



Highlights

in Canadian Dairy Cattle Research

2009



Dairy Farmers
of Canada



Les Producteurs laitiers
du Canada

Réseau laitier canadien
CUN
Canadian Dairy Network



Agriculture and
Agri-Food Canada

Agriculture et
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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 2007 to September 2008. 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).

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Animal Welfare





1

Effects of Bedding Quality on Lying Behavior of Dairy Cows

Journal of Dairy Science, December 2007, Volume 90, Number 12, pages 5468-5472.

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The objective of this study was to test the preferences of non-lactating cows for wet versus dry bedding by observing their standing and lying behaviour. Four groups of 6 cows each were housed in pens where they had access to 12 free stalls bedded with fresh sawdust over a geotextile mattress base. After a 5 day adaptation period, for the following 2 days all 12 stalls in 2 of the pens were bedded with sawdust that had been soaked in water, replaced twice daily. The 12 stalls in the other 2 pens continued to be maintained with a depth of approximately 10 cm of fresh, dry sawdust. Cows in the dry-bedded pens spent an average of 13.8 hours per day (h/d) lying down and 32 minutes per day (min/d) 'perched' with their 2 front feet in a stall. Cows in the wet-bedded pens lay down for only 8.8 h/d, perching in stalls for 92 min/day. During a second 2 day test period, 6 stalls in each pen were bedded with wet sawdust, 6 with dry, allowing the 6 cows in each pen a choice of stalls to use. On average, cows spent 12.5 h/day lying in the dry stalls and only 0.9 h/d lying in wet stalls. Half of the cows spent no time lying in the wet stalls. These observations clearly indicated the cows' preference for lying in dry stalls.

2

Validation of a System for Monitoring Individual Feeding and Drinking Behavior and Intake in Group-Housed Cattle

Journal of Dairy Science, December 2007, Volume 90, Number 12, pages 5732-5736.

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An automated feed intake monitoring system at the University of British Columbia's Dairy Education and Research Centre allows researchers to monitor the feed and water intake and behaviour of individual cows. Feed and water bins are fitted with antennae which detect the approach of animals fitted with transponders that uniquely identify each cow. If a cow is permitted access to a specific bin, a gate opens allowing her to feed or drink. The system records the cow and bin ID as well as the start and end times and bin weight at the beginning and end of each visit. These data are used to calculate visit frequencies and durations and the amount of feed or water consumed at each visit. The objective of this study was to validate the accuracy of this system by comparing its output with visual observations of cow behaviour and independent measures of feed disappearance. The system was virtually 100% accurate in identifying cows. Frequency and duration of visits recorded by the system were very highly correlated with the same measures calculated from observation of video activity recordings. Disappearance of feed from bins, calculated from independent measures of feed weights at 24 hour intervals, differed by less than 3.4% from weights recorded by the system itself. These results validate the use of this system for future use in cow feeding studies.



3

Dietary Forage Concentration Affects the Feed Sorting Behavior of Lactating Dairy Cows

Journal of Dairy Science, December 2007, Volume 90, Number 12, pages 5572-5579.

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Cows are commonly fed total mixed rations (TMRs) consisting of multiple ingredients of varying particle size. Most long particles are provided by forages which are generally higher in fibre than other ingredients. Although it may be assumed that cows consume ingredients in proportion to their presence in the TMR, this is often not the case — cows are capable of sorting the ingredients to access those they prefer. The objective of this study was to determine whether the proportion of forage in a TMR has an effect on sorting and to observe how cows respond to a change in TMR forage level. Two corn and grass silage-based TMRs were formulated: a low-forage (LF) diet containing 50.7% forage and a high forage (HF) diet containing 62.3% forage. Two groups of three cows were offered either the LF or the HF diet for 7 days then switched to the other diet for a further 7 days. On both diets, cows sorted in favour of short particles and against long particles and fibre. Overall, sorting was significantly greater on the LF diet. When switched from the HF to the LF diet, sorting behaviour changed to that characteristic of the LF diet within one day. The results indicate that cows rapidly adjust their sorting behaviour when subjected to a dietary change and they exhibit more sorting for short particles and against long particles and fibre when fed a LF diet.

4

Behavioural Indicators of Hunger in Dairy Calves

Applied Animal Behaviour Science, February 2008, Volume 109, Number 2-4, pages 180-189.

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The objective of this study was to compare the feeding behaviour of calves offered unrestricted (ad lib) access to milk with the behaviour of calves offered milk at a rate of 10% of body weight in 2 daily allotments. Calves, 8 to 14 days of age, were penned in groups of 4 with each pen providing access to an automated milk feeding stall which recorded milk intake and calf weight at each visit. Ad lib access to water, hay and calf starter was also provided to each group. Calves offered ad lib access to milk consumed, on average, approximately 8.5 kg of milk per day (kg milk/day) while calves whose intake was restricted consumed approximately 4.6 kg milk/day. Restricted-fed calves consumed all of their daily ration in 2 visits to the feeder, each lasting about 12 minutes, but these calves visited the feeder an average of another 24 times per day. Ad lib calves consumed milk during an average of 5.3 visits per day, each visit lasting 10.7 minutes—these calves also visited the feeder 2.1 times per day without feeding. Restricted-fed calves stood for 1 hour per day longer and were more aggressive, butting or pushing pen-mates occupying the feeder approximately seven times more often than calves in the ad lib-fed groups. The behaviours observed in restricted-fed calves are useful indicators of hunger.



5

Behavioural Responses by Dairy Cows Provided Two Hays of Contrasting Quality at Dry-Off

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Current knowledge suggests that a 40 to 60 day dry (non-lactating) period is desirable at the end of each lactation. This objective demands that lactation be terminated as quickly as possible while minimizing the distress response by the cow. To achieve this, common practice involves reducing nutrient intake by offering a diet of lower energy density and/or reducing milking frequency for a few days prior to complete termination (dry-off). In this study, cows were fed a late lactation total mixed ration (TMR) before being offered hay as the sole feed for 6 days before dry-off while milking frequency was gradually reduced. While one group of cows were offered an unlimited quantity (ad lib) of grass hay, a second group was offered ad lib access to oat hay of somewhat lower quality. Feed intake, production and behaviour were monitored before and during the 6-day dry-off treatment. Before treatment, cows consumed an average of 18.1 kg of feed dry matter (DM) and produced 16.4 kg of milk per day. By the day of dry-off, cows offered the oat hay were consuming 7 kg DM and producing 4.7 kg of milk/day; those offered grass hay consumed an average 12.9 kg DM and produced 7.8 kg of milk. Cows offered the oat hay showed an increase in vocalizations during the first 2 days after changing diets from TMR to hay suggesting that the behaviour may have been in response to hunger.



6

Effects of Sand and Straw Bedding on the Lying Behavior, Cleanliness, and Hoof and Hock Injuries of Dairy Cows

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In this study, researchers assessed cow preferences for free stall bedding materials and the effects of those materials on cow cleanliness, hock lesions and claw disease. Stalls had either a concrete base deep-bedded with straw (initially 6.5 kg of chopped barley straw with approximately 0.7 kg added each day) or a coarse sand base topped with approximately 20 cm of fine sand, cleaned and levelled twice daily. Groups of cows were provided one of the 2 stall types for at least 21 weeks then moved for a subsequent 5 days to a pen where they could choose either stall type. In the initial phase, cows with access to straw-bedded stalls spent 10% more time lying down than those housed with sand stalls. In the second phase, cows who had been housed with straw stalls preferred to continue lying in straw stalls, by a wide margin. Cows who had been housed with sand stalls showed no clear preference for straw or sand when given the choice but were not as averse to using the sand stalls as were the cows who had been housed with straw stalls. Hock injuries and claw lesions were recorded at the beginning and end of the initial 21 week stall trial period. The severity of hock lesions at the end of the trial was lower among the cows housed with sand stalls and claw health had improved to a greater extent among those animals. Although there was no difference in cleanliness between stall types, cows using straw stalls were not as clean as those using sand stalls.



7

Weaning Distress in Dairy Calves: Acute Behavioural Responses by Limit-Fed Calves

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In the context of current practice on most dairy operations, weaning simply means termination of the feeding of milk or milk replacer to calves who have had an opportunity to establish their consumption of solid feed. In spite of the fact that dairy calves have usually been separated from their dams at birth, the increased activity and vocalization they exhibit at weaning indicate some degree of distress. The objective of this study was to determine how these indicators of distress might be mitigated by changes in weaning management. Before weaning, individually-housed calves were offered unlimited (ad lib) access to a barley-based calf starter, chopped fescue hay and fresh water. Whole milk was provided twice daily in an open bucket at a daily rate of 10% of body weight. To test whether increased starter consumption at weaning might mitigate weaning distress, the daily milk ration offered to some of the calves was progressively diluted with water starting at 5 days preweaning such that the last feeding was pure water. Although this tactic doubled these calves' starter intake at weaning (to almost 2 kg/day) it did not mitigate their apparent distress when the twice-daily liquid feeding routine was discontinued. A second strategy was more successful: some calves were weaned abruptly, with the discontinuation of the liquid feeding routine, while for others the routine continued with warm water being offered twice daily. Higher rates of vocalization and activity among the abruptly weaned calves indicated that they experienced significantly more distress than the calves for whom the liquid feeding routine was continued.



8

Understanding Weaning Distress

Applied Animal Behaviour Science, March 2008, Volume 110, Number 1-2, pages 24-41.

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This paper reviews the literature on the distress responses exhibited by animals at weaning, including theoretical perspectives on the biological and psychological causes of these responses. In some cases, the weaning of young farm animals involves numerous stressors all imposed over a short period of time. Separation from the dam may be accompanied by a change in diet, introduction to new social partners and a change in physical environment. Each of these changes can negatively affect the young animal and their combined effects can be very detrimental to its welfare and future productivity. Temporal separation of each of these changes can reduce the acute distress response. For example, strategies to increase solid feed intake before terminating the availability of milk can reduce the impact of dietary change. Avoiding changes in the physical environment at the same time that milk feeding is terminated can also mitigate the distress response. The authors of the review advocate future research to develop weaning systems that are both beneficial in terms of animal welfare and practical in a commercial animal production setting.

9

Acute Behavioral Effects of Regrouping Dairy Cows

Journal of Dairy Science, March 2008, Volume 91, Number 3, pages 1011-1016.

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Current practice in many of today's milk production operations is to house lactating cows in free stall ('loose housing') facilities, managing them in groups according to parity, stage of lactation or level of production. This management model necessitates transferring one or more animals between groups as lactation progresses or production level changes. Because cows are social animals, social hierarchies become established within groups through both physical and non-physical interactions. The introduction of new animals into a group can destabilize these relationships. The objective of this study was to characterize behavioural and production responses when individual animals (focal cows) were introduced into established social groups of lactating cows. On average, when compared with their feeding behaviour during the 3 previous days, focal cows spent approximately 15 fewer minutes eating in the first hour after being introduced into a new group. They were displaced from the feeding area 25 times in the first day after introduction, compared with 10 times per day in the group from which they were transferred. Focal cows also spent less time lying down during the day after transfer, lay down less often and spent less time grooming themselves in the 4 days after introduction to the new group. Milk production of the focal cows declined on the first day after transfer but recovered in subsequent days.



10

Effect of Lameness on Dairy Cows' Visits to Automatic Milking Systems

Canadian Journal of Animal Science, March 2008, Volume 88, Number 1, pages 1-8.

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Automatic milking systems (AMS) require that animals voluntarily visit the milking robot multiple times per day. The objective of this study was to assess the effect of lameness on the frequency of their visits. Visit frequencies were monitored automatically for 578 cows by 12 AMS on eight farms. For each AMS, the 11 cows with the highest, and the 11 cows with the lowest visit frequencies were selected for evaluation of their locomotion status. Each of these 22 cows was assigned a gait score based on video recordings of their locomotion while walking in a straight line for 10 to 12 metres. The scoring method was based on evaluation of 6 components of locomotion resulting in the assignment of an overall score on a 5-point scale where a score of 1 is assigned to a cow with a normal gait; 5 to a cow judged severely lame. For 9 of the 12 AMS, significant differences in gait scores were found when the 11 low visit cows were compared to the 11 high visit animals. While only 4% of the high visit cows were judged slightly lame, 32% of the low visit cows were either slightly or severely lame. The authors conclude that monitoring their AMS visit frequencies might help to identify cows in the early stages of lameness.



11

A Barrier Can Reduce Competition over Teats in Pair-Housed Milk-Fed Calves

Journal of Dairy Science, April 2008, Volume 91, Number 4, pages 1607-1613.

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Although group rearing of milk-fed calves has some advantages, particularly in terms of animal welfare, the practice often results in competition between calves for access to the artificial teats through which the milk is fed. Calves are often aggressively displaced from a teat while they are feeding with the result that individual milk intakes can vary significantly. The objective of this study was to investigate ways to reduce displacement activity in group-housed, milk-fed calves. In one experiment, 6 pairs of calves were raised in pens that had a 46 cm barrier between 2 teat buckets; another 6 pairs were housed in pens with no such barrier. Three pairs of calves within each of the barrier treatments were offered 8 litres of milk per pair per day; the other 3 were offered only 5 litres. Although each pair of calves had access to 2 teats, calves switched from one teat to the other while feeding, often displacing their pen mate from the alternative teat. The amount of milk offered did not affect the frequency of switching but calves offered less milk switched teats more quickly after starting to nurse on each teat. The barrier between buckets reduced the frequency of switches but did not affect the time spent feeding from one teat before switching. A second trial further examined the effect of barriers. Pair pens had either no barriers or barriers on both sides and between the two teat buckets. All 3 barriers in each pen were either 46 cm or 100 cm long. The longer barriers prevented calves from displacing other calves who were feeding and doubled the length of time spent feeding before switching to the alternative teat. Thus, barriers between teat-buckets can reduce competition for milk among pair-housed calves, but the effects were strongest when using long barriers, which separate the teat-buckets as well as the front half of the calf during a feeding event.



12

The Concept of Social Dominance and the Social Distribution of Feeding-Related Displacements Between Cows

Applied Animal Behaviour Science, May 2008, Volume 111, Number 1-2, pages 158-172.

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Dairy cattle are commonly housed in free-stall ('loose housing') facilities and managed in groups. Because they are social animals, cows establish social relationships within those groups with some cows establishing dominance over others. In this study, dominance relationships were evaluated in terms of one cow displacing another from the feeding area. Each of six groups of 12 cows were observed over 3 days. A cow was considered to be dominant over another if the first displaced the second more often than the second displaced the first. Several types of bi-directional relationships can occur between 2 animals (a dyad): in a one-way dyad, one cow of a pair was observed to consistently displace the other; in a two-way dyad, both cows of a pair were observed to displace one another; a tied dyad was observed when both cows of a pair displaced each other the same number of times. A circular triad involved 3 cows where the first was dominant over the second, the second was dominant over the third and the third was dominant over the first. In 6 groups of 12 cows each, dominance relationships were linear, meaning that a basic, ranked hierarchy of animals was established. However, a large number of bi-directional relationships and circular triads were also observed. Animal rank in the dominance hierarchy was related to parity, cow behaviour and production. High ranking cows were older, produced more milk and spent more time at the feed barrier during the 2 hours after delivery of fresh feed. Displacements at the feed barrier appeared to be influenced by individual motivation and persistency as well as dominance rank.



13

Assessing Sleep State in Calves through Electrophysiological and Behavioural Recordings: A Preliminary Study

Applied Animal Behaviour Science, June 2008, Volume 111, Number 3-4, pages 235-250.

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A custom-designed equipment harness was fabricated which allowed the authors of this study to record electroencephalographic (EEG) data in group-housed calves that were able to move freely in their pens. Adhesive electrodes were attached at 10 locations on the top of the head and neck. Analysis of the EEG data made it possible to determine whether calves were awake or experiencing rapid eye movement (REM) or non-REM sleep during periods lasting 30 seconds. On average, calves were asleep during 25% of the time for which recordings were obtained. Approximately 55% of the total sleep time was spent in non-REM sleep; 45% in REM sleep. Both types of sleep were experienced by the calves in short bouts lasting two to three minutes. In addition to the EEG data, details of the calves' behaviour were recorded, based on visual observation, to determine whether these observations could be used to predict their EEG-determined state of wakefulness or sleep. The best behavioural predictors of sleep were calves lying on their sides or sternums with their heads either lifted up and still (resting with head lifted up still) or resting against body or ground (resting with neck relaxed). The first of these behaviours predicted 55% of the episodes that were identified as non-REM sleep by the EEG data. The second behaviour predicted 61% of EEG-identified REM episodes. EEG data indicated that calves were awake 81% of the time that they were either standing or lying down with their heads lifted up and moving.



14

Weaning Distress in Dairy Calves: Effects of Alternative Weaning Procedures

Applied Animal Behaviour Science, July 2008, Volume 112, Number 1-2, pages 33-39.

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Increased activity and vocalization are indicators of the distress experienced by calves when they are weaned. The objective of this study was to test a weaning strategy that might mitigate indicators of distress. Individually-housed calves were offered unlimited (ad lib) access to water and a barley-based calf starter. Whole milk was provided ad lib for 2 hour periods twice per day, at 0730 and 1800 hours, through a teat attached to the wall of each pen. At weaning, the teat was abruptly removed from the pens housing 8 calves. For another 8 calves, teats were left in place and warm water replaced milk during the 2 daily feeding periods for 2 days after weaning. Consumption of warm water (8 kg/day) by these calves was approximately the same as the amount of milk consumed before weaning (9 kg/day). At weaning, abruptly weaned calves vocalized at more than 3 times the rate and exhibited more activity and standing than calves that were offered warm water, indicating greater distress. Vocalizations for both groups were similar on the third day post-weaning when neither had access to teats. The authors of the study suggest that the increased distress experienced by the abruptly weaned calves may have been due to either the loss of their opportunity to suckle the artificial teat or the reduction in gut fill due to lower liquid intake.



15

Analgesics Improve the Gait of Lamé Dairy Cattle

Journal of Dairy Science, August 2008, Volume 91, Number 8, pages 3010-3014.

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Claw lesions often cause cows to limp or otherwise alter the way they walk. Researchers assessed lameness using 6 specific gait characteristics (back arch, head bob, tracking up, joint flexion, asymmetric steps and reluctance to bear weight; each scored separately on a scale of 0 to 100), as well as a numeric rating system (NRS) that assigns an overall gait score of 1 (sound) to 5 (clinically lame) based on a combined assessment of the 6 individual gait characteristics. The question asked in this study was: to what extent are gait abnormalities due to pain? To answer this question, the authors of the study administered pain relief medication (ketoprofen) to cows exhibiting gait impairment. Saline was administered to lame control cows. Cows were scored using both LCS and NRS before, during and after treatment. NRS improved in response to ketoprofen dose, with the greatest improvement occurring at the highest dose (3 mg per kg of body weight). None of the specific LCS gait characteristics showed a consistent response to treatment, suggesting that NRS provides a better indicator of pain due to lameness. However, even NRS improved by only 0.25 suggesting that more potent drugs are required to treat this pain, or that much variation in cow gait is due to factors other than pain.



16

Dominance in Free-Stall-Housed Dairy Cattle Is Dependent Upon Resource

Journal of Dairy Science, October 2008, Volume 91, Number 10, pages 3922-3926.

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Cows are social animals and thus when they are managed in groups, social relationships between animals are formed, leading to a social structure within each group. One aspect of social structure involves the dominance of some animals over others, resulting in a competitive hierarchy. In this study, the dominance rankings of cows within 6 groups of 12 animals were investigated by observing displacements at 3 different resources in their pens: a 7.2 meter feed bunk, a bedding area with 12 free stalls and a mechanical grooming brush. Each cow's dominance index (DI) was defined as the proportion of times that she initiated a displacement as a proportion of the total displacements in which she was involved at each resource. On this basis, 3 dominance groups were established for each resource: high-ranking cows with DI greater than 0.6; middle ranking cows with DI between 0.4 and 0.6; low ranking cows with DI less than 0.4. The primary objective was to determine whether DIs established by observing displacements at each resource would be highly correlated across resources. They were not, suggesting that other motivational factors also affect competition for each resource. The observation that 88% of total displacements occurred at the feed bunk, compared with 10% in the lying area and 3% at the brush, suggested that access to feed was of highest priority for these cows.

Environment





1

Impact of Single versus Multiple Policy Options on the Economic Feasibility of Biogas Energy Production: Swine and Dairy Operations in Nova Scotia

Energy Policy, September 2007, Volume 35, Number 9, pages 4597-4601.

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Concerns about the environmental impacts of livestock manure and energy costs have stimulated interest in on-farm anaerobic fermentation of manure and other organic wastes as a source of biogas energy which might both reduce energy costs and improve farm environmental sustainability. The objective of this study was to determine the economic feasibility of this technology for small-scale dairy farms in Nova Scotia with 50 to 500 cows and for swine (farrow-to-finish) operations having 200 to 800 sows. Economic feasibility was examined in terms of net present value, internal rate of return and payback period, and also included the effects of 3 potential government policy incentives: a 'green energy' credit policy, a capital cost-sharing policy and a low-interest loan policy. It is concluded that, without incentives, on-farm anaerobic biogas energy production was economically feasible for farrow-to-finish operations having 600 to 800 sows but not for dairy operations of the size ranges examined nor for smaller swine operations. A proposed green energy credit scheme was the only incentive program that made adoption of the technology feasible on dairy operations. Various combinations of incentive policy schemes were also examined to determine their effects on economic viability.



2

Long-Term Effects of Feeding Diets Without Mineral Phosphorus Supplementation on the Performance and Phosphorus Excretion in High-Yielding Dairy Cows

Canadian Journal of Animal Science, December 2007, Volume 87, Number 4, pages 639-646.

