Light Source at the University of Saskatchewan and is part of a series of studies by the authors that look at both thermal and synchrotron light for structural analysis of feed grains. Relationships between protein and starch as well as the 3-dimensional structure of those relationships and of the protein matrix itself were examined. Results showed marked differences between corn and barley and between the 4 barley varieties in terms of structural make-up and *in situ* degradation kinetics. Pooled over 4 barley varieties, positive correlations were found between the ratio of non-structural carbohydrate to protein for dry matter degradation rate, the soluble dry matter fraction and effective dry matter degradability.



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Milk Concentration of the Mammalian Lignan Enterolactone, Milk Production, Milk Fatty Acid Profile, and Digestibility in Dairy Cows Fed Diets Containing Whole Flaxseed or Flaxseed Meal

Journal of Dairy Research, August 2009, Volume 76, Number 3, pages 257-264

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Flaxseed is a rich source of secoisolariciresinol diglucoside (SDG), one of a number of estrogenic plant lignans which have been shown to have positive human health benefits, including possible roles in the prevention of menopausal symptoms, hormone-dependent cancers, cardiovascular disease, and osteoporosis. In monogastric animals, such plant lignans are converted by colonic bacteria into mammalian lignans, including enterodiol (ED) and enterolactone (EL). EL has been found in the milk of dairy cows, likely due to the conversion of SDG to EL in the rumen or colon. Higher concentrations of EL in milk would be beneficial in enhancing milk's nutraceutical value and, given the strong antioxidant properties of EL and other lignans, might serve to extend milk's shelf life. This study examined the effect of feeding either whole flaxseed (WF) or flaxseed meal (FM) on dry matter (DM) intake, digestion, milk yield and composition and on milk concentrations of EL and ED. From week 17 to week 21 of lactation, Holstein cows were offered total mixed rations containing either no flaxseed product (CTRL), 10% flaxseed meal (FM) or 10% whole flaxseed (WF; DM basis). DM intake, milk yield and milk composition were similar for cows fed all 3 diets. Milk EL concentration was higher in cows fed WF or FM, compared to CTRL. ED was not detected in milk from any of the cows.



51

Effects of Increasing Amounts of Dietary Wheat on Performance and Ruminal Fermentation of Holstein Cows

Journal of Dairy Science, August 2009, Volume 92, Number 8, pages 3825-3832

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Although feed wheat is often attractively priced in western Canada, dairy producers are usually reluctant to incorporate it into lactation diets because they believe it poses a greater risk for ruminal acidosis compared with barley. However, research evidence to support this belief is equivocal. Wheat contains a higher proportion of starch than barley and there is evidence that wheat starch is more rapidly degraded in the rumen but there are few practical trials demonstrating whether, or at what level, wheat might be used in lactation diets. This paper describes such a study. Holstein cows averaging 98 days in milk were offered total mixed rations (TMRs) based on alfalfa hay and barley silage with steam-rolled barley and corn grains (CTRL). In 2 treatment TMRs, steam-rolled wheat replaced barley at rates of either 10% or 20% of ration dry matter (DM). Six of the 12 cows in the study were fitted with rumen cannulae, allowing for assessment of rumen fermentation parameters and nutrient digestibility. DM intakes as well as milk volume and milk component yields were similar for cows on all diets as were whole-tract apparent digestibilities of DM, crude protein, acid detergent fibre and neutral detergent fibre. Average ruminal pH was lower (6.36 vs. 6.44) and rumen ammonia nitrogen concentration was higher (11.49 vs. 8.10 mg/dL) in cows fed wheat versus barley. Wheat diets also produced higher rumen volatile fatty acid concentrations and lower acetate:propionate ratios. The authors conclude that wheat can be fed at a rate of up to 20% of dietary DM without compromising production or increasing the risk of ruminal acidosis.



52

Grazing Cows are More Efficient Than Zero-grazed and Grass Silage-fed Cows in Milk Rumenic Acid Production

Journal of Dairy Science, August 2009, Volume 92, Number 8, pages 3874-3893

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Rumenic acid (RA: cis-9, trans-11 C18:2) is one of the conjugated linoleic acids (CLAs) found in milk fat that have been found to have beneficial health effects in animal models. RA arises from the ruminal biohydrogenation and isomerisation of linoleic (LA: C18:2) and linolenic (LNA: C18:3) acids. These conversions are influenced by dietary concentrations of LA and LNA, rumen pH and substrate passage rate through the rumen. RA and vaccenic acid (VA: trans-11 C18:1), another product of these reactions, are transported to the mammary gland where VA is converted to RA by a desaturase enzyme. Earlier research demonstrated that milk RA concentration was higher in cows allowed to graze grass pasture (GG) compared with those who were fed fresh-cut (GF) or ensiled grass (GS) from the same sward. The objective of the present study was to quantify the variables responsible for these differences. Cows were assigned to one of the 3 feeding strategies used in the previous study, as described above. There were significant differences in intakes of LA and LNA among cows on the 3 treatments (GG: 433; GF: 327; GS: 164 grams/day). Rumen pH was lowest for GG cows (6.32) with no difference between GF (6.79) and GS (6.71) cows. VA concentrations in rumen, blood and milk were all highest for the GG cows, intermediate for the GF cows and lowest for the GS cows. Milk RA concentrations followed the same pattern with blood VA concentration accounting for 95% of the variation in milk RA concentrations. The authors conclude that LA + LNA intake strongly influence milk RA concentration but was not the only determining factor.



53

Effect of Replacing Forage or Concentrate with Wet or Dry Distillers' Grains on the Productivity and Chewing Activity of Dairy Cattle

Animal Feed Science and Technology, August 2009, Volume 153, Number 1-2, pages 1-10

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Distillers' grains (DG) are by-products of the fermentation of grain to produce ethanol. Although corn DG have been used in dairy cattle diets for many years, the recent expansion of ethanol production in western Canada has resulted in the increased availability of wheat DG (WDG). This study evaluated the feeding value of WDG as a partial substitute for forage or concentrate in diets fed to lactating cows. Production parameters and chewing activity were evaluated in cows fed each of 4 total mixed rations (TMRs). The control diet (CTRL) was a typical western Canadian lactation ration based on alfalfa hay, barley silage and barley grain with canola meal, soymeal and corn gluten meal as protein supplements. In a second TMR, wet wheat/corn DG (WWCDG) primarily replaced barley silage at 10% of ration dry matter (DM). In two additional rations, concentrate was replaced by either dry corn DG (DCDG) or dry wheat DG (DWDG) at 10% of ration DM. There were no differences in DM intake among the 4 diets. Relative to CTRL, the WWCDG ration increased milk volume yield by 7% and milk protein yield by 9%; while milk fat yield did not change, milk fat test declined from 3.40% to 3.14% possibly related to a dilution effect associated with milk volume. Substitution of concentrate with either DCDG or DWDG had no effect on milk production parameters or chewing activity.

54

Effect of Propylene Glycol Supplementation on Microbial Protein Production in Transition Dairy Cows

Canadian Journal of Animal Science, September 2009, Volume 89, Number 3, pages 419-423

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Propylene glycol (PG) can be used to help correct negative energy balance in cows immediately after calving. Upon entering the rumen PG is converted to propionic acid which can be used by the liver to synthesize glucose, reducing the utilization of fatty acids and ketone bodies as energy sources. The success of this strategy has been variable, depending on method of delivery (drench versus top-dress), dosage and timing. A few studies have suggested that PG might inhibit rumen microbes, affecting volatile acid production or microbial protein synthesis. The objective of the present trial was to evaluate the latter possibility. Cows were fed a single transition diet from 7 days before expected calving date until 45 days in milk. Diets fed to one-half of the cows were top-dressed at twice daily feeding times with 312 grams of PG (624 grams PG/day). Daily urine output was collected on day 14 before expected calving and at days 15 and 38 after calving. Analysis of urinary output and metabolite concentrations indicated that microbial nitrogen supply increased on each subsequent sampling day, likely associated with increasing feed intake. However, the authors conclude that PG had no effect on rumen microbial protein production.



55

Apparent Ruminal Synthesis and Intestinal Disappearance of Vitamin B12 and its Analogs in Dairy Cows

Journal of Dairy Science, September 2009, Volume 92, Number 9, pages 4524-4529

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Previous studies have observed increased milk production in response to the intramuscular injection of vitamin B12 (B12) in combination with folic acid. These observations suggest that the cow's B12 supply, normally provided by rumen microbes, may at times be inadequate to support high production. The objective of the present study was to evaluate the metabolism of B12 and related cobalt (Co)-containing compounds (corrinoids) in the rumen and small intestine of cows when they were fed, or not fed, supplemental B12. Diets, fed to 4 lactating cows over 18 days were supplemented with Co at a rate of 0.76 mg/kg of dietary dry matter. The first 9 days served as a control period; 500 mg/day of B12 was added to the diets for the remaining 9 days. During the control period, 38% of the corrinoids found in the rumen were identified as B12. About 11% of daily Co intake was incorporated into corrinoids; 4% into B12. In the last 4 days of the B12 supplement period, 20% of the supplemental B12 was transferred to the duodenum. Although B12 was not the primary corrinoid synthesized by rumen microbes, it did appear to be the main one absorbed by the small intestine.

56

Maize Silage Particle Length Modulates Feeding Patterns and Milk Composition in Loose-housed Lactating Holstein Cows

Livestock Science, September 2009, Volume 124, Number 1-3, pages 33-40

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When total mixed rations (TMRs) are fed to high-producing dairy cows, the inclusion of long forage particles is considered important as a way of mitigating the risk of ruminal acidosis by stimulating chewing and the production of salivary buffers. On the other hand, cows are frequently able to sort long forage particles out of the TMR with the result that they do not consume the amount of fibre they require. The objective of this study was to determine the effect of corn silage particle size on intake, feeding behaviour and productive performance. Lactating cows averaging 91 days in milk were offered one of three TMRs containing corn silage (CS) harvested to yield theoretical average particle lengths of 5.5 (short), 8.1 (medium) or 14 mm (long). As CS particle length decreased, intakes of dry matter, energy and other dietary nutrients increased, including the amount of physically effective neutral detergent fibre. Yields of milk and milk components were not affected by particle length. As a result, energy efficiency of milk production (milk energy / feed energy) declined with decreasing particle length. CS particle length influenced feeding behaviour but most of the significant effects were non-linear. For example, the number of daytime meals was the same for cows fed short and long CS but higher for cows fed medium CS. Reducing CS particle length did reduce sorting against long particles.



57

Effects of Malic Acid on Feed Intake, Milk Yield, Milk Components and Metabolites in Early Lactation Holstein Dairy Cows

Livestock Science, September 2009, Volume 124, Number 1-3, pages 182-188

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Malic acid (MA) is a 4-carbon intermediate in the energy metabolism of rumen bacteria. In laboratory (*in vitro*) fermenters inoculated with rumen fluid, the addition of MA has increased concentrations of propionate and total volatile fatty acids, decreased methane production and increased substrate digestibility. However, previous whole-animal (*in vivo*) studies have yielded conflicting results, possibly due to varying diet composition and/or MA supplementation levels. In the present study, total mixed rations (TMRs) fed to early lactation Holstein cows (1-63 days in milk) were supplemented with 70, 140 or 210 grams of MA per cow per day. The TMR fed to control cows (CTRL) contained no supplemental MA. Milk yield increased significantly with increasing levels of MA supplementation while feed intake was not affected, resulting in significant improvements in feed efficiency. Milk component concentrations were similar to CTRL for all MA doses. MA-supplemented cows had higher concentrations of plasma glucose and serum insulin, lower concentrations of plasma beta-hydroxybutyrate and free fatty acids, and lower concentrations of urinary ketones, suggesting that nutrient digestibilities and energy availability may have been improved.



58

Effects of Feed Delivery Time on Milk Production and Sorting of Dairy Cows

Canadian Journal of Animal Science, September 2009, Volume 89, Number 3, pages 425-429

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A previous, short-term trial by these authors and their colleagues had demonstrated that that feeding dairy cows at 2100 hours improved milk fat and reduce sorting compared with feeding at 0900 hours. The objective of the present study was to determine whether the same effects would be observed in a longer, more statistically valid experimental design. Two groups of 14 cows each were fed once daily, at either 0900 or 2100 hours for 6 week periods with responses monitored during the last 3 weeks. Dietary dry matter consisted of 45% chopped alfalfa hay, 45% barley grain-based energy supplement and 10% protein supplement. Time of feeding did not affect either feed intake or milk production although milk fat concentrations for both treatments were very low, decreasing from an average of 2.14% during week 1 to 1.73% during week 3 of the monitoring period. The authors speculate that the low milk fat levels may have been due to the short particle lengths of the chopped hay, and may have prevented an effect of the time of feed delivery on milk fat. The decreasing milk fat concentrations during the study may have resulted from increased sorting against long and medium length feed particles which was greater in morning-fed than in evening-fed cows.

59

Nutrient Variation and Availability of Wheat DDGS, Corn DDGS and Blend DDGS from Bioethanol Plants

Journal of the Science of Food and Agriculture, September 2009, Volume 89, Number 10, pages 1754-1761

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Although dried distillers' grains with solubles (DDGS) have been commonly used in dairy diets for many years, the recent increase in bioethanol production in western Canada has increased the availability of both corn and wheat DDGS as well as blends of these 2 byproducts. The objective of this study was to compare the feeding values of these different DDGS and their source grains in terms of their chemical analyses, calculated nutrient fractions and *in situ* rumen degradabilities. Significant differences were found in all measures. Significant variation was also found between DDGS produced by different bioethanol plants. Energy values calculated for wheat DDGS were similar to wheat and corn suggesting wheat DDGS could be used as an alternative to wheat and corn in dairy and beef diets. Corn DDGS energy values were significantly higher than in corn while energy values of the blend DDGS were higher than those in wheat DDGS but similar to corn DDGS. Wheat DDGS had higher *in situ* crude protein degradability and lower NDF degradability than corn DDGS, but similar degradability to blend DDGS.



60

Evaluation of the Protective Effect of Probiotics Fed to Dairy Cows during a Subacute Ruminal Acidosis Challenge

Animal Feed Science and Technology, September 2009, Volume 153, Number 3-4, pages 278-291

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In early lactation, dairy cows are typically fed diets containing high levels of grain to provide the energy required to achieve their genetic potential for milk production. It is not uncommon for these cows to develop subacute ruminal acidosis (SARA), where ruminal pH falls to levels that negatively affect forage digestibility and overall rumen efficiency. Several strategies have been recommended to reduce the risk of SARA, including dietary supplementation with probiotics — microbial cultures or culture extracts capable of stabilizing ruminal pH and thus, attenuating the symptoms of SARA. This study tested the use of 2 different probiotics: a fermentation extract of the fungus *Aspergillus oryzae* (AO) and a combination (COMB) of 2 live organisms, *Saccharomyces cerevisiae* (yeast) and *Enterococcus faecium* (a bacterium). Total mixed rations (TMRs) balanced to meet their nutrient requirements were fed to lactating dairy cows averaging 212 days in milk. TMRs were supplemented with either 0.6 grams(g)/cow/day of AO (AO-0.6), 3 g/cow/day of AO (AO-3) or 2 g/cow/day of COMB. Control (CTRL) cows received no probiotic. After a 3 week adaptation period, SARA was induced by replacing a portion of each TMR with a pelleted blend of ground wheat and barley for 4 days. SARA induction lowered rumen pH in CTRL cows from an average of pH 6.1 during the adaptation period to a minimum of pH 4.4. In cows fed COMB, SARA induction resulted in a minimum pH of 5.0 and pH was maintained in the 5.6 to 6.0 range significantly longer than in CTRL cows. AO-0.6 cows experienced a minimum pH of 4.96 due to SARA induction; minimum pH was 4.88 in AO-3 cows. Neither level of AO supplementation significantly increased the time over which pH was maintained in the 5.6 to 6.0 range.

Genetics





1

Milk and Fat Production of Crossbred Holstein-Gir cows (*Bos taurus taurus*-*Bos taurus indicus*) in the Agreste Region of the Brazilian State of Pernambuco

Genetics and Molecular Biology, January 2008, Volume 31, Number 2, pages 468-474

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Pernambuco, one of the states in tropical north-eastern Brazil, is the second largest milk producing state in the region. Most of the dairy animals are crossbred as a result of a number of studies suggesting that these are more suitable than purebred cattle for tropical milk production. This study compared the milk production of cows produced by crossing Holsteins with cattle of the Gir breed, a Brahman-type (*Bos taurus indicus*). Cows were either first generation $\frac{1}{4}$ Holstein (QH), $\frac{1}{2}$ Holstein (HH) or $\frac{5}{8}$ Holstein (FH) or interbred $\frac{5}{8}$ Holstein (FHx). Over a period of 19 years (1980-99), average milk yields were 5.24, 7.15, 6.58 and 4.68 kg/day while fat yields were 0.23, 0.31, 0.27 and 0.19 kg/day for QH, HH, FH and FHx cows, respectively. Since the FHx crossbred was intended as a synthetic breed adapted to the region, the authors conclude that it will be necessary to review this decision in light of the observed superior production of the HH crossbred.



2

Genetic Characteristics of Japanese Holstein Cows Based on Multiple-lactation Random Regression Test-day Animal Models

Livestock Science, April 2008, Volume 114, Number 2-3, pages 194-201

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The Canadian test-day model (TDM) incorporates the statistical calculations used to derive estimated breeding values (EBVs) for dairy animals from test day data recorded by Dairy Herd Improvement organizations. While the current Canadian TDM analyzes milk, fat and protein yield as well as somatic cell score (SCS) from the cow's first 3 lactations, the current TDM used for Holstein genetic evaluations in Japan is the same TDM across the first 3 lactations. The objective of this study was to derive the statistical parameters required to switch from the same TDM across the first 3 lactations to a multiple-lactation TDM for Japanese national genetic evaluations. Statistical analysis of the first 3 lactation curves of Japanese Holstein cows revealed significant differences between parities. Persistency declined with increasing parity as did days to reach peak yield - 45, 40 and 36 days for first, second and third lactations, respectively. The heritability of milk yield, calculated daily, were lower for first versus second and third lactations. The authors suggest that differences between genetic characteristics for each parity should be taken into consideration when combining the proofs for multiple lactations to select for lifetime production.



3

Phenotypic Study of Body Condition Scores in Canadian Dairy Cattle

Canadian Journal of Animal Science, June 2008, Volume 88, Number 2, pages 213-224

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Immediately after calving the energy requirements of the dairy cow typically exceed her energy intake resulting in a period of negative energy balance in which she mobilizes body fat reserves. The amount of subcutaneous fat carried by the cow is referred to as her body condition. The most commonly-used method of assessing body fat reserves assigns a body condition score (BCS) of 1 to an extremely thin cow; 5 to a very fat cow. This paper reports a statistical analysis of BCS values collected in Québec for Holstein and Ayrshire cows that calved from January 1999 through May 2004. The objective was to evaluate environmental effects on BCS, including herd, year of calving, parity, month of calving, age at calving, and days in milk (DIM). The last 3 variables significantly influenced BCS. Average BCS for Holstein cows from first to fifth parity ranged from 2.90 to 2.95; for Ayrshires the range was from 2.99 to 3.15 with no significant differences between parities for either breed. A non-linear statistical model used to calculate BCS by days in milk showed that, for parities 1 to 3 of both breeds, BCS fell between 0.3 and 0.5 points between calving and 50-60 DIM before beginning to recover. At 305 days in milk, BCS was generally 0.2 to 0.3 points higher than the scores of 2.9 to 3.1 recorded at calving. The authors recommend routine recording of BCS for individual cows through the production cycle as an aid to monitoring energy balance.



4

A Critical Analysis of Production-associated DNA Polymorphisms in the Genes of Cattle, Goat, Sheep, and Pig

Mammalian Genome, September 2008, Volume 19, Number 9, pages 591-617

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New technologies that facilitate determining the nucleotide sequences in DNA have created the potential to enhance tradition genetic improvement strategies with the addition of information about the genes associated with particular desirable traits. Most economically important traits in livestock are variable in magnitude (e.g., milk yield, growth rate) and are influenced by multiple genes. They are referred to as quantitative traits to distinguish them from qualitative traits that are expressed as only one of 2 alternatives. Quantitative trait loci (QTLs) are the locations on the chromosomal DNA (genome) where these genes are found. Variations between animals in the level of expression of quantitative traits are due to slight differences in the DNA sequences of the genes influencing those traits. These differences are referred to as single nucleotide polymorphisms (SNPs - pronounced 'snips'); they are locations in the gene's DNA sequence where variations (polymorphisms) in a single base (nucleotide) are found. Associations between SNPs and observable traits are established through statistical methods by correlating performance records with the presence or absence of individual SNPs, permitting those SNPs to be identified as 'markers' for a particular trait. This facilitates the process of marker assisted selection (MAS) where a trait of interest is selected, not based on the trait itself, but on a marker linked to it. As of early 2008, some 1,123 QTL markers influencing 101 different traits had been identified on the bovine genome. This paper reviews information on these markers and on the strategies required to use this information effectively in MAS programs.