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Phosphorus (P) fed in excess of animal requirements is largely excreted in feces, increasing P loading on the environment. It has been common practice for most North American dairy farmers to feed higher than recommended levels of P in the belief that doing so would enhance reproductive performance. However, this belief has been disproven by several studies that have demonstrated the adequacy of the US National Research Council's recommended feeding level of 0.32 - 0.38% P in dietary dry matter. The objective of this study was to evaluate the long-term effects on production parameters and P excretion when typical Ontario diets were fed to high-yielding dairy cows. In one experiment, cows were offered corn silage/alfalfa haylage-based total mixed rations (TMR) containing either 0.35% or 0.42% P in dietary dry matter. The lower level was achieved with no P supplementation beyond that contained in basal diet ingredients. Cows received these diets over 2 lactations or until they were culled. The P concentration in feces from the cows fed 0.42% P was higher (0.84%) than that in the feces of the cows fed 0.35% P (0.62%). Although body weight, body condition score and milk urea nitrogen were all significantly lower in multiparous cows offered the lower P diet, milk yield, fat and protein yields were not influenced by dietary P concentration. Dry matter intake, as well as milk yield, fat and protein yields of the primiparous cows on the 0.35% P diet were significantly lower than those of the 0.42% P diet cows. These results suggest that a level of 0.35% is adequate to support high milk production in multiparous cows but that P supplementation would benefit primiparous cows, particularly in early lactation.



3

Phosphorus Utilization and Environmental and Economic Implications of Reducing Phosphorus Pollution from Ontario Dairy Cows

Journal of Dairy Science, January 2008, Volume 91, Number 1, pages 241-246.

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For many years, North American dairy farmers have supplemented lactation diets with levels of phosphorus (P) in excess of animal requirements. Several recent studies have demonstrated that a concentration of 0.35% P in dietary dry matter (DM) is adequate at all levels of milk production. Phosphorus fed in excess of requirements is excreted, leading to environmental degradation and increasing the cost of production. The first objective of this study was to validate a model of P metabolism using data from a feeding trial in which all P inputs (feed, water) and outputs (milk, feces, urine) were measured in 8 lactating cows. Values predicted by the model were very close to those measured in the trial with only a slight underprediction by the model of actual fecal P excretion. The second objective of the study was to use the validated model to predict P excretion from Ontario dairy farms, based on knowledge of diets commonly fed to both dry and lactating cows in that province. The model estimated that all Ontario dairy farms combined currently excrete approximately 7,000 tonnes of P annually, assuming an average concentration of 0.41% P in dietary DM. By lowering that concentration to 0.35%, it was estimated that producers could save \$20 per cow per year and reduce total P load on the environment by 1,300 tonnes.



4

Avian (IgY) Anti-Methanogen Antibodies for Reducing Ruminant Methane Production: In Vitro Assessment of their Effects

Australian Journal of Experimental Agriculture, January 2008, Volume 48, Number 1-2, pages 260-264.

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It has been estimated that between 12 and 30% of total atmospheric methane emissions originate from ruminant animals. Several strategies aimed at mitigating these emissions have been explored, including a previous study that demonstrated a 7.7% decrease in methane emissions from sheep after they received a vaccine against methane-producing rumen bacteria (methanogens). The objective of the present study was to attempt a similar approach, using passive rather than active immunization against rumen methanogens. Laying hens were immunized with crude cell preparations of 1 of 3 methanogens using 1 of 2 different adjuvants to enhance the immune response: complete Freund's adjuvant (CFA) or Montanide ISA 70 (Mon). Antibodies produced by the hens in response to immunization were harvested from eggs and partially purified. The effects of these antibodies on rumen microbe fermentation patterns were examined in laboratory (in vitro) incubations using inoculant from cows fed an early lactation total mixed ration (EL-TMR). Antibodies raised using CFA were incubated with ground EL-TMR as substrate; those raised using Mon were incubated with ground freeze-dried alfalfa. Antibodies from eggs of non-immunized hens were included in control incubations. In the CFA/EL-TMR incubations, there were no differences in methane production between controls and those containing anti-methanogen antibodies. In the Mon/alfalfa incubations, anti-methanogen antibodies produced short-duration reductions in methane production but by the end of the incubations, methane production was similar to controls.



5

Nutritional Management for Enteric Methane Abatement: A Review

Australian Journal of Experimental Agriculture, January 2008, Volume 48, Number 1-2, pages 21-27.

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This paper reviews current knowledge about methods to reduce methane emissions from ruminant livestock. Increasing the proportion of concentrates in the diet is, in many cases, a practical way to both reduce ruminal methane production and increase animal productivity. To estimate the contribution of ruminants to greenhouse gas (GHG) production, the International Panel on Climate Change (IPCC) recommends the use of a formula where dietary concentrate level is the only variable. Animals whose diets contain more than 90% concentrate are assumed to convert 3% of their dietary energy intake to methane. For diets containing 90% concentrate or less, it is assumed that 6.5% of dietary energy is converted to methane. The IPCC method fails to account for other dietary composition effects on ruminant methane production. Among these, there is good evidence to support the effects of some types of supplementary dietary lipids on mitigating methane production through changes in rumen fermentation patterns. Although high levels of lipid supplementation have been shown to decrease methane production by 40% or more, at practical levels of inclusion reductions of 10-25% may be more realistic. Dietary inclusion of monensin at levels commonly used in commercial cattle production has also been shown to reduce ruminal methane production, although the effect may not persist. This response is due to the combined effects of a reduction in ruminal protozoa numbers and a shift in fermentation resulting in decreased production of acetate in favour of propionate. Other nutritional strategies with potential to mitigate methane emissions include replacing grass silage with corn or small grain silage, improving pasture quality, replacing grass forages with legume forages and feeding plant extracts such as saponins and tannins. The potential application of these strategies requires further research before they can be considered commercially viable.



6

Aspects of Rumen Microbiology Central to Mechanistic Modelling of Methane Production in Cattle

Journal of Agricultural Science, April 2008, Volume 146, Number 2, pages 213-233.

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The goal of the Kyoto Protocol, signed by Canada and more than 170 other nations, is to reduce global emissions of 'greenhouse gases', including carbon dioxide, methane and nitrous oxide. The International Panel on Climate Change (IPCC) has estimated that 37% of methane emissions attributable to human activity are due to ruminant livestock. This estimate is based on the simple assumption that ruminants whose diets contain more than 90% concentrates emit 3% of their gross energy intake as methane; for all others, 6.5% of gross energy is emitted as methane. However, a sizeable body of research has demonstrated that gross energy intake conversion to methane can vary from 2 to 12%, depending on a number of dietary factors. This paper reviews current models of rumen metabolism that predict methane production. The objective is to identify areas for potential improvement in those models that might lead to more accurate predictions of diet-dependent methane production. The authors conclude that improved prediction of volatile fatty acid production in the rumen is likely to yield the greatest improvement in the current models. The production of acetic and butyric acids (which predominate when high forage diets are fed) leads to the production of hydrogen gas which is used by one group of rumen microbes to reduce carbon dioxide to methane. When larger quantities of propionic acid are produced, generally as a result of feeding more starch, less hydrogen becomes available for the production of methane. Other dietary factors affecting the prediction of methane production are also discussed.



7

Comparison of Energy Evaluation Systems and a Mechanistic Model for Milk Production by Dairy Cattle Offered Fresh Grass-Based Diets

Animal Feed Science and Technology, May 2008, Volume 143, Number 1-4, pages 203-219.

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In many parts of the world, the production of milk from cows grazing grass pastures is the system of choice for its low cost and perceived advantages in terms of animal welfare, preservation of rural landscapes and environmental sustainability. However, current energy evaluation systems do not accurately predict milk production responses to the consumption of grass forage. In most cases, in spite of the high digestibility of well-managed grass forages, production falls short of predictions. The objective of this study was to compare the predictions of three European energy evaluation systems with those of a mechanistic model of nutrient metabolism developed by the authors. The dataset for evaluation consisted of 41 treatments of grass-based diets. All 3 evaluation systems tended to overestimate the amount of energy provided by grass forage relative to requirements for milk production. The mechanistic model was able to identify that the main limitation of grass forages in support of high levels of production was their inadequate provision of glucogenic nutrients that would support milk production, in particular lactose production. For this reason, the mechanistic model was more accurate in predicting milk production response to consumption of grass forages. The authors propose further improvements to the mechanistic model to further improve its efficacy in evaluating grass forage energy values.



8

Meta-Analysis on the Effects of Lipid Supplementation on Methane Production in Lactating Dairy Cows

Canadian Journal of Animal Science, June 2008, Volume 88, Number 2, pages 331-334.

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The Intergovernmental Panel on Climate Change has estimated that as much as 16% of global methane production is due to ruminant livestock. The observation that methane losses from rumen fermentation can vary from 2 to 12% of gross energy intake suggests that there may be opportunities to reduce losses in some situations. One of the strategies that has been attempted to do this involves the supplementation of ruminant diets with lipids. In this paper, the authors summarize the results of 7 published lipid feeding trials, including 25 diets fed to lactating cows, by combining and re-analyzing the data from those trials. The average lipid concentration in the supplemented diets was 6.4%; unsupplemented diets contained an average of 2.5% lipid (expressed as ether extract). On average, lipid supplementation decreased dry matter intake by 6.4% while milk production and milk composition were not significantly affected. Methane production decreased by 9%, from 5.9 down to 5.3% of gross energy intake, mainly as a result of the decreased dry matter intake. Feed efficiency, expressed as kg of 4% fat-corrected milk per kg of dry matter intake, increased by an average of 7%. The authors conclude that supplementing lactating cow diets with lipids can mitigate methane losses and improve feed efficiency without any detrimental effects on milk production.

Feeding





1

Effects of Addition of Essential Oils and Monensin Premix on Digestion, Ruminal Fermentation, Milk Production, and Milk Composition in Dairy Cows

Journal of Dairy Science, November 2006, Volume 89, Number 11, pages 4352-4364.

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Essential oils (EO) are naturally occurring compounds that can be extracted from a variety of plant species by steam distillation. A number of short-term in vitro (laboratory-based) and in situ (feed contained in nylon bags suspended in the rumen) studies have demonstrated the ability of high doses of EO to kill bacteria and alter microbial metabolism. It has also been reported that EO can inhibit the activity of rumen bacteria that are sensitive to monensin (MO), an antimicrobial that is used in both dairy and beef cattle production. The objective of this study was to evaluate the effects of EO and MO, separately and together, on ruminal fermentation, feed digestion, milk production and milk composition. Although minor changes were observed in some of the variables measured, overall results failed to confirm the effects of EO on rumen metabolism reported in previous in vitro and in situ studies. MO supplementation produced effects similar to those reported in other whole-animal studies: decreased rumen ammonia concentration and lower milk fat test. MO also increased milk urea nitrogen level but this effect was negated when MO and EO were fed together.



2

Effects of Essential Oils on Digestion, Ruminal Fermentation, Rumen Microbial Populations, Milk Production, and Milk Composition in Dairy Cows Fed Alfalfa Silage or Corn Silage

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A number of studies have examined the potential of various plant extracts as 'natural' alternatives to conventional antimicrobials. Among these, recent interest has focused on essential oils (EO), naturally-occurring volatile compounds extracted from plants by steam distillation. In a number of short-term, laboratory-based (in vitro) incubations, EO have been shown to alter the growth and metabolism of several types of rumen bacteria. Few trials have been conducted to investigate the potential of EO to alter rumen fermentation in favour of improved nutrient utilization and performance of lactating cows. In this study, a mixture of EO was added to lactation diets based on alfalfa silage or corn silage to test their effects on digestion, ruminal fermentation, rumen microbial populations, milk production and milk composition. Although differences due to the different basal diets were observed for several variables, the only significant change attributable to EO was a slight increase in milk lactose concentration.



3

Assessment of the Effects of Cinnamon Leaf Oil on Rumen Microbial Fermentation Using Two Continuous Culture Systems

Journal of Dairy Science, May 2007, Volume 90, Number 5, pages 2315-2328.

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Essential oils, produced through the steam extraction of a variety of plant materials, are not actually oils but a diverse mixture of volatile aromatic compounds. Several short-term, laboratory-based (in vitro) batch and continuous culture fermentation studies have found that EO have antimicrobial activity while others have produced equivocal results. The objective of this study was to compare the effects of an EO extracted from cinnamon leaves (CIN) on microbial fermentation in 2 different in vitro continuous culture systems: Rusitec and dual-flow fermenter. Culture inocula were prepared from the rumen contents of lactating cows fed a total mixed ration of 51% forage and 49% concentrate. For most of the parameters measured, inconsistent results of CIN addition were obtained from each of the 2 culture systems. In both systems, CIN exhibited antimicrobial activity and reduced total volatile fatty acid (VFA) concentrations, feed fermentability and microbial protein synthesis. VFA production shifted away from propionate and toward butyrate production and protozoa numbers decreased. These negative effects argue against the potential use of CIN in ruminant diets.



4

Past Peak Lactational Performance of Iranian Holstein Cows Fed Raw or Roasted Whole Soybeans

Canadian Journal of Animal Science, September 2007, Volume 87, Number 3, pages 441-447.

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This study compared lactation and metabolic responses to feeding 3 different soy products to mid-lactation Holstein cows. Groups of 6 multiparous cows were offered total mixed rations (TMRs) containing 34% forage (21% alfalfa hay plus 13% corn silage) over a 7 week trial period. The concentrate portion of each TMR contained a variety of protein, mineral and vitamin sources including either 11.9% soybean meal (SBM), 13.3% raw soybeans (RawSB) or 13.3% roasted soybeans (RoasB). Dry matter intake was similar among the 3 TMRs while 3.5% fat-corrected milk yield was 40.0 kg/day for cows fed the RoasB diet, 39.3 kg/day for SBM and 38.0 for RawSB. Milk fat concentration was similar among treatments, but milk fat yield was significantly higher for cows fed the RoasB and SBM diets. Milk protein concentration was significantly lower for the RawSB and RoasB diets, while milk protein yield was not affected. Feeding RoasB significantly reduced rumen ammonia N, plasma urea N and milk urea N concentrations but ruminal pH, plasma glucose and beta-hydroxybutyrate concentrations were similar. Plasma concentrations of most essential amino acids, except leucine and phenylalanine, were increased by feeding RoasB.



5

Effects of Essential Oils and their Components on In Vitro Rumen Microbial Fermentation

Canadian Journal of Animal Science, September 2007, Volume 87, Number 3, pages 413-419.

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The perception that antibiotic use in animal production is contributing to antibiotic resistance among some pathogenic bacterial species has created interest in the discovery of alternative 'natural' treatments. Among these potential alternatives, essential oils (EO) have received attention due to several laboratory-based (in vitro) studies that have demonstrated their antimicrobial activity against some bacteria, protozoa and fungi. However, results have been inconsistent, probably as a result of the varied methods and EO preparations that have been used. Chemically, EO are not actually oils but a varied mixture of volatile aromatic compounds that can be extracted from various plant tissues by steam distillation. EO are responsible for the unique fragrances of these tissues. The objective of this study was to examine the effects of 5 EO and 4 isolated EO components on in vitro batch cultures simulating rumen fermentation. The EO tested were cinnamon leaf oil, clove leaf oil, sweet orange oil, oregano oil and thyme oil; the isolated EO components tested were the phenols carvacrol (CAR), cinnamaldehyde (CIN), eugenol (EUG) and thymol (THY). Among these, only CAR, EUG and THY had any effect on rumen fermentation. CAR and EUG increased the pH of the culture medium and shifted volatile fatty acid (VFA) production away from propionate and toward butyrate; they also reduced both dry matter and neutral detergent fibre (NDF) digestibility. THY reduced pH, decreased propionate as a proportion of total VFA and lowered NDF digestibility. The authors conclude that these effects would not be considered beneficial to lactating dairy cattle.



6

Hepatic Lipid Metabolism in Transition Dairy Cows Fed Flaxseed

Journal of Dairy Science, October 2007, Volume 90, Number 10, pages 4780-4792.

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It is not uncommon for dairy cows to accumulate liver fat in the transition from pregnancy to lactation, resulting in negative consequences for post-partum health, production and reproduction. The causes of fatty liver are not well established although cows carrying excess body fat in the dry period are more susceptible, leading to the belief that mobilization of body fat stores may be responsible. Previous studies have demonstrated that dietary polyunsaturated fatty acids (FA) can reduce liver fat accumulation and that omega-3 FA can increase the oxidation of liver fat. The objective of this study was to assess effects on lipid metabolism when either saturated or unsaturated FA are fed during the transition period. Thirty-three pregnant heifers and cows were assigned to one of 3 dietary treatments: (1) control pre-partum and post-partum diets containing no added lipids; (2) control diets supplemented with whole flaxseed (FS) at 3.3% and 11.0% of dry matter (DM) in pre- and post-partum diets, respectively, or; (3) control diets supplemented with Energy Booster (EB) at 1.7% and 3.5% of DM in pre- and post-partum diets, respectively. FS is a rich source of unsaturated FA, including linolenic acid, an omega-3 FA. EB is composed primarily of saturated FA. Diets had less significant effects on heifers than on cows that had previously calved. Among the latter, those that were fed EB diets had lower dry matter intake, milk production, plasma glucose and liver glycogen but higher plasma non-esterified fatty acids and β -hydroxy butyrate as well as higher liver triglycerides (TG) and total lipids than those fed FL or control diets. The authors conclude that feeding saturated FA in the transition period increased the risk of fatty liver while feeding unsaturated FA reduced the risk.



7

Effect of Raw or Roasted Whole Soybeans on Early Lactational Performance and Ruminal and Blood Metabolites in Iranian Cows

Journal of Agricultural Science, October 2007, Volume 145, Number 5, pages 529-537.

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This study compared production parameters of early lactation dairy cows offered 3 different total mixed rations (TMRs). All diets contained 38.7% forage dry matter (DM) of which 64% was alfalfa hay and 36% was corn silage. The concentrate portion of all diets contained 16.5% ground corn, 14.3% ground barley, 2.7% fish meal, 6.8% beet pulp and 1.0% mineral/vitamin supplement. Diet 1 contained 12% soybean meal and 8.2% whole cottonseed; diet 2 contained 12% raw, cracked soybeans and 8.2% cottonseed meal; diet 3 contained 12% roasted, cracked soybeans plus 8.2% cottonseed meal. Dry matter intake, uncorrected milk yield and 3.5% fat-corrected milk yield were significantly higher for cows offered diet 3 as was milk fat yield. Although milk protein yields were similar for all 3 diets, when diet 1 was fed milk protein test was 0.2 percentage points higher while rumen ammonia nitrogen (N), plasma urea N and milk urea N were lower. There were no differences in ruminal pH, or in plasma glucose or beta-hydroxybutyrate concentrations among diets. Plasma concentrations of essential amino acids (EAA), except leucine and phenylalanine, were higher in cows fed diet 3 than in those fed diets 1 and 2.



8

Effects of Rumen-Undegradable Protein Sources and Supplemental 2-Hydroxy-4-(Methylthio)-Butanoic Acid and Lysine-HCl on Lactation Performance in Dairy Cows

Journal of Dairy Science, November 2007, Volume 90, Number 11, pages 5176-5188.

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Current doctrine suggests that high-producing dairy cattle require the intestinal absorption of higher levels of essential amino acids (EAA) than can be provided by microbial protein flowing from the rumen. Before the advent of bovine spongiform encephalopathy (BSE), these were typically provided by supplementing lactation diets with high quality animal protein sources that are relatively undegradable in the rumen but well digested in the small intestine. More recently, the prohibition on feeding some animal protein sources has created interest in the use of modified plant-based protein. Since it is generally assumed that lysine and methionine are the most limiting EAA for high levels of milk production, another alternative has been to provide supplementation of these EAA directly. The objective of this study was to compare milk production responses of early-lactation cows offered diets containing animal versus plant-derived protein sources with and without the addition of supplemental lysine and methionine sources. Two diets (A and B) were supplemented with heat- and lignosulfonate-treated canola meal; in two others (C and D), a blend of animal protein sources was used. Diets B and D were also supplemented with sources of lysine and methionine. Protein source (animal vs. plant) had no effect on dry matter intake (DMI) or 3.5% fat-corrected milk (FCM) production during the first 17 weeks of lactation. Although supplementation with lysine and methionine reduced DMI during the first 4 weeks of lactation, milk production was not affected. EAA supplementation did not improve production over that obtained with either plant- or animal-based protein supplementation.



9

Extent of Processing of Barley Grain Did Not Affect Productivity of Lactating Dairy Cows

Animal Feed Science and Technology, November 2007, Volume 138, Number 3-4, pages 272-284.

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When included in diets for cattle, barley grain requires processing to break the hull, exposing the kernel to rumen microbial digestion. However, the optimum extent of processing required for inclusion in lactation diets for dairy cows is controversial. Thorough processing is thought to favour both the rate and maximum extent of barley starch fermentation, increasing microbial growth and protein synthesis. In contrast, minimal processing is thought to reduce the risk of acidosis due to a slower rate of organic acid production from starch. Researchers at Agriculture and Agri-Food Canada's Lethbridge Research Centre developed the concept of processing index (PI) to describe extent of processing. PI is the volume weight of barley after processing divided by its volume weight before processing. Results from a limited experiment by this group suggested an optimum PI of 0.64 for steam-rolled barley fed to lactating dairy cows. However, the authors of the present study have questioned that recommendation on the basis that the barley used had a higher fibre level than that typically used in diets for lactating cows. Therefore, this study examined the production effects of feeding a more typical low fibre barley, steam-rolled to lots having a PI of either 0.83 or 0.69. Incubation of nylon-bagged samples of the 2 lots in the rumen demonstrated a more rapid disappearance of starch from the 0.69 PI sample. However, no practical differences between the 2 lots were seen in terms of either feed intake or milk production parameters. The authors speculate that the optimum extent of processing required may be less for the low fibre barleys typically used in diets fed to lactating dairy cows.



10

Long-Term Effects of Feeding Monensin on Milk Fatty Acid Composition in Lactating Dairy Cows

Journal of Dairy Science, November 2007, Volume 90, Number 11, pages 5126-5133.

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Monensin is routinely included in diets fed to growing beef cattle and lactating dairy cows for its beneficial effects on digestion and microbial metabolism. By selectively inhibiting the growth of gram-positive bacteria, monensin shifts ruminal fermentation toward the production of end products that are more energy efficient while reducing the risk of bloat and acidosis. Monensin has also been shown to reduce biohydrogenation by rumen microbes, resulting in more unsaturated fatty acids flowing into the lower digestive tract, being absorbed into the bloodstream and appearing in milk. Results of a few previous studies have suggested that these effects of monensin may dissipate over time. The present study was designed to determine the long-term effects of feeding monensin on milk fatty acid profile in lactating dairy cows. Twenty-four lactating Holstein cows were assigned to either a typical total mixed ration (TMR) containing a 60:40 ratio of forage:concentrate dry matter (DM), including a placebo premix, or the same TMR where the placebo premix was replaced by a monensin premix providing 24 mg monensin per kg DM. Milk samples were collected before the introduction of treatments and monthly thereafter for 6 months. Dietary monensin inclusion increased total monounsaturated fatty acids (FA) in milk fat by 5%, total omega-6 polyunsaturated FA (PUFA) by 19%, total omega-3 PUFA by 16%, total cis-18:1 by 7%, and total conjugated linoleic acid (CLA) by 43%. Concentrations of docosahexaenoic acid (22:6 omega-3), docosapentaenoic acid (22:5 omega-3), and cis-9, trans-11 CLA in milk fat increased by 19, 13, and 43%, respectively, compared with the control. These effects persisted over the course of the study. These results suggest that monensin was at least partly effective in inhibiting the biohydrogenation of unsaturated FA in the rumen and consequently increased the percentage of n-6 and n-3 PUFA and CLA in milk, thus enhancing the nutritional properties of milk with regard to human health.



11

Effects of Photoperiod and Glucose-Supplemented Drinking Water on the Performance of Dairy Calves

Journal of Dairy Science, November 2007, Volume 90, Number 11, pages 5199-5207.

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Previous studies have demonstrated positive production effects in growing and lactating dairy cattle when photoperiod is controlled. A 16-18 hour daily light period has been shown to stimulate weight gain, advance sexual maturity and increase first lactation milk yield in heifers and to increase milk yield in cows. In contrast, little information has been published on the effects of photoperiod on the growth of neonatal calves. The present study examined the effects of photoperiod length and glucose supplementation (GS) in water on the growth of calves from birth to 8 weeks of age. Twenty-four individually-penned calves were assigned to one of 4 treatments: (1) a short-day photoperiod (SDPP) with 10 hours of light and no GS; (2) SDPP with GS at 50 grams/litre of water; (3) a long day photoperiod (LDPP) with 18 hours of light with no GS, and; (4) LDPP with GS at 50 grams/litre of water. All calves were bucket-fed 2 litres of whole milk twice daily and all had unlimited access to their GS or non-GS water. Although calves offered GS had higher blood levels of glucose and sodium, GS had no effect on any of the developmental parameters measured. Between 29 and 56 days of age, the LDPP calves consumed more starter and grew faster than the SDPP calves. As a consequence, LDPP calves were significantly heavier and were wider across the hips at the end of the study. Measures of rumen microbial activity indicated that rumen maturation was also more advanced in the LDPP calves.



12

Chemical Composition, Ensiling Characteristics and Ruminal Degradability of Forage Soybean Cultivars

Canadian Journal of Animal Science, December 2007, Volume 87, Number 4, pages 623-629.

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The objective of this study was to compare the ensiling characteristics, chemical composition and in situ rumen degradability of 2 forage soybean cultivars, Kodiak and Mammouth, grown in Québec. Samples of crops harvested at the R6 stage and wilted to 50% moisture were ensiled for 45 days in mini silos measuring 7.6 cm in diameter by 25 cm in height. Both cultivars ensiled well as indicated by a gradual increase in lactic acid concentration accompanied by steadily declining pH. Chemical analysis revealed that, compared with Kodiak, the Mammouth silage had higher neutral detergent fibre (NDF, 49.0 versus 44.4%), acid detergent fibre (37.1 vs. 35.3%) and acid detergent lignin (8.1 vs. 6.4%) as well as higher neutral and acid detergent insoluble protein levels. Kodiak silage had higher crude protein (CP) and buffer soluble protein concentrations than Mammouth. Rumen (in situ) incubation of samples contained in nylon bags indicated that Kodiak had higher degradability of ruminal dry matter (60.6 vs. 54.9%), CP (82.8 vs. 75.2%) and NDF (27.2 vs. 22.7%) compared with Mammouth.

13

Effects of Garlic and Juniper Berry Essential Oils on Ruminal Fermentation and on the Site and Extent of Digestion in Lactating Cows

Journal of Dairy Science, December 2007, Volume 90, Number 12, pages 5671-5681.

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Essential oils (EO) are the products of steam distillation of various plant components. Although they are generally poorly defined chemically, several studies have observed antimicrobial activity when they have been included in laboratory (in vitro) rumen fermentations. Such observations have led to the idea that EO might provide 'natural' alternatives to the use of antimicrobials in livestock feeds. The objective of the present study was to evaluate the effects of EO from garlic (GAR) or juniper berries (JUN) on rumen and digestive parameters in lactating dairy cows. These effects were also compared with those of monensin (MON), an antimicrobial commonly used in ruminant diets. Mid-lactation cows were provided unlimited (ad libitum) intakes of one of four total mixed rations (TMRs) containing either GAR, JUN, MON or no additives (CON). Dry matter (DM) intakes, milk production, ruminal microbial protein synthesis, ruminal pH, and ruminal concentrations of volatile fatty acids and ammonia were similar for all diets as were total tract digestibilities of dry matter, organic matter, fibre, and starch. GAR and JUN increased ruminal protein digestion by 11% over CON. As a result, ruminal digestion of DM and organic matter were also 13% higher. In contrast, MON reduced ruminal digestion of dietary protein by 11% compared with CON and the MON diet produced the lowest milk fat concentration. Measures of inflammatory response indicators in blood suggested that none of the additives had an effect on immune status.



14

Milk Production, Milk Composition, Blood Composition, and Conception Rate of Transition Dairy Cows Fed Different Profiles of Fatty Acids

Canadian Journal of Animal Science, December 2007, Volume 87, Number 4, pages 591-600.

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In this study, 153 cows in 2 locations were offered diets containing one of 3 different lipid sources starting at 6 weeks before their expected calving dates. The lipid sources were Megalac® (MEG), a source rich in oleic acid (18:1); micronized soybeans (SOY), a source rich in linoleic acid (18:2); and whole flaxseed (FLA), a source rich in alpha linolenic acid (18:3). Both pre-partum and postpartum diets were designed to contain similar lipid concentrations and were offered to allow unlimited (ad libitum) intakes. Lipid supplementation was terminated at day 50 of pregnancy for cows that conceived or at 120 days postpartum for cows that did not. Fat source did not significantly affect pre-partum dry matter (DM) intake, milk production or milk composition. First service conception rate was highest for cows fed FLA (54.3%), intermediate for those fed MEG (40.0%) and lowest for those fed SOY (26.9%). Cows fed MEG had higher total embryo mortality after 2 inseminations 35.3% than those fed FLA (9.5%) or SOY (9.1%). These data support the findings of early studies that showed a beneficial effect of feeding flaxseed on reproductive function.



15

Effects of Lasalocid or Monensin Supplementation on Digestion, Ruminal Fermentation, Blood Metabolites, and Milk Production of Lactating Dairy Cows

Journal of Dairy Science, December 2007, Volume 90, Number 12, pages 5714-5725.

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Monensin (MON) and lasalocid (LAS) are ionophores, so called because they interfere with ion transfer across the cell membranes of gram-positive bacteria, inhibiting their growth. When included in ruminant diets, ionophores shift rumen fermentation such that more propionate and less acetate are produced, increasing energetic efficiency. In addition, inclusion of these products results in improved nitrogen (N) efficiency due to reduced protein and amino acid degradation in the rumen. However, results of previous studies have suggested that these 2 ionophores may differ in the ways that they modify N metabolism. Although many studies have characterized the metabolic effects of MON in lactating dairy cattle, few have examined the use of LAS. The objective of this study was to compare the actions of the 2 ionophores on digestion, rumen fermentation, metabolism and milk production. Two groups of cows were offered red clover silage based total mixed rations (TMR) supplemented at a rate of 24 mg/kg of TMR dry matter (DM) with either MON or LAS. No differences were observed between treatments in daily DM intake, or in yields of milk, milk fat or milk protein. Both ionophores produced similar changes in rumen fermentation patterns and nutrient digestibility and both increased fecal N excretion. Compared with those fed the LAS diet, cows consuming the MON diet had higher levels of plasma and milk urea N, excreted more urinary and total N, and tended to have lower blood levels of non-essential amino acids.



16

The Effect of Glyphosate on Digestion and Horizontal Gene Transfer during In Vitro Ruminal Fermentation of Genetically Modified Canola

Journal of the Science of Food and Agriculture, December 2007, Volume 87, Number 15, pages 2837-2843.

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Glyphosate is the active ingredient in Roundup®, the trade name of a herbicide marketed by Monsanto. Many commercial crops have been genetically modified to confer tolerance to glyphosate (Roundup-Ready®) which makes it possible to use glyphosate to control weeds in these crops without damaging the crop itself. Glyphosate acts by inhibiting the plant enzyme, 5-enolpyruvylshikimate-3-phosphate synthase (*epsps*) which is involved in the biosynthesis of aromatic amino acids, vitamins and other secondary metabolites in plants and micro-organisms. Tolerance is conferred by transferring the gene *cp4-epsps* which allows the plant to overcome *epsps* inhibition. Since products derived from glyphosate-tolerant crops (e.g., alfalfa, canola meal) are fed to livestock, it is possible that the *cp4-epsps* gene could be transferred to rumen bacteria during the digestion process. Another possibility is that the products of plants treated with glyphosate may carry herbicide residues which could inhibit rumen microbial *epsps* activity. The objective of this study was to examine these possibilities using laboratory bench-top (in vitro) cultures simulating rumen fermentation. Cultures included both Roundup-Ready® canola meal and one of 3 concentrations of glyphosate. Results demonstrated that high concentrations of glyphosate could inhibit *epsps* activity in rumen bacteria which could place selective pressure on some species. However, there was no evidence that this selective pressure would promote the transfer of *cp4-epsps* from glyphosate-tolerant canola meal to rumen microbes.



17

Effect of Heat Processing on Ruminal Degradability and Intestinal Disappearance of Nitrogen and Amino Acids in Iranian Whole Soybean

Livestock Science, January 2008, Volume 113, Number 1, pages 43-51.

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This study was designed to evaluate the effects of 2 heat treatments on the digestibility of protein in 2 varieties of soybeans commonly cultivated in Iran. Simple roasting was achieved by heating to 140-145 degrees Celsius in a drum roaster followed by gradual cooling. Steep-roasting involved the same heating procedure followed by transfer to 100 litre covered barrels for 45 minutes before being allowed to cool. Samples of raw (RawSB), roasted (RosSB) and steep-roasted (StrSB) were subjected to chemical analysis of nitrogen fractions and to rumen degradability and intestinal digestibility evaluation using in situ nylon bag techniques. Chemical analysis revealed that, in both RosSB and StrSB, the non-protein N and buffer soluble N fractions were reduced compared to RawSB. Rumen incubations demonstrated similar soluble and insoluble potentially degradable N fractions for all 3 samples, but the rate of nitrogen disappearance was significantly higher for RawSB. Calculated rumen N degradability for heat processed soybeans was significantly lower than that for RawSB. The same pattern was observed for the degradability of most amino acids (AA) although some variation was found among individual AA. For RosSB and StrSB, intestinal digestibility of both N and AA was improved significantly over RawSB. No significant differences were found in total tract disappearance of N between raw and roasted seeds although total tract disappearance of total AA was higher for RawSB than for roasted seeds. No significant differences were found between the 2 soybean varieties tested in any of the variables measured. The authors conclude that roasting and steep-roasting are effective for changing the site of soybean protein digestion from rumen to small intestine.



18

Metabolic Changes and Net Portal Flux in Dairy Cows Fed a Ration Containing Rumen-Protected Fat as Compared to a Control Diet

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It is generally assumed that one of the primary factors limiting milk production in cows with high yield potential is the availability of glucose for milk lactose synthesis. Since very little is absorbed from the gut, most of the mammary gland's glucose supply must be provided by the synthesis of glucose in the liver (gluconeogenesis). The precursors for this process are primarily starch that has escaped rumen degradation, propionic acid produced by rumen fermentation and amino acids absorbed through the small intestine. A common practice, designed to increase energy density, is to supplement lactation diets with lipids. However, this often involves substituting lipid for a portion of dietary starch, possibly reducing the amount of glucogenic precursors reaching the liver. The objective of this study was to determine the metabolic and milk production consequences of this practice. Cows with catheters in their portal and mesenteric veins and mesenteric arteries were fed a diet containing a high level of starch or a diet where a portion of the starch was replaced by rumen-protected lipid. Dry matter intakes were lower when cows were fed the lipid-supplemented diet although energy intake was unaffected due to the higher energy density of the lipid. Substituting part of the starch by lipid only numerically decreased net portal absorption of glucose but had no effect on whole body rate of appearance of glucose. Although arterial glucose concentrations were lower on the lipid diet, suggesting that less glucose was reaching the mammary gland, milk and lactose yields were higher on this diet than on the starch diet. Lipid supplementation raised plasma free fatty acid, triglyceride and glucagon concentrations and increased the glucagon-to-insulin ratio, suggesting a more efficient utilization of available glucose on this diet.



19

Effects of Feeding Forage Soybean Silage on Milk Production, Nutrient Digestion, and Ruminal Fermentation of Lactating Dairy Cows

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This study compared the feeding value of forage soybean silage (SS) harvested at the full-pod stage with that of fourth-cut alfalfa silage (AS) in terms of chemical composition and production responses. Two total mixed rations (TMRs) containing 48% forage were formulated; 72% of the forage in each was either SS or AS, the remainder was corn silage. Urea was added to the SS ration to bring its crude protein (CP) concentration up to that of the AS ration. The 2 TMRs were each offered to 10 early lactation Holsteins, 2 of which were fitted with rumen cannulae to allow for sampling and analysis of rumen fermentation patterns. SS contained higher concentrations of neutral detergent fibre (NDF) (+15%) and acid detergent fibre (+28%) but less CP (-25%) than AS. Cows offered the SS ration consumed less dry matter (DM) (23.5 versus 25.1 kg/day) and produced less milk (35.5 vs. 37.2 kg/d) than those offered the AS ration. However, because SS cows had higher milk fat test (3.8 vs. 3.6%), energy-corrected milk yields on both rations were similar as were milk protein, lactose and total solids tests. Rumen ammonia nitrogen and milk urea nitrogen concentrations were higher for the SS cows (15.6 vs. 14.3 mg/dL) while rumen pH was lower. Total tract digestibilities of DM, CP, and NDF were similar for both diets.



20

Interactions Between Barley Grain Processing and Source of Supplemental Dietary Fat on Nitrogen Metabolism and Urea-Nitrogen Recycling in Dairy Cows

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Urea is synthesized in the liver of the cow in order to detoxify ammonia released into the bloodstream from rumen fermentation and from deamination of amino acids in the liver itself. It is estimated that 40 to 80% of this urea may be recycled back into the rumen where 35-55% of the urea nitrogen (N) entering the rumen may be used by rumen microbes to synthesize microbial protein. The amount of urea that returns to the rumen depends on the relative concentrations of urea in blood and ammonia in rumen fluid as well as the rate of fermentation of organic matter in the rumen—higher rates are expected to result in more rapid use of ammonia N. Another factor that appears to influence urea flow into the rumen is the concentration of unsaturated fatty acids (UFA) in the diet. It is suggested that UFA increase the efficiency of microbial protein synthesis by reducing rumen protozoa counts, thereby lowering rumen ammonia concentration and increasing urea transfer from the blood into the rumen. This study was designed to assess the effects of barley processing and dietary fat source on rumen N metabolism and urea recycling into the rumen. Urea production and urea entering the rumen were higher in cows fed dry-rolled barley compared with those fed pelleted barley. However, the proportion of the urea entering the rumen that was used for microbial protein synthesis and the proportions excreted in urine and feces were not affected by method of barley processing. Urea and ammonia kinetics were similar when supplemental fat was supplied as either whole canola seed or whole flaxseed.



21

Ruminal Temperature May Aid in the Detection of Subacute Ruminal Acidosis

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Lactating dairy cows often experience subacute ruminal acidosis (SARA) as a result of the rapid fermentation of high starch diets which produces high concentrations of organic acids and low rumen pH. SARA is characterized by repeated 3 to 5 hour periods of rumen pH below 5.6. Although valuable in a research setting, continuous measurement of rumen pH is not practical on-farm. Several other diagnostic indicators of SARA have been proposed, but only instantaneous measurement of pH using rumenocentesis and measurement of rumen propionate concentration have yielded reliable results. These methods are also impractical for routine use. The objective of this study was to determine whether measurement of rumen temperature might prove to be a practical indicator of SARA. Six rumen-fistulated lactating cows were assigned to 1 of 2 dietary treatments. Three cows were offered a control total mixed ration (TMR) formulated to avoid risk of SARA; the other 3 were offered a SARA-inducing TMR. After 1 week of adaptation to diets, rumen pH and temperatures were recorded every minute over a 4 day period using an indwelling electrode. Cows offered the SARA diet had rumen pH readings below 5.6 and temperatures above 39.2°C for more minutes per day than the cows fed the control TMR. The lowest daily rumen pH readings correlated very well with the highest temperatures suggesting that measuring rumen temperature may have potential application for diagnosing SARA.



22

Supplementation with Whole Cottonseed Reduces Methane Emissions and Can Profitably Increase Milk Production of Dairy Cows Offered a Forage and Cereal Grain Diet

Australian Journal of Experimental Agriculture, January 2008, Volume 48, Number 1-2, pages 73-76.

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During the winter, when rainfall is normally adequate to support pasture growth, dairy cattle in the state of Victoria, Australia receive most of their nutrients from grazing. In the summer months, however, dry conditions demand that supplementary feeds provide a larger proportion of the cows' energy and protein requirements. At the particular time that this study was conducted, a complete lack of pasture meant that the animals' total requirements had to be provided by stored feeds. Fifty lactating cows, at an average 200 days in milk, were offered alfalfa hay in the morning, grass silage in the afternoon and cracked triticale grain at a rate of 1.5 kg dry matter (DM) at each of 2 milking times. One half of the cows were also offered whole cottonseed (WCS) at 2.7 kg DM/day with their grain supplement. WCS supplementation increased milk yield by 15%, milk fat yield by 19% and milk protein yield by 16%. Milk protein concentration decreased by 3% while milk fat and lactose concentrations were not affected. Methane emissions, measured using the sulfur hexafluoride dilution technique, were reduced by 12% per cow and by 21% per kg of milk solids produced.



23

Feeding High Proportions of Barley Grain Stimulates an Inflammatory Response in Dairy Cows

Journal of Dairy Science, February 2008, Volume 91, Number 2, pages 606-614.

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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, characterized by low rumen pH and the death of gram-negative bacteria resulting in the release of endotoxin, a cellular component of these bacteria. Ruminal acidosis is thought to predispose lactating cows to other metabolic disorders such as fatty liver, laminitis, liver abscesses, displaced abomasum and bloat. However, the mechanism through which acidosis might lead to these other disorders is unclear. One explanation, favoured by the authors of this paper and others, is that the release of endotoxin from the rumen results in an acute systemic inflammatory response leading to alterations in immune function. The objective of this study was to assess responses to the feeding of increasing quantities of barley grain to lactating dairy cows. Total mixed rations (TMRs) containing 0%, 15%, 30% or 45% rolled barley grain were offered to 8 rumen-cannulated cows. Cows consuming higher quantities of grain had slightly reduced rumen pH while total feed dry matter intake and milk production increased. Ruminal endotoxin concentrations in cows fed 30% and 45% barley grain increased 7.7- and 13.6-fold, respectively, over the concentration in cows fed no barley grain. Blood plasma concentrations of proteins produced in response to an inflammatory reaction to endotoxin by the liver increased significantly, lending support to the idea that ruminal acidosis may provoke changes in immune function.



Towards Non-Invasive Methods to Determine the Effect of Treatment of Soya-bean Meal on Lysine Availability in Dairy Cows

Animal, February 2008, Volume 2, Number 2, pages 224-234.

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Several techniques have been applied to the estimation of rumen degradation and intestinal digestion and absorption of feed protein in lactating cows. Some of the more informative of these techniques involve surgical preparation of animals which is both expensive and of concern in terms of animal welfare. The primary objective of this study was to compare results obtained from a technique requiring the surgical installation of ruminal or duodenal cannulae with 2 techniques that do not require surgical preparation of experimental animals. The second objective was to compare the post-ruminal supply of the amino acid lysine from diets containing conventional solvent-extracted soybean meal (SBM) with those containing 2 'rumen-protected' SBM products: either expeller-processed SBM or lignosulphonate-treated SBM. Although each of the 3 techniques tested yielded different numerical estimates of post-ruminal supply of lysine, based on lysine recovery, the authors conclude that the 2 that did not require surgical intervention yielded the more reliable results. In particular, the Lysine Flux method, which employs ^{15}N to determine the rate at which absorbed amino acids dilute blood nitrogen, proved most acceptable. Within each technique, no differences were found in the post-ruminal availability of lysine among diets containing different types of SBM. The increase in availability of 'bypass' lysine in diets containing the rumen-protected SBM products was offset by the increased availability of lysine from microbial protein in diets containing the conventional SBM.



25

Effects of Monensin and Dietary Soybean Oil on Milk Fat Percentage and Milk Fatty Acid Profile in Lactating Dairy Cows

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Many previous studies have demonstrated the milk fat depressing effect of monensin (MON) inclusion in diets fed to lactating cows. However, other studies have reported no effect. These inconsistent results suggest that the response to MON may be influenced by factors such as MON dose level, inclusion method, dietary composition or stage of lactation. The objective of this study was to evaluate the effects of MON in combination with soybean oil (SBO) supplementation. Seventy-two multiparous, lactating Holsteins were each offered one of 6 total mixed rations (TMRs) formulated with (at 22 grams/kg TMR dry matter) or without MON and including supplementation of SBO at 0, 1.7 or 3.4% of TMR dry matter. MON inclusion with no SBO supplementation significantly decreased milk fat and 4% fat-corrected milk (FCM) yields and milk fat percentage. SBO-supplemented diets decreased both milk fat and protein yields and concentrations as well as milk and 4% FCM yields. SBO also lowered total saturated fatty acids (FA) in milk in favour of monounsaturated FA. The combination of MON and SBO significantly decreased milk fat percentage but the effect on fat yield was not significant. MON plus SBO also increased the concentrations of trans-18:1 FA, polyunsaturated FA and conjugated linoleic acid isomers in milk fat while decreasing concentrations of short- and medium chain length FA.