5

Detection of QTL for Milk Protein Percentage in Italian Friesian Cattle by AFLP Markers and Selective Genotyping

Journal of Dairy Research, November 2008, Volume 75, Number 4, pages 430-438

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Quantitative traits are those whose level of expression (e.g., milk yield) are influenced by the expression of more than one gene. Quantitative trait loci (QTL) are the locations on the chromosomal DNA (genome) where these genes are found. Variations between animals in the level of expression of quantitative traits are due to slight differences in the DNA sequences of the genes influencing those traits. These differences are referred to as single nucleotide polymorphisms (SNPs - pronounced 'snips'); they are locations in the gene's DNA sequence where variations (polymorphisms) in a single base (nucleotide) are found. The objective of this study was to identify QTLs associated with estimated breeding value for milk protein percentage (EBVP%) in the genomes of Italian Holstein sires from 2 families. Genotyping of 64 sires with extreme high or low EBVP% identified 305 and 291 EBVP%-associated SNPs in the two sire families, respectively. Seventeen of these SNPs were found to have statistically significant associations with EBVP%. The locations (loci) of 11 of these SNPs were identified, 10 of which were close to loci previously attributed to QTL associated with milk protein percentage.



6

Linkage Disequilibrium and Signatures of Selection on Chromosomes 19 and 29 in Beef and Dairy Cattle

Animal Genetics, December 2008, Volume 39, Number 6, pages 597-608

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Individual genes that carry the genetic code defining the specific characteristics of an individual exist in pairs, one on each of the 2 paired chromosomes. The genes could have alternate forms called alleles. If two alleles exist for a gene, then there are 4 possible allele combinations for each gene, e.g. AA, BB, AB or BA. Alleles of other genes, located somewhere else on the genome, can match up in a similar way and therefore could be CC, DD, CD or DC. If there was no linkage between these 2 genes, the probabilities of each of the 4 possible combinations occurring for each gene would be independent of one another and the 2 genes would be said to be in linkage equilibrium. In contrast, if certain combinations (e.g., AA/CD) were found to occur more often than expected by random combination, the 2 genes are said to be in linkage disequilibrium (LD). The different versions of the DNA code carried by each allele can be characterized by variations in the nucleotides present at a single point in the DNA sequence (a single nucleotide polymorphism, SNP). This study examined the extent of LD for 370 SNPs on chromosome 19 and 186 SNPs on chromosome 29 from Angus and Holstein cattle. It was found that LD became insignificant when SNPs were separated by more than about 20 million nucleotides. 'Signatures' refer to regions on the genome that underlie variation in economically important traits. This study identified several such signatures on the chromosomes examined.



7

Reliability of Genomic Predictions for North American Holstein Bulls

Journal of Dairy Science, January 2009, Volume 92, Number 1, pages 16-24

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The objective of this study was to determine whether the addition of genomic data would improve the prediction of daughter performance of Holstein sires. Parent average (PA) genetic evaluations published in 2003 for 3,576 US and Canadian sires born before 1999 were used to predict the progeny performance of 1,759 bulls born between 1999 and 2002. Official 2003 PA values were compared with PA values calculated with the inclusion of genomic evaluations (GPAs) in terms of their predictive ability. Genotypes were determined on DNA extracted from semen samples and subjected to analysis with the Illumina BovineSNP50 BeadChip which detects over 54,000 genetic variants. Predictions based on GPAs were more accurate for all 27 traits examined, including 5 yield traits, 5 fitness traits, 16 conformation traits, and net merit. GPA predictions accounted for 5 to 38% more of the variation in these traits, compared with predictions based on PAs without genomic data; the greatest improvement was in the prediction of fat percentage due to the influence of a single identified gene with a large effect on the trait. Across all traits, the reliabilities of GPA-based predictions averaged 50%, versus 27% for PA-based predictions. The authors conclude that genetic progress will increase when breeders begin to use genotype data in combination with pedigrees and phenotypes when making mating decisions.



8

Immune Responses of Holstein and Norwegian Red × Holstein Calves on Canadian Dairy Farms

Journal of Dairy Science, February 2009, Volume 92, Number 2, pages 518-525

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Until recently, Canadian dairy cattle selection indexes have emphasized production traits, with less emphasis placed on health and fertility. This has resulted in rapid progress in production at the expense of animal health and fertility. While recent adjustments to selection indexes favouring these traits have been made, other strategies for their improvement are also attracting interest, including crossbreeding. While the heterosis (hybrid vigour) effect of crossbreeding alone is expected to have positive effects on fertility traits, the identification of an appropriate breed to cross with the highly productive Holstein (H) has been a challenge. This paper describes part of a study where the Norwegian Red (NR) has been crossed with H cattle. The NR was chosen both for its production potential and for the fact that both udder health and fertility have been emphasized in NR selection indexes for the past 30+ years. Identification of animals with superior immune defence mechanisms may help decrease the prevalence of mastitis and other infectious diseases. The present study compared antibody responses of purebred H calves with NR x H crossbred calves vaccinated with a hen egg white protein. To assess cell-mediated immune response, the vaccine also contained a yeast-derived antigen. Primary antibody-mediated immune response was highest with the NR x H calves but no difference was observed between the two breed groups for secondary antibody response or for cell-mediated immune response. Nonetheless, high and low immune responders could be identified in both breed groups suggesting that variation in these traits exists and that they may offer some potential to identify more disease resistant animals.



9

Differences in Udder Health and Immune Response Traits of Holstein-Friesians, Norwegian Reds, and Their Crosses in Second Lactation

Journal of Dairy Science, February 2009, Volume 92, Number 2, pages 749-757

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Until recently, dairy cattle selection indexes in many countries have emphasized production traits, with less emphasis placed on functionality. This has resulted in rapid progress in production at the expense of fertility and animal health, including the incidence of mastitis. The Norwegian Red (NR) is a breed in which selection for udder health and fertility traits as well as production have been emphasized for the past 30+ years. The present study compared immune responses and udder health of second-lactation Holstein-Friesian (HF), NR and NR x HF (NRX) cows. Animals were vaccinated with a hen egg white protein combined with a yeast-derived antigen to assess both antibody-mediated (AMIR) and cell-mediated (CMIR) immune responses. NR cows produced a greater AMIR 14 days after vaccination but AMIR after 21-days and CMIR were similar for all 3 breed groups. Each group had similar numbers of high and low responding individual cows. Udder health was assessed as average somatic cell score (SCS) over the lactation, average SCS within 30 days before vaccination, peak SCS for each individual cow during lactation and incidence of mastitis. By these criteria, NR cows had significantly better udder health than either HF or NRX cows. Udder health measures were similar among low and high immune responders.

10

Phenotypic Analysis of Pregnancy Effect on Milk, Fat, and Protein Yields of Canadian Ayrshire, Jersey, Brown Swiss, and Guernsey Breeds

Journal of Dairy Science, March 2009, Volume 92, Number 3, pages 1300-1312

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Pregnancy reduces milk production. Although the effect of pregnancy is minor in the first few months, hormones released by the fetus and placenta as gestation progresses cause regression of the mammary gland. And later in gestation, the nutrient demands of the growing fetus draw nutrients away from lactation. The current Canadian Test-Day Model, which is used to calculate genetic evaluations for production traits, does not account for pregnancy. If these effects significantly influence production records, they should be accounted for in the calculation of estimated breeding values since they are non-genetic. The objective of this study was to examine the production and reproduction records of cows of 4 breeds to determine whether days open or stage of pregnancy influences milk or milk component yields. In all 4 statistical models applied to the data, the effect of pregnancy was significant. The model that provided the most realistic results indicated that production began to progressively decline after about 4 months of gestation. Estimates of the effects of late pregnancy were hindered by the lack of records available during the dry period.



11

Influence of Stearoyl-coenzyme A Desaturase 1 Genotype and Stage of Lactation on Fatty Acid Composition of Canadian Jersey Cows

Journal of Dairy Science, March 2009, Volume 92, Number 3, pages 1220-1228

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Research evidence suggests that consumption of some saturated fatty acids (SFA), particularly those with 12 to 16 carbon chain lengths (C12–C16) poses a health risk for humans, due to their effect of increasing blood cholesterol concentration. In contrast, monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) have been shown to decrease concentrations of both cholesterol and low-density lipoproteins, reducing the risk of atherosclerosis and coronary heart disease. Several attempts, mainly nutritional, have been made to improve the fatty acid profile of cow's milk which typically contains about 70% SFA, 25% MUFA and 5% PUFA. The present study examines the possibility of decreasing the degree of saturation of cow's milk fatty acids through the application of genomics. One of the enzymes involved in converting SFA to MUFA in the cow's mammary gland is stearoyl-CoA desaturase 1 (SCD1). The level of activity of SCD1 is referred to as the desaturase index, the ratio of MUFA to SFA for each of the fatty acids on which SCD1 acts. It has been shown that 2 different forms of SCD1 exist, due to a single nucleotide polymorphism (SNP) in the gene that codes for the enzyme. SNC1A contains alanine at position 293 of its amino acid sequence; SCD1V contains valine at that position. In this study, the SCD1 genotypes of 525 Canadian Jersey cows were determined along with the fatty acid composition of their milk. Cows of the SCD1A genotype produced higher milk and protein yields and their desaturase indexes for C10, C12 and C14 fatty acids were higher. Desaturase indexes for C16 and C18 fatty acids were similar for both SCD1A and SCD1V variants.



12

Development and Characterization of a High Density SNP Genotyping Assay for Cattle

PLoS One, April 2009, Volume 4, Number 4, pages 1-13

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Genetic variation between individuals is due to differences in the sequences of nucleotides in their DNA. Most of these differences occur as single nucleotide polymorphisms (SNPs), where a single nucleotide is replaced by another. For a few traits, a SNP in a single gene can determine the observable expression of the trait but for most traits, the magnitude of their expression is influenced by a combination of SNPs in a number of genes that may be widely distributed across the genome. These are termed quantitative traits and the locations of the genes influencing these traits are called quantitative trait loci (QTLs). This paper describes the development of a method for characterizing 54,000 SNPs approximately evenly spaced across the bovine genome simultaneously. The method was tested by analyzing the genomes of (genotyping) 576 animals from 21 cattle breeds and six related species. The analysis revealed that, within individual breeds, from 39,765 to 46,492 SNPs may involve more than one possible nucleotide substitution. The value of the method was demonstrated by identifying QTLs for coat color and the presence or absence of horns. The method described has been commercialized in the form of the BovineSNP50 BeadChip assay produced by Illumina Inc.



13

Genome-Wide Survey of SNP Variation Uncovers the Genetic Structure of Cattle Breeds

Science, April 2009, Volume 324, Number 5926, pages 528-532

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Researchers have recently determined the complete sequence of nucleotides in the DNA (genome) of several animal species. Although the vast majority of the nucleotides found at specific locations (loci) on the genome are common among species, mutations have resulted in the replacement of one nucleotide by another at specific loci. These replacements lead to nucleotide variations at these loci among species and individuals within species, referred to as single nucleotide polymorphisms (SNPs, pronounced 'snips'). This paper reports on work aimed at characterizing SNPs among cattle breeds. Methods were developed to detect differences among 19 breeds in terms of the nucleotides present at 37,470 SNP loci on their genomes. Results suggest that domestication and selection for economically valuable traits such as milk production and resistance to disease has resulted in a reduction in diversity among these breeds. The study also detected a number of 'signature' regions on the genome – relatively stable DNA sequences resulting from selection for favourable traits common to all breeds. The authors point out that genetic diversity within breeds is no less than that found in the human population.

NOTE: the authors listed are the Canadian researchers that participated in this research among 150 members of the HapMap Consortium who are not included because of the restricted space available.



14

Effect of Preadjusting Test-day Yields for Stage of Pregnancy on Variance Component Estimation in Canadian Ayrshires

Journal of Dairy Science, May 2009, Volume 92, Number 5, pages 2270-2275

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Ideally, all measurable environmental variables affecting observed (phenotypic) production records should be included in the statistical model used to calculate genetic evaluations. However, the current Canadian Test-Day Model (CTDM) does not account for pregnancy. Direct adjustment for pregnancy in the CTDM would add an additional level of complexity to the model requiring reprogramming and significantly greater computing power. An alternative is to pre-adjust test-day records for stage of pregnancy before using those records in the CTDM. The objective of this study was to compare these 2 approaches using a data sample of 981 Canadian Ayrshire cows from 18 herds. Pre-adjustments were based on estimates of the effects of pregnancy on production traits established in a previous study by these authors. Overall, the two approaches yielded very similar results although pre-adjusting records did result in a decline in estimated breeding values (EBVs) for some non-pregnant elite cows. Results of the study suggested that records pre-adjusted for pregnancy effects could be used in the CTDM without altering the current model. As an alternative to inclusion of pregnancy effects in the CTDM, pre-adjustment improves the estimation of breeding values by accounting for the negative impacts of pregnancy in genetic evaluations, improving EBVs.



15

Effect of Pregnancy on Production Traits of Canadian Holstein Cows

Journal of Dairy Science, June 2009, Volume 92, Number 6, pages 2947-2959

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Pregnancy reduces milk production. Although the effect of pregnancy is minor in the first few months, hormones released by the fetus and placenta as gestation progresses cause regression of the mammary gland. And later in gestation, the nutrient demands of the growing fetus draw nutrients away from lactation. The current Canadian Test-Day Model, which is used to calculate genetic evaluations for production traits, does not account for pregnancy. This paper describes the evaluation of 7 different statistical models to account for the effect of pregnancy on production traits in Canadian Holstein cows. More than 22 million milk, milk component yield and somatic cell count records from 2.7 million cows were analyzed. Models varied in terms of the effects used, including days open, month of pregnancy, stage of pregnancy, days pregnant, stage of lactation and terms representing interactions between stage of pregnancy and stage of lactation. The objective was to identify the model that most appropriately accounted for the effects of pregnancy on production records. Models that included month of pregnancy or stage of pregnancy effects were most successful in achieving this objective. Using these models to adjust production records for pregnancy effects resulted in changing the ranking of cows based on estimated breeding values but did not change the ranking of sires.



16

A Whole Genome Scan to Map QTL for Milk Production Traits and Somatic Cell Score in Canadian Holstein Bulls

Journal of Animal Breeding and Genetics, June 2009, Volume 126, Number 3, pages 216-227

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Quantitative traits are those whose level of expression (e.g., milk yield) are influenced by the expression of more than one gene. Quantitative trait loci (QTLs) are the locations on the chromosomal DNA (genome) where these genes are found. Variations between animals in the level of expression of quantitative traits are due to slight differences in the DNA sequences of the genes influencing those traits. These differences are referred to as single nucleotide polymorphisms (SNPs - pronounced 'snips'); they are locations in the gene's DNA sequence where variations (polymorphisms) in a single base (nucleotide) are found. In this study, a total of 462 Canadian Holstein bulls were used to test associations between 1,536 SNPs and QTLs for milk production traits and somatic cell score (SCS) on the bovine genome. Twenty-one SNPs were found to be associated with milk production; of these, 5 were associated with QTLs not previously reported in other dairy cattle populations. Associations of 4 of the 21 SNPs identified had statistically significant associations with several milk production traits, including milk, fat and protein yield. Twelve SNPs were associated with QTLs known to affect SCS. The authors propose that the chromosome regions identified should be further investigated to identify the causative mutations underlying these QTLs associated with production and SCS.



17

Spermatozoal Transcriptome Profiling for Bull Sperm Motility: a Potential Tool to Evaluate Semen Quality

Reproduction, July 2009, Volume 138, Number 1, pages 65-80

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Current routine methods of assessing the quality of bovine semen depend on measurement of a sire's testicle size along with visual observation of his semen, including ejaculate volume and sperm concentration as well as sperm morphology and motility. Other microscopy methods can be used, including examination of various sperm cell structures and of dynamic responses to stimuli but these methods have not been highly successful in predicting the field performance of particular samples of semen. In a previous study, these authors examined the messenger RNA (mRNA) profiles (transcriptomes) of sperm cells as an indication of which genes were being expressed. They found that the presence of some specific transcripts (mRNA species) were associated with high non-return rates (NRR), the percentage of cows that were inseminated but not re-inseminated within a specified time period, usually 56 days. In the present study, a similar technique was used to determine whether an association could be found between motility and the presence of particular transcripts in sperm cells. Using a different and very specific technique for extracting mRNA, it was found that some of the transcripts previously found to be associated with high NRR were also associated with high sperm motility.



Genetic Parameters of Milking Frequency and Milk Production Traits in Canadian Holsteins Milked by an Automated Milking System

Journal of Dairy Science, July 2009, Volume 92, Number 7, pages 3422-3430

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As more Canadian herd owners adopt automatic milking system (AMS) technology, genetic parameters must be estimated to adjust production records to account for wide variations in milking frequency and milking intervals among cows milked in an AMS. In this study, 2 different statistical models were used to estimate those genetic parameters from 141,927 daily AMS visit and production records of 953 first lactation Holstein cows from 14 farms in Ontario and Québec. A 2-trait model included daily milking frequency and daily milk yield. A multiple-trait model incorporated effects of milk, fat and protein yields, somatic cell score (SCS) and milking frequency. The parameters estimated were heritabilities of each trait and genetic correlations between them. Heritability (H) is an estimate of the proportion of the phenotypic (observed) variation of a trait that is due to genetics, as opposed to environment. Genetic correlation is a measure of the degree of genetic relationship between 2 traits. In the 2-trait model, the H value for milking frequency ranged between 0.02 and 0.08 while that for milk yield was between 0.14 and 0.20. Genetic correlations between the 2 traits were 0.80 at the end of lactation and 0.27 in mid-lactation. H values from the multiple-trait model were 0.14, 0.26, 0.20, 0.21, and 0.20 for milking frequency, milk, fat and protein yields and SCS, respectively. Genetic correlations between milking frequency and milk, fat, and protein yields were positive while that between SCS and milking frequency was negative.



19

Selection of Single-Nucleotide Polymorphisms and Quality of Genotypes used in Genomic Evaluation of Dairy Cattle in the United States and Canada

Journal of Dairy Science, July 2009, Volume 92, Number 7, pages 3431-3436

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Determination of the sequence of DNA nucleotides across the entire bovine genome has led to the development of a method of genotyping cattle based on the use of the Illumina BovineSNP50 BeadChip (IBB). The IBB can determine the alleles of an animal at nearly 57,000 single-nucleotide polymorphisms (SNPs) – locations in the sequence where an alternative nucleotide may be found. However, not all SNPs provide useful contributions to genomic evaluations. The objective of this study was to identify SNPs that do not contribute or are unreliable in order to simplify genomic evaluation by eliminating them from future consideration. The IBB was used to genotype 10,690 bulls and 1,901 cows. A subsample of 5,503 bulls for which complete SNP profiles were available was chosen for determining which SNPs should contribute to genomic evaluations. Of the 56,947 SNPs characterized, 16,073 were deleted for their failure to contribute significantly to accuracy of genomic evaluation. The selected 40,874 SNPs provide a reliable basis for genomic prediction of genetic merit.



20

Modeling Milk Urea of Walloon Dairy Cows in Management Perspectives

Journal of Dairy Science, July 2009, Volume 92, Number 7, pages 3529-3540

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The objective of this study was to develop a statistical model to account for variation in test-day milk urea (MU) concentrations using the results of over 600,000 MU tests from first lactation cows in 632 dairy herds in southern Belgium. The heritability of MU concentration (the proportion of the total variance accounted for by genetics) was calculated as 0.13. A high degree of variation between herds on specific test-days suggested that herd management had a strong influence on MU. Comparison of individual MU test results with MU concentrations predicted by the model made it possible to establish target ranges for both herds and individual cows which can be used to help troubleshoot management problems. For individual cows, a target range of between 200 and 400 mg/litre was established. Cows with MU outside this range or whose MU deviated from the predicted value by more than 50 mg/litre were considered candidates for further evaluation. About 7.5% of the MU tests used to construct the model were outside these thresholds. For a herd to warrant concern, the difference between its predicted and actual average test-day MU level would have to exceed the standard deviation of its average test-day MU concentration. Of the herd average MU values calculated from the model dataset, 6.7% were outside this limit; many of these occurred during the grazing season.



21

Genetic Parameters of Production Traits in Chinese Holsteins Using a Random Regression Test-day Model

Journal of Dairy Science, September 2009, Volume 92, Number 9, pages 4697-4706

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The Chinese dairy industry is in a state of rapid evolution. At the end of 2007, there were an estimated 13.9 million dairy cattle in China, an increase of 182% over the population in 2000. A dairy performance testing program (PTP), initiated in the 1990s, was applied in only a few major centres until 2006 when it was expanded to include 8 of China's 23 provinces. In 2008 it was expanded again to include an additional 8 provinces. The PTP currently in use in China employs a lactation animal model, using regional performance data for local genetic evaluations. The primary objective of a cooperative research agreement between China and Canada is to establish a nation-wide test-day model for dairy genetic evaluations. This paper describes the derivation of genetic parameters for production traits to be used in the test-day model for evaluation of Chinese Holsteins. Data for the derivations were drawn from the complete records of 54 herds comprising 109,005 test-day records of 9,706 cows. Traits included were milk, fat and protein yield and somatic cell score (SCS). Heritabilities (H: the proportion of total variation due to genetics) of the production traits ranged from 0.222 to 0.346; the H for SCS ranged from 0.092 to 0.187. All H were higher in third lactation cows. Genetic correlations (GCs) between yield traits within parities were all greater than 0.8; GCs between yield traits and SCS were close to zero.