Effects of Replacing Corn Dry Distillers' Grains with Triticale Dry Distillers' Grains on Lactation Performance and Plasma Metabolites of Dairy Cows

Canadian Journal of Animal Science, March 2008, Volume 88, Number 1, pages 129-132.

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Distillers' grains are a by-product of ethanol production from grain, the residual solids remaining after the digestion of starch. The liquid fraction remaining after distilling off the ethanol from the fermentation mixture is referred to as distillers' solubles. Distillers' dry grains plus solubles (DDGS) consist of the 2 by-product fractions mixed together and dried. Although a great deal of knowledge and experience has been gained on the feeding of corn DDGS, less is known about the feeding value of by-products of ethanol production from other grains. These are becoming increasingly available with the expansion of fuel ethanol production. This study compared the production responses when lactating cows were fed diets containing either corn DDGS or triticale DDGS at a level of 21% of dietary dry matter. No significant differences between DDGS sources were found in terms of dry matter intake, milk yield or milk composition although blood levels of urea nitrogen and some essential amino acids were higher with corn DDGS feeding. The authors conclude that triticale DDGS can replace corn DDGS in diets fed to lactating cows with no negative impacts on production.



27

Chemical Composition and Ruminal Degradability of Grain Pearl Millet Grown in Southwestern Québec

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The discovery that pearl millet (PM) can suppress root lesion nematodes has led to cultivation of the crop as an alternative to soil fumigation in some areas of Canada where potatoes are grown. Several new high-yielding PM hybrids adapted to growing conditions in eastern Canada have recently been released by Agriculture and Environmental Renewal Canada. Because of the potential availability of PM for livestock feeding in these areas, knowledge of its nutritional value is of interest. An earlier study showed that PM grain had a higher protein level with a slightly better amino acid profile than corn. The grain also has a higher energy value due to its 3 to 6% fat content, including a significant contribution from alpha-linolenic acid, giving it a higher content of omega-3 fatty acid than other cereal grains. For these reasons, PM grain has successfully replaced corn in diets fed to broilers. This study was undertaken to evaluate the potential of PM grain in ruminant diets. Chemical composition and in situ ruminal nutrient degradabilities were determined for five of these new PM hybrids. Starch levels varied from 63.2 to 66.8% of PM grain dry matter (DM), neutral detergent fibre concentrations were in the 13.4 to 14.5% range and crude protein (CP) ranged from 14.1 to 17.3%. In situ rumen degradabilities were estimated by incubating nylon-bagged samples in the rumens of lactating Holsteins. Ranges for effective degradabilities of DM, CP and starch were 69.2-70.3%, 73.7-76.0%, and 76.0-78.9%, respectively. While there was significant variation among hybrids, their chemical composition and rumen degradability characteristics suggest potentially high feeding values for lactating dairy cows.



28

The Effects of Feed Bunk Competition on the Feed Sorting Behavior of Close-Up Dry Cows

Journal of Dairy Science, March 2008, Volume 91, Number 3, pages 1115-1121.

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When offered total mixed rations (TMRs) dairy cows are frequently able to sort and selectively consume ration ingredients on the basis of their diverse particle sizes. When they select against the long particles, typically representing the fibre-rich forage component of the diet, they increase their risk of ruminal acidosis. In addition, selective consumption changes the composition of the remaining ration, a result that may be detrimental to animals of low rank in the group dominance hierarchy who do not have an opportunity to consume the ration as it was originally offered. The primary objective of this study was to characterize sorting by dry cows fed a TMR during their final 3 weeks of gestation (close-up period). Sorting was determined by separating long, medium, short and fine particles with a particle separator having three screens (18, 9 and 1.8 mm openings) and a bottom pan. Feed was offered in individual feed bins with one bin allocated to each cow. A second objective was to determine how increased competition for feed would affect feeding and sorting behaviours. In this case, each bin was shared by 2 cows. Whether sharing a bin or not, cows sorted against long particles allowing them to consume more short and fine particles. Competition had no significant effects on sorting, dry matter intake or time spent eating. Competitively-fed cows had fewer meals per day, and tended to have larger and longer meals. The results suggest that increased competition at the feed bunk promotes feeding behaviour patterns that will likely, due to feed sorting, increase the between-cow variation in composition of TMR consumed.



29

Use of Monensin Controlled-Release Capsules to Reduce Methane Emissions and Improve Milk Production of Dairy Cows Offered Pasture Supplemented with Grain

Journal of Dairy Science, March 2008, Volume 91, Number 3, pages 1159-1165.

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The primary objective of this study was to determine whether monensin, provided in a controlled release capsule (CRC), would reduce methane production by lactating dairy cattle. A second objective was to determine the effects of monensin CRC on milk production. In an initial experiment, 60 lactating cows grazed a ryegrass pasture in the state of Victoria, Australia and were provided a supplement of 5 kg/day (as-fed) of cracked barley grain. One half of the cows received a monensin CRC at the beginning of the trial and another 130 days later. Using the sulfur hexafluoride technique, methane production was measured for 4-day periods starting at 25 and 81 days after administration of the first CRC and 83 days after administration of the second. Milk production was measured for 100 days after insertion of each CRC. In a second experiment, 7 pairs of cows were fed fresh ryegrass pasture harvested daily, supplemented with 5 kg/day of grain, as in the first experiment. At staged intervals, monensin capsules were placed in one of each pair of cows. Seventy-five days after CRC placement both cows of each pair were confined in individual respiration chambers for 3 days while dry matter (DM) intake and methane production were measured. In both experiments, the calculated rate of monensin release from CRCs was approximately 240 mg/day resulting in an average dosage rate of between 12 and 14.5 mg per kg of DM intake. Monensin treatment had no effect on methane production in either experiment although it did increase milk fat and protein production by cows in the grazing study.



30

Effects of Essential Oils on Proteolytic, Deaminative and Methanogenic Activities of Mixed Ruminal Bacteria

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The perception that the use of antimicrobials, such as monensin, in animal production may contribute to antibiotic resistance among some pathogenic bacterial species has created interest in the discovery of alternative 'natural' treatments. Among these potential alternatives, essential oils (EO) have received attention due to several laboratory-based (in vitro) studies that have demonstrated their antimicrobial activity. However, little information exists about the effects of EO on the metabolic activity of mixed rumen microbes. The objective of this study was to characterize the effects of 3 EO and 2 isolated EO compounds (EOC) on protein breakdown, deamination of amino acids and the production of methane by rumen microbes maintained in a laboratory (in vitro) batch culture system. The EO tested were cinnamon leaf oil (CIN), garlic oil (GAR) and juniper berry oil (JUN); the isolated EO components were anethol (AN) and p-cymene (PC). While CIN and GAR significantly reduced the production of propionate as a proportion of total volatile fatty acids (VFA), none of the other additives affected relative VFA proportions. At most levels of addition, EO and EOC increased amino acid deamination and, consequently, ammonia concentrations in the culture medium. None of the additives affected protein breakdown. Methane production and methane concentration were decreased by all additives but AN. The authors conclude that GAR, JUN, AN and PC would not likely prove beneficial in ruminant diets due to their effects on deamination which would reduce the efficiency of nitrogen utilization. CIN and GAR might be good alternatives to antimicrobials such as monensin due to their effect on reducing methane synthesis.



31

Outcome of Bt Transgenes and Protein in Corn Silage, Processed Grains, and Rumen Content

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Silage and grain from transgenic Bt corn hybrids are widely grown and fed to livestock. Genes transferred (transgenes) from the bacterium *Bacillus thuringiensis* (Bt) enable the corn crop to produce a Bt endotoxin which protects plants from damage by the European corn borer. The objective of this study was to evaluate the extent to which Bt transgenes might be transferred from corn silage or corn grain to rumen microbes when these ingredients are used in dairy diets. Three Bt corn hybrids were grown and ensiled in both laboratory-scale mini-silos and in larger (500 kg) silos for feeding trials. Silage in the mini-silos was sampled at intervals up to 64 days after initial ensiling. Transgenes were undetectable after 32 days. In the larger silos, transgenes were still detectable 198 days after ensiling. After feeding these silages to lactating dairy cows for 7 days, Bt endotoxin was detectable at very low levels in rumen digesta but was undetectable in rumen fluid. Commercial samples of Bt corn grain were subjected to micronization, extrusion and flaking to evaluate the effect of processing on the persistence of Bt transgenes. Bt endotoxin concentrations in the 3 Bt hybrids decreased by 35, 74, and 67%, respectively, after micronization; by 92, 98, and 89% after extrusion; and by 100% in all hybrids after flaking. After ensiling, grain processing, and passage through the ruminant forestomach, Bt protein is unlikely to have any secondary impact on the environment.



32

Assessment of the Potential of Feed Enzyme Additives to Enhance Utilization of Corn Silage Fibre by Ruminants

Canadian Journal of Animal Science, March 2008, Volume 88, Number 1, pages 97-106.

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Several researchers have investigated the use of exogenous fibrolytic (fibre-digesting) enzymes to enhance the utilization of feeds by ruminant animals. However, results have been inconsistent, largely because the specific enzymatic activities required to digest specific fibre fractions have not been established. Most of the enzyme preparations tested to date contain varying proportions of endoglucanase (EG) and xylanase (XL) activity. In this study, 8 enzyme preparations with varying EG and XL activities were tested for their ability to enhance the digestion of corn silage by a mixed culture of microbes in a laboratory (in vitro) fermentation system. Only the preparation with the highest activity of EG significantly improved the degradation of corn silage fibre fractions. To test whether the higher EG activity was the key to enhance corn silage fibre degradation, a second experiment tested the same 8 enzyme preparations added to the in vitro fermentation system at equal EG or XL activity levels. All but one of the cultures equalized for EG activity produced an increase in fibre digestibility while equalization for XL activity had little effect. It was concluded that exogenous enzyme preparations with high EG and low XL activities have the potential to improve corn silage digestibility by ruminants.



33

Effects of Planting Date on Fiber Digestibility of Whole-Crop Barley and Productivity of Lactating Dairy Cows

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Diets fed to lactating cows must contain enough physically effective fibre to stimulate chewing and the secretion of salivary buffers which dampen the pH depressing effects of rapid organic acid production from fermentable carbohydrates. However, the digestibility of fibre is also important—slowly digested fibre remains in the rumen longer, increasing distension and decreasing intake. Forage fibre digestibility can also be influenced by the ambient temperature during the growing season. This study was designed to assess the effect of average growing season temperature on the feeding value of barley silage. Barley crops were planted on May 5 (early) and June 7 (late). The crops were harvested on July 26 and August 25, respectively, at the late-dough stage. The average temperature during development of the early crop was 14.5°C; for the late crop it was 16.4°C. Contrary to expectations, freshly-harvested samples of the late crop had higher in-vitro fibre digestibility than the early crop (61.2 versus 51.9%, respectively). Dry matter crude protein (CP) and neutral detergent fibre (NDF) concentrations in the late crop were also higher (CP: 12.4 vs. 8.7%; NDF: 52.6 vs. 50.4%). After ensiling for at least 7 weeks, each forage was fed to mid- to late-lactation Holstein cows at an inclusion rate of 58.5% of dietary dry matter. Although cows fed the late crop had greater total tract dry matter digestibility (68.9 vs. 66.1%) and tended to increase body weight gain (864 vs. 504 g/d) compared with those fed the early crop, neither dry matter intake nor milk yield were affected by dietary treatment, averaging 20.2 and 27.2 kg/day, respectively.

34

Using the Unique Degradation Ratio System (DRS) as an Alternative Method for Feed Evaluation and Diet Formulation: A Review

Animal Science Journal, April 2008, Volume 79, Number 2, pages 143-151.

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Efficient production of microbial protein in the rumen depends on the balance between the rates of degradation of feed carbohydrates and nitrogen-containing substrates such as feed proteins and urea. Excessive rumen degradation of proteins and non-structural carbohydrates is less efficient than digestion of those compounds in the small intestine. Optimization of digestive efficiency demands accurate prediction of rates and sites of digestion. This paper reviews current commonly used models that attempt to predict rumen degradation characteristics and promotes the degradation ratio system (DRS) as an improvement over current models. The DRS facilitates the prediction of nutrient supply to the animal based on feed characteristics that are influenced by variety, maturity, processing and climate. The author recommends the use of the DRS to more accurately account for feed characteristics and ruminant digestive processes and provides examples of its application.



35

A Meta-Analysis of the Impact of Monensin in Lactating Dairy Cattle. Part 1. Metabolic Effects

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Monensin, a product of the bacterium *Streptomyces cinnamonensis*, inhibits the growth of gram-positive bacteria by interfering with the transport of ions across their cell membranes. As a result of this effect, when fed to ruminants, monensin causes a shift in rumen microbial populations which affects rumen metabolism in ways that improve the efficiency of energy and nitrogen utilization. Other positive effects on digestion include reduced risk of bloat and lactic acidosis. Many studies have examined the effects of including monensin in diets fed to lactating dairy cows but results have not always been consistent. The purpose of the work reported in this paper was to combine and re-evaluate the results of those studies that published metabolic data. Overall, monensin inclusion in lactation diets significantly reduced blood concentrations of acetoacetic acid, beta-hydroxybutyric acid (BHBA) and non-esterified fatty acids (NEFA). Blood glucose and urea concentrations were increased while cholesterol, calcium, milk urea and insulin levels were not affected. The changes observed are indicative of improved energy metabolism. Method of delivery, timing of administration, stage of lactation and diet all affected responses. Reduction of blood BHBA concentration was greatest when monensin was mixed into a total mixed ration (TMR) but was more variable compared to either topdress or controlled release capsule. Greater BHBA reductions were also noted when monensin was administered in early lactation or fed to cows on pasture. Blood glucose increase was reduced when monensin was delivered in a TMR or from a controlled-release capsule versus administration as a ration top-dress. Monensin reduced blood NEFA when administered both pre- and post-calving but had less effect on blood glucose in the pre-calving period.



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A Meta-Analysis of the Impact of Monensin in Lactating Dairy Cattle. Part 2. Production Effects

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Monensin is routinely included in diets fed to growing beef cattle and lactating dairy cows for its beneficial effects on digestion and microbial metabolism. By selectively inhibiting the growth of gram-positive bacteria, monensin shifts rumen fermentation toward the production of end products that result in more efficient energy metabolism while reducing the risk of bloat and lactic acidosis. Although many studies have examined the effects of monensin on lactating dairy cows, results have varied. The purpose of this paper was to combine and re-analyze data from 77 trials in 36 studies that published data on the production of milk and milk components. Overall, monensin use decreased dry matter intake by 0.3 kg and increased milk yield by 0.7 kg, resulting in a 2.5% increase in production efficiency. Both milk fat and milk protein tests were decreased, by 0.13% and 0.03%, respectively. Milk protein yield increased by 0.016 kg/day but milk fat yield, lactose test and lactose yield were not affected. Analyses of fatty acid (FA) profiles in milk fat revealed an overall decrease in short-chain FA and stearic acid accompanied by a significant 22% increase in conjugated linoleic acids. Monensin also improved both body weight change and body condition score. Some of these effects of monensin use were influenced by diet. For example, a higher dietary concentration of stearic acid enhanced the effect of monensin on decreasing milk fat yield while increasing rumen peptide balance increased its effect on increasing milk protein yield.



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Hay to Reduce Dietary Cation-Anion Difference for Dry Dairy Cows

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Hypocalcaemia is a problem encountered by high producing dairy cows at calving mainly because of the new need for calcium for milk production. Lowering dietary cation-anion difference (DCAD) has been shown to decrease the risk of the clinical (milk fever) and subclinical forms of hypocalcemia. The DCAD of a ration is a measure of acidification potential. The most common method for its calculation is to subtract 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 commercial products treated with hydrochloric acid (HCl). The objective of this trial was to test the use of low-DCAD hay for its efficacy in achieving metabolic effects considered indicative of successful DCAD treatment. Low-DCAD timothy hay was produced by growing a crop on low potassium (K) soil enriched with calcium chloride (CaCl_2); high-DCAD hay was grown on soil with higher K and no CaCl_2 enrichment. A control total mixed ration (TMR) with a DCAD of 296 meq/kg DM was compared with a TMR containing low-DCAD hay as the only forage (DCAD = -24 meq/kg DM). A third diet contained the same ingredients as the control TMR but was acidified with the addition of HCl (DCAD = -19 meq/kg DM). Compared with the control, the low-DCAD hay diet reduced urine pH from 8.21 to 5.89, decrease blood base excess (-0.4 vs. 3.8 mM) and bicarbonate (23 vs. 27 mM), increased urinary Ca excretion from 1.2 to 13.4 grams/day and did not affect dry matter intake (DMI) or digestibility. Metabolic indicators were similar for low-DCAD and HCl-acidified diets, although DMI and blood pH were higher for cows on the low-DCAD diet. The authors conclude that feeding low-DCAD hay was effective in achieving the objectives of pre-partum dry cow DCAD management.



38

Modelling the Implications of Feeding Strategy on Rumen Fermentation and Functioning of the Rumen Wall

Animal Feed Science and Technology, May 2008, Volume 143, Number 1-4, pages 3-26.

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Mathematical models of physiological systems are valuable in helping to understand the mechanisms operating in complex systems and in predicting responses to changes in system inputs. Most existing mechanistic rumen models adequately account for the effects of changes in microbial activity, particulate and fluid passage rates and absorption by the rumen wall on the digestibility of feed and the production of microbial mass, volatile fatty acids and other end products. However, few have attempted to model either the influence of the rumen wall on the absorption of nutrients or the proliferation of these tissues in response to the products of microbial fermentation. This paper describes the development of a model that accounts for the contribution of the tissues of the rumen wall to overall rumen physiology. Model development was based on recent experimental data, but the authors stress that new experimental data are required for further evaluation and development. They identify a number of specific areas where more data is required to improve the model. The model may be combined with existing models of rumen fermentation, aiming at the improvement of their ability to accurately describe whole rumen function, including functionality of the host tissues surrounding the rumen contents. Such a model would be applicable under a broad range of circumstances. It would be particularly applicable with studying rumen acidification.



39

Timothy Hay with a Low Dietary Cation-Anion Difference Improves Calcium Homeostasis in Periparturient Holstein Cows

Journal of Dairy Science, May 2008, Volume 91, Number 5, pages 1959-1968.

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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, its application 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 feeds such as soy or canola meals treated with hydrochloric acid (HCl), but these treatments often reduce dry matter intake. This study examined the possibility that feeding low-DCAD hay during the dry period might be an alternative strategy. Timothy hay was obtained from an established stand grown under a pivot irrigation system. Low-DCAD hay was produced by adding 224 kg of calcium chloride (CaCl_2) per hectare to the circular area between the second and third pivot towers. Control (high-DCAD) hay was grown between the fourth and fifth towers with no CaCl_2 treatment. Forty-one non-lactating, pregnant dairy cows were randomly assigned to diets containing low- or high-DCAD hay at a rate of 63% of dietary dry matter (DM) beginning 30 days before their expected calving dates. DCAD values for the diets containing the low- and high-DCAD hays were 1.6 versus 14.5 mEq/100 grams of DM, respectively. The low-DCAD diet significantly reduced urine pH and blood bicarbonate concentration while increasing the concentration of blood ionized calcium during the periparturient period without depressing dry matter intake.



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Modeling the Adequacy of Dietary Fiber in Dairy Cows Based on the Responses of Ruminal pH and Milk Fat Production to Composition of the Diet

Journal of Dairy Science, May 2008, Volume 91, Number 5, pages 2046-2066.

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To support high levels of milk production, diets for dairy cows in early lactation must provide an optimum balance of fermentable organic matter (FOM - mainly starch) and structured roughage, typically measured as physically effective neutral detergent fibre (peNDF). While FOM provides energy to support high milk yields, its rapid fermentation results in the release of high levels of organic acids in the rumen. Salivary buffer secretion, resulting from chewing peNDF, dampens the pH depression that these acids might provoke. Excessive dietary FOM or inadequate peNDF often leads to the pH depression characteristic of subacute ruminal acidosis (SARA). The objective of this paper was to use published data to construct mathematical models that would help to predict optimum peNDF levels in diets for lactating cows. The resulting models demonstrate that the risk of SARA increases when daily average ruminal pH is lower than 6.16 and when rumen pH remains below 5.8 for more than 5.24 hours/day. The models also predicted that rumen pH rises as the concentration of peNDF in the diet is increased, reaching a plateau of pH 6.20-6.27 at a peNDF concentration of about 31.2% of dietary dry matter (DM). Although increasing peNDF levels up to about 32% of dietary DM slightly decreased DM intake and actual milk yield, 3.5% fat-corrected milk yield rose due to an increased milk fat test. Based on model predictions, it was concluded that a dietary peNDF level of about 30 to 33% is optimal for minimizing the risk of SARA without sacrificing production efficiency. The optimum for any specific diet will depend on the particular FOM sources included in the diet, on feed intake level, and on stage of lactation.



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Effects of the Chop Lengths of Alfalfa Silage and Oat Silage on Feed Intake, Milk Production, Feeding Behavior, and Rumen Fermentation of Dairy Cows

Journal of Dairy Science, May 2008, Volume 91, Number 5, pages 1942-1958.

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Lactating dairy cows require adequate physically effective fibre in their diets to promote chewing and salivation. Salivary buffers dampen the pH depressing effect of rapid organic acid production from high dietary levels of fermentable carbohydrates. One of the important variables affecting the physical effectiveness of a diet is its particle size distribution—longer particles require more chewing before they can pass out of the rumen. The objective of this study was to compare responses to feeding forages with different particle size distributions in terms of dietary intake, rumen fermentation patterns, feeding behaviour and milk production. Alfalfa (A) and oat (O) crops were each processed with a forage harvester set up to produce nominal chop lengths of either 6 (S) or 19 mm (L). Geometric mean particle lengths after ensiling were: AL - 14.2 mm, AS - 10.9 mm, OL - 13.4 mm, OS - 10.4 mm. Four total mixed rations (TMRs) were formulated to contain alfalfa silage and oat silage, each at 24% of TMR dry matter (DM), as follows: AL + OL, AL + OS, AS + OL and AS + OS. Each TMR was offered to mid-lactation Holstein cows to allow unlimited (ad libitum) intake. While reducing the nominal chop length of alfalfa did not affect feed intake, reducing the oat silage chop length increased DM intake from 19.4 to 21.2 kg/d. No other differences were observed between dietary treatments for milk production, rumen fermentation, feeding behaviour or blood metabolites. All diets resulted on low rumen pH and milk fat levels indicative of ruminal acidosis.



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A Model of Phosphorus Digestion and Metabolism in the Lactating Dairy Cow

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This paper describes a mathematical model designed to simulate the flow of different forms of phosphorus (P) through and within the lactating dairy cow. The model was validated through comparison with data described in 2 published studies. P exists in 3 forms: as phytic acid (Pp), as organic P, not including Pp (Po), and as inorganic P (Pi). All of these forms were assumed to be present in the diet and it was also assumed that Pi was the form in which P was absorbed into the bloodstream. Several variables affecting flow through the digestive system were assumed constant, including total P intake (75 grams/day), salivation rate (239 litres/day) and the passage rate of liquid from the rumen (198 litres/day). Given these assumptions, the model predicted the digestibility of total P across the digestive tract to be 38%. Of the Pp in feed, 74% was digested in the rumen, the remainder passing through the tract undigested. The net amounts of Po and Pi flowing from the rumen were respectively 2.4 and 3.0 times the amounts consumed. Microbial growth accounted for the increase in Po. The increase in Pi was due to its secretion into the rumen in saliva—65% of absorbed Pi was recycled back into the rumen via this route. Secretion in milk accounted for 30% of absorbed P; excretion in urine accounted for 1%. The authors propose several improvements to the model to account for bone turnover, Pp digestibility and the regulation of Pi absorption.



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Salivary Secretion During Meals in Lactating Dairy Cattle

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Lactating cows fed large quantities of grain are subject to ruminal acidosis due to the rapid production of volatile fatty acids by rumen microbial fermentation of starch. The potential reduction in rumen pH resulting from high acid concentration is tempered by buffers contained in saliva. A basic element of current dairy nutrition doctrine is the belief that coarse forages with high fibre levels provoke chewing which, in turn, promotes increased saliva production which helps to prevent acidosis. The objective of this study was to determine to what extent forages with differing fibre content, particle size and moisture content affect salivation when they are eaten by lactating cows. Four different diets were formulated to provide nutrients required for 25 kg of daily milk production. Concentrate was provided separately from the forage portion of each diet which was either barley silage, alfalfa silage, long-stemmed alfalfa hay or chopped barley straw. At each meal, boluses of chewed and swallowed feed were collected as they entered the rumen and analysed to determine the amount of saliva they contained. The rate at which saliva was secreted during eating was not significantly different among forages. However, the rates at which the different forages were eaten affected total saliva secretion because the time required to consume a fixed quantity of each forage varied considerably. In terms of the amount of saliva secreted per unit of forage dry matter, forages were ranked as follows: barley straw > alfalfa hay > barley silage > alfalfa silage. Eating rates, and therefore total saliva production, did not differ among the concentrate portions of each diet.



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The Relationships Between Intake and Net Portal Fluxes of Energy Metabolites in Ruminants: A Meta-Analysis

Animal Feed Science and Technology, May 2008, Volume 143, Number 1-4, pages 27-58.

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The purpose of this paper was to compile and collectively re-analyze by meta-analysis statistical methods, published data relating effects of intake on absorption of energy in ruminants. Data examined were from studies using adult sheep (17 data sets), growing cattle (9 data sets) and lactating dairy cows (1 data set). Measures of intake examined were dry matter intake (DMI) and digestible organic matter intake (DOMI). Energy absorption was characterized by flow of metabolites through the portal vein to the liver, including oxygen, volatile fatty acids, beta-hydroxybutyrate, glucose and lactate. The calculation of relationships between intake measures and portal metabolite flows indicated straight line, species-dependent associations for most. The authors advocate further work to improve our understanding of these associations, that could be related to differences in dietary composition between species.



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The Effect of Dietary Linola Oil on CLA and Other Fatty Acids in Bovine Milk

Canadian Journal of Animal Science, June 2008, Volume 88, Number 2, pages 321-324.

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Ruminant tissues are unique in their content of conjugated linoleic acids (CLA), a mixture of isomers of an 18-carbon fatty acid containing 2 double bonds (18:2) separated by one single bond. The primary isomers found in milk are cis-9, trans-11 CLA and trans-10, cis 12 CLA, where cis and trans refer to the spatial configuration of the double bond and the associated numbers identify the first carbon atom (counting from the acid end) where the double bond occurs. A great deal of interest in these particular isomers has arisen from animal and cell culture studies suggesting that they may have health-promoting properties. In a number of feeding trials, milk CLA concentrations have been increased by feeding vegetable oils containing linoleic (cis-9, cis-12 18:2) or linolenic (cis-9, cis-12, cis-15 18:3) acids. The inclusion of oilseeds in lactating cow diets has also increased milk CLA levels but results have been inconsistent. In particular, studies on the feeding of linola seeds have reported mixed responses in milk CLA concentration. The objective of this study was to evaluate the milk CLA response to pure linola oil to eliminate the possibility that the mixed responses to linola seed may have been due to differences in seed processing affecting digestibility. Eleven multiparous Holstein cows were offered a total mixed ration (TMR) containing 3% linola oil on a dry matter basis; a second group of 11 were offered a TMR containing 3% pork lard for comparison. Over a 21-day feeding period, lipid source did not significantly affect feed intake, milk volume or milk component yields. While milk fat percentage was significantly lower for cows fed the linola diet (3.54 vs. 3.88%) their average milk CLA level was significantly higher (1.58 vs. 0.77% of fatty acid methyl esters).



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Evaluation of the Response of Ruminal Fermentation and Activities of Nonstarch Polysaccharide-Degrading Enzymes to Particle Length of Corn Silage in Dairy Cows

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The primary objective of this study was to evaluate the effects of corn silage (CS) particle size (PS) on feed intake and rumen fermentation when CS was offered in total mixed rations (TMRs) with fibre-rich hay to lactating dairy cows. Three lots of CS were processed with a forage harvester set to deliver nominal average particle sizes of 14 (long), 8.1 (medium) and 5.5 mm (short). After ensiling, each CS lot was incorporated into a TMR at an inclusion rate of 40% of TMR dry matter together with 10% grass hay and 50% concentrate. Each of the 3 TMRs were offered to lactating cows with no limit placed on intake. PS distributions for each of the 3 diets were determined with the Penn State Particle Separator having 19, 8 and 1.18 mm screens. Physically effective neutral detergent fibre (peNDF) levels in the diets were calculated as the proportions of particles retained on single or multiple screens multiplied by dietary NDF concentration. Cows sorted the short CS TMR less than the other 2 diets. Although there were no differences among diets in terms of rumen pH or total volatile fatty acid production, consumption of the short CS TMR resulted in a lower proportion of propionate, a higher proportion of butyrate and a higher acetate-to-propionate ratio. Decreasing PS also increased the activities of rumen enzymes that degrade non-starch polysaccharides. Ruminal and total-tract digestion of carbohydrates were higher with the medium PL diet leading the authors to conclude that a moderate PS reduction of CS maximized feed efficiency and energy supply.



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A Meta-Analysis of the Impact of Monensin in Lactating Dairy Cattle. Part 3. Health and Reproduction

Journal of Dairy Science, June 2008, Volume 91, Number 6, pages 2328-2341.

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Monensin is commonly fed to both growing beef and lactating dairy cattle for its positive effects on production efficiency. In addition, by selectively inhibiting the proliferation of gram-negative bacteria, thus altering rumen fermentation patterns, monensin reduces the risk of bloat and lactic acidosis. Although many studies have examined the metabolic and production effects of including monensin in diets fed to lactating dairy cattle, fewer have documented consequences for health and reproduction. The purpose of this paper was to review these effects by combining and re-analysing the data published in 16 available studies. Among its positive health effects, monensin was found to decrease the relative risk (RR) of ketosis, displaced abomasum and mastitis. Overall, the RR of milk fever, lameness, dystocia, retained placenta and metritis were not affected. However, RR of dystocia and retained placenta increased with days of monensin treatment before calving while RR of retained placenta and metritis were lower when monensin was delivered by a controlled-release device rather than incorporated in, or top-dressed on, a mixed ration. Reproductive measures indicated no effects on either first-service conception rate or days to pregnancy.



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The Histamine H1 Receptor Is Not Involved in Local Control of Mammary Blood Flow in Dairy Cows

Journal of Dairy Science, June 2008, Volume 91, Number 6, pages 2461-2468.

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The results of a number of studies have suggested that blood flow to the mammary gland can change in response to the concentrations of nutrients in the blood supply. For example, in one study, mammary blood flow decreased by 16% when the plasma concentration of glucose was increased by 78%. In another experiment, mammary blood flow increased by 33% when the circulating concentration of the amino acid histidine was reduced by 89%. It has been proposed that these changes in blood flow are regulated by the release of biochemical agents that either dilate or constrict arteries carrying blood to the gland. In the case where lower histidine levels increased blood flow, it was hypothesized that a reduced secretion of histamine was responsible for the observed effect. Histamine, synthesized from histidine by the enzyme histamine decarboxylase, can cause arterial (vaso-) constriction when it binds to a receptor (H1) in the arterial wall. A reduction in the supply of histidine, leading to reduced histamine synthesis, might result in a release of this vasoconstrictive effect, thus increasing blood flow. To test this hypothesis, the authors of this study infused the H1 receptor blocker, chlorpheniramine (CPA), into the mammary artery along with low or normal concentrations of histidine. Neither CPA nor low histidine concentration affected mammary blood flow, suggesting that histamine is not involved in the regulation of mammary blood flow.



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Effects of Lasalocid or Monensin on In Situ Biohydrogenation of Flaxseed and Sunflower Seed Unsaturated Fatty Acids

Canadian Journal of Animal Science, June 2008, Volume 88, Number 2, pages 335-339.

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Unsaturated fatty acids entering the rumen are 'biohydrogenated' by rumen microbes. The rate at which this process occurs has an effect on whether it proceeds to completion or is only partially completed before substrate fatty acids pass out of the rumen. For example, linolenic acid (C18:3) is converted stepwise to C18:2, C18:1 and C18:0 but variable amounts of C18:2 and C18:1 may escape from the rumen when the rate of passage exceeds the rate of biohydrogenation. Earlier feeding experiments suggested that monensin feeding might reduce the rate of rumen biohydrogenation. The objective of the present study was to further evaluate this possibility using both monensin and lasalocid, another antimicrobial ionophore having similar effects on rumen metabolism. Multiparous lactating dairy cows, fitted with rumen cannulae, were fed total mixed rations supplemented with monensin, lasalocid or no ionophore (control). Nylon bags containing either ground sunflower seed (rich in C18:2) or ground flaxseed (rich in C18:3) were incubated in the rumens of cows on each ionophore treatment to assess rates of biohydrogenation. Neither ionophore affected the biohydrogenation rate of 18:2 but monensin decreased the biohydrogenation rate of 18:3 compared with control or lasalocid.



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Nutrient Intake and Feeding Behavior of Growing Dairy Heifers: Effects of Dietary Dilution

Journal of Dairy Science, July 2008, Volume 91, Number 7, pages 2786-2795.

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Overfeeding prepubertal heifers may negatively effect future milk production, likely as a result of fat accumulation in the developing mammary gland. A target growth rate in the 0.9 to 1.0 kg/day range is recommended to optimize growth without negatively affecting mammary development. To achieve this objective, a common strategy is to limit-feed a nutrient-dense diet, taking advantage of a slower passage rate of feed through the rumen, improving feed efficiency. However, the practice of limit feeding can have some negative effects in terms of animal welfare. In several studies, limit-fed animals have shown behavioural signs of being hungry, including consuming tree bark, spending more time standing while not eating and increasing vocalization. An alternative to limit feeding is to offer unlimited (ad lib) access to diets of lower nutrient density which allow animals to satisfy their appetites while providing sufficient nutrients to achieve growth targets. The objective of this study was to offer total mixed rations (TMRs) of 3 different nutrient densities to prepubertal heifers (average 7.4 months of age; 250 kg body weight) to assess their feeding and feed sorting behaviour. A control diet was 17% corn silage, 52.1% grass silage and 30.9% concentrate. Two test diets consisted of the control diet plus 10 or 20% rye straw. On all 3 diets, heifers sorted against long particles (retained on a 19 mm screen) in favour of short particles (passed by an 8 mm screen, retained on a 1.18 mm screen). On the 10 and 20% straw diets, they sorted in favour of medium particles (passed by 19 mm screen, retained on 8 mm screen). Increasing quantities of straw in the TMR increased sorting for medium, short and fine (passed by 1.18 mm screen) particles, increased feeding time and meal duration and decreased feeding rate, meal size and meal frequency. All diets were able to support the target growth rates suggested above. The results suggest that a low-quality feedstuff may be included in the diet to target nutrient intake and reduce feed costs without negatively affecting feeding behavior or growth potential.



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Influence of Carbohydrate Source on Ruminal Fermentation Characteristics, Performance, and Microbial Protein Synthesis in Dairy Cows

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Many of the diets fed to lactating dairy cattle in North America contain corn, barley, wheat or oats, or a combination of these grains as the primary source of rapidly fermentable carbohydrate, in the form of starch. However, the starch contents of these grains vary from around 57-58% for barley and oats to about 77% for wheat. In addition, the rumen fermentation rates of the starch contained in these grains vary considerably. A slower starch fermentation rate combined with a high passage rate of digesta through the rumen results in a higher proportion of the starch escaping fermentation. Since the rate and extent of rumen carbohydrate fermentation influences the rate of microbial protein synthesis, different grain sources can have varying effects on protein supply to the cow. The objective of this study was to evaluate diets containing each of these 4 grains in terms of their effects on rumen fermentation, microbial protein synthesis and milk production. Diets contained 50% forage dry matter (DM) in the form of alfalfa hay and barley silage. Between 28 and 31% of dietary DM was dry-rolled corn, barley, wheat or oats. Diets were fed to 8 multiparous, mid-lactation Holstein cows, 4 of whom were fitted with rumen cannulae. Dietary DM intakes (24.0 - 26.2 kg/d) and uncorrected milk yields (36.8 - 40.2 kg/day) were not significantly different among diets although the wheat diet tended to suppress both. Cows fed the barley and wheat diets had lower milk fat tests compared with those fed the corn-based diet; those fed the oats diet had intermediate milk fat tests. Starch source did not affect rumen fermentation characteristics but estimated microbial nitrogen flow out of the rumen was higher in cows fed the barley-based diet compared with those fed the oats-based diet. Total tract apparent digestibility (AD) of DM, organic matter or neutral detergent fibre were not affected by diet but AD of starch was higher in cows fed the oats diet compared with those fed the corn or wheat diets. The efficiency of nitrogen use for productive purposes tended to be lower in cows fed the wheat diet than in those fed the other diets.



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Distribution of ^{15}N in Amino Acids During ^{15}N -Leucine Infusion: Impact on the Estimation of Endogenous Flows in Dairy Cows

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The nitrogen (N) found in the gastrointestinal (GI) tract of the dairy cow comes from both exogenous (feed) and endogenous sources. Endogenous sources include urea which enters the rumen either directly across the rumen wall from the bloodstream or in the form of secretions from salivary glands. Other nitrogenous compounds enter the GI tract as digestive secretions or as cells sloughed from its inside surface. When determining nutrient requirements for maintenance and production it is important to account for these endogenous sources as they can contribute significantly to overall nutrient balance. The experiment described in this study was designed to estimate flows of endogenous N at various points along the GI tract. To do this, labelled [^{15}N]leucine was infused into the bloodstream over 200 hours, resulting in an enrichment of this stable isotope (^{15}N) in all nitrogenous compounds in the body, including endogenous N sources. Dilution of isotope by feed N as it passed through the GI tract made it possible to estimate endogenous N flows at the entrance to (duodenum) and exit from (ileum) the small intestine and into feces. Endogenous N represented between 14 and 30% of the total flow of N through the duodenum and 18 to 31% of the total N found in feces. Endogenous N flowing through the ileum was 18% higher than the flow into feces, indicating absorption of this difference by the large intestine. Of the endogenous N flowing through the duodenum, approximately half was incorporated in microbial protein whereas the remainder was present as free proteins (enzymes, desquamated cell, etc).



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Robust Expression in Yeast Cells of a Reporter Gene Driven by Rumen Protozoal Promoter Sequences

World Journal of Microbiology and Biotechnology, August 2008, Volume 24, Number 8, pages 1529-1537.

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Under normal circumstances, the population of microbes in the rumen of a mature animal consists of a mix of bacteria, fungi and protozoa. Although they are not essential for the survival of host animals, protozoa play important roles in contributing to fibre digestion and reducing the risk of acidosis. However, some species of protozoa that are efficient fibre digesters also feed on rumen bacteria, reducing the availability of bacterial protein to the host. Other species, although they do not feed on bacteria, are not as effective in digesting fibre. The objective of the research described in this paper is to explore the possibility that species among the second class of protozoa can be genetically altered to enhance fibre digestion. In this initial phase of the work, the researchers were able to clone a gene that was very actively expressed in the protozoan *Isotricha intestinalis*. The DNA sequence responsible for promoting expression of this gene was isolated and used in the construction of a DNA shuttle/expression vector capable of transforming yeast cells. Once inside yeast cells, the promoter was able to direct the high level expression of a recombinant protein. The authors conclude that their results address a major hurdle in the engineering of *Isotricha* for the production of recombinant proteins, and thereby underscore the technical feasibility of genetically altering rumen protozoa to enhance the production of enzymes that contribute to rumen health and productivity.



54

A Review of Plant-Derived Essential Oils in Ruminant Nutrition and Production

Animal Feed Science and Technology, August 2008, Volume 145, Number 1-4, pages 209-228.

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The perception that antibiotic use in animal production has contributed to antibiotic resistance among some bacterial species pathogenic to humans has created interest in the discovery of alternative 'natural' treatments. Among these potential alternatives, essential oils (EO) have received attention due to several studies that have demonstrated their antimicrobial activity. Chemically, EO are not actually oils but a varied mixture of volatile aromatic compounds that can be extracted from various plant tissues by steam distillation. EO are responsible for the unique colours and essences of these tissues. This paper reviews the results of studies that have evaluated the effects of EO on microbial fermentation in both short-term laboratory (in vitro) culture systems and in whole animal feeding trials. Results from in vitro culture systems have been inconsistent but suggest that high doses of EO have the potential to improve nitrogen and/or energy utilization efficiency. However, such high doses have also been shown to reduce total volatile fatty acid production, an indicator of fermentation inhibition. Some EO and EO components have been shown to inhibit methane production by rumen microbes, an effect that could be applied to the mitigation of greenhouse gas emissions. Several studies have also demonstrated the bactericidal activity of EO against pathogenic bacterial species such as *E. coli* 0157:H7 and certain *Salmonella* species. The authors of the review conclude that EO have the potential to improve feed efficiency and decrease pathogen loads in ruminants but that, to realize this potential, more research is required to identify specific components of EO that have beneficial effects.



55

Relationship Between Enzymic Activities and In Vitro Degradation of Alfalfa Hay and Corn Silage

Animal Feed Science and Technology, August 2008, Volume 145, Number 1-4, pages 53-67.

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The potential of exogenous enzyme preparations to enhance the digestion of forages has been investigated by a number of researchers, including the authors of this paper. Results have been inconsistent, partly because the specific enzymic activities in these preparations have not been well characterized. This paper reports the results of a re-analysis of 14 previously reported studies where laboratory (in vitro) culture systems were used to test the ability of various enzyme preparations to enhance the fermentation of alfalfa hay and corn silage. Preparations included varied activities of several enzymes, including endoglucanase, exoglucanase, xylanase and protease. Measures of fibre digestion included gas production, dry matter degradability and neutral detergent fibre degradability. Associations between the activities of specific enzymes and measures of fibre digestion varied, depending on the statistical analysis employed. The strongest positive association with fibre digestion for both forages was with the exoglucanase activity of the respective preparations.

56

Effects of Phlorotannins from *Ascophyllum nodosum* (Brown Seaweed) on In Vitro Ruminal Digestion of Mixed Forage or Barley Grain

Animal Feed Science and Technology, August 2008, Volume 145, Number 1-4, pages 375-395.

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Tannins are naturally occurring compounds found in both terrestrial and marine plants. In some studies, the inclusion of terrestrial tannins in ruminant diets has resulted in reduction of pasture bloat, enhanced protein utilization and improved productivity. However, responses have been inconsistent, likely due to the diversity and structural complexity of the tannins used. Phlorotannins (PT), produced by marine brown algae, have less complex structures than most terrestrial tannins. PT have been reported to have both antimicrobial and antioxidant properties. When fed to cattle, PT-containing extracts of brown algae have been shown to reduce the fecal shedding of *E. coli*, including the human pathogenic strain 0157:H7. This study examined the effects of PT in laboratory (in vitro) cultures of mixed forages and barley grain inoculated with rumen fluid. PT reduced the rate of fermentation of both substrates but the effect on mixed forages occurred at a lower PT concentration, suggesting that fibre-digesting bacteria may be more sensitive to PT than starch digesters. A reduction in ammonia production by the cultures indicated that PT might also reduce the deamination of amino acids.



57

Effects of Peripartum Propylene Glycol Supplementation on Nitrogen Metabolism, Body Composition, and Gene Expression for the Major Protein Degradation Pathways in Skeletal Muscle in Dairy Cows

Journal of Dairy Science, September 2008, Volume 91, Number 9, pages 3512-3527.