Stearoyl-CoA Desaturase 1 Genotype and Stage of Lactation Influences Milk Fatty Acid Composition of Canadian Holstein Cows

Animal Genetics, October 2009, Volume 40, Number 5, pages 609-615

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This study examined the role that genotype and stage of lactation might have on the fatty acid (FA) profile of cow's milk. Previous research had identified mutations (single nucleotide polymorphisms, SNPs) in a gene (stearoyl-CoA desaturase 1, SCD1) that codes for an enzyme involved in the synthesis of unsaturated FA (UFA) and conjugated linoleic acids (CLA). Depending on the SNP, the enzyme can have either a valine (V) residue or an alanine (A) residue at location 293 of its amino acid sequence. Cows of the AA genotype produce 2 copies of the A-type of SCD1 each time the gene is expressed; those of the AV genotype produce one each of the V-type and the A-type; the VV genotype produces 2 copies of the V-type enzyme. Milk FA profiles were assessed in terms of an index which expressed the ratio of mono-UFA to total FA within each FA chain length. It was found that cows of the AA genotype had significantly higher C10 (i.e., 10-carbon FA), C12 and C14 indexes than did cows of the AV or VV genotypes. In early lactation (up to 100 days in milk), C18 and total FA indexes were higher while C10, C12, C14 and CLA indexes were lower than in later stages of lactation. Concentrations of a number of unsaturated C18 FA as well as total UFA were higher in early lactation while those of saturated and unsaturated C10 to C16 and total saturated FA were lower than in later lactation. CLA concentrations were not influenced by either SCD1 genotype or stage of lactation.

Health





1

Comparison of Bacterial Culture, Histopathology, and Immunohistochemistry for the Diagnosis of Johne's Disease in Culled Dairy Cows

Journal of Veterinary Diagnostic Investigation, January 2008, Volume 20, Number 1, pages 51-57

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Johne's Disease (JD) is caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP), a slow-growing bacterium that infects the small intestine (ileum) and lymph nodes of cattle and other species. Efforts to reduce the incidence of JD have been hampered by the difficulty of identifying infected animals, due to the way in which MAP establishes itself within infected tissues. A number of diagnostic tests for MAP exist. Those that identify antibodies to MAP include enzyme-linked immunosorbent assay (ELISA), agar gel immunodiffusion and the complement fixation test. Other tests, including fecal/tissue culture, detect the bacterial agent directly. Direct fecal or tissue culture of MAP is the 'gold standard' for diagnosis but, due to the bacterium's slow growth, may take 4-16 weeks to provide definitive results. The objective of this study was to evaluate 2 more rapid techniques for identifying MAP in tissues: staining of tissue slices for microscopic examination (histopathology: HP) and detection of MAP-specific antigens in tissue slices using antibodies labelled with peroxidase (immunohistochemistry: IHC). HP identified MAP-like organisms in only 7 of 78 (8.97%) and 6 of 106 (5.61%) samples of culture-positive ileal and lymph node tissues, respectively. IHC analysis of the same tissues identified MAP in the ileum of 9 of 78 (11.54%) and in the lymph nodes of 5 of 106 (4.67%) culture-positive tissues. Both HP and IHC gave negative results for all culture-negative tissues. The authors conclude that culturing MAP from potentially infected tissues was far superior to either HP or IHC for diagnosing MAP infection.



2

Passive Immunity in Ontario Dairy Calves and Investigation of Its Association with Calf Management Practices

Journal of Dairy Science, October 2008, Volume 91, Number 10, pages 3840-3849

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Calves acquire protection from infectious disease through the passive transfer of antibodies (immunoglobulins, Ig) from their dams in the form of colostrum. Calves that fail to acquire sufficient protection (failure of passive transfer, FPT) often perform poorly and are subject to higher rates of illness and death. This paper reports the results of 2 studies designed to evaluate the extent of FPT among 1,340 calves on 123 dairy farms in southern Ontario. In both studies, blood samples were collected from calves between birth and 8 days of age. Serum total protein (STP) concentration, measured with a refractometer, was used to estimate the quantity of Ig acquired. STP concentrations ranged from 3.5 to 9.8 grams per decilitre (g/dL). Using a cutoff point of 5.2 g/dL as a minimum adequate STP level, 17% of the 1,340 calves studied experienced FPT. Participating producers were asked to complete a questionnaire on calf management practices including methods used to collect, store, and feed colostrum. The objective was to determine whether calf STP levels were associated with specific management practices. Risk of FPT was significantly higher for calves on farms where they were allowed to remain with their dams for more than 3 hours after birth. Providing a larger quantity of colostrum within 6 hours of birth significantly reduced the calves' risk of FPT. STP concentrations were significantly higher in calves cared for by female caregivers than in those tended by males.



3

Herd- and Cow-Level Prevalence of Foot Lesions in Ontario Dairy Cattle

Journal of Dairy Science, October 2008, Volume 91, Number 10, pages 3888-3895

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This study was designed to determine the prevalence of hoof lesions among dairy cattle housed in tie-stall (TS) and free-stall (FS) facilities in Ontario. Five professional hoof trimmers recorded the incidence of lesions among 13,530 cows during routine visits to 204 herds. Trimmers were trained to recognize 11 lesions according to standardized definitions. Abnormal walking or standing behaviour, non-hoof lesions and the application of blocks and wraps were also noted. Overall, FS cows had a markedly higher incidence of lesions, at 46.8% of cows, than TS cows, at 25.7%. Sole and white line hemorrhages and sole ulcers were the most common lesions recorded across both types of housing; their incidence in TS herds, at 7.7 and 4.7% of cows, was lower than in FS herds where 11.0% and 9.2% of cows were affected, respectively. The most common lesion in TS herds was digital dermatitis (DD) affecting 9.3% of cows and 69.7% of herds. DD was found in 96.7% of FS herds and affected 22.7% of the cows in those herds. Non-foot lesions were recorded in less than 1% of cows in either housing type. On average, 3.3% of cows in TS herds and 7.5% of cows in FS herds were judged lame by the hoof trimmers based on abnormal standing or walking.



4

T lymphocyte Proliferative Capacity and CD4+/CD8+ Ratio in Primiparous and Pluriparous Lactating Cows

Journal of Dairy Research, November 2008, Volume 75, Number 4, pages 457-465

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T cells are one of the broad classes of white blood cells (lymphocytes) that play a role in cell-mediated immune responses. Sub-populations of T lymphocytes are distinguished by molecular 'markers' on their surfaces. T 'helper' (Th) cells carry the CD4 marker; these cells release chemical messengers that regulate other immune cells. 'Cytotoxic' T cells carry the CD8 marker; their role is to lyse tumour cells and pathogen-infected cells. Each of these T lymphocyte sub-populations contain 'memory' cells that have previously encountered the specific antigen to which they are programmed to respond. On subsequent encounters with these antigens, they proliferate rapidly, producing a more effective response than was mounted at the first encounter. The objective of this study was to evaluate differences in T lymphocyte populations and their ability to respond to an antigen challenge (proliferative capacity: PC) in primiparous (PP) versus multiparous (MP) cows. The blood of MP cows had higher numbers of CD4 cells and lower numbers of CD8 cells compared with that of PP cows. T cells from MP cows had lower PC than those from PP cows. The authors suggest that the observed changes in the CD4/CD8 populations along with the lower T cell PC could lead to a weaker T cell immune response resulting in increased susceptibility to infection in MP cows.



5

Characterization of a *Staphylococcus aureus* Small Colony Variant (SCV) Associated with Persistent Bovine Mastitis

Foodborne Pathogens and Disease, December 2008, Volume 5, Number 6, pages 785-799

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Staphylococcus aureus (SA) accounts for up to 30% of all cases of bovine mastitis. There are no available successful vaccines against SA infection and antibiotic treatment is often not effective. SA infections are often persistent due to the ability of the pathogen to modify its form, resulting in a slow-growing, small-colony variant (SCV). This variant can survive inside host cells without triggering a protective immune response. The objective of this study was to evaluate the possible role of SA SCV in chronic bovine mastitis. Foremilk samples from 11 cows with chronic mastitis yielded 6 that contained SA, 5 of which formed colonies of the normal size and were otherwise characterized as typical SA cultures. The sixth isolate from the chronic mastitis cows contained both typical SA colonies and small non-haemolytic colonies which grew very slowly. When the culture medium contained antibiotic, 3 (50%) of *S. aureus* positive cultures developed SCVs which, when returned to antibiotic-free media, reverted to normal SA cultures. Characterization of one SCV strain (SCV Heba3231) revealed marked difference between Heba3231 and its parent strain in the expression of several metabolic activities typical of SA. This metabolic profile was similar to that found in human SCV strains of SA. This is the first report implicating an SCV strain of SA in bovine mastitis and demonstrating a similarity between bovine and human strains.



6

Application of Infrared Thermography as an Indicator of Heat and Methane Production and Its Use in the Study of Skin Temperature in Response to Physiological Events in Dairy Cattle (*Bos taurus*)

Journal of Thermal Biology, December 2008, Volume 33, Number 8, pages 468-475

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The objective of this study was to evaluate the use of infrared thermography (IT) to estimate heat and methane production by dairy cattle. IT is a simple, relatively inexpensive technique involving indirect temperature measurement by recording the wavelength of infrared radiation emitted by, in this case, body surfaces. Previous studies had demonstrated that cattle that were more efficient in converting feed energy into animal products had lower heat production and methane emissions, which can affect the amount of radiation dissipated by the animals' body surface. Since routine measurement of either of these variables is not practical on commercial farms, it was thought that IT might provide a realistic alternative. Lactating dairy cows were fed with one of two different total mixed ration formulations typically used in Canada and the measurements were collected monthly on six occasions. Oxygen consumption (to determine heat production) and methane production were monitored with an open-circuit indirect calorimetry system while IT was used to scan several body locations at the same time. A strong correlation ($r = 0.88$) was found between heat production and the surface temperature of the feet. Temperature difference between left and right flanks, within 100 minutes after feeding the cows, was found to provide the best correlation ($r = 0.77$) with methane production. Feeding and milking also provoked marked changes in surface temperature, suggesting that IT might be applied to the assessment of other physiological changes.



7

Comparing Network Analysis Measures to Determine Potential Epidemic Size of Highly Contagious Exotic Diseases in Fragmented Monthly Networks of Dairy Cattle Movements in Ontario, Canada

Transboundary and Emerging Diseases, December 2008, Volume 55, Number 9-10, pages 382-392

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Highly infectious diseases, such as foot-and-mouth disease (FMD), become epidemic through the movement of animals from farm to farm, especially in the period between initial introduction and confirmation of the first case. The speed at which a disease spreads depends on connectedness of farms and the frequency of animal movements between them. The purpose of this study was to estimate the magnitude of a potential epidemic among Ontario dairy herds should one of the herds become infected with a disease such as FMD. The movements of adult dairy cows within the network of Ontario farms enrolled in the Dairy Herd Improvement (DHI) program were examined over a period of 3 years (2004-2006) based on DHI-recorded animal transfers. The networks defined by these movements were highly fragmented. Three different network analysis measures were compared. The out-degree (OD) of a farm represents the number of other farms one infected farm could expose through a direct animal transfer. The infection chain (IC) is the number of farms connected either directly (one step) or indirectly (>1 step) to a source farm by animal movements. Strong and weak component (SWC) analysis measures links between farms created by animal transfers without taking into consideration the order of transfers in time. Farm OD and IC analysis gave similar and more reasonable estimates of potential maximal epidemic size than SWC analysis. The authors suggest that further studies should be conducted to verify the value of using these analyses to predict maximum epidemic size in large connected farm networks.



8

Repeat-based Subtyping and Grouping of *Staphylococcus aureus* from Human Infections and Bovine Mastitis Using the R-domain of the Clumping Factor A gene

Diagnostic Microbiology and Infectious Disease, January 2009, Volume 63, Number 1, pages 24-37

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Clumping factor A (clfA) is a protein found on the surface of *Staphylococcus aureus* (SA) cells which is responsible for the clumping of blood plasma observed when adding SA to human plasma. The objective of this study was to demonstrate a method of differentiating human SA strains from those causing bovine mastitis based on differences in the gene that codes for clfA. Previous studies had demonstrated that one section (the R-domain) of the clfA gene contains numerous repeats of an 18 nucleotide sequence that codes for 3 repeating pairs of the amino acids serine and aspartic acid (Ser-Asp). Results of the present study showed that, among human strains of SA, there were large differences in both the length of the clfA gene (and its product protein) and in the number of Ser-Asp code repeats in those genes. In contrast, there was much less variability in bovine mastitis SA isolates; 16 of 19 of these had clfA genes of identical length containing exactly 52 repeats, implying that specific selection of strains had occurred in the mammary gland. The authors conclude that this method, termed 'variable number tandem repeat-based typing' is a useful technique for differentiating SA strains.

9

Risk Factors Associated with *Mycobacterium avium* subspecies *paratuberculosis* Seropositivity in Canadian Dairy Cows and Herds

Preventive Veterinary Medicine, January 2009, Volume 88, Number 1, pages 32-41

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Mycobacterium avium subspecies *paratuberculosis* (MAP) is the bacterium that causes Johne's Disease (JD), a debilitating disease of ruminants. This study evaluated variables that affected the prevalence of antibodies to MAP in 315 Canadian dairy herds. The data were drawn from a number of surveys conducted in 6 provinces. In addition to a number of farm management risk factors, the influence of concurrent infections by bovine leukemia virus (BLV), bovine viral diarrhea virus (BVDV) and *Neospora caninum* (NC) were examined. Farm management factors that were found to significantly increase or decrease the proportion of MAP antibody-positive (seropositive) cows in a herd included: more than one cow sharing the maternity pen at the same time, group-housing of preweaned calves in winter, open heifers purchased during the last 12 months, and having beef cattle in direct, 'nose-to-nose' contact with dairy animals. Herds with cows that carried antibodies to BVDV and those where calves were not properly vaccinated for BVD were also more likely to have MAP seropositive cows. In contrast, herds where calves were properly vaccinated for BVD (including a booster after 6 months of age), had 40% fewer MAP seropositive cows.



10

Assessment of Bovine Mammary Chemokine Gene Expression in Response to Lipopolysaccharide, Lipotechoic Acid 1 Peptidoglycan, and CpG Oligodeoxynucleotide 2135

Canadian Journal of Veterinary Research, January 2009, Volume 73, Number 1, pages 49-57

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When the mammary gland is infected with pathogenic bacteria, mammary cells that recognize molecules on the surface of the pathogen respond with the secretion of cytokines, signalling chemicals that initiate the inflammatory response recognized as mastitis. Cytokine release also results in the secretion of chemokines, molecules responsible for directing the cell-mediated immune response to infection. A variety of different chemokines may be released, depending on the specific pathogen involved. The purpose of this study was to characterize the pattern of chemokines secreted in response to Gram-negative bacteria (e.g., *E. coli*) and Gram-positive bacteria (e.g., *Staph. aureus*) pathogen-associated molecular patterns (PAMPs) that include lipopolysaccharide, lipotechoic acid, and bacterial DNA. Chemokine response was determined by examining the expression of genes that code for various chemokines in mammary gland tissue cultures incubated with surface recognition molecules typical of these 3 different PAMPs. Results indicated that PAMPs associated with different mastitis-causing pathogens induce different chemokine secretion patterns suggesting that the mammary gland's immune response may vary depending on the specific pathogen involved. This may explain why *E. coli* infections are usually acute, while *Staph. aureus* infections typically lead to chronic, subclinical mastitis.



11

Comparison of Milk Culture, Direct and Nested Polymerase Chain Reaction (PCR) with Fecal Culture Based on Samples from Dairy Herds Infected with *Mycobacterium avium* subsp. *paratuberculosis*

Canadian Journal of Veterinary Research, January 2009, Volume 73, Number 1, pages 58-64

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Johne's Disease (JD) is caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP), a slow-growing bacterium that infects specific sites in the small intestines (ileum) of cattle and other species. Efforts to reduce the incidence of JD have been hampered by the difficulty of identifying infected animals. A number of diagnostic tests for MAP exist. Direct fecal culture of MAP is considered the 'gold standard' but, due to the bacterium's slow growth, this method may take 8-16 weeks to provide definitive results. The objective of this study was to evaluate 2 alternative methods using the polymerase chain reaction (PCR) to detect MAP DNA in milk samples. PCR results were compared with results of fecal and milk culture of samples from 146 cows in 14 MAP-positive herds in southern Ontario. Milk PCR results were significantly correlated with milk culture results but none of the milk analysis results were related to those of fecal culture. One of the PCR tests proved more sensitive than the other; this test could be used in place of milk culture. On average, more MAP were found in feces than in milk. The observation that fecal and milk culture results were poorly correlated means that animals with high numbers of MAP in milk culture may not be detected by fecal culture, and vice versa. The authors suggest that, to identify suspected MAP-infected animals using the tests in this study, both milk and fecal samples should be collected in duplicate to improve the diagnostic rate.

12

Association of *Escherichia coli* J5-Specific Serum Antibody Responses with Clinical Mastitis Outcome for J5 Vaccinate and Control Dairy Cattle

Clinical and Vaccine Immunology, February 2009, Volume 16, Number 2, pages 209-217

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Escherichia coli (EC) is one of the major environmental pathogens causing clinical mastitis (CM) in dairy cattle. J5 vaccine, which contains a poorly defined mix of antigens prepared from a mutant EC species (J5) is commonly used to improve the cow's defences to this pathogen. The objective of this study was to evaluate the effectiveness of J5 vaccination. Cows in 2 commercial herds in New York State were vaccinated with J5 approximately 60 and 28 days before their expected calving dates. A similar number of cows in each herd served as untreated controls. Before the initial vaccination, cows in both groups had similar blood levels of J5-specific antibodies (immunoglobulins: Ig), including IgM, IgG1 and IgG2. Immediately after calving, the J5 cows had significantly higher levels of J5-specific IgG1 and IgG2. However, after experiencing CM, no significant differences in Ig levels were detectable between J5 and control cows. CM caused by EC resulted in 75% less milk loss in J5-vaccinated cows compared with controls and J5 cows were at significantly lower risk of being culled, particularly in early lactation.



13

Evaluation of the California Mastitis Test as a Precalving Treatment Selection Tool for Holstein Heifers

Veterinary Microbiology, February 2009, Volume 134, Number 1-2, pages 136-142

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The California Mastitis Test (CMT) is a simple cow-side test for subclinical mastitis. It operates by disrupting the cell membrane of somatic cells present in a milk sample, allowing the DNA in those cells to react with the test reagent, forming a gel. In this study, the CMT was evaluated for its ability to detect intramammary infections (IMI) in pre-partum Holstein heifers, in comparison with a portable electrical conductivity meter (ECM). Mammary secretions from all 4 quarters of 428 dairy heifers were sampled between 6 and 12 days before their expected calving dates. Samples were subjected to testing by both the CMT and ECM. Results were compared to those from conventional laboratory bacterial culture (BC) to determine the sensitivity and specificity of CMT and ECM. Sensitivity measures a test's ability to correctly identify a positive culture; specificity measures its ability to confirm a negative-culture sample. BC revealed that 69% of the heifers sampled had pre-partum IMI; 16.8% had IMI involving major mastitis pathogens. The sensitivity and specificity of CMT for detecting any IMI in individual quarters were 68.9% and 68.4%, respectively. In contrast, CMT sensitivity and specificity for detecting IMI by major pathogens in any of a heifer's 4 quarters were 91.0% and 27.5%, respectively. Depending on the conductivity cut-off point chosen, ECM sensitivities and specificities ranged from 25.2% to 41% and 65.2% to 83.3%, respectively, for detection of all IMI at the quarter level. For detection of major pathogens at the heifer level, ECM sensitivities and specificities ranged from 53.7% to 68.7% and 44.1% to 59.5%, respectively. These results indicate that the CMT and ECM are not sufficiently accurate to be used for the routine detection of IMI in pre-partum heifers. However, a negative CMT or ECM result could be used pre-calving to identify heifers or quarters that were not infected.



14

Impact of Hyperketonemia in Early Lactation Dairy Cows on Health and Production

Journal of Dairy Science, February 2009, Volume 92, Number 2, pages 571-580

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When dairy cows make the transition from pregnancy to lactation, metabolic energy demand increases rapidly and significantly. This often leads to mobilization of body fat reserves and increased blood concentrations of the ketone bodies beta-hydroxy butyrate (BHB) and acetoacetic acid. This hyperketonemia (HK) may lead to the diagnosis of ketosis and other related metabolic disorders as well as loss of milk production. The objective of this study was to establish a threshold serum BHB concentration at which HK has a significant negative effect on production and animal health. Serum samples, obtained from 1,010 Ontario Holstein cows during the first and second weeks after calving, were analysed for BHB concentration. Health information was collected from various sources; Dairy Herd Improvement (DHI) records provided first-test day production data. Serum BHB concentrations at or above 1,200 micromoles per litre ($\mu\text{mol/L}$) in the first week after calving were associated with increased risks of subsequent displaced abomasum (DA) and metritis. The critical week 2 BHB threshold for increased risk of DA was 1,800 $\mu\text{mol/L}$; there was no association between week 2 serum BHB concentration and risk of metritis. A threshold of 1,400 $\mu\text{mol BHB/L}$ was the minimum threshold for predicting clinical ketosis risk from week 1 or week 2 serum samples. No association was found between week 1 or 2 serum BHB concentrations and risk of clinical mastitis. First DHI test day milk yields were reduced when week 1 BHB serum concentration was at or above 1,200 $\mu\text{mol/L}$ and when week 2 concentration was at or above 1,400 $\mu\text{mol/L}$. Higher week 1 or 2 serum BHB was also associated with higher milk fat percentage and lower milk protein percentage on first DHI test day.