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After calving, milk production usually increases more rapidly than nutrient intake, resulting in a period of negative nutrient balance when body stores of fat and protein must be mobilized to fill the deficit. Amino acids derived from the mobilization of body protein are primarily used by the liver to synthesize glucose which is required in large quantities for the production of lactose by the mammary gland. Propylene glycol (PG) is often used to treat cows which are experiencing a glucose deficiency after calving since PG can also be used by the liver to synthesize glucose. This study was designed to determine whether feeding PG during the transition period could reduce the mobilization of protein from muscle. Sixteen non-lactating, pregnant cows were fed total mixed rations (TMRs) twice daily. Eight were treated with 300 mL of PG as a top-dress on their TMR at each feeding; the other 8 cows served as controls, receiving the same diets without PG supplementation. To assess effects on protein mobilization, several indicators of protein breakdown were measured. PG supplementation had no effect on any of these. However, results of the study did demonstrate increased activity of metabolic pathways involved in protein breakdown during early lactation in both control and PG-supplemented cows.

58

Rearing Strategy and Optimizing First-Calving Targets in Dairy Heifers: A Review

Animal, September 2008, Volume 2, Number 9, pages 1393-1404.

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It is generally accepted that targets for the growth of replacement heifers require recognition of a number of factors, including genetics, cost of rearing, a balance between frame size and body weight and potential for future milk production. Although rapid growth before puberty hastens sexual maturity, mammary development and future productivity can be impaired when gain in weight and frame size are not balanced. Similarly, heifers carrying excessive body condition at calving are subject to dystocia and other periparturient disorders, particularly if structural development is inadequate. Underfeeding in late gestation can also lead to calving difficulties such as retained placenta. This paper reviews a large body of information about heifer rearing practices and makes recommendations for optimizing growth targets for heifers of various genetic types and environmental conditions, particularly relevant to France, but also to several other countries.



59

***Prevotella bryantii* 25A Used as a Probiotic in Early-Lactation Dairy Cows: Effect on Ruminal Fermentation Characteristics, Milk Production, and Milk Composition**

Journal of Dairy Science, September 2008, Volume 91, Number 9, pages 3536-3543.

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In early lactation, many dairy cows develop ruminal acidosis when high starch intakes result in the rapid production of organic acids, depressing rumen pH. One of the most acidifying of these is lactic acid, produced at low pH by secondary fermenters. A large number of trials have examined the effects of probiotics (direct-fed microbials: DFM) on mitigating this effect. In this study, the authors administered a strain of bacteria (*Prevotella bryantii*) that is able to utilize starch and produce end-products other than lactic acid. Since *Prevotella* species are among the most numerous inhabitants of the rumen, it was speculated that *P. bryantii* would, unlike some other DFM, survive and thrive in the rumen environment. Inoculum was administered daily from 3 weeks prepartum until 7 weeks post-partum, directly into the rumen of 6 cows through a cannula. An equal number of control cows received no DFM treatment. Feed intake, milk production and rumen pH were similar in treated and control cows. Milk fat test tended to be higher in DFM-treated cows. Although rumen lactic acid concentrations were no different in the 2 groups of cows before treatment, within 2 to 3 hours after feeding lactation diets, average rumen lactate levels in the DFM-treated cows fell to half of that in the controls. This difference persisted through the 7 week lactation test period. Rumen concentrations of ammonia nitrogen, acetic, butyric and branched-chain 4-carbon fatty acids were higher in the cows who received the *P. bryantii* culture.

Genetics





1

Whole Genome Linkage Disequilibrium Maps in Cattle

BMC Genetics, October 2007, Volume 8, Number 74, pages 1-12.

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Genes that carry the genetic code defining the specific characteristics of an individual exist as a pair of alleles, one allele on each of 2 paired chromosomes. Each allele can carry the code for 1 of 2 different versions of a DNA sequence (e.g., A or B). If the 2 alleles are identical, the individual is homozygous for that gene (AA or BB); if the alleles are different the individual is heterozygous for that gene (AB or BA). Any of the roughly 30,000+ alleles represented on the cow's 30 pairs of chromosomes (genome) can match up in this way, so a gene coding for a second characteristic, located somewhere else on the genome, could be CC, DD, CD or DC. If there were no linkages between these 2 genes, the probability of each of the 4 possible combinations occurring for each gene would be 0.25 (1 in 4) 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). This paper describes work which established the degree of LD between 2,670 markers (genetic sequences) across the entire genomes in 8 breeds of cattle. The authors conclude that LD predominantly exists among genes that are in closer proximity to one another than previously reported. Understanding which areas of the genome are in high LD will assist in the identification of genes underlying economically relevant traits.

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2

Single Nucleotide Polymorphisms in the Open Reading Frame of the Stearoyl-CoA Desaturase Gene and Resulting Genetic Variants in Canadian Holstein and Jersey Cows

DNA Sequence - Journal of DNA Sequencing and Mapping, October 2007, Volume 18, Number 5, pages 357-362.

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Stearoyl-CoA desaturase (SCD) is an enzyme found in the ruminant mammary gland and adipose tissue that is responsible for introducing a single cis double bond between carbons 9 and 10 of fatty acids having chain lengths of 12 to 19 carbons. Interest in SCD is due to its ability to convert vaccenic acid (trans-11 C18:1) to cis-9, trans-11 conjugated linoleic acid (9,11 CLA). The concentration of 9,11 CLA in milk varies widely among cows and it has been speculated that this variation may in part be due to variation in the activity of mammary SCD. Therefore, the objective of this study was to determine whether such variations in SCD activity might be due to differences in the amino acid sequence of SCD due to variations (single nucleotide polymorphisms, SNPs) in the DNA sequence of the SCD gene. Examination of the DNA sequences of SCD genes from 44 Holstein and 48 Jersey cows revealed 3 SNPs present in both breeds and a fourth SNP unique to Holsteins. These SNPs resulted in 4 different SCD genotypes in Holsteins but only 2 in Jerseys. However, only 2 genotypes (the same 2 in both breeds) coded for enzymes with different amino acid sequences. The authors speculate that these enzyme variants may contribute to the variations seen in 9,11 CLA milk levels.



3

Identification of Novel Host Defense Peptides and the Absence of Alpha-Defensins in the Bovine Genome

Proteins: Structure, Function and Bioinformatics, November 2007, Volume 73, Number 2, pages 420-430.

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Host defence peptides (HDPs) are short sequences of amino acids produced by the innate immune systems of organisms ranging from amoeba to humans. Observations that HDPs can both influence the strength of the immune response and directly kill pathogenic organisms has provoked interest in their potential use in treating infections by antibiotic resistant pathogens. Although several different classes of HDPs have been identified, one class, the defensins, have attracted particular interest. The amino acid sequences of 3 sub-families within this class are closely related: the alpha-, beta and theta-defensins. It is speculated that the similarities among these sub-families are due to their evolution from a single ancestral beta-defensin. The objective of this study was to discover new candidate HDPs produced by cattle by looking for DNA sequences in the bovine genome that would be expected to code for proteins similar to the 34 known bovine HDPs. A further 68 potential HDPs were found, 66 of which were beta- and theta-defensins; 2 were of another class, the cathelicidins. No alpha-defensins were found suggesting that cattle do not produce HDPs from this sub-family.



4

Identification of Single Nucleotide Polymorphisms in Bovine CARD15 and their Associations with Health and Production Traits in Canadian Holsteins

BMC Genomics, November 2007, Volume 8, Number 421, pages 1-11.

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When a pathogenic organism enters an organ such as the mammary gland or intestine, receptor molecules on the surface of cells lining the organ (epithelial cells) recognize molecules on the surface of the pathogen and initiate responses designed to destroy it. For example, when *Staph. aureus* enters the mammary gland, a complex molecule (peptidoglycan, PGN) associated with its cell wall is detected by a number of epithelial recognition molecules, including toll-like receptor-2 (TLR-2) and caspase recruitment domain 15 (CARD 15). Recognition leads to the secretion of signalling molecules (cytokines and chemokines) that recruit immune system cells to the site of infection, resulting in inflammation and engulfment of the pathogen by white blood cells (WBC). WBC that are released into the mammary gland of the dairy cow are referred to as somatic cells (SC). This sequence of events operates more effectively in some cows than in others, resulting in individuals being more or less resistant to chronic infection. To some extent resistance is controlled by genetics and an effort is being made to identify and breed for resistant genotypes. The objective of this study was to identify variations (single nucleotide polymorphisms, SNPs) in the genes that code for TLR-2 and CARD 15 in order to evaluate relationships between these SNPs and SC scores in lactating cows. No SNPs were identified in the TLR-2 gene but 4 were identified in the CARD 15 gene, 2 of which were found to be associated with estimated breeding values (EBVs) for SC score or milk and milk protein yields. The authors speculate that one of these SNPs in particular may play a role in the cow's response to intramammary infection.



5

A Physical Map of the Bovine Genome

Genome Biology, November 2007, Volume 8, Number 8, pages 165-182.

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Current dairy genetic improvement schemes are based on selection of animals based on observable characteristics (phenotypes) of individuals, their parents and their progeny. As progress is made in identifying the specific genes responsible for various production, health and conformation traits, it is anticipated that future schemes will be based on some combination of phenotype- and genome-based selection. Ultimately, 'genome-wide' selection can be enhanced by a map to align variation in individuals' DNA sequence with the genes causing variation in economically important traits. Until recently, however, genome mapping techniques have not been able to provide complete maps. This paper describes the methods used to construct complete maps of the genomes of animals from 3 different cattle breeds. Mapping was based on the use of bacterial artificial chromosome (BAC) clones, where short, overlapping segments of genomic DNA are incorporated into a BAC from which multiple copies are cloned (replicated) and sequenced. After sequencing, the genome is reassembled using a computer program that matches identical end sequences in overlapping regions of adjacent segments. In this study, 422,522 end sequences facilitated the ordering and assembly of sequences determined using 290,797 BAC clones.

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6

**Differentially Expressed Genes Associated with
Staphylococcus aureus Mastitis of Canadian Holstein Cows**

Veterinary Immunology and Immunopathology, December 2007, Volume 120, Number 3-4, pages 201-211.

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Infection by a pathogenic organism activates the immune system, resulting in the increased expression (up-regulation) of some genes and the decreased expression (down-regulation) of others. The objective of this study was to identify genes in blood mononuclear cells (BMC) and milk somatic cells (MSC) whose expression was different in cows infected with *Staph. aureus* (SA) compared with cows not infected with SA. BMC are a class of white blood cells, including monocytes and lymphocytes, involved in the immune response. MSC are mainly neutrophils, macrophages and lymphocytes expressed into the alveoli of the mammary gland in response to infection. An array of DNA sequences representing 167 genes known to be involved in the immune response was used to detect the abundance of corresponding messenger RNA (mRNA) sequences in extracts from these cells. mRNA extracts from 7 cows known to be chronically infected with SA were compared with extracts from healthy cows of similar age, parity and stage of lactation. In the BMCs, 14 of the 167 genes were up-regulated and 8 were down-regulated in the SA-infected compared with the non-infected cows. In contrast, 14 genes were up-regulated and 2 down-regulated in MSC cells from SA-infected cows, with few similarities between these and the genes affected in the BMC. Array results were confirmed using real-time polymerase chain reaction (RT-PCR) amplification of mRNA. The authors interpret the results as evidence of the complexity of gene expression patterns in response to infection.



7

Estimation of Genome-Wide Haplotype Effects in Half-Sib Designs

Journal of Animal Breeding and Genetics, December 2007, Volume 124, Number 6, pages 356-361.

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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 the a combination of SNPs in a number of genes that may be widely distributed across the genome. These are termed quantitative traits and the location of the genes influencing these traits are called quantitative trait loci (QTLs). Methods have been developed that currently allow the characterization of more than 50,000 SNPs approximately evenly spaced across the bovine genome. The combination of specific nucleotides found at each of these SNPs is unique for every individual (except identical twins), defining the individual's genotype. Statistical correlations between the distribution of variation in SNPs and observed (phenotypic) traits makes it possible to identify the locations of QTLs. Once these associations are established, it becomes possible to predict the expression of a quantitative trait from the animal's genotype and to calculate a genome-wide estimated breeding value (GEBV) for that trait. In this study, a simulation study evaluated factors that might influence the accuracy of GEBV estimates. Comparison of GEBVs with true breeding values yielded correlations between 0.60 and 0.79. The authors speculate that these values will improve as further development of technology increases the number of SNPs that can be simultaneously characterized.

8

Genetic Modification of the Lactation Curve by Bending the Eigenvectors of the Additive Genetic Random Regression Coefficient Matrix

Journal of Dairy Science, December 2007, Volume 90, Number 12, pages 5753-5758.

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Peak milk yield and lactation persistency are two important characteristics of a lactation curve that determine a cow's lactation milk yield. Peak milk yield describes the highest daily milk yield during a cow's lactation and lactation persistency is a measure of the rate of decline of test day milk yields after the peak. Estimated breeding values for production traits are calculated from test day yields using sophisticated mathematical transformations. This paper proposes an improved method which facilitates the simultaneous improvement of both milk production and lactation persistency by modifying the shape of the lactation curve.



9

Polymorphisms in the 5' Upstream Region of the CXCR1 Chemokine Receptor Gene, and Their Association with Somatic Cell Score in Holstein Cattle in Canada

Journal of Dairy Science, January 2008, Volume 91, Number 1, pages 407-417.

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When the cow's mammary gland is infected by gram-positive bacteria such as *Staph. aureus*, molecules associated with the bacterial membrane are recognized by receptor molecules on the surface of the epithelial cells lining the gland. The interaction of these 2 molecules initiates the secretion of signalling molecules (cytokines) by the epithelial cells. One particular cytokine, interleukin-8 (IL-8) exerts a very strong attraction for a receptor (CXCR1) on the surface of neutrophils, one of a number of different types of white blood cells (WBC). The IL-8/CXCR1 attraction results in the recruitment of a large number of neutrophils to the site of infection where they release free oxygen radicals and enzymes designed to destroy the pathogen. A second wave of WBC (macrophages) rapidly replace the neutrophils, minimizing their potential damaging effects to the mammary epithelium itself. These and other WBC (lymphocytes) make up what are referred to as somatic cells (SC). The strength of the attraction between IL-8 and CXCR1 is thought to influence the efficacy of the immune response to intramammary infection. The objective of this study was to investigate the possibility that variations (single nucleotide polymorphisms, SNPs) in the gene that codes for CXCR1 might be responsible for individual variation in the strength of the IL-8/CXCR1 interaction. Four SNPs were identified in the genomes of 338 Canadian Holstein bulls. Analysis of correlations between the presence of these SNPs and estimated breeding values (EBVs) for SC scores revealed an association between one of the SNPs and SC score EBVs across all 3 lactations.



10

Transcriptome Analysis of Bull Semen with Extreme Nonreturn Rate: Use of Suppression-Subtractive Hybridization to Identify Functional Markers for Fertility

Biology of Reproduction, January 2008, Volume 78, Number 4, pages 618-635.

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In cattle breeding, the most commonly-used measure of a sire's fertility potential is his non-return rate (NRR). NRR is defined as the percentage of cows that were inseminated but not re-inseminated within a specified time period, usually 56 days. Although semen quality is routinely evaluated using both visual and laboratory techniques, there is currently no test capable of predicting NRR. In this study, the researchers explored the possibility that differences in sperm cell (spermatozoa) messenger RNA (mRNA) profiles (transcriptomes) between high and low NRR sires might lead to a method of predicting NRR. Much of the mRNA found in the mature spermatozoa is considered residual, having no known function. Therefore, a second objective of this study was to identify sources and functions for these molecules (transcripts). Transcriptomes were analyzed in semen samples from 10 sires with known NRRs. Five had the lowest ($\leq 65\%$) and 5 others the highest ($\geq 71\%$) NRRs among 180 sons sired by the same bull. Significant differences were found in the mRNA transcriptomes of the 2 groups. While 29% of the transcripts found in the high-NRR group had known cellular functions, only 10% of those from low-NRR group did. In addition, 17% of the mRNA transcripts in the high-NRR group were identified with DNA segments on the bovine genome compared with only 3% of the low-NRR transcripts. The authors suggest that the analysis of mRNA transcriptome profiles might be a useful tool in fertility assessment of bull semen and in advancing knowledge about the causes of infertility.



11

Efficiency of Quantitative Trait Loci-Assisted Selection in Correlations Between Identified and Residual Genotypes

Animal Science Journal, February 2008, Volume 79, Number 1, pages 22-28.

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Characteristics determined by a single gene are usually referred to as qualitative traits, e.g., genetic anomaly, BLAD (Bovine Leukocyte Adhesion Deficiency). Conversely, most production traits are quantitative in nature and are influenced by multiple genes each with a small effect. Quantitative trait loci (QTLs) are locations on the genome where these genes are found. Recent developments in genomic technologies have facilitated the identification of QTLs for a number of important production traits. However, so far only a small proportion of the relevant QTLs for each trait have been identified. Therefore, the genotype of a trait is characterized as a combination of the QTL-identified genotype and a residual genotype which is assumed to be influenced by as-yet undetected QTLs. There is good evidence to suggest that interactions exist between the known and unknown QTLs. This is the phenomenon of epistasis where the expression of one gene is modified by the other genes. The objective of this study was to assess the potential impact of such interactions on the accuracy of QTL-assisted selection in comparison with conventional phenotypic selection. A method for improving the accuracy of QTL-assisted selection is described.

12

Comparison of Models for Genetic Evaluation of Survival Traits in Dairy Cattle: A Simulation Study

Journal of Animal Breeding and Genetics, April 2008, Volume 125, Number 2, pages 75-83.

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The contribution of each cow to herd profitability partly depends on her longevity, particularly her length of survival after first calving. For purposes of genetic improvement, several different measures of longevity are used: age at disposal, survival (or not) to a given age, survival (or not) to a given state of production (e.g., second lactation) or months after first calving. The objective of this study was to compare actual survival data for Canadian Jersey cows with results predicted by 3 different mathematical simulation models. Each model was run for 20 generations of random mating, including selection for a trait unrelated to longevity. Three different levels of heritability for survival, 2 female population sizes and 2 sire progeny group sizes were examined for their effects on model predictions. Estimated breeding values (EBVs) were calculated for each sire used. The 3 different models each ranked sire EBVs differently with the relative rankings being independent of heritability, female population size or sire progeny group size. One model, the random regression model, was favoured for its ability to accurately predict the survival of sire daughters to fixed ages after first calving.



13

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

Mammalian Genome, April 2008, Volume 19, Number 4, pages 226-245.

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Individual susceptibility or resistance to disease is affected by many factors, including nutrition, current state of general health, host-pathogen interactions and host genetics. Genetics exerts its influence through variations in gene expression which affect the function of proteins involved in the immune response to infection. One source of these variations may be differences in the DNA sequences that code for specific proteins, resulting from single nucleotide polymorphisms (SNPs), nucleotide deletions or insertions, gene or whole chromosome rearrangements, gene duplications or copy number variations. These variations in the genetic code can result in significant differences in the amino acid sequences of proteins, affecting their function. In some cases, they may result in the complete absence of a functional protein or in more or fewer copies of a protein being synthesized. This paper reviews genetic variations that underlie several diseases of cattle, sheep, goats and pigs. Knowledge of the genetic bases of some livestock diseases has led to control through selective breeding and may also lead to control through genomic modification.

14

Relationship Between Reproduction Traits and Functional Longevity in Canadian Dairy Cattle

Journal of Dairy Science, April 2008, Volume 91, Number 4, pages 1660-1668.

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A cow's productive life ends when she dies or when the herd manager decides to cull her. Culling for low productivity is generally referred to as voluntary culling. Involuntary culling is attributed to death, poor health, poor temperament, reproductive failure or other causes. Herd productivity generally increases when a higher proportion of culling decisions are voluntary. More involuntary culling is associated with higher veterinary and replacement costs, reduced cow longevity and lower profitability. The objective of this study was to evaluate relationships between measures of reproductive performance and longevity in Canadian dairy cattle. Functional longevity was defined as the number of days from first calving to culling, death or leaving the herd for some other reason (censoring). Records of 1.7 million Holsteins, 67,000 Ayrshires and 33,000 Jerseys were used in the analysis. Cows that required a hard pull at calving or gave birth to small or dead calves were at higher risk of culling. Functional longevity was also shorter for cows that require more services per conception, had a longer interval between first service to conception, or had a calving to first service interval of more than 90 days.



15

Extent of Linkage Disequilibrium in Holstein Cattle in North America

Journal of Dairy Science, May 2008, Volume 91, Number 5, pages 2106-2117.

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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). In this study, 5,564 SNPs were characterized in the genomes of 821 North American Holstein bulls. For 497 haplotypes coming from dams of those bulls, the extent of LD between each pair of SNPs was determined. LD was highest for SNPs that were close to one another but declined rapidly with distance between SNPs. The pattern of LD across the genome was very irregular. The authors suggest that a larger number of SNPs are required to capture the LD needed for successful genomic selection or QTL mapping, and also for better assessment of the pattern of LD along the chromosomes.



16

An Assessment of Population Structure in Eight Breeds of Cattle Using a Whole Genome SNP Panel

BMC Genetics, May 2008, Volume 9, Number 37, pages 1-9.

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Examination of DNA sequences can establish relationships between individuals and populations. Earlier efforts to determine the origins of and relationships between different breeds of cattle have studied differences in mitochondrial DNA or segments of nuclear DNA with repetitive sequences (microsatellites) that are subject to high rates of mutation. In the present study, genetic relationships between 8 different breeds of cattle were evaluated by profiling the occurrence of 2,641 single nucleotide polymorphisms (SNPs) across the bovine genome. The largest differences in SNP occurrences were found when comparing *Bos taurus* with *Bos indicus* breeds. Within the *Bos taurus* breeds, significant differences were found between beef versus dairy breeds and between European versus Asian breeds. Population structure among the breeds was constructed by determining linkages between SNPs. The authors conclude that a large number of SNPs are required to correctly identify the genome of an individual with a particular breed.

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17

Differential Gene Expression of High and Low Immune Responder Canadian Holstein Dairy Cows

Developments in Biologicals: Animal Genomics for Animal Health, June 2008, Volume 132, Number 1, pages 315-320.