15

Prevalence and Herd-level Risk Factors for Intramammary Infection with Coagulase-negative *Staphylococci* in Dutch Dairy Herds

Veterinary Microbiology, February 2009, Volume 134, Number 1-2, pages 37-44

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Bacteria of the *Staphylococcus* (Staph.) genus are classified by their ability to produce coagulase, an enzyme that causes blood clot formation. The primary mastitis-causing coagulase-positive Staph. species is *Staph. aureus*. Although coagulase-negative staphylococci (CNS) are often considered to be minor mastitis pathogens, this study found that they are the most frequently isolated group of pathogens from cows with subclinical mastitis in the Netherlands. In a survey of 4,220 quarter samples from cows in 49 Dutch dairy herds, 10.8% of quarters and 34.4% of cows were infected with CNS. While the prevalence of CNS infections ranged from 20 to 33% in multiparous cows, 49.4% of heifers had one or more CNS-positive quarters. Somatic cell counts (SCC) in CNS-positive quarters averaged 109,000 cells/ml, compared with 58,000 cells/ml in CNS culture-negative quarters. Fourteen CNS species were isolated, 4 species (*Staph. chromogenes*, *Staph. epidermidis*, *Staph. capitis* and *Staph. simulans*) accounting for 65.2% of CNS isolates. Herd-level risk factors associated with increased prevalence of CNS infection included source of drinking water being other than tap water, housing of dry cows in one group rather than multiple groups, measurement of individual cow SCC every month, udder health monitoring by the veterinarian, pasturing during outdoor season, percentage of stalls contaminated with milk, and bulk milk SCC greater than 250,000 cells/ml.



16

Impact of Intramammary Infections in Dairy Heifers on Future Udder Health, Milk Production, and Culling

Veterinary Microbiology, February 2009, Volume 134, Number 1-2, pages 113-120

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Intramammary infections (IMI) have been detected in heifers as early as 9 months before calving and it is very common for these animals to contract clinical mastitis early in their first lactation. This paper summarizes current knowledge about the nature of late gestation IMI in heifers and how they affect future udder health, milk production and risk of culling. The most common bacteria involved are coagulase-negative staphylococci (CNS) but *Staphylococcus aureus* (SA) and environmental pathogens are also found. Heifers calving with IMI are more likely to be culled in their first lactation than their non-infected herdmates. Culling risk increases with the early detection as well as severity and persistence of infection. High production reduces culling risk. IMI due to CNS tend to be cleared early in lactation with little long-term effect on udder health or milk production. In contrast, SA infections can do significant damage to mammary tissue; the longer they persist, the greater their negative effects on future udder health and production.



17

Factors Associated with the Risk of Antibiotic Residues and Intramammary Pathogen Presence in Milk from Heifers Administered Prepartum Intramammary Antibiotic Therapy

Veterinary Microbiology, February 2009, Volume 134, Number 1-2, pages 150-156

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Intramammary (IM) infusion of antibiotics at dry-off is commonly recommended to reduce the incidence of mastitis in early lactation. Results of recent trials have led to the recommendation that prepartum IM antibiotic treatment of heifers would likewise reduce IM infections in these animals. This study was designed to evaluate this practice in terms of the risks of milk antibiotic residues and the incidence of IM infections in early lactation primiparous cows. Heifers were treated with IM antibiotic (cephapirin sodium) at 10 to 21 days before anticipated calving dates. Milk samples, collected at the third, sixth and tenth milkings postpartum, were analysed for antibiotic residues. Mammary secretions were collected before antibiotic treatment and milk samples were taken during the first 3 weeks of lactation for bacterial culturing and somatic cell counts (SCC). Antibiotic residues were detected in 28.0, 8.82 and 3.68% of milk samples from milkings 3, 6 and 10, respectively. The risk of residues being present in milk decreased with the length of time between antibiotic treatment and calving and with time after calving. Higher concentrations of antibiotic in postpartum milk samples were associated with reduced pathogen presence and SCC in those samples as well as increased milk production in the first 200 days of lactation. The authors recommend antibiotic screening of milk from primiparous cows that have been treated with IM antibiotic prepartum.



18

Antibody to Ovalbumin and Delayed-type Hypersensitivity to *Candida albicans* and Mycobacteria in Lactating Holstein Cows using Quil A or Freund's Complete Adjuvant

Veterinary Immunology and Immunopathology, February 2009, Volume 127, Number 3-4, pages 220-227

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The objective of this study was to develop a method for testing the immune responsiveness (IR) of dairy cows in order to use IR as a phenotypic trait to which genetic selection could be applied. Although several tests for both antibody- (AMIR) and cell-mediated immune responses (CMIR) are available, these cannot be used in a commercial setting for a number of reasons. For example, use of the standard tuberculin skin test cannot be used because this would compromise its use as a test for tuberculosis (TB). Likewise, some of the agents commonly used to enhance the immune response (adjuvants) cannot be used in commercial herds because they contain heat-killed bacteria which may also interfere with the TB test. In the present study, ovalbumin (OVA) and a yeast-cell antigen (YC), combined with either a conventional (FCA) or a novel (Quil A) adjuvant, were tested for their ability to elicit AMIR and CMIR in lactating Holstein cows. Antibody responses to both initial and secondary immunization with OVA were statistically significant and similar for both FCA and Quil A adjuvants. YC combined with Quil A produced a CMIR (assessed as delayed-type hypersensitivity, DTH) similar to that seen when FCA was used as an adjuvant. The authors conclude that the combination of OVA and YC with Quil A provides a safe and effective way to induce AMIR and DTH for phenotyping dairy cattle based on their immune responsiveness.



19

Analytical Specificity and Sensitivity of a Real-time Polymerase Chain Reaction Assay for Identification of Bovine Mastitis Pathogens

Journal of Dairy Science, March 2009, Volume 92, Number 3, pages 952-959

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Mastitis is the most frequently occurring and economically the most important infectious disease in dairy cattle. Reliable and fast identification of the bacteria causing mastitis is important for disease management and for targeting antimicrobial therapy in the herds. Identification of the organism responsible has traditionally relied on bacterial culture, a method having major limitations. Culturing is slow and up to 50% of milk samples yield negative results due to no growth in culture. This paper describes the validation of an assay that simultaneously identifies 11 major mastitis pathogens in milk as well as the gene that confers resistance to penicillin and other beta-lactamase antibiotics in *Staphylococcus* organisms. The PCR (polymerase chain reaction) assay detects unique DNA sequences in the genomes of the bacterial species being tested for. Analytical sensitivity (the ability to identify bacteria correctly in positive samples) and specificity (the ability to provide negative test results for negative samples) were 100% for the complete set of 454 bovine milk samples tested. The authors conclude that the high sensitivity, specificity and speed of the PCR assay, in comparison to conventional bacterial culturing, has potential to provide tremendous improvements for the efficacy of mastitis testing programs. In addition to providing results more quickly and accurately, the PCR test is able to detect pathogen DNA in many samples where culturing yields no growth.



20

Presence of PPE Proteins in *Mycobacterium avium* subsp. *paratuberculosis* Isolates and their Immunogenicity in Cattle

Veterinary Microbiology, March 2009, Volume 135, Number 3-4, pages 294-400

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Mycobacterium avium subspecies *paratuberculosis* (MAP) is the bacterium that causes Johne's Disease (JD), a debilitating disease of ruminants resulting from chronic MAP infection of the small intestine. The aim of this study was to detect a particular family of proteins (PPE proteins) on the cell wall of live MAP bacteria and to investigate the immunogenicity of these proteins in MAP infected cattle. PPE proteins are known to play an important role in tuberculosis, a disease due to infection by a close relative of MAP. The genetic code for 36 variants of PPE have been identified on the MAP genome. Surface proteins were stripped from live MAP cells and analysed by liquid chromatography and mass spectroscopy. Two PPE proteins, Map3420c and Map1506, were detected. Antibodies to these 2 proteins were prepared and incubated with MAP cells to confirm that the proteins were exposed on the cell surface. Serum samples from naturally infected Holstein cows were found to contain specific antibodies against Map3420c, demonstrating the surface exposure and immunogenicity of PPE proteins of MAP during natural MAP infection.



21

Ultrasonographic Fetal Well-being Assessment, Neonatal and Postpartum Findings of Cloned Pregnancies in Cattle: A Preliminary Study on 10 Fetuses and Calves

Canadian Veterinary Journal, March 2009, Volume 50, Number 3, pages 261-269

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Cloned embryonic, fetal and newborn calves are subject to high risk of mortality due to developmental abnormalities, including underdevelopment of the placenta, abnormal uterine fluid accumulation and large offspring syndrome. The objective of this study was to determine whether transabdominal ultrasonography (TU) could be used to assess the status of cloned fetuses in utero. Ten third trimester cloned fetuses carried by Holstein heifers were assessed in the third trimester of pregnancy, using TU to record fetal heart rate (FHR), uterine fluid volume and placentome size. An attempt was then made to relate these indicators with pregnancy outcomes. One heifer had severe abdominal distension due to excessive uterine fluid accumulation resulting in rupture of the fetal membranes on day 227 of pregnancy and fetal death. Three others also had premature rupture of the fetal membranes between days 264 and 266; their fetuses were delivered early. Two heifers were referred to the veterinary hospital for poor general status and suspected fetal fluid accumulation on day 255 of pregnancy. The remaining 4 heifers had parturition induced, with cesarean sections performed between days 277 and 279 of pregnancy. By 1 week after calving, 7 of the 10 fetuses or newborn calves had died. Third trimester FHR varied from 92 to 128 beats per minute. There was no correlation between FHR and fetal activity but fetal hyperactivity was a possible sign of fetal distress as was the presence of particulate matter in the uterine fluids. Having demonstrated that TU can be used to image the bovine fetus in utero, the authors suggest that additional studies should be conducted to establish criteria for assessing fetal welfare with this method.



22

Degenerative Disease of the Distal Interphalangeal Joint and Sesamoid Bone in Calves: 9 Cases (1995–2004)

Journal of the American Veterinary Medical Association, March 2009, Volume 234, Number 6, pages 794-799

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The interphalangeal joint (IPJ) is on the rear of each claw, where the distal phalanx (commonly called the coffin or pedal bone) articulates with the navicular bone and the middle phalanx. Lameness-causing diseases affecting the IPJ are typically infectious, resulting from ulceration or perforation of the sole. This study examined the clinical signs, diagnosis, treatment and outcome of a degenerative form of disease affecting the IPJ in 9 calves. All calves were lame with no external evidence of hoof lesions. In 5 cases, a single forelimb was affected; in 4 cases both forelimbs were involved. In 12 limbs, the inside claw was affected; in 9 it was the outside claw. Examination by x-ray revealed extra bone growth in the joint. Surgical removal of this tissue in 3 calves resulted in resolution of lameness within 3 months. In 4 calves, pain relief was applied in the form of a block on the adjacent normal claw or through administration of a non-steroidal anti-inflammatory analgesic. Two of these calves improved during the 4- to 6-month period after discharge and survived to complete at least 2 lactations, although they continued to show signs of lameness. The other 2 were culled before 24 months of age because of delayed growth. One of the remaining 2 calves that were not treated continued to deteriorate and was eventually culled 6 months after discharge; the other gradually improved over several months. The authors suggest that degenerative joint disease should be considered in calves exhibiting lameness originating from the IPJ.



23

Evaluation of Prepartum Serum Cholesterol and Fatty Acids Concentrations as Predictors of Postpartum Retention of the Placenta in Dairy Cows

Journal of the American Veterinary Medical Association, March 2009, Volume 234, Number 6, pages 790-793

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According to the results of recent surveys by the US National Animal Health Monitoring System, retained placenta (RP) is the third most common health disorder in dairy cows, affecting 7.8% of lactating cows. In addition to its negative effects on subsequent conception rates and calving intervals, RP predisposed cows to other post-partum metabolic disorders. The objective of this study was to determine whether RP could be predicted by monitoring pre-partum blood metabolite levels or white cell counts. Blood samples were drawn 10 days before their expected calving dates from 1,083 cows in 20 commercial Holstein herds in southern Ontario. White blood cell (WBC) counts and serum concentrations of fatty acids (FA), betahydroxy butyrate (BHB), cholesterol, glucose, urea and calcium were measured. Farm staff recorded cases of RP, defined as failure of a cow to expel fetal membranes by 24 hours after calving. Comparing cows that experienced RP with those that passed their membranes normally, no significant differences were found in pre-partum concentrations of any of the serum metabolites or WBC examined, except for serum FA and cholesterol which were significantly higher in RP cows. Each 0.1 millimole per litre increase in pre-partum serum FA or cholesterol concentration predicted a 5% increase in the likelihood that a cow would experience RP. The authors conclude that pre-partum energy metabolism contributes to the development of RP. They recommend using pre-partum serum concentrations of FA and cholesterol to identify cows that might be predisposed to RP.



24

Challenges in Mucosal Vaccination of Cattle

Veterinary Immunology and Immunopathology, March 2009, Volume 128, Number 1-3, pages 192-198

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The term mucosa refers to the mucous membranes that line the respiratory, intestinal and urogenital tracts. Since most of the pathogens that infect animals either colonize or enter the body through the mucosa, there is interest in fortifying the immune response at those sites. Antibody and immune cell responses generated by mucosal exposure to antigens differ from those generated by systemic immunization. For example, mucosal epithelia (surface cells) produce antibodies of the IgA family whereas systemic (e.g., intramuscular, subcutaneous) vaccination mainly provokes IgG production. This paper reviews the challenges inherent in attempting to provoke a satisfactory mucosal immune response to vaccines delivered either orally or through the nasopharyngeal route. The oral route poses problems in ruminant animals because unprotected vaccine antigens can be degraded by rumen microbes. However, an example is given where an antigen was delivered through genetically altered alfalfa to take advantage of the ruminant's cud-chewing activity. This would initiate responses in the tonsil, which enhances both respiratory and intestinal immunity, before degradation. Mucosal vaccines must be delivered in dosages sufficient to overcome clearance along with the mucous that coats these tissues. In addition, mechanisms are needed to facilitate antigen adherence to mucosal epithelia. Among other challenges in developing mucosa-specific vaccines is the difficulty in assessing their success because antibody production is short-lived, there is little IgA in serum, and the IgA in mucus does not persist in the absence of ongoing antigenic stimulation.



25

Prevalence and Molecular Characterization of *Cryptosporidium* spp. in Dairy Calves from 11 Farms in Prince Edward Island, Canada

Veterinary Parasitology, March 2009, Volume 160, Number 3-4, pages 323-326

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Cryptosporidium are intestinal protozoan parasites that can infect and cause diarrhea in humans and many wild and domestic animals, including cattle. Hosts excrete large numbers of *Cryptosporidium* oocysts in their feces. Infections can spread rapidly when others come into direct contact with feces or consume oocyst contaminated water or food. *Cryptosporidium parvum* (CP) is the most common species in pre-weaned dairy calves; older animals can be infected with several other species, including *Cryptosporidium andersoni* (CA). CP is also commonly reported in humans, while CA has only rarely been reported. Previous studies have reported *Cryptosporidium* infection rates among Canadian dairy calves of 15% in British Columbia; 28, 41 and 46% in Ontario and 89% in Québec. The objective of the present study was to estimate the prevalence of *Cryptosporidium* species among dairy calves in Prince Edward Island. Fecal samples, collected from 183 dairy calves on 11 farms, were examined for *Cryptosporidium* oocysts using immunofluorescence microscopy. Species were identified by testing for the presence of DNA segments known to be unique to each species. Only 10 calves (6.2%) from 4 of the 11 farms surveyed were found to be infected. CP was the only species detected. The authors suggest that the presence of this species implies a potential risk for infection of humans.



26

Herd-level Risk Factors for Seven Different Foot Lesions in Ontario Holstein Cattle Housed in Tie Stalls or Free Stalls

Journal of Dairy Science, April 2009, Volume 92, Number 4, pages 1404-1411

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Based on observations of cow locomotion, it has been estimated that 20 to 22% of North American cows housed in tie-stalls (TS) and 23 to 27% of those in free-stalls (FS) are lame. Estimates based on examination of hooves suggest that actual lameness prevalence may be much higher. European studies reported that 50% of cows in TS facilities had hoof lesions; estimates of 70-80% have been reported for FS herds. The objective of the present study was to estimate the prevalence of hoof lesions in Ontario Holstein herds housed in TS and FS facilities and to evaluate factors associated with the level of lesion prevalence within herds. Hoof lesions were recorded by 5 professional hoof trimmers in 134 TS and 38 FS herds. Lesions recorded were digital dermatitis (DD), sole ulcer (SU), sole hemorrhage (SH), heel horn erosion (HE), white line separations (WLS), white line abscess (WLA), and interdigital fibroma (IF). In FS herds, more frequent alley scraping was associated with higher prevalence of SU and DD; having the herd trimmed in summer or fall was associated with a higher prevalence of DD; intermediate stall bedding depth was associated with a decreased level of IF and trimming heifers before calving was associated with a decreased level of WLA. TS herds that allowed year-round access to outside areas were associated with a higher level of DD, WLS and IF, compared with herds kept inside year-round or allowed only seasonal access to outside areas. Routine spraying of feet was associated with an increased level of DD, and the use of wood bedding material (vs. straw) was associated with an increased level of IF in TS herds. TS herd size in excess of 40 cows was also associated with an increased level of IF.



27

Reference Limits for Biochemical and Hematological Analytes of Dairy Cows One Week Before and One Week After Parturition

Canadian Veterinary Journal, April 2009, Volume 50, Number 4, pages 383-388

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A number of diagnostic tests are used to assess the metabolic state of the cow as she makes the transition from gestation to lactation. Interpretation of the results of these tests requires their comparison to established benchmarks. The objective of this study was to propose such benchmarks (reference limits) for blood levels of β -hydroxybutyrate, fatty acids, glucose, cholesterol, urea, calcium, and phosphorus as well as blood cell counts, based on analysis results from a number of previous studies. Different reference limits were established for pre-calving and post-calving periods, respectively, as follows: β -hydroxybutyrate, 218–884 and 216–1177 micromoles per litre; fatty acids, 0.0–1.0 and 0.1–1.4 millimoles per litre (mmol/L); cholesterol, 1.3–3.0 and 1.9–2.9 mmol/L; glucose, 2.64–4.75 and 2.3–5.2 mmol/L; urea, 2.1–8.0 and 1.9–7.8 mmol/L; calcium, 2.18–2.65 and 1.64–2.61 mmol/L; phosphorus 1.48–2.65 and 1.04–2.73 mmol/L. Reference limits proposed for pre- and post-calving blood cell counts, respectively (all counts expressed $\times 10^9/L$, i.e. billion per litre), were: leukocytes, 4.8–14.6 and 3.9–17.0; neutrophils, 1.7–6.2 and 0.7–9.0; lymphocytes, 1.9–9.0 and 2.1–10.0; monocytes, 0.0–0.3 and 0.0–0.3; eosinophils, 0.1–1.2 and 0.0–0.7.

28

Neonatal Blood Lactate Concentration and Calf Morbidity

Veterinary Record, April 2009, Volume 164, Number 17, pages 533-534

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The objective of this study was to evaluate associations between blood lactate concentrations in newborn calves, indicators of their vitality at birth and disease incidence in the first 2 weeks of life. An overall vitality score was based on assessment of reaction to cold water placed on the head, interdigital reflex, mucosal membrane colour and respiration. Each of these indicators was assigned a score of 0, 1 or 2. Lactate concentrations were measured in blood samples taken within 5 minutes of birth using an inexpensive, transportable lactate meter which facilitated rapid, on-site testing. Calving difficulty (dystocia) was scored 0 for an unassisted delivery; 1 for light assistance; 2 when a mechanical puller was required. Of the calves born to the 377 heifers enrolled in the study, 96 were not included in the statistical analysis due to stillbirth, early mortality or missing data. Blood lactate concentrations were significantly higher in calves requiring assistance at birth and in those with lower vitality scores. However, many of the calves with the highest vitality score (8) also had high blood lactate concentrations. Neither blood lactate level nor vitality score were significantly associated with disease incidence from birth to 14 days of age. The authors conclude that the predictive value of a single blood lactate assessment at birth was limited due to the wide variability of the lactate values recorded.



29

Neutrophils as One of the Major Haptoglobin Sources in Mastitis Affected Milk

Veterinary Research, May 2009, Volume 40, Number 3, pages 1-12

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Haptoglobin (Hp) is a protein that binds hemoglobin when it is released from red blood cells as a result of infection and inflammation. In cows with mastitis, somatic cell counts (SCC) and milk Hp levels are markedly elevated but the source of this Hp is not known. The objective of this study was to establish that source. Examination of the relationship between milk SCC and Hp levels in 50 mastitic cows revealed a strong positive correlation ($r = 0.742$). Gene expression analysis of somatic cells (neutrophils) from cows with high milk Hp concentrations showed that these cells are capable of synthesizing Hp and that they can markedly increase Hp synthesis by increasing the production of the mRNA that carries the genetic code for its production. Further analysis demonstrated that neutrophils were directly responsible for releasing Hp into milk. Hp was also elevated in mammary gland epithelial (surface) cells in cows with high milk Hp levels suggesting that these cells are another source of milk Hp.



Impact of Postpartum Milking Frequency on the Immune System and the Blood Metabolite Concentration of Dairy Cows

Journal of Dairy Science, May 2009, Volume 92, Number 5, pages 1900-1912

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The transition from gestation to lactation places considerable stress on the dairy cow due to the significant hormonal, metabolic and immunological adjustments that occur. As a result, many cows develop metabolic disorders such as milk fever, ketosis and fatty liver which reduce their productivity and increase their risk of being culled. The objective of this study was to evaluate the effect of reducing the stress of high production by milking a group of cows only once per day (1x) compared with herd mates milked twice per day (2x) during the week after calving. Blood concentrations of nonesterified fatty acids (NEFA), β hydroxybutyric acid (BHBA), urea, and bilirubin increased in all cows after calving but increases in NEFA and BHBA were greater in 2x cows than in 1x cows. Serum glucose concentration also decreased at this time but remained higher in 1x cows. These differences persisted until 24 days in milk (DIM) for NEFA and glucose and until 14 DIM for BHBA suggesting that the 1x cows were able to maintain a more favourable (less negative) energy balance during this period. 1x cows produced 31% less milk volume during the first week of lactation and 8.1% less over the following 13 weeks. However, milk fat and protein concentrations were higher for the 1x cows, resulting in similar component yields. Assessment of blood hormone levels and white blood cell immune functions revealed only minor differences between 1x and 2x cows.