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It would be advantageous for animal breeders to be able to selectively breed for animals with high resistance to disease. However, there are currently no measurable criteria on which to base such selection decisions. The objective of this study was to identify genes whose level of expression might serve to identify cows that express a greater response to an immune challenge. Holstein cows were immunized to provoke both an antibody-mediated immune response (AMIR) and a cell-mediated immune response (CMIR). The strength of the AMIR was evaluated by measuring blood antibody concentrations; the CMIR was assessed using an intradermal hypersensitivity test. For each response type, cows were classified as high (HR), average (AR) or low (LR) responders. Blood mononuclear cells (BMC) were isolated from each group and messenger RNA (mRNA) was extracted from the cells. An array of DNA sequences representing genes known to be involved in the immune response was used to detect the abundance of corresponding mRNA sequences in these extracts. Several significant differences in gene expression were found between the LR and HR cows in both AMIR and CMIR categories.



18

Genotype-Specific IL8RA Gene Expression in Bovine Neutrophils in Response to *Escherichia coli* Lipopolysaccharide Challenge

Animal Genetics, June 2008, Volume 39, Number 3, pages 298-300.

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In the context of mammary gland health in the lactating dairy cow, somatic cells (SC) are white blood cells (WBC) that are released through the lining of the gland in response to infection. One type of WBC, the neutrophils, are recruited to the site of infection in response to the release of signalling molecules (cytokines) by the epithelial cells that line the gland. When the infection is caused by gram-negative bacteria, a component of the bacterial cell wall (lipopolysaccharide, LPS—also referred to as endotoxin) is recognized by molecules on the surface of epithelial cells. In response, the epithelial cells secrete interleukin-8 (IL-8), a cytokine that strongly attracts CXCR1, a protein on the cell surface of neutrophils. In previous work, the authors of this study found 4 different variants of the gene which codes for the synthesis of CXCR1, due to the presence of variations in base pairs (single nucleotide polymorphisms, SNPs) at particular locations in the gene's DNA code. They also found correlations between the presence of these SNPs and estimated breeding values for SC scores among a sample of 338 Holstein bulls. The objective of the present study was to compare responses to LPS in neutrophils from blood samples from cows carrying the 3 different genotypes resulting from alternative base pair combinations at one of these SNPs. It was found that, based on the abundance of messenger RNA produced, one particular genotype did produce a much stronger response than the others. These findings add support to the possibility of selecting animals for their ability to elicit a stronger immune response to infection by gram-negative bacteria such as *Escherichia coli*.



19

Functional Characterization of a Single Nucleotide Polymorphism in the 5' UTR Region of the Bovine Toll-like Receptor 4 Gene

Developments in Biologicals: Animal Genomics for Animal Health, June 2008, Volume 132, Number 1, pages 331-336.

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Bacterial infections of bodily tissues are rapidly detected by both the tissue cells and tissue-associated immune cells. Molecules in the outer membranes of these body (somatic) cells recognize and bind molecules on the surfaces of pathogenic bacteria. This binding results in the release of signalling molecules (cytokines) that recruit other immune cells to the site of infection. When gram-negative bacteria infect the mammary gland, lipopolysaccharide (LPS) molecules in their outer membranes are recognized and bound by the somatic cell surface protein, toll-like receptor 4 (TLR4). In previous work, the authors of this study discovered the existence of 2 different variants of the gene that codes for TLR4, due to the presence of alternative base pairs at one specific site (single nucleotide polymorphism, SNP) in the DNA code. The present study was designed to compare TLR4 gene expression in response to an LPS challenge in white blood cells from cows carrying the 2 different versions (genotypes) of the SNP. Significant differences were found in the abundance of messenger RNA produced by the 2 genotypes, suggesting that the SNP variants affected the regulation of TLR4 gene expression. The results add support to the idea that genetic selection for specific genotypes might affect susceptibility or resistance to infection by gram-negative pathogens.



20

Identification of Polymorphisms in Bovine TLR2 and CARD15, Associations between CARD15 Polymorphisms and Milk Somatic Cell Score in Canadian Holsteins, and Functional Relevance of SNP c.3020A>T

Developments in Biologicals: Animal Genomics for Animal Health, June 2008, Volume 132, Number 1, pages 247-253.

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The inflammation seen in infectious diseases such as mastitis, metritis, laminitis and Johne's Disease is the result of a series of well-ordered responses by the immune system. The first step is the recognition of molecules on the surface of the pathogen by receptors in tissue-associated immune cells. For example, caspase recruitment domain 15 (CARD15) is an intracellular receptor that recognizes a molecule (muramyl dipeptide, MDP) in the bacterial cell wall. Likewise, toll-like receptor-2 (TLR2) is a receptor that recognizes cell wall components of gram-positive bacteria. Recognition leads to the secretion of signalling molecules (cytokines) that recruit immune system cells to the site of infection, resulting in inflammation and engulfment of the pathogen by white blood cells (WBC). WBC that are released into the mammary gland of the dairy cow are referred to as somatic cells (SC). This sequence of events operates more effectively in some cows than in others, resulting in individuals being more or less resistant to chronic infection. To some extent resistance is controlled by genetics and an effort is being made to identify and breed for resistant genotypes. The objective of this study was to identify variations (single nucleotide polymorphisms, SNPs) in the genes that code for TLR-2 and CARD 15 in order to evaluate relationships between the presence of these SNPs and health and production traits in lactating cows. No SNPs were identified in the TLR-2 gene but 4 were identified in the CARD 15 gene, 2 of which were found to be associated with estimated breeding values (EBVs) for SC score or milk and milk protein yields.



21

Genetic Improvement of Total Milk Yield and Total Lactation Persistency of the First Three Lactations in Dairy Cattle

Journal of Dairy Science, June 2008, Volume 91, Number 6, pages 2836-2843.

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As statistical methods improve and computing power increases, it becomes possible to introduce new, more accurate genetic calculations using more data than has been possible in the past. Currently, Canadian national genetic evaluations of dairy cattle are based on a test day model which calculates estimated breeding values (EBVs) for milk, fat and protein yields as well as somatic cell score for each of 3 lactations. This paper compares the efficiency of various selection criteria in terms of genetic gain in both total milk and total persistency of the first three lactations. The use of multiple-lactation test day animal model does not prolong the generation interval as compared with the use of only the first lactation EBV. The results suggest the use of first three-parity selection index for genetic improvement of total economic returns comprising of both total milk and total persistency.

22

High Density Linkage Disequilibrium Maps of Chromosome 14 in Holstein and Angus Cattle

BMC Genetics, July 2008, Volume 9, Number 45, pages 1-12.

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Genes that carry the genetic code defining the specific characteristics of an individual exist as a pair of alleles, one allele on each of 2 paired chromosomes. In most cases, each allele can carry the code for 1 of 2 different versions of a DNA sequence (e.g., A or B). Therefore, there are 4 allele combinations possible for each gene - AA, BB, AB or BA. Alleles of other genes can match up in the same way, so a gene coding for a second characteristic, located somewhere else on the genome, could be CC, DD, CD or DC. If there were no linkages between these 2 genes, the probability of each of the 4 possible combinations occurring for each gene would be 0.25 (1 in 4) 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). This study determined the locations of genes in LD on chromosome 14 from Holstein and Angus cattle. The genes were identified by the presence of single nucleotide polymorphisms (SNPs) within their DNA sequences. Understanding which areas of the genome are in high LD will assist in the identification of genes underlying economically relevant traits.



23

A Whole-Genome Scan to Map Quantitative Trait Loci for Conformation and Functional Traits in Canadian Holstein Bulls

Journal of Dairy Science, July 2008, Volume 91, Number 7, pages 2844-2856.

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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 the association between QTL and 1,536 SNPs on the bovine genome. Forty-five SNPs were found to be associated with the QTLs of 17 conformational traits; 151 SNPs were associated with functional trait QTLs. Of these 196 SNPs, 169 of them were newly detected in this study; 27 have been previously reported. The authors suggest that 161 of these SNPs were located in genes but are unlikely to be responsible for differences in expression of the traits with which they are associated. They recommend further investigation to potentially identify the mutations underlying differences in quantitative trait expression.



24

A Genome Scan to Detect Quantitative Trait Loci for Economically Important Traits in Holstein Cattle Using Two Methods and a Dense Single Nucleotide Polymorphism Map

Journal of Dairy Science, August 2008, Volume 91, Number 8, pages 3225-3236.

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Quantitative traits are those traits whose level of expression (e.g., milk yield) are influenced by a large number of genes. 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 may be associated with differences in single bases (nucleotides) in the DNA sequences of the genes influencing the traits, which are known as single nucleotide polymorphisms (SNPs; pronounced 'snips'). The objective of this study was to identify QTL for milk, fat and protein yields; somatic cell score; herd life; interval from calving to first service; and age at first service, using estimated breeding values (EBVs) of 484 Holstein sires genotyped for around 10,000 SNPs. Two methods were used, the first method identified 102 potential QTL and the second method found 144 SNPs associated with the traits examined. Both methods found QTL for milk yield on chromosomes 3, 5 and 16; for fat yield on 14 and 19; for protein yield on 1, 3, 16 and 28; for calving to first service on 2 and 13 and for age at first service on chromosome 14.



25

Bovine CD14 Gene Characterization and Relationship Between Polymorphisms and Surface Expression on Monocytes and Polymorphonuclear Neutrophils

BMC Genetics, August 2008, Volume 9, Number 50, pages 1-11.

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In response to infection, host cell recognition molecules bind with molecules presented by the pathogen to elicit an immune response. CD14 is a recognition protein found in both the membrane and cytoplasm of monocytes and neutrophils, white blood cells that function as part of the innate immune system. Cytoplasmic CD14 is also found in cells lining the intestine and mammary gland. When a gram-negative pathogen infects the mammary gland, a molecule in its outer cell wall (lipopolysaccharide, LPS - also referred to as endotoxin) is recognized by CD14. CD14/LPS binding results in the release of messenger molecules (cytokines) which recruit other immune cells to the site of infection in a concerted effort to destroy the pathogen. It has been speculated that variations in CD14 binding may be responsible for the variation seen among cows in their responses to intramammary infection. The objective of this study was to investigate the possibility that variations in the gene that codes for CD14 might result in amino acid sequence variations in CD14 that could affect its binding to LPS. Examination of the DNA sequences in the CD14 genes of 106 Canadian Holsteins and 46 Jersey cows revealed variations (single nucleotide polymorphisms, SNPs) at 5 different locations. However, only one of these SNPs affects the amino acid sequence in the CD14 protein. The authors speculate that this substitution may particularly affect the expression of CD14 in the cell membranes of neutrophils. Examination of the promoter region of the CD14 gene suggested that expression of the gene may be regulated in a number of different ways.



26

Comparison of Random Regression Models with Legendre Polynomials and Linear Splines for Production Traits and Somatic Cell Score of Canadian Holstein Cows

Journal of Dairy Science, September 2008, Volume 91, Number 9, pages 3627-3638.

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The Canadian test-day model (CTDM) incorporates the statistical calculations used to derive estimated breeding values (EBVs) for dairy animals from test day data recorded by Dairy Herd Improvement organizations. The current CTDM analyzes milk, fat and protein yield as well as somatic cell score (SCS) from the cow's first 3 lactations, recorded from 5 to 305 days in milk (DIM). However, because about 60% of cows are still lactating beyond 305 DIM, the inclusion of data from later test days might improve the accuracy of EBV calculations. The objective of this study was to evaluate alternative statistical methods that might be used in the CTDM for calculating EBVs from test data extending up to 365 DIM. Whereas current and previous methods have been based on fitting continuous mathematical functions to data, this study examined the application of linear splines. In this technique, a number of linear segments (splines) joined by 'knots' are fitted to sequential segments of the test day yield and SCS data. The spline methods yielded predictions which had lower deviations from the observed data at either end of the 305-365 day lactations compared with the current calculation. A spline model with 7 segments joined by 6 knots gave the best performance.

Health





1

Helenalin Reduces *Staphylococcus aureus* Infection In Vitro and In Vivo

Veterinary Microbiology, January 2007, Volume 119, Number 2-4, pages 330-338.

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Staphylococcus aureus (SA) is one of the most problematic causes of bovine mastitis. When cells lining the mammary gland (epithelial cells) detect the presence of SA, they respond with the production of signalling molecules (cytokines) such as tumour necrosis factor-alpha (TNF- α). These cytokines recruit white blood cells (WBC) to the site of infection resulting in inflammation. While the role of the WBC is to engulf and destroy the pathogen, in the case of SA, the inflammatory response actually enhances bacterial viability. The authors of this study hypothesized that treatment with an anti-inflammatory agent might reduce the severity of SA infection. Helenalin (HLN) is one of a number of natural products of medicinal plants that have anti-inflammatory properties. In an initial experiment, bovine mammary epithelial cells grown in culture with or without TNF- α were treated with HLN. HLN significantly reduced the growth of SA. In a second trial, the mammary glands of lactating mice were infected with SA with or without HLN treatment. Again, HLN treatment reduced the proliferation of SA. Examination of mammary gland tissue indicated that HLN also reduced the number of WBC that were recruited in response to SA infection. The authors speculate that HLN may have potential in the treatment of bovine mastitis due to SA infection.



2

Protective Immune Responses to a Multi-Gene DNA Vaccine Against *Staphylococcus aureus*

Vaccine, January 2007, Volume 25, Number 5, pages 814-824.

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Staphylococcus aureus (SA) is one of the most troublesome pathogens causing intramammary infection (IMI) in dairy cattle. Several SA vaccines based on the use of bacterial extracts or purified antigens have been developed. However, their use has been restricted because of their limited efficacy, high production costs and stability. Research by these authors has focussed on the development of DNA vaccines which use the animal's own synthetic machinery to produce a modified version of specific bacterial virulence proteins (VPs). A fragment of DNA (gene) which codes for the protein is attached to a loop of bacterial DNA (a plasmid). The plasmid serves as a vehicle (vector) to carry the VP gene into host animal cells which subsequently synthesize the VP. The immune system responds by producing antibodies to the VP and activating the mechanisms responsible for cellular immunity. This paper describes the development of a DNA vaccine against SA incorporating a combination of 3 VP genes. When used to immunize mice, all animals produced a strong immune response, including the production of antigen (VP)-specific antibodies. Fifty-five percent of immunized mice survived a challenge with a virulent strain of SA compared with only 15% of non-immunized mice. Another group of immunized mice were unaffected when challenged with a strain of SA that caused arthritis after administration to control animals. The authors conclude that this multi-gene DNA vaccine was effective in protecting against the two different types of infection caused by SA.

3

The Effect of Season on Somatic Cell Count and the Incidence of Clinical Mastitis

Journal of Dairy Science, April 2007, Volume 90, Number 4, pages 1704-1715.

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The objective of this study was to characterize seasonal variation in bulk milk somatic cell count (BTSCC), individual cow SCC (ICSCC) and incidence rate of pathogen-specific clinical mastitis (IRCM). Data were collected from 300 Dutch dairy farms over a 4-year period. BTSCC peaked in August and September each year. While the probability of cows getting a high ICSCC was highest in August, the probability of maintaining a high ICSCC was highest in May. IRCM was highest in December to January for all pathogens but *Strep. uberis* which was highest in August, apparently associated with being on pasture. Herds that had no access to pasture had higher *E. coli* IRCM in summer than in winter. Seasonal differences in IRCM were smaller for minor pathogens than for the major ones.



4

Lack of Protection of Mice Against *Staphylococcus aureus* Despite a Significant Immune Response to Immunization with a DNA Vaccine Encoding Collagen-Binding Protein

Vaccine, June 2007, Volume 25, Number 27, pages 5053-5061.

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Staphylococcus aureus (SA) is one of the most common pathogens causing mastitis in dairy cattle. SA is a particularly difficult pathogen to control because of its many different strains and the ability of individual strains to express different virulence proteins (VP) under varying conditions. One class of VP that is essential in the establishment of many SA infections is the group of surface adhesins which facilitate the binding of SA cells to host tissues. Collagen-binding protein (CNA) is the main adhesin responsible for attachment to collagen. Based on this knowledge, the present study was designed to test the efficacy of a CNA-based DNA vaccine against SA. DNA vaccines use the animal's own synthetic machinery to produce a modified version of specific bacterial virulence proteins (VPs). A fragment of DNA which codes for the VP is attached to a loop of bacterial DNA (a plasmid). The plasmid serves as a vehicle (vector) to carry the VP gene into host animal cells which subsequently synthesize the VP. The immune system responds by producing antibodies to the VP and activating the mechanisms responsible for cellular immunity. A CNA-based DNA vaccine administered to mice provoked a strong antibody response and evidence of a cell-mediated immune response. However, the vaccine not only failed to protect the mice when they were challenged with an intraperitoneal injection of SA but appeared to promote the spread of infection.



5

Effect of Herd Characteristics, Management Practices, and Season on Different Categories of the Herd Somatic Cell Count

Journal of Dairy Science, September 2007, Volume 90, Number 9, pages 4137-4144.

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In a previous study, these authors determined that the arithmetic average of individual cow somatic cell counts (HSCC) was a better indicator of a herd's subclinical mastitis situation than either bulk milk SCC or the average of individual yield-corrected SCC. The objective of the present study was to evaluate effects of herd characteristics, management practices and season on HSCC among 246 Dutch dairy herds. Herds were categorized into 3 HSCC groups: low (less than 150,000 cells/ml), medium (150,000 - 200,000 cells/ml) and high (more than 200,000 cells/ml). Management practice data were derived from herd manager responses to 3 questionnaires administered by the researchers. None of the variables examined were associated with HSCC across all 3 HSCC categories but associations were found within categories. Dry pre-milking treatment was associated with lower HSCC in the low HSCC category; feeding calves high SCC milk was associated with high HSCC in the medium HSCC category. Average parity of cows correlated with lower HSCC in the low HSCC category but higher HSCC in the medium category. Feeding calves with fresh milk was associated with higher HSCC in low category herds but lower HSCC in high category herds. The authors suggest that, given the differences found in associations across the 3 HSCC categories, care must be taken in drawing general conclusions about the effects of herd characteristics and management changes on individual farms.

6

Comparison of Bulk Milk, Yield-Corrected, and Average Somatic Cell Counts as Parameters to Summarize the Subclinical Mastitis Situation in a Dairy Herd

Journal of Dairy Science, September 2007, Volume 90, Number 9, pages 4145-4148.

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Although bulk tank milk somatic cell count (BMSCC) is often used as an indicator of the subclinical mastitis in a herd, it can yield erroneous results when milk from cows with high SCC is withheld from the bulk tank. The objective of this study was to identify a better indicator which correlates well with the percentage of cows in the herd having SCCs greater than 250,000 cells/ml. Two alternative measures were examined: the arithmetic average test-day SCC (HSCC) and the yield-corrected average test-day SCC (CHSCC) across all cows. Test-day milk recording and BMSCC data were collected from 300 Dutch farms. Correlations between % of cows having SCCs greater than 250,000 cells/ml and BMSCC, CHSCC or HSCC were 0.80, 0.88 and 0.94, respectively. The authors conclude that the simple arithmetic average test-day SCC (HSCC) is the best of the 3 parameters to summarize the average herd subclinical mastitis situation in a dairy herd.



7

Effect of Precalving Intramammary Treatment with Pirlimycin in Nulliparous Holstein Heifers

Canadian Journal of Veterinary Research, October 2007, Volume 71, Number 4, pages 283-291.

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The results of several studies have indicated significant rates of intramammary infection (IMI) in heifers during the pre-calving period. The objective of the present study was to evaluate the efficacy of antibiotic treatment in this period on the incidence of IMI in early lactation and on 305-day first lactation milk yield. Quarter milk samples were drawn from 428 Holstein heifers between 6 and 12 days before their expected calving dates for evaluation of IMI. IMI was found in 69% of the heifers and 33% of the quarters sampled. *Staphylococcus aureus* (SA) was cultured from 10% of heifers and 3% of quarters. After sampling, 219 of the heifers were treated with a dose of the antibiotic pirlimycin in each quarter; the remaining 209 heifers were untreated, serving as controls. Two days after calving, quarters of all animals were sampled again. IMI rates at this time were 45% for control heifers; 31% for treated heifers. SA accounted for 10% of the IMIs in the control group; 5.6% in the treated group. Although antibiotic treatment reduced the post-calving incidence of IMI due to gram-positive pathogens, the incidence of new IMIs caused by yeasts and gram-negative bacteria was higher in the treated heifers. Overall, treatment did not affect milk production or somatic cell counts on the first 3 post-partum test days. However, heifers who were treated more than 1 week before calving produced, on average, 302 kg more milk in the first 305 days of their first lactations.



8

Comparative Analysis of Innate Immune Responses Following Infection of Newborn Calves with Bovine Rotavirus and Bovine Coronavirus

Journal of General Virology, October 2007, Volume 88, Number 10, pages 2749-2761.

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Bovine rotavirus (BRV) and bovine coronavirus (BCV) frequently infect neonatal calves, causing diarrhea and death. These 2 viruses belong to different virus families and have different courses of infection. BRV is a non-enveloped, double-stranded RNA virus that normally causes acute infections of short duration in neonates; BCV is an enveloped, single-stranded RNA virus that can cause more chronic infections. Given these differences between the 2 viruses and the infections they produce, the authors of this study postulated that the pattern of innate immune responses to each might be different. To examine this hypothesis they isolated 4 short loops of small intestine in each of 6, 1-day old male Holstein calves, then infected 2 of the 4 with either BRV or BCV—the other 2 loops in each calf served as controls. Eighteen hours after infection, tissue samples were taken for analysis of immune response by examining their messenger RNA (mRNA) profiles using complementary DNA microarrays and quantitative real-time polymerase chain reaction. mRNA profiles, in tissues infected with BRV, indicated stronger activation of several immune system components (toll-like receptor 3, interleukin-6, the p65 subunit of the transcription factor NF- κ B) than in tissues infected with BCV. While both viruses showed no significant differences in cell cycle regulation of host system following infection they evolved to elicit different innate immune responses.



9

Somatic Cell Count in Milk of Selenium-supplemented Dairy Cows after an Intramammary Challenge with *Staphylococcus aureus*

Journal of Veterinary Medicine Series A, November 2007, Volume 54, Number 9, pages 478-483.