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Laboratory Evaluation of 3M Petrifilms and University of Minnesota Bi-plates as Potential On-farm Tests for Clinical Mastitis

Journal of Dairy Science, May 2009, Volume 92, Number 5, pages 2297-2305

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When dairy farmers encounter a case of mastitis, they frequently treat the infection with an antibiotic (AB) although they have no information about its potential to eliminate the causative organism. Since mastitis treatment accounts for more than half of the AB use on Canadian dairy farms, a more targeted approach should reduce treatment cost, the cost of discarding AB-contaminated milk and the public health concern about the perceived over-use of AB in animal agriculture. The objective of this study was to evaluate the potential of 2 different commercial on-farm culture systems to aid in mastitis AB-treatment decisions. The 2 systems tested were the Minnesota Easy Culture System II Bi-plate (BP) and 3M Petrifilm (PF). Mastitic milk samples from 282 cows were tested using both systems to determine their ability to differentiate appropriate treatment groups. Cows whose milk samples yielded growth of Gram-positive bacteria (e.g. *Staph. aureus*) were considered candidates for AB treatment. Samples yielding no growth or growth of Gram-negative organisms (e.g., *E. coli*) indicated that AB-treatment was not appropriate. Test system results were compared with results from conventional laboratory culture to evaluate the sensitivity and specificity of each system. Sensitivity measures the test's ability to correctly identify a positive culture; specificity measures its ability to confirm a negative-culture sample. The BP system had a sensitivity of 97.9% and a specificity of 68.6% while the PF system had a sensitivity of 93.8% and a specificity of 70.1%. Differences between the tests were not statistically significant. The authors conclude that both systems were useful in helping to decide which mastitis cases should be treated with antibiotic.



Journal of Dairy Research, May 2009, Volume 76, Number 2, pages 129-136

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This paper reports the results of a 2003 survey of pathogen-specific mastitis prevalence in Dutch dairy herds and compares these results to those of previous surveys. Quarter milk samples were collected from 408 multiparous cows having a somatic cell count (SCC) of more than 250,000 cells/ml at their most recent test and from 145 primiparous cows with a previous test SCC exceeding 150,000 cells/ml. Another 2046 quarter samples were taken from 519 cows with previous test SCCs below these limits. Bacteria were isolated from 37.7% of samples from high-SCC cows and from 21.1% of samples from low-SCC cows. Coagulase-negative staphylococci (CNS) were found in all herds and in 10.8% of quarter samples. National Prevalences of *Staphylococcus aureus*, *Streptococcus uberis* and *Streptococcus dysgalactiae* were all less than 2%. While *Streptococcus agalactiae* was not found in this survey, it was the predominant pathogen identified in surveys conducted in 1973 and 1975, a distinction attributed to *Staphylococcus aureus* in the 1980s and to CNS in the 2003 survey. Prevalences of *Streptococcus uberis* and *Streptococcus dysgalactiae* infections remained at similar low levels from 1973 until 2003.



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Performance of API Staph ID 32 and Staph-Zym for Identification of Coagulase-negative Staphylococci Isolated from Bovine Milk Samples

Veterinary Microbiology, May 2009, Volume 136, Number 3-4, pages 300-305

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Bacteria of the *Staphylococcus* (Staph.) genus are classified by their ability to produce coagulase, an enzyme that causes blood clot formation. The primary mastitis-causing coagulase-positive Staph. species is *Staph. aureus*. Several recent studies have found that coagulase-negative staphylococci (CNS) are the most frequently isolated group of pathogens from heifers and from cows with high somatic cell counts. A recent study involving several of the present authors identified 14 CNS species among intramammary infections in Dutch dairy cattle, many of which differ in their antimicrobial susceptibility, virulence factors, host response to infection and transmissibility. Current culture-based methods for differentiating these CNS species are laborious and time-consuming. Commercial test kits that are used to differentiate human CNS isolates may provide an alternative but require validation for use with bovine isolates. The purpose of the present study was to test the applicability of 2 of these test kits to the differentiation of CNS bovine mastitis pathogens. Compared with identification of CNS species based on DNA-sequencing, both test kits misidentified a large proportion of bovine CNS isolates and were, therefore, judged unsuitable for use in the diagnosis of intramammary infections in dairy cattle.



34

Induction of Delayed-type Hypersensitivity and Interferon-gamma to *Candida albicans* and Anti-Hen-Egg White Lysozyme Antibody as Phenotypic Markers of Enhanced Bovine Immune Response

Veterinary Immunology and Immunopathology, May 2009, Volume 129, Number 1-2, pages 93-100

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In species other than dairy cattle, responsiveness to immunization has been used as a phenotypic trait in genetic selection programs aimed at improving disease resistance. It has been demonstrated that both antibody- (AMIR) and cell-mediated (CMIR) immune responses should be evaluated for this purpose. Protocols to elicit AMIR are relatively easy to implement. However, evaluating CMIR is more challenging, the most common method being the assessment of delayed-type hypersensitivity (DTH) in response to an intradermal injection of antigen. DTH response is currently used to assess exposure of cattle to *Mycobacterium bovis*, the pathogen responsible for bovine tuberculosis (TB). The objective of the present study was to develop a method to elicit both AMIR and CMIR that could be routinely used as a phenotypic marker in genetic selection for disease resistance in dairy cattle without interfering with current diagnostic tests such as that for TB. Hen-egg white lysozyme (HEWL) was tested for its ability to induce AMIR. Two antigen preparations derived from *Candida albicans* (CA) cells were used to elicit DTH. HEWL and CA antigens combined with an immune response amplifying agent (adjuvant: Quil A) were administered intramuscularly to 26 lactating and 5 dry Holstein cows. Twenty-one days later, the cows were given an intradermal injection of CA antigens to test for DTH. The protocol elicited significant AMIR and CMIR that could be used as phenotypic markers of immune responsiveness in dairy cattle.



35

Kinetics and Residues after Intraperitoneal Procaine Penicillin G Administration in Lactating Dairy Cows

Journal of Veterinary Pharmacology and Therapeutics, June 2009, Volume 32, Number 3, pages 289-295

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When abdominal surgery is performed in dairy cattle on-farm, the risk of infection is high due to the non-sterile operating environment. In addition to observing practices to minimize infection, veterinarians will usually administer antibiotics either before or after surgery to reduce infection risk. A survey of western Canadian bovine veterinarians revealed that 98% of respondents used antibiotics in this way and that 56% occasionally administered antibiotics directly into the peritoneum before closing. Because the intraperitoneal (IP) route of administration constitutes extra-label use of these products, there is a need to characterize the pharmacokinetics (PK) of IP-administered antimicrobials, including their blood concentrations, rate of clearance from the body and residue levels. This study was designed to establish the PK of procaine penicillin G (PEN) administered by the IP route at a dosage of 21,000 IU/kg in lactating cows. Results demonstrated that IP PEN is rapidly absorbed into the bloodstream and subsequently eliminated from the body. Milk from treated cows contained detectable PEN for a minimum of 31 hours and a maximum of 52 hours after administration. By 10 days post-administration, it was no longer possible to detect PEN in muscle, liver or kidney. The authors emphasize that, due to the limited scope of this study, the PEN elimination rates observed should not be used to recommend generally applicable withdrawal times.



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Risk Factors for Bacteriological Quality of Bulk Tank Milk in Prince Edward Island Dairy Herds. Part 1: Overall Risk Factors

Journal of Dairy Science, June 2009, Volume 92, Number 6, pages 2634-2643

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This study was designed to identify farm management (risk) factors that influence bacteria counts in bulk tank milk (BTM). All 235 dairy farms on Prince Edward Island had their BTM tested for bacteria every 2 weeks between March 2005 and March 2007. Two smaller groups of farms were used to assess the association between BTM bacteria counts and risk factors. One group of 39 (case) farms were chosen based on their having high BTM bacteria counts on multiple occasions. A second group of 30 farms served as low-count controls. Recorded risk factors included information on basic hygiene and farm management practices, evaluation of milking and cleaning systems, and scoring of environmental and cow hygiene. Factors significantly associated with high BTM bacteria counts included highly alkaline wash water and poor teat-end cleanliness. High detergent wash water temperature and the use of a water softener were significantly associated with low BTM bacteria counts. The authors suggest that, although only these 4 factors had statistically significant associations with BTM bacteria counts, there are many other factors related to animal and environmental hygiene that should be considered when seeking improvement in bacteriological milk quality.

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Risk Factors for Bacteriological Quality of Bulk Tank Milk in Prince Edward Island Dairy Herds. Part 2: Bacteria Count-specific Risk Factors

Journal of Dairy Science, June 2009, Volume 92, Number 6, pages 2644-2652

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A number of different tests are used to assess bacterial contamination of milk. The total aerobic count (TAC) estimates the total number of aerobic (oxygen-requiring) bacteria in raw milk samples. The preliminary incubation count (PIC) quantifies bacteria that grow at low temperature (psychrotrophs), as might be the case when refrigeration is inadequate. Bacteria that are likely to survive batch pasteurization (thermoduric bacteria) are assessed with the laboratory pasteurization count (LPC). The coliform count (CC) measures intestinal bacteria that are present in the environment due to fecal contamination. This study evaluated risk factors for high bulk tank milk (BTM) bacteria counts for each of the tests described above. For each test, a case group consisted of herds that had multiple high counts over the course of the previous 6 biweekly tests; a control group had consistently low tests. Recorded risk factors included information on basic hygiene and farm management practices, evaluation of milking and cleaning systems, and scoring of environmental and cow hygiene. Risk factors for high TAC and PIC included washing the teats with water before milking, not using teat pre-dip, and poor udder hygiene. High LPC and CC were associated with low temperature cleaning solution, high water hardness score, and highly alkaline detergent wash.



38

Sole Ulcers in Finnish Dairy Cattle

Preventive Veterinary Medicine, June 2009, Volume 89, Number 3-4, pages 227-236

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The Healthy Hooves project in Finland was initiated to establish a national recording system for hoof disease in dairy cattle. A pilot study carried out in 2002 identified laminitis and laminitis-like lesions such as sole ulcers (SU), white line problems and sole haemorrhages as the most common hoof lesions in Finnish dairy herds. Of these, SU is assumed to be the most costly because its long and painful duration usually makes cows lame and negatively affects milk yield, reproductive performance and udder health. This paper reports the results of a study which merged 2 years of hoof lesion data recorded by hoof trimmers with production data from the Finnish Agricultural Data Processing Centre. Data from a single lactation from each of 16,792 cows were used to evaluate SU incidence in 554 tie-stall (TS) and 149 loose-housed (LH) herds. Cows examined only once had an SU risk of 5.23% in TS herds and 7.58% in LH herds. The odds of detecting SU increased substantially with the number of times cows were examined. Holsteins were 2.89 times more likely to be affected than Ayrshires in TS herds; 2.94 times more likely in LH herds. In TS herds, the presence of other hoof lesions such as haemorrhages, heel-horn erosions and corkscrew claw increased the risk of having SU. In LH herds, only haemorrhages were associated with higher risk of SU and only for second lactation cows. TS herds that used rubber mats had lower risk of sole ulcers than those where stalls had concrete surfaces. TS-housed, fourth lactation cows had higher risk of SU than did cows of other parities. Although SU incidence was not associated with production level in LH herds, production decreased with increasing SU in TS herds.



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Relationship Between *in vitro* Susceptibility Test Results and Treatment Outcomes for Gram-positive Mastitis Pathogens following Treatment with Cephapirin Sodium

Journal of Dairy Science, June 2009, Volume 92, Number 6, pages 2589-2597

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The purpose of laboratory bacterial culture of milk samples from mastitic cows is to identify the primary causative organism and to determine its susceptibility to a variety of candidate antibiotics. *In vitro* susceptibility tests (IVST) also estimate the minimum inhibitory concentration (MIC) of these antibiotics required to control the pathogen. However, several previous studies have indicated that the results of IVST are poor predictors of on-farm antibiotic performance. The objective of the present study was to test the association between IVST results and on-farm treatment outcomes for a range of mastitis pathogens treated with the antibiotic cephapirin sodium (CPS). Pathogens were isolated from mammary gland quarters that were treated with CPS. The overall cure rate achieved by CPS treatment of mastitis was 82% irrespective of pathogen type. IVST indicated that 94.8% of pathogens recovered from quarters classified as cures were susceptible to CPS. However, IVST also predicted a 91.2% CPS susceptibility for pathogens recovered from quarters where treatment failed. The probability of treatment success when the isolate was classified by IVST as susceptible was 82% while the probability of treatment failure when the isolate was classified as resistant was 27%. These observations led to the conclusion that the on-farm outcomes of mastitis treated with CPS were not associated with IVST results or MIC values.



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The Association between Foot Lesions and Culling Risk in Ontario Holstein Cows

Journal of Dairy Science, June 2009, Volume 92, Number 6, pages 2572-2579

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The objective of this study was to evaluate the associations between the occurrence of specific hoof lesions and the length of time affected animals remained in the herd after trimming. Five professional hoof trimmers recorded lesions during routine trimming of 6,513 cows in 157 Ontario herds. Cows were more likely to be culled if they had hoof horn lesions rather than infectious lesions. Across all herds, cows with lesions were culled between 149 and 168 days (95% confidence interval; median 157 days) after a lesion was identified while those with no identified lesions left the herd between 175 and 198 days (95% confidence interval; median 188 days) after the same trimming day. Compared with cows with no identified lesions, those with white line lesions, sole ulcers and sole hemorrhage were 1.72, 1.26 and 1.36 times more likely to be culled, respectively. The authors suggest that early detection of hoof horn lesions could potentially reduce their effect on cow longevity.

41

Providing Probability Distributions for the Causal Pathogen of Clinical Mastitis Using Naïve Bayesian Networks

Journal of Dairy Science, June 2009, Volume 92, Number 6, pages 2598-2609

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A Bayesian network (BN) is a mathematical model of relationships between variables, a statistical method for discovering valid, novel and potentially useful patterns in data, including cause and effect relationships. Naïve BN (NBN) are the simplest such models. The objective of this study was to determine the accuracy of an NBN to predict the probability that an individual clinical mastitis (CM) case is caused by a specific pathogen or class of pathogens, in the absence of bacteriological culture results. Data used to derive the NBNs were from 3,833 CM cases recorded on 274 Dutch dairy farms. Two-thirds of the data were used for NBN construction; the other third were used for NBN validation. One model was constructed to predict the probabilities of a new CM being caused by either a Gram-positive or Gram-negative pathogen. Testing this NBN demonstrated an accuracy of 73% in correctly classifying Gram status of a CM case. The second was constructed to predict probabilities of CM being caused by streptococci, *Staphylococcus aureus*, or *Escherichia coli*. The accuracy of this NBN was 52%. Accuracies of both models improved when considering only CM cases with a high probability of being caused by a single pathogen. The authors suggest that, by providing input variables such as season, parity, stage of lactation, somatic cell count, mastitis history and clinical signs, NBN could be used to assist in CM treatment decisions in the absence of definitive culture identification of pathogens.



42

Somatic Cell Scores and Clinical Signs following Experimental Intramammary Infection of Dairy Cows with a *Staphylococcus aureus* Small Colony Variant (*S. aureus* SCV) in Comparison to Other Bovine Strains

Veterinary Microbiology, June 2009, Volume 137, Number 3-4, pages 326-334

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Intramammary infections (IMI) caused by *Staphylococcus aureus* (SA) are difficult to cure, often resulting in persistent infections that are resistant to antibiotic treatment. It is thought that SA is able to persist through its ability to modify its form, resulting in a slow-growing, small-colony variant (SCV) that can survive inside immune cells without triggering a cell-mediated immune response. The objective of this study was to evaluate differences in the characteristics of IMI as well as host responses to IMI caused by 2 SA SCV strains versus IMI caused by their parent strains exhibiting normal colony morphology. An infective dose of each of the 4 pathogens was infused into 3 quarters of clinically healthy lactating cows. The fourth quarter of each cow was infused with pathogen-free carrier to serve as a control. IMI caused by the SCV strains were mild and produced less tissue damage compared with those caused by the parent strains. SCV infections were followed by chronic subclinical mastitis while the parent strains produced IMI with both acute and chronic phases. The authors conclude that the pathogenicity of SCVs is related to their resistance to host defences and antimicrobial drugs and their ability to revert into their rapidly growing parent forms resulting in recurrent and relapsing infections.

43

SNPs in the Bovine IL-10 Receptor are Associated with Somatic Cell Score in Canadian Dairy Bulls

Mammalian Genome, July 2009, Volume 20, Number 7, pages 447-454

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Genetics play an important role in an individual cow's susceptibility and response to infectious disease. In the case of mastitis, previous studies have demonstrated that a large number of sites on the bovine genome (quantitative trait loci, QTLs) are associated with both mastitis susceptibility and somatic cell count (SCC) response to infection. SCC is a heritable trait that is positively correlated with mastitis incidence. The objective of this study was to identify genetic variants (single nucleotide polymorphisms, SNPs) in several immunoregulatory genes and associate them with estimated breeding values (EBVs) for SCC. The authors hypothesized that SNPs in genes that regulate inflammation may affect a cow's risk of developing mastitis. The genes examined included bovine interleukin-10 (IL-10) and its receptor (IL-10R) as well as transforming growth factor β 1 (TGF- β 1) and its receptor (TGF- β R). IL-10 and TGF- β 1 are signalling molecules (cytokines) that attenuate inflammation in tissues like those lining the alveoli of the mammary gland. SNPs were identified in IL-10, IL-10R and TGF- β 1 genes and these were subsequently genotyped in 500 Holstein, 83 Jersey and 50 Guernsey bulls. Results indicated that SNPs in IL-10R were associated with variation in EBVs for SCC.



44

Age Related Variation in Expression of CD21 and CD32 on Bovine Lymphocytes: A Cross-sectional Study

Veterinary Immunology and Immunopathology, July 2009, Volume 130, Number 1-2, pages 70-78

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Although it would be advantageous to vaccinate young calves against a number of potential pathogens, the immaturity of their immune systems and the inhibitory effects of maternal antibodies (Ab) passively acquired from colostrum often result in poor responses to vaccination. B lymphocytes (B cells) are Ab-producing immune cells that circulate in the blood and through lymphoid tissues. CD21 and CD32 are receptors on B cells involved in regulation of Ab responses. CD21 is an activating receptor on B cells, whereas CD32 is an inhibitory receptor. Complement component C3, after activation can bind covalently to antigen; C3d (a breakdown product of C3) can bind CD21 on B cells, leading to cell activation and eventual production of Ab. The concentration of C3 in the serum of newborn calves is low; providing C3 fragments as components of vaccines may enhance Ab responses. In contrast, CD32 binds complexes of vaccine antigen with maternal Ab, leading to suppressed B cell responses. One objective of this study, therefore, was to evaluate expression of CD21 on B cells from young calves. A second objective was to determine whether the inhibitory receptor CD32 was present on neonatal B cells as well. Blood samples were drawn from calves ranging from 1 to 90 days of age for measurement of cellular expression of CD21 and CD32. The vast majority of circulating B cells expressed both receptors from birth, suggesting that these cells are subject to both activating and inhibitory influences from birth. The authors conclude that expression of CD21 does not appear to be a factor limiting Ab responses in young calves.



45

Relationships between Rumen Lipopolysaccharide and Mediators of Inflammatory Response with Milk Fat Production and Efficiency in Dairy Cows

Journal of Dairy Science, August 2009, Volume 92, Number 8, pages 3800-3809

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Lactating dairy cows are often fed substantial quantities of grain to increase dietary energy availability in support of high milk production. Most of the energy provided by grain is derived from starch which is rapidly fermented to volatile fatty acids by rumen microbes. In addition, because grain consumption does not provoke significant chewing and rumination, salivary buffer secretion is minimal. These two effects of grain feeding frequently lead to ruminal acidosis (RA), characterized by low rumen pH and the death of gram-negative bacteria resulting in the release of endotoxin, a cellular component of these bacteria also referred to as lipopolysaccharide (LPS). RA is thought to predispose lactating cows to other metabolic disorders such as fatty liver, laminitis, liver abscesses, displaced abomasum and bloat. The authors of the present study hypothesized that LPS released as a result of RA might also be implicated in the milk fat depression (MFD) often observed in cows fed high grain diets. The mechanism proposed involved an acute systemic inflammatory response to LPS absorption from the rumen leading to alterations in immune function. Total mixed rations (TMRs) containing 0%, 15%, 30% or 45% rolled barley grain were offered to 8 rumen-cannulated lactating cows. Ruminal LPS concentrations increased significantly while milk yield, milk fat yield, milk fat concentration and milk energy efficiency declined with increasing dietary barley content. These changes in milk and milk fat yields were also associated with concentrations of plasma C-reactive protein, one of the mediators of the acute inflammatory response to elevated LPS.



46

Effect of Prepartum Dry Cow Antibiotic Treatment in Dairy Heifers on Udder Health and Milk Production

Journal of Dairy Science, September 2009, Volume 92, Number 9, pages 4395-4403

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Contrary to the belief of many dairy farmers, a large proportion of heifers may harbour intramammary infections (IMI) before calving. These IMI are commonly first noticed when first test results indicate elevated somatic cell counts (SCC). Heifers calving with IMI are at risk of increased udder health problems, lower production and early culling. The objective of this study was to determine whether prepartum treatment of heifers with a dry cow antibiotic would decrease the incidence of IMI at calving and improve measures of health and productivity in their first lactation. Cloxacillin dry cow treatment was administered to 184 heifers on 13 Dutch dairy farms, 8 to 10 weeks before their expected calving dates. Another 185 heifers served as untreated controls. Milk samples were taken from all animals at calving and at 10 to 14 days in milk (DIM). The proportion of treated heifers that were culture-negative on these 2 occasions was significantly higher than for their untreated herdmates (52.7% versus 41.6% at calving; 75.5% versus 68.8% at 10-14 DIM). The risk of developing clinical mastitis at some point during first lactation was 9% in the treated heifers; 18% in the controls. Cloxacillin treatment also reduced average test day SCC in early lactation, from 71,000 cells/ml in controls to 55,000 cells/ml in treated heifers. Average first lactation test-day milk production was increased from 23.6 kg/day in controls to 24.5 kg/day in treated heifers. Prepartum treatment of heifers with cloxacillin may temporarily help in solving the mastitis problem, but dairy farmers with heifer mastitis problems need to analyze their mastitis management.



47

Heifer and Quarter Characteristics Associated with Periparturient Blood and Milk Neutrophil Apoptosis in Healthy Heifers and in Heifers with Subclinical Mastitis

Journal of Dairy Science, September 2009, Volume 92, Number 9, pages 4330-4339

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Polymorphonuclear neutrophilic leukocytes (PMNL) are immune cells that play an important early role in the body's response to infection. Previous research had found that the activity of PMNL may be impaired before and after calving in both heifers and multiparous cows. It was speculated that this may partially account for the high prevalence of intramammary infections in fresh heifers. The reason for lower PMNL activity was thought to be due to an increase in programmed cell death (apoptosis) before calving. The objective of this study was to evaluate rates of PMNL apoptosis in heifers around calving and to assess the effects of management, environmental and animal variables on this phenomenon. Heifers that were fed supplementary minerals and vitamins pre-partum had lower rates of PMNL apoptosis in both blood and milk than herd mates that did not receive supplement. The authors speculate that this improvement was due to higher selenium intake. Milk and blood PMNL apoptosis was also found to be lower between April and June than from January to March. Heifers losing body condition before calving had higher blood PMNL apoptosis. Milk PMNL apoptosis was lower in quarters whose teat orifices were colonized with non-aureus staphylococci before calving.

48

Microbiological Quality of Bulk Tank Raw Milk in Prince Edward Island Dairy Herds

Journal of Dairy Science, September 2009, Volume 92, Number 9, pages 4239-4248

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This study was designed to assess bulk tank milk (BTM) quality using 4 different bacteriological tests as well as somatic cell count (SCC), to assess correlations between results of the 4 bacteriological tests and to evaluate the effect of season on test results. All 235 dairy farms on Prince Edward Island had their BTM sampled every 2 weeks between March 2005 and March 2007. The 4 bacteriological tests used were the total aerobic count (TAC), the preliminary incubation count (PIC), the laboratory pasteurization count (LPC), and the coliform count (CC). The TAC estimates the total number of aerobic (oxygen-requiring) bacteria in raw milk samples. The PIC quantifies bacteria that grow at low temperature (psychrotrophs), as might be the case when refrigeration is inadequate. Bacteria that are likely to survive batch pasteurization (thermoduric bacteria) are assessed with the LPC. The CC measures intestinal bacteria that are present in the environment due to fecal contamination. Although the correlation between TAC and PIC was moderate (0.57), correlations between other test results were all low indicating the value of using multiple tests to assess the bacteriology of BTM. All test results tended to be lower in winter; TAC, CC and SCC were notably higher in summer.



49

Reliability of the Bulk Milk Somatic Cell Count as an Indication of Average Herd Somatic Cell Count

Journal of Dairy Research, November 2009, Volume 76, Number 4, pages 490-496

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The objective of this study was to evaluate the validity of using bulk tank somatic cell count (BTSCC) to assess the prevalence of subclinical mastitis in a herd. An initial evaluation of BTSCC sampling variation involved 53 Dutch dairy farms selected on the basis of their BTSCC records for the previous 2 years; low, medium and high BTSCC herds were chosen to ensure a wide range of values for this study. Five consecutive samples were taken from each farm's bulk tank to estimate sampling variation. Average BTSCC ranged from 56,000 to 441,000 cells/ml. At the low end of that range, average absolute sampling variation was in the order of 1,800 cells/ml; at the high end it was around 182,000 cells/ml. Sampling variation results were used to correct BTSCC values in a comparison with herd average SCC calculated from individual cow SCCs corrected for milk yield (CHSCC) on 300 farms. On average, BTSCC was 49,000 cells/ml lower than CHSCC, ranging from 10,000 cells/ml lower to 182,000 cells/ml higher. To determine possible reasons for discrepancies between BTSCC and CHSCC, producers were asked to respond to questionnaires regarding their mastitis prevention and control practices. It was determined that farms with a small (less than 20%) differences between BTSCC and CHSCC were more aggressive in their management of mastitis. Farms that fed high-SCC milk or milk with antibiotic residues to calves were 2.4-times more likely to have a large difference since the high-SCC milk did not enter the bulk tank.

Milk Production





1

Serum Levels of Stanniocalcin-1 in Holstein Heifers and Cows

Domestic Animal Endocrinology, February 2009, Volume 36, Number 2, pages 105-109

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Stanniocalcin (STC) derives its name from the fact that it is produced by the 'corpuscles of Stannius', glands found uniquely in fish, where STC regulates calcium and phosphorus metabolism, opposing the effect of prolactin (PRL). Since its original discovery in fish, genes coding for 2 unique forms of STC have been found in mammals. Earlier experiments demonstrated that STC-1 had a role in lactation and gestation in mice. These findings, along with the observed interaction of STC and PRL in fish, have stimulated interest in the possibility of a role for STC in bovine lactation since PRL is also known to play a role in lactation regulation. Previous studies by the present authors demonstrated that expression of the STC-1 gene in the cow's mammary gland as well as the concentration of STC-1 in milk increased as milk production declined with increasing lactation length. These observations suggest that STC-1 may act to suppress milk production. The objective of the present study was to measure serum STC-1 concentrations in dairy animals at various stages of development and metabolic status. The highest levels (9-13 nanograms per ml, ng/ml) of STC-1 were found in pregnant heifers. In young calves, serum STC-1 concentrations fell from the 8 ng/ml range at 1 month of age to 5-6 ng/ml from 4 months of age to conception. During mid- to late-lactation, levels were in the 6-8 ng/ml range, rising sharply to the 12 ng/ml range during the dry period before declining after calving.



2

Fatty Acid Profile of Bovine Milk Naturally Enhanced with Docosahexaenoic Acid

Journal of Agricultural and Food Chemistry, February 2009, Volume 57, Number 4, pages 1366-1371

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In order to improve the nutritional quality and potential health benefits of milk fat, several research groups have demonstrated ways of adjusting dairy cow diets to decrease concentrations of saturated fatty acids (SFA) in favour of polyunsaturated FA (PUFA). Of particular interest, the present authors and others have demonstrated increases in milk fat content of very long chain omega-3 PUFA (VLCω3PUFA) by feeding sources of fish oil. The VLCω3PUFA eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) have been shown to have positive cardiovascular effects for adults and to be essential for nervous system development in children. The objective of this study was to compare concentrations of FA in regular homo milk (HM) to those in a commercial milk product (FMHM) produced by cows fed a diet containing fish meal. Significant differences were found for many individual saturated and unsaturated FA although total SFA in the FMHM was only very slightly lower than that in the HM. EPA, DPA and DHA concentrations were 2.78, 1.76 and 23.8 times higher in FMHM than in HM. Increased VLCω3PUFA concentrations were largely at the expense of omega-6 PUFA.

3

Local Control of Mammary Involution: Is Stanniocalcin-1 Involved?

Journal of Dairy Science, May 2009, Volume 92, Number 5, pages 1998-2006

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First discovered in fish, genes coding for 2 forms of stanniocalcin (STC-1 and STC-2) have been discovered in mammals. Results of earlier studies in both fish and mice have created interest in the possibility that, by operating in opposition to prolactin, STC-1 may have a role in reducing milk synthesis after peak lactation. The present study was designed to assess the possible role of STC-1 in terminating milk production at dry-off. In 9 late lactation Holstein cows, two quarters were milked (MQ) while the other two quarters were left unmilked (UQ, dried off) for 14 days. Tissue, blood and milk samples were taken at intervals from 7 days before until 14 days after the beginning of the non-milking period. Drying-off 2 quarters resulted in a 30% increase in milk yield from the MQ. Milk STC-1 concentration increased in the UQ but not in the MQ and, at day 2 of dry-off, the rate of programmed cell death (apoptosis) in UQ was 3 times that in MQ. Mammary epithelial tissue (MET) cultured in the presence of milk taken from the UQ on day 14 had 3 times as many apoptotic cells as MET cultured with 14-day milk from MQ. The metabolic rate of MET cultured with 7-day or 14-day milk from UQ was reduced by 14.6% and 23.6%, respectively, effects that were negatively correlated with the STC-1 concentration in the milk. These results suggest that STC-1 may be involved in the physiology of dry-off although the exact mechanism of its action is unclear.



4

Selenomethionine Stimulates Expression of Glutathione Peroxidase 1 and 3 and Growth of Bovine Mammary Epithelial Cells in Primary Culture

Journal of Dairy Science, June 2009, Volume 92, Number 6, pages 2670-2683

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It has been suggested that programmed cell death (apoptosis), mediated by reactive oxygen species (ROS) may be partly responsible for the gradual decline in milk production after peak yield has been achieved. ROS can cause oxidative damage to the milk producing cells of the mammary gland (mammary epithelial cells, MEC). Glutathione peroxidases (GPx) are a family of enzymes responsible for breaking down ROS to reduce this effect and selenium (Se) is an essential component of these enzymes. Two members of the GPx family have been identified in bovine mammary tissue: GPx1 which acts inside the cell and GPx3 which acts outside the cell. The objectives of this study were to determine the locations of these 2 enzymes in cultured MEC cells and to test their responses to various concentrations of organic Se (selenomethionine, SeMet) in the growth medium. An additional objective was to determine whether MEC cells would be able to utilize SeMet as a selenium source since the Se must be cleaved from SeMet and converted to selenophosphate before being incorporated into the GPx. It was found that both GPx1 and GPx3 were synthesized by MEC cells and that the 2 enzymes were localized in the cell nucleus and cytoplasm. Increasing SeMet concentrations from 0 up to 50 nanomoles per litre linearly increased MEC number and viability as well as GPx1 and GPx3 abundance over 5 days of culture.

5

An Analysis of the Relationship between Bulk Tank Milk Quality and Wash Water Quality on Dairy Farms in Ontario, Canada

Journal of Dairy Science, August 2009, Volume 92, Number 8, pages 3714-3722

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Water used in cleaning and disinfecting milking equipment is one of several potential sources of bacterial contamination of raw (bulk tank) milk. In this study, water samples were drawn from 'point-of-use' (e.g., initial flow from tap or hose in milk house) on over 5,000 Ontario dairy farms. The degree of contamination by *E. coli* and coliform bacteria in these samples was compared with routine bacterial counts in bulk tank milk on those farms. Although 13.6% and 53.8% of water samples were contaminated with *E. coli* and coliforms, respectively, only 2.8% of bulk tank milk samples had elevated bacteria counts, suggesting that bacterial contamination of wash water had a minor effect on raw milk quality. Other risk factors found to be associated with high raw milk bacteria counts included high average monthly somatic cell counts, high production and seasonally cooler temperatures. Examination of the geographic distribution of contaminated water sources revealed 3 clusters of *E. coli* contamination and 1 of coliform contamination. No such clusters were identified for elevated bacteria counts in raw milk.



6

Cyclic Adenosine Monophosphate (cAMP)-specific Phosphodiesterase is Functional in Bovine Mammary Gland

Journal of Dairy Science, August 2009, Volume 92, Number 8, pages 3757-3765

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A research report published in 1991 demonstrated that caffeine administered to mice during pregnancy could increase milk production in their subsequent lactation. The mechanism of action of caffeine involves inhibition of a family of enzymes called phosphodiesterases (PDEs) that inactivate cyclic AMP and cyclic GMP, so-called 'second messengers' that promote several aspects of milk synthesis. Based on this knowledge, the authors of the present study hypothesized the presence of one or more PDEs in the bovine mammary gland. A large number of PDEs had been described. Each of 11 distinct PDE families is identified on the basis of its substrate affinity, biochemical properties, regulation, and sensitivity to inhibitors. From 1 to 4 distinct genes code for each of those families and each of those genes can be transcribed in a number of different ways to produce multiple PDE 'splice variants'. In the present study, a number of different genomics techniques were used to demonstrate the presence of the enzyme PDE4D1, its gene and its mRNA transcript in mammary gland tissue from culled dairy cows. PDE4D1 is a member of PDE family 4, coded by the D (fourth) gene coding for that family and transcribed to splice variant 1. Analysis of enzyme activity and inhibition demonstrated that PDE4D1 accounted for 20% of the total PDE enzymatic activity in mammary gland tissue.

Reproduction





1

Expression of Fibroblast Growth Factor Receptors during Development and Regression of the Bovine *Corpus Luteum*

Reproduction, Fertility and Development, July 2008, Volume 20, Number 6, pages 659-664

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During the bovine estrous cycle, ovulation is initiated by a surge of luteinizing hormone (LH) secretion from the pituitary which causes an ovarian follicle to shed its ovum into the oviduct. The remaining follicular tissue transforms into a corpus luteum (CL) that develops over the course of 15-18 days before it regresses (in the absence of conception) under the influence of prostaglandin- $f_{2\alpha}$ (PGF). Although LH and PGF are the main external hormones influencing the formation and regression of the CL, a number of other factors are involved in the local control of CL development. Among these are members of a family of 22 fibroblast growth factors (FGFs). The expression of both FGF1 and FGF2 has been shown to change over the CL life cycle. These FGFs are able to influence various cellular events, depending on the receptors with which they interact. FGF receptors (FGFR) are encoded by 5 different genes, 3 of which (FGFR1, FGFR2 and FGFR3) are able to transcribe alternative forms (splice variants) of their respective FGFs. This study examined the expression of messenger RNA encoding 'B' and 'C' FGFR splice variants in bovine CLs recovered from an abattoir at 4 stages of development and regression. Expression of these FGFR1 and FGFR2 splice variants was readily detected. While FGFR1C and FGFR2C expression did not change throughout the CL lifespan, FGFR1B expression was increased in the final stage of development before regression. The authors conclude that FGFR1 and FGFR2 are the main FGF receptors in the bovine CL.



2

Precocious Mammary Development in an 8-month-old Holstein Heifer

Canadian Veterinary Journal, August 2008, Volume 49, Number 8, pages 803-805

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This paper is a case report describing the unusual observation of a 10 month old Holstein heifer with a mammary gland resembling that of an adult cow. Although her height and weight were within the normal range, her 23 cm deep udder had the consistency of a lactating gland with teats measuring 5 cm in length. In attempting to find an explanation for this anomaly, the authors considered the possibilities of an ovarian tumour, phytoestrogen toxicity or a follicular cyst. Although no cysts were present on examination, the explanation favoured by the authors was that estrogen production by an earlier follicular cyst had stimulated mammary gland growth. Subsequent luteinisation of the cyst, resulting in elevated progesterone secretion may have stimulated further development. Examination of secretions from the gland revealed a protein concentration of 14.9% with fat and lactose levels of 0.12% and 0.2%, respectively. Somatic cell count was 3.9 million cells/ml although no bacterial infection was detected. The heifer conceived to artificial insemination at 15 months of age but was voluntarily culled 2 months later.

3

Pregnancy Rates and Peripheral Progesterone Levels following Ovsynch or CIDR Ovulation Synchronization/ Timed Artificial Insemination Protocols in Postpartum Dairy Cows

Canadian Journal of Animal Science, September 2008, Volume 88, Number 3, pages 457-461

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The objective of this study was to compare two different protocols currently used to achieve timed artificial insemination (TAI) in lactating dairy cows. The Ovsynch protocol involves an initial injection of gonadotropin releasing hormone (GnRH) followed by a dose of prostaglandin F2 α (PGF2 α) 7 days later. A second injection of GnRH 48 hours after PGF2 α is followed by TAI 16 hours later. The CIDR protocol consists of initial injections of progesterone (P4) and estradiol (E2) accompanied by vaginal insertion of a controlled internal drug release (CIDR) device. Seven days later, an injection of PGF2 α is followed by CIDR removal the next day and a second injection of E2 on the following day. TAI is carried out 28 hours after this second E2 injection. Lactating Holstein cows were randomly assigned to one of the two TAI protocols: 111 to Ovsynch, 116 to CIDR. Pregnancy was diagnosed by ultrasound at day 35 after TAI and confirmed by rectal palpation at day 60. Milk samples were taken from the beginning of treatment until day 35 after TAI for P4 determination. Pregnancy rates (PR) for Ovsynch and CIDR groups of cows were 31 and 41%, respectively. Among those that ovulated in response to treatment, PR were 49 and 69% for Ovsynch and CIDR cows, respectively. Milk P4 concentrations were similar for cows in both treatment groups.



4

Effects of Dim Light at Night on Milk Yield, Milk Composition and Endocrine Profile of Lactating Dairy Cows

Canadian Journal of Animal Science, October 2008, Volume 88, Number 4, pages 609-612

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A number of studies have demonstrated that extending the photoperiod during lactation can increase milk production by dairy cows. Although this effect is associated with elevated blood levels of prolactin, a milk production enhancing hormone, it is probably mediated by increased activity of insulin-like growth factor-I (IGF-I). A longer photoperiod has also been shown to decrease circulating levels of melatonin, an inhibitor of IGF-I secretion, suggesting another possible mechanism for the effect of extended photoperiod on milk production. Although increased production is clearly beneficial, it has been suggested that continuous lighting might interfere with essential diurnal endocrine patterns. Therefore, the objective of this study was to evaluate the effect of low light intensity on plasma hormones and milk production as a compromise between darkness and recommended higher light intensities. Lactating cows averaging 139 days in milk were exposed to light intensities of either 0.5 lux (dark) or 40-60 lux (dim) between 1800 and 0400 hours over 28 day periods. Milk production and plasma levels of melatonin and IGF-I were similar in cows exposed to the 2 lighting regimens. The diurnal rhythm of prolactin release was slightly altered by exposure to the dim light regimen.



5

Regulation of Angiotensin Type 2 Receptor in Bovine Granulosa Cells

Endocrinology, October 2008, Volume 149, Number 10, pages 5004-5011

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In addition to its role as a potent vasoconstrictor, angiotensin II (AngII) is also thought to play a role in the development of ovarian follicles. During the bovine estrous cycle, 'waves' of small antral follicles emerge every 8-10 days. Most of these will undergo atresia (collapse and death) while a single dominant follicle will develop to the ovulatory stage every 19-22 days. Follicles consist of a number of cell types, including thecal cells that form the follicular wall and granulosa cells that surround the interior cavity (antrum) and oocyte (egg). In rodents, AngII plays a role in follicular atresia, acting through receptors (AGTR2) found in granulosa cells. In cattle, AGTR2 had previously been reported to occur only in thecal cells. The objective of this study was to determine whether AngII has an effect on follicular atresia in cattle as it does in rodents. Both AGTR2 protein and messenger RNA (mRNA) coding for AGTR2 were found in both thecal and granulosa cells. Although the abundance of AGTR2 mRNA in thecal cells was similar in both healthy and atretic follicles, healthy granulosa cells had higher levels than atretic granulosa cells. Treatment of cultured granulosa cells with hormones expected to increase estrogen secretion resulted in increased AGTR2 mRNA and protein levels. Fibroblast growth factors had opposite effects. The addition of AngII itself did not affect estrogen secretion or cell proliferation. Because estrogen secretion is assumed to be an indicator of granulosa cell health, the authors conclude that AngII is not associated with follicular atresia in cattle but may have other specific roles during follicle growth.



6

Role of Transforming Growth Factor- β 1 in Gene Expression and Activity of Estradiol and Progesterone-generating Enzymes in FSH-stimulated Bovine Granulosa Cells

Reproduction, October 2008, Volume 136, Number 4, pages 447-457

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In the bovine estrous cycle, multiple ovarian follicles begin developing every 7-12 days. As development progresses, most of these follicles will undergo atresia (regression and death) while a single dominant follicle continues to develop to the ovulatory stage. These events are controlled by many chemical signals, including pituitary-released gonadotropins, steroid hormones and local stimulatory and inhibitory growth factors. Luteinizing hormone (LH) stimulates follicular thecal cells (TC) to produce androgens while follicle stimulating hormone (FSH) promotes conversion of androgens into estradiol (E2) by granulosa cells (GC). LH also stimulates conversion of cholesterol to progesterone (P4) by GC. The objective of this study was to evaluate the role of transforming growth factor β 1 (TGFB1) in modifying the synthesis of E2 and P4 in cultured bovine GC. FSH in the culture medium increased the production of E2 and the expression of the messenger RNA (mRNA) coding for the enzymes responsible for E2 synthesis. The addition of TGFB1 inhibited FSH-stimulated E2 synthesis and decreased the expression of mRNA coding for E2-synthetic enzymes and FSH receptor. P4 production by GC was not influenced by the presence of FSH in the culture medium but P4 synthesis increased with incubation time, an effect that was inhibited by TGFB1. Interestingly, TGFB1 inhibited conversion of androgens to E2 but did not affect the capacity to produce E2 from estrone. Similarly, in spite of inhibiting the expression of enzymes responsible for the supply of pregnenolone from cholesterol, TGFB1 did not affect the capacity to produce P4 from pregnenolone. These findings indicate that the main pathways leading to FSH-stimulated E2 and P4 synthesis from androgens and cholesterol are inhibited by TGFB1 yet some E2 and P4-producing capacity appears to be retained. Such selective inhibitory effects would concur with a physiological role for TGFB1 in limiting FSH-induced growth of ovarian follicles during selection of the dominant ovulatory follicle.



7

Challenges of Functional Genomics Applied to Farm Animal Gametes and Pre-hatching Embryos

Theriogenology, November 2008, Volume 70, Number 8, pages 1277-1287

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This paper describes the rapidly developing science of genomics and the related fields of phenomics and functional genomics, focusing on the potential and challenges inherent in their application to studies of gametes (sex cells) and early-stage embryos. The application of genomics has facilitated the sequencing of the entire bovine genome – all of the hereditary information contained in its DNA. This has led to expectations of the discovery of causes of disease and of rapid increases in the rate of genetic improvement of cattle. In pursuit of the latter, genomics has led to the identification of regions of the genetic code containing alternative nucleotide sequences (polymorphisms). The association of single nucleotide polymorphisms (SNPs) with phenotypic traits (observable characteristics) has given rise to the science of phenomics, where the results of genomic studies are combined with traditional breeding strategies. While genomics is primarily aimed at characterizing nucleotide sequences, functional genomics describes gene expression patterns as well as gene (and protein) functions and interactions. Although functional genomics has already been applied to the study of gene expression in gametes and embryos, future applications face important technical challenges due to the unique nature of gamete and early embryo development as well as the scarcity of tissues available for study.



8

Evaluation of the DG29 Test for Early Detection of Pregnancy in Cattle

Canadian Veterinary Journal, November 2008, Volume 49, Number 11, pages 1119-1121

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The need to get high-producing dairy cows pregnant within a few months after calving presents a challenge for most milk producers. A high proportion of cows will typically not conceive to first insemination and it is important to diagnose these 'open' cows as soon as possible so they can be re-bred. Current methods for detecting open cows involve rectal palpation by a veterinarian after 35 days of potential gestation or ultrasound examination after day 28. Both of these methods require off-farm expertise. In the past, a number of blood tests have been proposed to detect pregnancy. Primarily based on the detection of progesterone or an 'early conception factor', these tests proved to be less accurate than rectal palpation or ultrasound. More recently proposed methods have been based on the detection of placental proteins in blood. This paper reports on the evaluation of one such test (DG29) which is based on a specific pregnancy-related protein in blood. First-calf heifers were allocated to 1 of 2 groups: non-inseminated (n=18) or inseminated by artificial insemination or embryo transfer (n=202). Blood samples were taken at days 5-9 (D7), days 12-16 (D14), days 17-23 (D21), days 29-36 (D30), and days 44-66 (D60) after insemination. Heifers that came into heat before D30 were rebred and removed from the trial. Test accuracy on D30 blood samples was compared with pregnancy diagnosis performed by ultrasound on the same day. The DG29 test correctly identified 99.4% of the heifers pregnant by ultrasound and 66.7% of heifers non-pregnant by ultrasound. The latter is likely attributable to early embryonic deaths. In non-inseminated heifers, 100% were correctly identified as non-pregnant. DG29 may complement owner's observations and the veterinarian's services of rectal palpation and ultrasound.



9

Regulation of Bovine Tumor Necrosis Factor- α -Induced Protein 6 in Ovarian Follicles during the Ovulatory Process and Promoter Activation in Granulosa Cells

Endocrinology, December 2008, Volume 149, Number 12, pages 6213-6225

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Tumor Necrosis Factor- α -Induced Protein 6 (TNFAIP6) is an inflammatory-associated protein that is produced in many pathological and physiological contexts, where it plays important roles in inflammation and tissue remodelling by binding to various protein ligands and complex carbohydrates contained in the extracellular matrix (ECM). Ovulation is a complex process induced by luteinizing hormone (LH)/human chorionic gonadotropin (hCG), during which in rodents and horses the marked production of TNFAIP6 is associated with proper formation of the ECM that surrounds the oocyte (the cumulus-oocyte complex), protects the oocyte from environmental damages during extrusion, and facilitates sperm attachment, penetration and fertilization. The objective of this study was to evaluate levels and regulation of TNFAIP6 expression induced by hCG in bovine follicles. Concentrations of both TNFAIP6 messenger RNA and protein were initially low but increased significantly in follicular theca and granulosa cells in response to the hCG treatment. To investigate the mechanisms involved in increasing TNFAIP6 synthesis, the promoter region of the TNFAIP6 gene was studied in primary granulosa cell cultures, and factors involved in the promoter activation were identified.

10

Development and Characterization of a Simian Virus 40 Immortalized Bovine Endometrial Stromal Cell Line

Endocrinology, January 2009, Volume 150, Number 1, pages 485-491

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In the cow, ovulation involves the shedding of an oocyte from an ovarian follicle into the oviduct. The remaining follicular tissue is transformed to become a corpus luteum (CL) which produces increasing amounts of progesterone (P4) from the day of ovulation (day 0) until a plateau is reached 5-6 days later. If the oocyte is not fertilized, CL P4 synthesis starts to decline rapidly around day 15 when development of a new ovulatory follicle begins, followed by ovulation 5-6 days later. If fertilization does occur, the CL continues to produce P4 which suppresses further follicle development. The signal that fertilization has occurred is interferon- τ (IFN- τ) which is produced by the new embryo. IFN- τ inhibits the action of oxytocin which stimulates uterine epithelial (inside surface) cells (EC) to produce prostaglandin F2 α (PGF2 α) which can provoke destruction of the CL (luteolysis). In addition to its action on EC, IFN- τ stimulates the production of prostaglandin E2 (PGE2) in uterine stromal cells (SC) which are closely associated with EC. It had previously been difficult to determine the timing and location of IFN- τ activity in these cells because available cell culture lines contained both EC and SC. This paper describes the development of a pure SC line which was rendered immortal (not subject to aging) by viral infection of the nuclei of cells isolated from the lining of the bovine uterus.



11

Retrospective Case Study of Fetal Mummification in Cows that Did Not Respond to Prostaglandin F_{2α} Treatment

Canadian Veterinary Journal, January 2009, Volume 50, Number 1, pages 71-76

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Fetal mummification occurs in only about 2% of bovine pregnancies. Jersey and Guernsey cows and cows of any breed that previously had a similar event are at higher risk. In most cases, mummification occurs between the third and eighth month of gestation without spontaneous expulsion of the fetus. In some cases, treatment with prostaglandin F_{2α} (PGF_{2α}) results in lysis of the corpus luteum (luteolysis) accompanied by a fall in circulating progesterone levels, cervical opening and abortion. This study examined the consequences of 14 cases where PGF_{2α} treatment was not effective. In 11 cases, the mummified fetus was removed by hysterotomy (cesarian section); in 3 others, treatment with estrogen, oxytocin and PGF_{2α} was followed by manual dilation of the cervix and fetal extraction. Five of the 11 cows subjected to hysterotomy became pregnant again while none of the 3 subjected to hormone treatments and manual extraction conceived. The authors conclude that hysterotomy is the preferred treatment for cows carrying a mummified fetus.

12

Spatiotemporal Expression of Transcriptional Regulators in Concert with the Maternal-to-Embryonic Transition during Bovine *in vitro* Embryogenesis

Reproduction, January 2009, Volume 137, Number 1, pages 13-21

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Virtually all of the cellular machinery necessary for development of the bovine embryo up to the morula stage is transcribed from maternal DNA to messenger RNA (mRNA) during the germinal vesicle (GV) stage of the oocyte's development before ovulation occurs. Although some transcription of the embryo's own DNA (genome) occurs as early as the 2-cell stage, major transcription activation of the embryonic genome begins at the transition from 8 to 16 cells – the maternal-embryonic transition (MET). The objective of this study was to characterize the roles of 3 known regulators of DNA transcription to messenger RNA (mRNA) and of mRNA translation to protein in control of the MET. Based on their known involvement in the MET of other species, the expression and localization of Y box binding protein 2 (YBX2), TATA box-binding protein (TBP), and activating transcription factor 2 (ATF2) were studied from the GV stage of the oocyte to the embryonic blastocyst. Although ATF2 mRNA expression remained constant from the GV to the four-cell embryo before declining through to the blastocyst stage, ATF2 protein levels remained constant through all stages. YBX2 protein levels decreased while TBP levels increased throughout development. TBP protein was localized in the nucleus of 8- to 16-cell embryos, whereas YBX2 was exclusively found in the cytoplasm and was not found after the 16-cell stage.



13

Control of Ovarian Function for Assisted Reproductive Technologies in Cattle

Animal Reproduction, January 2009, Volume 6, Number 1, pages 114-124

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The paper reviews current technologies that are being used to control the estrous cycle in cattle, allowing for superstimulation, fixed-time artificial insemination (FTAI) or fixed-time embryo transfer (FTET) to recipients, without the need for estrus detection. These programs are dependent on controlling the timing of the development of waves of antral follicles, either pharmacologically with combinations of hormones, or through ultrasound-guided follicle ablation (removal). However, ultrasound-guided follicle ablation is difficult to apply in the field, and the use of GnRH or LH to induce ovulation and synchronize follicle wave emergence is efficacious in only 60 to 70% of cases. The administration of estradiol benzoate in progestin-treated cattle effectively synchronizes follicle wave emergence for both superovulation and estrus synchronization, but estradiol benzoate is not available in many countries. The authors suggest that recent knowledge of follicle wave dynamics in cattle will allow them to design alternatives that are just as efficacious.

14

Elevated Testicular Temperature Modulates Expression Patterns of Sperm Proteins in Holstein Bulls

Molecular Reproduction and Development, January 2009, Volume 76, Number 1, pages 109-118

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To achieve normal sperm development (spermatogenesis), the testes of bulls must be maintained in a temperature range 4-5°C below core body temperature. Increased testicular temperature results in abnormal spermatogenesis, impaired sperm function and lower fertilization rates. Since sperm DNA is not transcribed, the authors of this study hypothesized that the negative effects of increased testicular temperature must be mediated through the activity of proteins synthesized in the early stages of spermatogenesis. To test this hypothesis, ejaculates were collected from Holstein bulls before, during and after a 3-day period in which their scrota were insulated to increase testicular temperature. Sperm concentration and motility decreased significantly from pre-treatment values until approximately 36 days after the start of treatment, then began to rise again. Concentrations of several sperm proteins followed a similar pattern, including angiotensin converting enzyme (ACE), hexokinase-1, and a subunit of Na⁺/K⁺ ATPase. Conversely, another protein, identified as tissue inhibitor of metalloproteinase-2 (TIMP-2) was present at a low concentration before treatment, rising to day 28 after treatment began, and subsequently declining to pre-treatment concentrations. The authors proposed that concentrations of these proteins could be used as indicators of impaired sperm function due to elevated testicular temperature.



15

Analysis of Bovine Sexed Sperm for IVF from Sorting to the Embryo

Theriogenology, January 2009, Volume 71, Number 1, pages 30-38

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Cattle producers are now able to purchase bull semen which is more likely to result in female calves as a result of cell sorting technologies that increase the proportion of sperm carrying X chromosomes. However, the use of sexed semen (SS) in both artificial insemination and *in vitro* fertilization (IVF) programs has resulted in reports of low fertility rates. It has been suggested that the sorting process might induce partial capacitation, the process by which sperm are prepared for fertilization. This study compared the use of SS and non-sexed semen (NS), either fresh or frozen, in terms of their ability to provoke blastocyst (early embryo) development after IVF of bovine oocysts (eggs). To promote capacitation, heparin was added to IVF media at 3 alternative concentrations when SS was used but only at the highest level with NS. SS produced significantly fewer blastocysts than NS but freezing had a more negative effect on fertilization and blastocyst development than sexing. Maximum blastocyst production required less heparin when SS appeared initially more capacitated.

16

Unveiling the Bovine Embryo Transcriptome during the Maternal-to-Embryonic Transition

Reproduction, February 2009, Volume 137, Number 2, pages 245-257

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Virtually all of the cellular machinery necessary for development of the bovine embryo up to the morula stage is transcribed from maternal DNA to messenger RNA (mRNA) during the germinal vesicle (GV) stage of the oocyte's pre-ovulatory development. Although some limited transcription of the embryo's own DNA (genome) occurs as early as the 2-cell stage, major transcription activation of the embryonic genome begins at the transition from 8 to 16 cells – the maternal-embryonic transition (MET). The objective of this study was to characterize the variety of messenger RNA (mRNA) transcripts produced at the MET in bovine embryos using a method (suppression subtractive hybridization) by which complementary DNA (cDNA) is generated from each unique mRNA transcript. An expression profile for over 300 different transcripts was compared with a profile of transcripts found in embryos treated with a transcription inhibitor. The latter represented transcripts present before the MET. The majority of post-MET transcripts identified were for proteins involved in gene transcription, RNA processing, or protein biosynthesis. Others were potentially involved in the maintenance of pluripotency – the ability to differentiate into any cell type.



Oxytocin Receptor Down-Regulation is Not Necessary for Reducing Oxytocin-Induced Prostaglandin F_{2α} Accumulation by Interferon- τ in a Bovine Endometrial Epithelial Cell Line

Endocrinology, February 2009, Volume 150, Number 2, pages 897-905

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This paper describes the development of a unique, immortalized (not subject to aging) line of culturable bovine uterine epithelial (inside surface) cells (EC) which permitted the elucidation of molecular mechanisms controlling pregnancy recognition. Interferon tau (IFN- τ) is the molecular signal which prevents destruction of the corpus luteum (CL). CL maintenance is required to prevent subsequent estrous cycles and to prepare and maintain a uterine environment conducive to embryo development. In the absence of fertilization, the pituitary hormone oxytocin (OT) stimulates EC to produce prostaglandin-F_{2α} (PGF_{2α}) which is responsible for CL destruction (luteolysis). When conception does occur, IFN- τ produced by the early-stage embryo (trophoblast) inhibits OT-stimulated PGF_{2α} synthesis. Although it had been suggested that this inhibition was due to IFN- τ preventing the synthesis of endometrial OT receptors, a number of experiments showed that inhibition could occur much more quickly than could be explained by this mechanism. Using the newly-developed cell line, these authors were able to demonstrate that the mechanism likely also involves direct interference by IFN- τ in the signalling pathway through which OT stimulates PGF_{2α} synthesis or in the transcription of genes coding for PGF_{2α} synthesis.



In vitro Culture and Somatic Cell Nuclear Transfer Affect Imprinting of SNRPN Gene in Pre- and Post-implantation Stages of Development in Cattle

BMC Developmental Biology, February 2009, Volume 9, Number 9, pages 1-13

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Somatic cell nuclear transfer (SCNT) has been used to create clones (identical individuals) of several animal species. The technique involves removing the nucleus from a body cell of a donor animal and implanting it into an enucleated oocyte (egg cell) of the same species. The embryo that arises from the new zygote is then transplanted into a surrogate dam for further development. Because fertilization is bypassed, the process requires that control mechanisms remaining in the oocyte's cytoplasm will reprogram the genetic material in the donor nucleus back to the embryonic state. However, the low success rate of SCNT suggests that this reprogramming is seldom completely successful. The objective of this study was to compare the programming of a specific gene in bovine embryos produced by artificial insemination (AI), *in vitro* fertilization (IVF) and somatic cell nuclear transfer (SCNT). The gene examined encodes SNRPN (small nuclear ribonucleoprotein), a molecule that plays an important role in several critical physiological processes. The SNRPN gene is one of a small number of genes that have been identified as 'imprinted', meaning that the genetic code from only one of the parents is expressed. Expression of imprinted genes is regulated by methylation of the promoter region of the gene. This study found that the degree of SNRPN methylation was significantly lower in SCNT and IVF embryos than in embryos derived from AI, demonstrating the type of faulty programming that might be involved in the poor viability of SNCT-derived embryos.



19

Expression of Fibroblast Growth Factor 13 (Fgf13) mRNA in Bovine Antral Follicles and Corpora Lutea

Animal Reproduction, April 2009, Volume 6, Number 2, pages 409-415

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The bovine estrous cycle involves the initial development of a number of ovarian follicles which eventually yield to the maturation of a single 'antral' follicle (AF). At ovulation, the AF sheds its oocyte and the remaining follicular tissue, consisting of thecal and granulosa cells, is transformed into a corpus luteum (CL). Although these events are ultimately controlled by pituitary hormones, a number of local factors are also involved, including a family of 22 peptides known as fibroblast growth factors (FGFs). Although most of these FGFs exert their influence through extracellular FGF receptors (FGFR), FGFs 11, 12, 13 and 14 act only within the cell in which they are produced and do not bind to FGFR. Based on a previous study which demonstrated the expression of FGF13 in the developing gonads of mice, the objective of the present study was to determine whether FGF13 might play a role in the development of bovine AF and CL by measuring FGF13 messenger RNA expression (mRNA) patterns in those tissues. FGF13 mRNA was found in the CL and in AL thecal and granulosa cells but not in oocytes. Thecal cell FGF13 mRNA increased as the follicle grew but was unrelated to follicular estrogen concentration while FGF13 mRNA expression in granulosa cells and CL was unrelated to stage of development.

20

Modulation of Bovine Sperm Signalling Pathways: Correlation between Intracellular Parameters and Sperm Capacitation and Acrosome Exocytosis

Reproduction, Fertility and Development, April 2009, Volume 21, Number 4, pages 511-524

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Capacitation (CAP) is the process by which sperm are prepared for fertilization after they have entered the female reproductive tract. In preparation for binding to the outer wall of the oocyte and ejection of sperm nucleus into the oocyte (acrosome exocytosis, AE), the outer membrane of the sperm cell undergoes a number of modifications. These are accompanied by numerous biochemical changes inside the cell, including increases in intracellular pH, calcium (Ca) and cyclic AMP (cAMP) concentrations, elevated activity of enzymes that activate various proteins (phosphorylases, PPL), and the production of reactive oxygen species (ROS) that activate signals controlling enzyme activity. The objective of this study was to evaluate these changes and their interactions in relation to progress toward CAP and AE of freshly ejaculated bull spermatozoa. Increasing concentration of ROS (specifically, hydrogen peroxide) increased cAMP concentration and promoted AE. CAP was positively correlated with PPL activity and Ca concentration while AR was positively correlated with pH and Ca concentration.



Cryopreservation Affects Bovine Sperm Intracellular Parameters Associated with Capacitation and Acrosome Exocytosis

Reproduction, Fertility and Development, April 2009, Volume 21, Number 4, pages 525-537

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Capacitation (CAP) is the process by which sperm are prepared for fertilization after they have entered the female reproductive tract. In preparation for binding to the outer wall of the oocyte (the acrosomal reaction, AR) and ejection of the sperm nucleus into the oocyte, the outer membrane of the sperm cell undergoes a number of modifications. These are accompanied by numerous biochemical changes inside the cell, including increases in intracellular pH, calcium (Ca) and cyclic AMP (cAMP) concentrations and elevated activity of enzymes that activate various proteins (phosphorylases, PPL). When bull semen is frozen for use in artificial insemination, many of the sperm do not survive and most that do survive have experienced alterations that affect their ability to inseminate. The objective of this study was to evaluate how freezing and thawing affects the biochemical events involved in CAP and AR. Immediately after thawing and washing frozen semen, almost 50% of the sperm had undergone CAP and more than 20% had lost their acrosomes. cAMP concentrations were lower while Ca concentrations were higher in frozen-thawed sperm than in freshly ejaculated sperm but pH was in the expected range. Positive correlations were found between CAP, cAMP and Ca levels, and PPL activity while AR was positively correlated with pH and Ca concentration. The authors conclude that sperm CAP and AR mechanisms are affected by freezing and thawing, but that frozen-thawed sperm still retain their ability to regulate the biochemical events necessary to achieve insemination.



22

Effects of Dietary Energy and Protein Density on Plasma Concentrations of Leptin and Metabolic Hormones in Dairy Heifers

Journal of Dairy Science, April 2009, Volume 92, Number 4, pages 1430-1441

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Leptin is a peptide hormone secreted by adipose tissue (body fat) which is thought to be involved in communicating the status of body energy reserves to the brain. The brain then integrates this information in its control of appetite and reproductive function. The objective of this study was to measure the effects of varying nutrient intake levels by pre-pubertal heifers on blood concentrations of metabolites and hormones, including leptin. At 102-106 days of age (101-105 kg body weight, BW) 30 Holstein heifers were assigned to 1 of 3 dietary treatments formulated to achieve BW gains of 1.1 (H), 0.8 (M) or 0.5 (L) kg/day by adjusting dietary energy and protein densities. Approximately 40% of the total variation in plasma leptin concentrations was accounted for by differences in dry matter intake (18%), BW (17%) and back-fat thickness (5%). Plasma insulin concentrations increased after eating and the magnitude of these increases was proportional to dietary energy density. No such increases were observed for plasma leptin. Although plasma leptin concentrations increased with age, levels at puberty were similar for heifers on all 3 dietary treatments. The authors interpret results to indicate that plasma leptin concentrations may not be a critical trigger for puberty in rapidly growing heifers, but a certain threshold of leptin concentrations may be important for puberty in heifers with moderate or restricted growth rates.



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Plasma Luteinizing Hormone Concentrations in Cows Given Repeated Treatments or Three Different Doses of Gonadotropin Releasing Hormone

Theriogenology, April 2009, Volume 71, Number 6, pages 984-992

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When timed artificial insemination (TAI) strategies are used to breed dairy cows, gonadotropin releasing hormone (GnRH) is used to induce a preovulatory release of luteinizing hormone (LH) from the pituitary gland. The resulting LH 'surge' stimulates the ovarian follicle to release its egg (ovum). The remaining follicular tissue transforms into a corpus luteum which produces increasing amounts of progesterone (P4). The typical 100 microgram (μg) dose of GnRH used in TAI programs produces an LH surge lasting 4 to 5 hours (h) in contrast to a spontaneous preovulatory surge which normally lasts approximately 10 h. Because several studies have observed higher conception rates in cows that ovulated spontaneously, compared with TAI-treated cows, the objective of the present study was to evaluate the effects of GnRH treatment timing, frequency and dose on the magnitude and duration of the LH surge. In a first experiment, 7 or 8 days after estrus cows were given 2 doses of prostaglandin (PGF) to lyse the corpus luteum followed by treatment with 100 μg of GnRH once at 36 (control), or twice, either 2 h apart at 36 and 38 (GnRH38), or 4 h apart at 36 and 40 (GnRH40) h after the first dose of PGF. Average plasma LH concentration was highest in the GnRH38 cows, lowest in controls and intermediate in the GnRH40 group. The LH surge lasted longer in GnRH40 cows (8.0 h) than in either GnRH38 (7.0 h) or control (7.1 h) groups but average plasma P4 concentration was highest in controls. In a second experiment, ovariectomized cows received 50, 100, or 250 μg of GnRH. The 250 μg dose resulted in the greatest release of LH and the LH surge lasted longer (6.8 h) compared with the lower doses (5.1 h).



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Transforming Growth Factor- β 1 Inhibits Luteinization and Promotes Apoptosis in Bovine Granulosa Cells

Reproduction, June 2009, Volume 137, Number 6, pages 969-977

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In the bovine estrous cycle, under the influence of FSH, successive waves of follicle development occur at regular intervals every 7-12 days. During this process, a single follicle will continue to grow and ovulate while the majority of recruited follicles will undergo regression and death due to atresia. These events are controlled by many chemical signals, including pituitary-released gonadotropins, steroid hormones and local stimulatory and inhibitory growth factors. Luteinizing hormone (LH) stimulates follicular thecal cells (TC) to produce androgens (A4) while follicle stimulating hormone (FSH) promotes conversion of A4 into estrogen (E2) by granulosa cells (GC). LH also stimulates conversion of cholesterol to progesterone (P4) by GC. A previous study by these authors demonstrated that transforming growth factor β 1 (TGFB1) inhibits E2 and progesterone (P4) production in FSH-stimulated cultured GC by inhibiting key synthetic enzymes. The objective of the present study was to evaluate the effects of TGFB1 on these synthetic pathways in GC cells cultured without the addition of FSH. Effects of TGFB1 on cell proliferation and apoptosis (programmed cell death) in the presence and absence of FSH were also evaluated. P4 secretion increased with time in culture. Addition of TGFB1 decreased P4 secretion and the expression of messenger RNA (mRNA) coding for enzymes responsible for P4 synthesis. E2 synthesis decreased with time in culture but the addition of TGFB1 partially reversed this decline by stimulating the activity of E2 synthetic enzymes and the expression of mRNA coding for those enzymes. In the presence or absence of FSH, TGFB1 decreased the amount of granulosa cells in the quiescent G0/G1 phase of the cell cycle and committed a higher proportion of GC to apoptosis. This study indicates that TGFB1 inhibits P4 differentiation of developing bovine granulosa cells while preserving E2-producing capacity. TGFB1 could have a critical role in directing subordinate follicles towards atresia and that escape from this inhibition may be a mechanism that determines continued growth of the ovulatory follicle.



Reproductive Performance of Dairy Cows is Influenced by Prepartum Feed Restriction and Dietary Fatty Acid Source

Journal of Dairy Science, June 2009, Volume 92, Number 6, pages 2562-2571

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In the last few weeks before calving, dairy cows often reduce their feed intake, resulting in a period of negative energy balance (NEB) that usually extends into early lactation. NEB is commonly thought to lead to various metabolic disorders after calving and to negatively affect breeding success. However, it has also been suggested that post-partum feed intake increases more rapidly in cows experiencing more severe prepartum NEB. One strategy for reducing NEB at this time has been to increase dietary energy density by adding lipids to both pre- and post-partum rations. It has also been demonstrated that supplementary dietary lipids enriched in some specific fatty acids can have more direct effects on reproductive performance although it is unclear whether feeding these lipids pre-partum will have these beneficial effects. The objective of this study was to evaluate the effects of pre-partum feed intake and lipid supplementation on reproductive health and performance. Beginning 34 days before expected calving, Holstein cows were subjected to one of 2 levels of feed intake: unrestricted (ad libitum) dry matter intake (DMI) or DMI restricted to 76% of ad libitum DMI (feed-restricted). Within these 2 DMI treatments, diets were supplemented with either canola, linola or flax seeds at 8% of dry matter. Ad libitum cows had more uterine infections (10/37 vs. 2/35) but fewer ovarian cysts (2/37 vs. 7/35) than feed-restricted cows. Interval from calving to first ovulation was longer in cows fed canola than in those fed either linola or flax (34.7 vs. 23.7 and 21.0 days, respectively). More of the ad libitum cows conceived to the first artificial insemination (47 vs. 19%) and these cows had 34 fewer days open than the feed-restricted cows.



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Postpartum Anestrus in Dairy Cattle

Theriogenology, June 2009, Volume 71, Number 9, pages 1333-1342

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One of the main determinants of dairy enterprise profitability is the success with which cows can be re-bred within 2 to 3 months after calving. A number of factors can negatively affect that success, including negative energy balance (NEB) due to increasing energy demands for milk production as well as a number of common post-partum metabolic disorders. These factors can inhibit a normal return to estrous activity through their influence on any of 3 stages of follicular development: emergence of follicles from the pool of primordial follicles, development of a group of selected ovulatory follicles or establishment of a dominant follicle which goes on to ovulate. The authors advocate prevention of anestrus resulting from abnormal follicle development through management practices aimed at reducing NEB and post-partum metabolic disorders. They also suggest that a better understanding of the role of genes involved in ovulation and the influence of signals linking metabolic state with reproductive activity is required.

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Revealing the Bovine Embryo Transcript Profiles during Early *in vivo* Embryonic Development

Reproduction, July 2009, Volume 138, Number 1, pages 95-105

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Virtually all of the cellular machinery necessary for development of the bovine embryo up to the morula stage is transcribed from maternal DNA to messenger RNA (mRNA) during the germinal vesicle (GV) stage of the oocyte's pre-ovulatory development. Although some limited transcription of the embryo's own DNA (genome) occurs as early as the 2-cell stage, major transcription activation of the embryonic genome begins at the transition from 8 to 16 cells – the maternal-embryonic transition (MET). As development progresses from oocyte to 2-cell and 8-cell embryo through to the blastocyst stage, the pattern of mRNA transcripts that orchestrate that development undergoes marked changes. The objective of this study was to characterize mRNA expression patterns at those stages. Because of the limited and variable sample sizes available and the short time frames over which expression patterns change, a hybridization microarray was developed, containing over 1,000 complementary DNA (cDNA) sequences created from mRNA transcripts found in previous studies of early bovine embryonic development. Results demonstrated marked changes in expression patterns, particularly between oocyte and blastocyst profiles.



BH4 Peptide Derived From Bcl-xL and Bax-Inhibitor Peptide Suppresses Apoptotic Mitochondrial Changes in Heat Stressed Bovine Oocytes

Molecular Reproduction and Development, July 2009, Volume 76, Number 7, pages 637-646

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In vitro fertilization is a commonly-used reproductive technology involving the removal of oocytes from the ovary followed by fertilization in an artificial medium. After fertilization, embryos are allowed to mature to the blastocyst stage and are then implanted into the uteri of foster cows. Oocysts exposed to heat stress (HS) are subject to damage which resembles the programmed cell death (apoptosis) that occurs in other tissues, reducing the success of IVF in terms of blastocyst formation. Previous studies demonstrated that apoptosis-like damage (ALD) is mediated by a number of enzymes of the caspase family and that inhibition of these enzymes could reduce ALD. Caspase activity is regulated by a family of Bcl-2 proteins of which one group inhibits and another group promotes caspase activity. Of those that inhibit, all contain an amino acid sequence (domain) labelled BH4 peptide. An additional mechanism regulating caspase activity involves Bax-inhibitor peptide (BIP) which inhibits the activity of Bax, one of the caspase-promoting Bcl-2 proteins. The objective of this study was to determine whether either BIP or BH4 peptide derived from one of the caspase-inhibitory Bcl-2 proteins would attenuate HS injury in cultured bovine oocytes. While HS-treatment induced changes indicating damage to oocytes resulting in impaired embryo development, development of HS-treated oocytes into blastocysts was improved when BIP or BIP + BH4 were added to the culture medium. These results confirmed the involvement of Bcl-2 proteins in heat-induced ALD of bovine oocytes.



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The Effect of Porcine Luteinizing Hormone in the Synchronization of Ovulation and Corpus Luteum Development in Nonlactating Cows

Theriogenology, July 2009, Volume 72, Number 1, pages 120-128

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Timed artificial insemination (TAI) programs are used by an increasing number of dairy farmers, largely because they eliminate the need for heat detection. Although several different TAI protocols have been recommended, they all involve the administration of gonadotropin releasing hormone (GnRH) to stimulate luteinizing hormone (LH) release from the pituitary gland which, in turn, induces ovulation and the development of a corpus luteum (CL). The CL produces progesterone (P4) which is responsible for maintaining a uterine environment conducive to embryo viability. Previous research showed that administration of 25 mg of porcine LH (pLH) was significantly more effective than the standard 100 microgram (μ g) dose of GnRH in inducing ovulation in dairy heifers. The present authors had also demonstrated that pLH produced higher P4 concentrations than GnRH, suggesting that the additional P4 might enhance embryo survival. In spite of these potential advantages in favour of pLH, it is significantly more costly than GnRH. This study was designed to evaluate the possibility of using pLH at doses lower than the recommended 25 mg to replace GnRH in a TAI program. Cows whose estrous cycles had been synchronized using 2 different TAI protocols were treated with either 100 μ g GnRH or 8, 12.5 or 25 mg pLH. The 25 mg dose of pLH produced higher plasma LH levels than any of the other 3 treatments but ovulation rate was similar to that in cows given GnRH. Responses to the lower doses of pLH were not as favourable, to the extent that the authors could not recommend their use.



Pregnancy Rates to Timed Artificial Insemination in Dairy Cows Treated with Gonadotropin-releasing Hormone or Porcine Luteinizing Hormone

Theriogenology, July 2009, Volume 72, Number 2, pages 262-270

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The application of timed artificial insemination (TAI) protocols allows dairy farmers to inseminate cows without requiring estrus detection. Although several complex strategies have been recommended, a simple TAI protocol involves administration of gonadotropin releasing hormone (GnRH) to trigger ovulation and/or new follicular growth on day 0 followed by prostaglandin (PG) on day 7 to induce lysis of the corpus luteum, a second dose of GnRH on day 9 and insemination on day 10. The objective of this study was to evaluate the substitution of GnRH by porcine luteinizing hormone (pLH) in such a protocol, since a number of previous trials had suggested that, although it is more costly, pLH might yield better results. Using GnRH at both days 0 and 9 was compared with replacing GnRH with pLH on either or both of those days. After the day 0 treatment, 62% of pLH cows versus 44% of GnRH cows ovulated. Pregnancy rate after TAI was highest (42%) in cows treated with GnRH on day 0 (G0), followed by pLH on day 9 (P9). G0/G9, P0/G9 and P0/P9 treatments resulted in pregnancy rates of 28%, 30%, and 26%, respectively. Plasma progesterone concentrations after insemination, considered important for the maintenance of embryo viability, were similar among the 4 treatment protocols.



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The Dynamics of Gene Products Fluctuation During Bovine Pre-Hatching Development

Molecular Reproduction and Development, August 2009, Volume 76, Number 8, pages 762-772

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Virtually all of the cellular machinery necessary for development of the bovine embryo up to the morula (8-cell) stage is transcribed from maternal DNA to messenger RNA (mRNA) during the germinal vesicle (GV) stage of the oocyte's development prior to ovulation. Expression of this stored mRNA is controlled by a number of mechanisms, including the length of the 'poly-A' tail on each transcript. The poly-A tail is a sequence of adenosine nucleotides normally added to the end of each mRNA transcript after transcription. The tail is shortened over time and when it reaches a critical length, the mRNA is enzymatically degraded. In the case of developing embryo, stored mRNA transcripts are stabilized by the removal of their poly-A tail and by the RNA-protein complexes within which they are enclosed. Transcription activation of the embryonic genome begins at the transition from 8 to 16 cells – the maternal embryonic transition (MET). The objective of this study was to characterize fluctuations in total RNA, mRNA, poly-A bearing mRNA and protein concentrations from the GV stage of the oocyte to the blastocyst stage of the embryo. Total RNA and poly-A RNA levels declined steadily from the GV stage to the 8-cell stage after which they rose sharply in the morula and blastocyst. Total mRNA concentration declined up to the morula stage before increasing rapidly in the blastocyst. The sharp increases observed were attributed to the transcription activation that occurs at the MET.



Biology of Reproduction, August 2009, Volume 81, Number 2, pages 415-425

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In mammals, the production of female sex cells (oocytes) begins before birth but the process (meiosis) is arrested before it is complete until the animal reaches puberty. However, when arrested oocytes are removed from the ovary, they resume their development, suggesting that meiosis arrest is controlled by regulatory molecules secreted into the ovarian environment. Previous studies had suggested that cyclic adenosine monophosphate (cAMP) was the signal responsible for meiosis arrest through its inhibition of maturation-promoting factor (MPF) activity. Phosphodiesterases (PDEs) are a family of 23 enzymes that inactivate cAMP and other cyclic nucleotides. The objective of this study was to characterize the presence and distribution of PDEs in the bovine ovary as a first step in determining whether inhibition of PDE activity might delay the release of meiosis arrest in oocytes removed from ovaries for use in *in vitro* fertilization applications. Previous research with rodents had suggested that PDE3a was present in oocytes while PDE4d was predominant in ovarian granulosa and cumulus cells. The present study demonstrated that PDE3 was predominant in bovine oocytes with about 20% of PDE activity attributable to PDE8. In cumulus cells, PDE8 accounted for 60% of the PDE activity; 5% was due to PDE4. The authors suggest that the finding of PDE8 in these cells challenges the hypothesis that PDE4 was the primary PDE in the ovarian follicle.



33

Regulation of MMP2 and MMP9 Metalloproteinases by FSH and Growth Factors in Bovine Granulosa Cells

Genetics and Molecular Biology, September 2009, Volume 32, Number 3, pages 516-520

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During the bovine estrous cycle, 'waves' of primordial follicles emerge every 8-10 days. Most of these will undergo atresia (collapse and death) while a single dominant follicle will develop to the ovulatory stage every 19-22 days. Developing follicles consist of a number of cell types held together by an extracellular matrix (ECM) consisting of protein and other structural components. In the process of follicular atresia, enzymes that degrade ECM proteins are activated by factors released within the follicle cells. Matrix metalloproteinases (MMP) are enzymes thought to have an important role in this process. The objective of this study was to evaluate the effects of various hormones and growth factors on MMP activation in cultured follicular granulosa cells. MMP activation was assessed by measuring the abundance of messenger RNA (mRNA) transcripts coding for 2 MMP proteins, MMP2 and MMP9. Follicle-stimulating hormone (FSH) and insulin-like growth factor-1 (IGF1) both stimulated estrogen secretion and reduced MMP2 and MMP9 mRNA abundance. In contrast, epidermal growth factor (EGF) and fibroblast growth factor-2 (FGF2) inhibited estrogen secretion but had no effect on MMP mRNA expression. None of these hormones or growth factors affected the rate of programmed cell death (apoptosis). The authors interpret the effects of FSH and IGF-1 as suggesting that excess MMP2 and MMP9 expression is neither required nor desired for follicle development.



Journal of Endocrinology, September 2009, Volume 202, Number 3, pages 347-353

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The bovine estrous cycle involves the initial development of a number of ovarian follicles which eventually yield to the maturation of a single 'antral' follicle (AF). The AF consists of an oocyte (egg) and a fluid-filled cavity (antrum), both surrounded by 'granulosa' cells (GC), all encapsulated within 2 layers of 'thecal' cells (TC). Although development of the follicle is ultimately controlled by gonadotropic and steroid hormones, a number of local factors are also involved, including a family of 22 peptides known as fibroblast growth factors (FGFs). One of these, FGF8 has been found in rodent oocytes and in bovine follicles while messenger RNA (mRNA) coding for FGF17, a close relative of FGF8, has been detected in human prostate and placental tissues and in mouse oocytes and embryos. The objective of this study was to ascertain the presence of FGF17 in bovine follicular cells and determine whether the expression of FGF17 mRNA in these cells was responsive to follicle stimulating hormone (FSH) and/or insulin-like growth factor I (IGF-I). FGF17 was found in the oocytes of pre-antral follicles and in both oocytes and GC of antral follicles. FGF17 mRNA was also evident in oocytes and, to a lesser extent, in GC and TC of healthy follicles. Addition of FSH or IGF-I to cultured GC decreased FGF17 mRNA abundance. Treatment with FGF17 inhibited both estradiol and progesterone secretion by these cells. The authors conclude that FGF17 is involved in the control of GC differentiation in bovine follicles.



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Copy Number Variation of Testis-Specific Protein, Y-Encoded (TSPY) in 14 Different Breeds of Cattle (*Bos taurus*)

Sexual Development, September 2009, Volume 3, Number 4, pages 205-213

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Cattle have 60 chromosomes, 30 matching (homologous) pairs, of which 58 are called autosomes and 2 are sex chromosomes, labelled XX in female cattle; XY in males. When egg and sperm (sex) cells are formed in the ovaries and testes, respectively, the autosomes undergo a process of recombination, where each of 2 homologous pairs exchange segments of DNA, ensuring that no 2 sex cells are identical. The two X chromosomes found in females undergo the same process but, in males, a large proportion of the length of the Y chromosomes (the male-specific region, MSR) does not pair with its X partner, with the result that recombination only occurs at the tips of 2 chromosomes, known as the pseudoautosomal regions. The length of the MSR varies among species and breeds, all having several regions containing multiple copies of specific genes. It is speculated that exchange of genetic material between these copies provides for genetic diversity in the absence of recombination between chromosomes. In addition, recombination within the MSR can influence the number of active copies of the genes involved (copy number) which can affect the phenotypic (observable) characteristics of individual animals. This study was designed to evaluate copy number variation of the testis specific protein Y-encoded (TSPY) gene among cattle breeds. Among the common Canadian dairy cattle breeds, Brown Swiss and Guernsey had the highest average TSPY copy numbers, at 161 and 166, respectively. Average copy numbers for Holsteins, Jerseys and Ayrshire were 89, 128 and 86, respectively. Considerable variation between individuals within breeds was also observed.



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Differential mRNA Expression in In Vivo Produced Pre-Implantation Embryos of Dairy Heifers and Mature Cows

Molecular Reproduction and Development, December 2009, Volume 76, Number 12, pages 1165-1172

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Although average pregnancy rates (PR) achieved in mature cows has declined from about 66% down to around 40% over the past 50 years, PR in heifers have remained relatively constant at around 70%. The major cause for low PR in mature cows is thought to be early embryo mortality which may be due to reduced developmental competence of the embryo, chromosomal defects, alterations in the maternal uterine environment, lack of synchrony between embryo and dam, or failure of the dam to respond to embryonic signals. Pregnancy recognition by maternal tissues is dependent upon secretion by the embryo of interferon-tau (IFN- τ) while successful embryonic development depends on the coordinated expression of various enzymes within the embryo. Among the latter, sodium/potassium-ATPase (Na/K-ATPase), facilitative glucose transporters (GLUT5) and heat shock protein 70 (HSP70) are important. Other factors such as BAX and BCL2 also play key roles in the regulation of growth and function in embryos. To elucidate differences in embryo viability, this study compared the abundance of messenger RNA (mRNA) transcripts for these factors in embryos from heifers versus those from mature cows. Within each parity group, embryo quality was also graded by morphological characteristics whereby grade I or II embryos are considered of good quality. Among grade I embryos, HSP70 mRNA abundance was higher in those from heifers than in those from cows. For pooled embryos of both grades, expression of IFN- τ mRNA was also greater in heifers than in cows. Expression of GLUT5 and Na/K-ATPase mRNA was higher in grade I than in grade II cow embryos.

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Sylvestre, E.	Reproduction	31204	Vasseur, E.	Animal Welfare	1638
Talbot, B.G.	Health	30157	Veiga, A.M.	Reproduction	5184
	Milk Production	1175		Reproduction	33206
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Taniguchi, M.	Feeding	4088		Animal Welfare	1033
Taylor, J.F.	Genetics	6112		Animal Welfare	1335
	Genetics	7113		Animal Welfare	1436
	Genetics	12117	Verkerk, G.A.	Animal Welfare	932
	Genetics	19124	Vernet, J.	Feeding	1566
Teixeira, N.A.	Reproduction	19194	Verschoor, C.P.	Health	43167
Thangavelu, G.	Feeding	962	Vigneault, C.	Reproduction	12189
	Feeding	3685		Reproduction	16191
Therrien, J.	Reproduction	18193				



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