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Selenium (Se) has important roles in the immune response to infection and, therefore, to an individual's disease resistance. Se deficiency in dairy cattle has been associated with retained placenta, infertility, reduced milk production and impaired growth. These effects are related to the role of Se in the activation of the antioxidant enzyme glutathione peroxidase (GPx1). Inadequate activity of GPx1 results in the impaired ability of white blood cells to fight infection. The objective of this study was to characterize responses of lactating cows to Se deficiency in terms of somatic cell scores (SCS) and blood GPx1 activity. Twelve multiparous Holstein-Friesian cows were fed a Se-deficient diet starting 2 months before scheduled calving. At 45 days before calving, 6 cows received a single intramuscular injection of barium selenate at a dose of 1 ml per 50 kg of body weight; the other 6 received no supplemental Se. Blood samples were collected at 15 day intervals from the day of Se injection until 150 days in milk (DIM). At 140 DIM, 2 mammary quarters of each cow were challenged with an infusion of *Staph. aureus* (SA) after which milk samples were drawn aseptically for 7 days. Se-supplemented cows had higher blood GPx1 activities throughout the experiment but lower SCS after the SA challenge. The authors conclude that a single dose of barium selenate given 45 days pre-partum was effective until at least 150 DIM.



10

Factors Affecting Canadian Veterinarians' Use of Analgesics when Dehorning Beef and Dairy Calves

Canadian Veterinary Journal, November 2007, Volume 48, Number 11, pages 1129-1136.

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Dairy calves are dehorned to prevent injury to their handlers or to other animals. Because dehorning by any means is clearly painful to the calf, producers and veterinarians are being encouraged to use analgesic (pain-killing) drugs (AD) during the procedure. This paper describes a Canada-wide survey of veterinarians designed to determine their attitudes toward and use of AD when dehorning beef and dairy calves. Results of the survey indicated that veterinarians are more likely to be AD users the more they perceived that dehorning was painful to calves. Veterinarians working in British Columbia or Alberta were 5.9 times more likely to use AD than those in other provinces. The authors attribute this observation to the higher level of animal welfare outreach in the western provinces. Those primarily in dairy practice were 3.7 times more likely to use AD than those primarily in beef practice, except for those veterinarians who perceived that their dairy clients were unwilling to incur the additional cost of AD. Overall, responses to the survey suggest that use of AD by veterinarians is heavily influenced by concerns about cost. The authors suggest, however, that the use of AD is not expensive and that continuing education should help to alleviate veterinarians' reluctance to adopt the routine use of AD when dehorning calves.



11

Calf-Level Risk Factors for Neonatal Diarrhea and Shedding of *Cryptosporidium parvum* in Ontario Dairy Calves

Preventive Veterinary Medicine, November 2007, Volume 82, Number 1-2, pages 12-28.

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Cryptosporidium parvum (CP) is an intestinal parasite that infects calves in the first few weeks of life, often causing diarrhea or death. The infection can be detected by examining feces for CP oocysts (eggs) or by using an antibody test to measure CP antigen. This study was designed to identify management factors associated with increased risk of CP oocyst shedding and diarrhea in dairy calves. Four weekly fecal samples were collected from each of 919 calves under 30 days of age on 11 Ontario dairy farms that had a history of diarrhea or CP infection (cryptosporidiosis). Samples were examined for the presence of CP oocysts; their consistency was used to assess diarrhea. Farm managers completed a questionnaire designed to provide information about calf management practices. CP oocysts were detected in the feces of 78% of the calves; diarrhea was diagnosed in at least one of the 4 samples taken from 73% of the calves. Management factors that significantly decreased the risk of calves shedding CP oocysts were the treatment of dams with calf scour prevention medications and the inclusion of coccidiostats in calf feed. Calves born in the summer and those left with their dams for more than an hour after birth had an increased risk of diarrhea. Calves shedding CP oocysts were 5.3 times as likely to experience diarrhea than calves that were not shedding.



12

Prevalence and Distribution of Mastitis Pathogens in Subclinically Infected Dairy Cows in Flanders, Belgium

Journal of Dairy Research, November 2007, Volume 74, Number 4, pages 478-483.

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This study was designed to determine the prevalence of intramammary infections (IMI) and their causative pathogens in Flanders, Belgium. Data were obtained from quarter milk samples collected during 3 consecutive annual bacteriological screenings of dairy herds with an AA-milk designation. Seventeen percent of quarters were culture-positive while 41% of cows tested had at least 1 culture-positive quarter. Fifty-seven percent of the pathogens identified were non-aureus staphylococci, 18% were *Staphylococcus aureus* and 16% were aesculin-positive cocci; only 0.3% were *Streptococcus agalactiae*. Among 6,390 cows having a geometric mean composite somatic cell count during the last three monthly test-days greater than 250,000 cells/ml, nearly 65% had at least one culture positive quarter. Non-aureus staphylococci were identified in 41% of quarter cultures from these cows; *Staphylococcus aureus* were isolated from 25%; aesculin-positive cocci were found in 18%.



13

Exogenous Glucagon Effects on Health and Reproductive Performance of Lactating Dairy Cows with Mild Fatty Liver

Animal Reproduction Science, December 2007, Volume 102, Number 3-4, pages 194-207.

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Post-partum cows often experience an accumulation of liver lipid (fatty liver, FL), thought to be due to the mobilization of adipose lipid during a period of negative energy balance. The objective of this study was to determine the health and reproductive effects of mild FL and to test whether glucagon administration could alleviate these detrimental effects. The liver lipid status of 32 multiparous Holstein cows was assessed at 8 days post-partum. Twenty-four cows whose liver triglyceride levels exceeded 1% of liver biopsy wet weight were assigned to an FL-susceptible group; the remaining 8 cows were assigned to a control group. Control cows and 8 FL-susceptible cows received saline subcutaneously every 8 hours for 14 days. Two other groups of 8 FL-susceptible cows received either 2.5 mg or 5 mg of glucagon in saline subcutaneously on the same schedule. FL-susceptible cows had an increased number of days with elevated body temperature during the injection period, increased incidence of mastitis after glucagon treatment, increased days to first estrus and insemination, increased days to conception, and decreased conception rate. Subcutaneous administration of 5 mg of glucagon at 8 hour intervals decreased the number of days with elevated body temperature and the incidence of mastitis after treatment.

14

An Investigation into the Practices of Dairy Producers and Veterinarians in Dehorning Dairy Calves in Ontario

Canadian Veterinary Journal, December 2007, Volume 48, Number 12, pages 1249-1254.

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Dehorning dairy calves is considered necessary to reduce the risk of injury to both the people handling the cattle and other animals. Several procedures can be used but it is clear that all inflict pain on the calf. While the 1990 Canadian Code of Practice for dairy cattle recommends the use of anaesthetic when dehorning, the revised Code will likely propose the practice as a requirement. The objectives of this study were to determine the prevalence of anaesthetic use when dehorning among Ontario dairy farmers and veterinarians and to identify ways to increase their use. Of the 207 farmers surveyed, 78% dehorned their own calves; 22% of these used local anaesthetic; 13% were not aware that pain relief was an option when dehorning. On average, the 65 veterinarians who completed the survey dehorned calves for 31% of their dairy clients, using local anaesthetics 92% of the time. Both farmers and veterinarians indicated that they used anaesthetics primarily for pain management. Among farmers, the main reasons given for not using anaesthetics were time and cost; for veterinarians time was the main consideration. The authors conclude that veterinarians should take the lead in educating producers about the advantages of using pain relief when dehorning.



15

Association Between Management Practices and Within-Herd Prevalence of *Cryptosporidium parvum* Shedding on Dairy Farms in Southern Ontario

Preventive Veterinary Medicine, January 2008, Volume 83, Number 1, pages 11-23.

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Cryptosporidium parvum (CP) is an intestinal protozoan parasite that causes diarrhea and sometimes death in calves that are typically 1 to 4 weeks of age. Infection spreads when CP oocysts (eggs) are excreted by infected calves then ingested by pen mates. The objective of this study was to identify management practices associated with the prevalence of CP shedding in Ontario dairy farms. Fecal samples collected from 1,089 1-4 week-old calves in 119 herds were examined for the presence of CP oocysts. Herd managers completed a questionnaire about their management practices. CP oocysts were found in fecal samples from 30% of the calves; 77% of the herds had a least one calf that was shedding. Within individual herds, the proportion of calves that were shedding CP oocysts ranged from 0 to 80%. Management factors that increased the prevalence of shedding within herds included treatment of either dams or calves with calf scour prevention medications and feeding milk replacer in the first week of life. Concrete floors in calf housing areas and the use of soap or detergent in washing calf feeding equipment reduced the risk of shedding.



16

Radiographic Detection of Thoracic Lesions in Adult Cows: A Retrospective Study of 42 Cases (1995–2002)

Canadian Veterinary Journal, March 2008, Volume 49, Number 3, pages 261–267.

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X-ray imaging (radiography) can be used to detect lesions in the heart, lungs and lower trachea (thoracic region) of cattle. This study was designed to determine the reliability of thoracic radiography (TR) in correctly diagnosing thoracic lesions by comparing radiographic findings with lesions found on post-mortem examination (necropsy). The records of cows that had been subject to both TR and necropsy within a period of 7 days were reviewed. Although 27 of the cows had clinical signs of respiratory disease, 40 had visible thoracic lesions at necropsy. Twelve cows showed clinical signs of heart problems. TR correctly identified cows with respiratory lesions in 92% of cases (positive predictive value) but in only 57% of cases did TR correctly identify cows with no visible lesions at necropsy (negative predictive value). To assist in the interpretation of TR of cows, the authors created an evaluation grid which facilitates standardization in the reading of bovine thoracic radiographs.



17

Comparison of Flow Cytometry and Immunofluorescence Microscopy for the Detection of *Giardia duodenalis* in Bovine Fecal Samples

Journal of Veterinary Diagnostic Investigation, March 2008, Volume 20, Number 2, pages 178-185.

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Giardia duodenalis (GD) is a protozoan parasite that infects the small intestines of most domestic and wild animals. It is commonly found in adult dairy cattle and GD cysts shed in manure can infect both humans and other animals. Accurate estimates of fecal GD cyst numbers are important for estimating prevalence and infectivity. This study compared 2 methods for detecting GD in fecal samples from 36 dairy cows and 208 calves. The method most commonly used involves concentration fecal cysts by flotation and centrifugation followed by detection with conventional or immunofluorescence microscopy (IM). Both methods require the manual counting of cysts. IM was compared with flow cytometry (FC) which, after initial sample preparation, automatically detects and counts particles suspended in a stream of fluid as they pass through a beam of light. IM detected GD in 69.7% and 48.1% of cows and calves, respectively. GD detection by FC depended upon equipment settings required to discriminate GD cysts from other suspended particles. The authors conclude that FC is less sensitive than IM for detection of fecal GD cysts.



18

Mammary Tissue Damage During Bovine Mastitis: Causes and Control

Journal of Animal Science, March 2008, Volume 86, Number 13, pages 57-65.

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Intramammary infection (IMI) by bacterial pathogens is the primary cause of mastitis in dairy cows. Mastitis is a costly disease due to the costs of treatment, the value of discarded milk during infection and the long-term damage caused to milk secretory tissue. This paper reviews current knowledge about mammary tissue damaged caused by IMI. Tissue damage caused by pathogens and by the cow's own immune response to infection reduces both the number and the activity of secretory cells lining the mammary gland (epithelial cells, EC). Initial damage is due to EC membrane destruction by bacterial toxins and to invasion of bacteria into EC followed by multiplication and EC death. Recognition of pathogens by EC and tissue-associated white blood cells results in an influx of immune cells (neutrophils) at the site(s) of infection. Neutrophils release enzymes and reactive oxygen compounds in an effort to destroy the pathogen but, in doing so, also cause damage to mammary tissue. Breakdown of the blood-milk barrier and the extracellular matrix supporting alveolar structure leads to further damage and death of EC. A number of studies suggest that treatment of mastitis with antioxidants and other protective medications may reduce damage to EC and mitigate future milk loss.



19

Bacterial Lipopolysaccharide Induces Increased Expression of Toll-Like Receptor (TLR) 4 and Downstream TLR Signaling Molecules in Bovine Mammary Epithelial Cells

Veterinary Research, March 2008, Volume 39, Number 2, pages 11-22.

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Most cases of dairy cow mastitis are caused by bacterial intramammary infection (IMI). When a pathogen enters the mammary gland, molecules on its cell surface are recognized by receptor molecules on and in host (cow) cells lining the gland, including epithelial cells and tissue-associated immune cells. Toll-like receptor-4 (TLR-4) is one of those receptors, specifically recognizing lipopolysaccharide (LPS, also called endotoxin) associated with the outer cell membrane of gram-negative bacteria. Binding of LPS by TLR-4 initiates a chain of signalling reactions culminating in the recruitment of white blood cells (WBC) whose role is to destroy the pathogen with enzymes and reactive oxygen compounds. To limit damage to host tissues, a negative feedback mechanism is initiated which modulates the response to LPS/TLR-4 binding. Toll interacting protein (TOLLIP) and caspase 8 (CASP8) are two molecules that are involved in these processes. This study examined the expression of these components of the innate immune system in response to LPS challenge in mammary epithelial (MAC-T) cells maintained in tissue culture. Exposure to LPS provoked increased transcription of genes coding for both TLR-4 and TLR-2, another similar receptor. Levels of both proteins on MAC-T cell surfaces were also increased. Production of signalling molecules involved in the WBC recruitment pathways responded to the increases in TLR synthesis as did production of TOLLIP and CASP8.



20

Utilization of Lactoferrin to Fight Antibiotic-Resistant Mammary Gland Pathogens

Journal of Animal Science, March 2008, Volume 86, Number 13, pages 66-71.

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Lactoferrin (Lf) is an iron-binding protein, produced by the mammary gland, that has both antibacterial and anti-inflammatory activities. Its antibacterial activity is thought to be mainly due to its sequestration of the iron which is essential to microbial growth. However, it has also been found that Lf enhances the efficacy of penicillin (PEN) 4-fold in treating infections by PEN-susceptible strains of *Staphylococcus aureus* (SA) and 4- to 16-fold in treating infections by PEN-resistant SA strains. PEN is one of a number of antibiotics that contain a β -lactam nucleus. These antibiotics inhibit the synthesis of bacterial cell walls by blocking the synthesis of a protein essential to their structure. Bacterial strains that are resistant to β -lactam antibiotics produce the enzyme β -lactamase which breaks down β -lactam, nullifying its effect. It is known that Lf reduces the activity of β -lactamase in resistant strains of SA by repressing the expression of the β -lactamase gene. The objective of this study was to test the synergistic effect of Lf in the treatment of SA mastitis with PEN. In a first experiment, lactating cows were infected in all 4 quarters of the mammary gland with a β -lactam resistant strain of SA. Quarters were then treated with saline (control), Lf, PEN or PEN plus Lf. Cure rates were 0, 11.1, 9.1 and 45.5%, respectively. In a second trial, cows with chronic SA infections from a previous lactation were treated with PEN or PEN plus Lf. Cure rates were 12.5% versus 33.3%, respectively. These results clearly demonstrate a potential application for Lf in treating cases of PEN-resistant SA mastitis.



21

Subacute Ruminal Acidosis in Dairy Cows: The Physiological Causes, Incidence and Consequences

The Veterinary Journal, April 2008, Volume 176, Number 1, pages 21-31.

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This paper reviews current knowledge about subacute ruminal acidosis (SARA) in dairy cows. SARA is characterized by extended periods of low rumen pH due to the excessive production of organic acids by rumen microbes, relative to the ability of salivary buffers to maintain stable rumen pH. Estimates of the incidence of SARA among early- and mid-lactation cows range from 19% to 26%. The primary cause of SARA is the consumption of large quantities of fermentable carbohydrates, usually starch contributed by the grain portion of the diet. The simultaneous consumption of fibrous feeds can mitigate pH depression through the secretion of salivary buffers in response to chewing and rumination. Low rumen pH for extended periods causes the death of fibre-digesting, gram-negative bacteria resulting in the release of endotoxin. Absorption of endotoxin into the bloodstream can result in inflammation in a number of tissues. Although endotoxin is clearly an important factor in this inflammatory response, results of studies where SARA has been experimentally induced indicate that other factors may also be involved. The direct consequences of the death of fibre-digesting microbes include reduced fibre digestibility, depressed feed intake and lower milk fat test. In addition, the systemic effects of SARA can lead to diarrhea, laminitis and liver abscesses.

22

Modelling Bovine Spongiform Encephalopathy

Journal of Agricultural Science, April 2008, Volume 146, Number 2, pages 183-194.

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This paper describes a simple mathematical model that simulates the spread of bovine spongiform encephalopathy (BSE, 'mad cow disease') following introduction into a cattle population. Earlier modelling attempts were limited by the lack of availability of a confirmatory test for BSE in individual animals. Three 'pools' are assumed in the present model, corresponding to susceptible, infected and sick animals. Model equations describe the spread of the disease from one pool to the next. Four factors were sufficient to describe the time course of a BSE epidemic: infectivity, time of introduction, delay before control begins and incubation period. Infectivity affects the rate of spread from susceptible through infected to sick. The predicted intensity of a BSE epidemic depends on the time of introduction and the delay before control is exercised. Incubation period is predicted to affect both the rate of one-way transfer between pools and the duration of the epidemic. When used to simulate the BSE epidemic in the UK, the model provided results similar to actual experience.



23

Incidence Rate of Clinical Mastitis on Canadian Dairy Farms

Journal of Dairy Science, April 2008, Volume 91, Number 4, pages 1366-1377.

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This paper reports the results of a Canada-wide study of the incidence rate of clinical mastitis (IRCM) and the association of pathogen-specific IRCM with bulk milk somatic cell count (BMSCC). A total of 3,149 cases of clinical mastitis were recorded among 106 dairy farms in 10 provinces over 1 year. *Staphylococcus aureus* (SA), *Escherichia coli* (EC), *Streptococcus uberis* (SU), and coagulase-negative staphylococci (CNS) were the most commonly identified pathogens. Average IRCM across all herds was 23.0 cases per 100 cow years, ranging from 0.7 to 97.4 within individual herds. No association was found between BMSCC and overall IRCM, but low and medium BMSCC herds had the highest EC and culture-negative IRCM and the lowest SA IRCM. While SA, SU and *Streptococcus dysgalactiae* (SD) IRCM were lowest in the Western provinces, SA and SD IRCM were highest in Québec. SA, SU, CNS and other streptococcal IRCM were higher while *Klebsiella* and EC IRCM were lower for cows in tie-stalls than for those housed in free stall facilities.

24

The Influence of Cow Factors on the Incidence of Clinical Mastitis in Dairy Cows

Journal of Dairy Science, April 2008, Volume 91, Number 4, pages 1391-1402.

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This study examined associations between incidence rates of clinical mastitis (IRCM) and cow factors, including season, monthly somatic cell counts (SCCs) and clinical mastitis (CM) history. Primiparous and multiparous cows were analysed separately. Two lactation stages were examined within each parity group: the first month of lactation and the second month onward. The statistical model employed was also used to predict the probability of a CM case being caused by a gram-positive (G+) or gram-negative (G-) pathogen, with quarter position included as an additional variable. Data were from 274 Dutch dairy herds, including 28,137 lactations of 22,860 cows collected over 18 months. A total of 5,363 CM cases were recorded, with 2,525 classified as either G+ or G-. IRCM varied between 0.0002 and 0.0074 for individual cows. Among the cow factors examined, previous CM cases, SCC in the previous month, and mean SCC in the previous lactation increased the IRCM in the current month of lactation. It was not possible to predict whether an individual case was caused by a G+ or a G- pathogen based on cow factors alone.



25

Comparison of 2-Step Laparoscopy-Guided Abomasopexy versus Omentopexy via Right Flank Laparotomy for the Treatment of Dairy Cows with Left Displacement of the Abomasum in On-Farm Settings

Journal of the American Veterinary Medical Association, June 2008, Volume 232, Number 11, pages 1700-1706.

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Left displaced abomasum (LDA) is a common disorder of dairy cows in early lactation, reducing productivity and increasing the cow's risk of being culled. Several alternative surgical procedures are used to correct the displacement and anchor the abomasum into its normal position. The most commonly used technique requires that an incision be made in the side or bottom of the body cavity, followed by anchoring the abomasum (abomasopexy) or the membranes attached to it (omentopexy) to the inside of the body wall. Potential complications include infection of the wound, herniation through the wound and generalized infection inside the body cavity (peritonitis). Alternatives include blind-tack suture or toggle pin applied through the skin. These methods are often unsuccessful because they are done without being able to visualize the abomasum. This paper describes a procedure for abomasopexy using a laparoscope, a tiny video camera at the end of a fibre optic cable which allows internal organs to be viewed. The laparoscope is used to guide the placement of sutures through the skin. This technique was compared with the commonly used omentopexy procedure. Success rates of the 2 procedures were similar in terms of the cows' appetites, comfort and milk yield 7 days post-surgery. After 60 days, milk yields and risks of relapse or being culled were also similar. The laparoscopic procedure was completed more quickly and required antibiotic support less frequently than the omentopexy procedure.



26

Estimate of the Direct Production Losses in Canadian Dairy Herds with Subclinical *Mycobacterium avium* Subspecies *paratuberculosis* Infection

Canadian Veterinary Journal, June 2008, Volume 49, Number 6, pages 569-576.

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Mycobacterium avium subspecies *paratuberculosis* (MAP) is the bacterium responsible for Johne's Disease (JD), a contagious, progressive infection that causes abnormal thickening of the lining of the intestinal tract, restricting the absorption of nutrients. Animals become infected as calves, through ingestion of contaminated manure. Although overt signs of the infection are rarely seen before 3-5 years of age, JD can cause significant production losses as it develops due to decreased milk production and reproductive efficiency; increased mortality and susceptibility to other diseases; and premature culling. The objective of this study was to determine the annual direct production losses for the Canadian dairy industry as a whole and for an average Canadian dairy herd infected with MAP. Estimates of MAP prevalence, derived from a survey of 373 dairy farms in 8 provinces, were combined with known impacts of subclinical MAP infection. For the Canadian dairy industry as a whole, the average economic loss per 100 cows was estimated to be \$1,196 annually (\$12/cow and \$385/MAP-positive cow), assuming that 3.1% of this group of 100 cows is MAP-positive. In an average Canadian MAP-positive dairy herd of 61 cows, the mean economic loss was estimated to be \$2,992 annually (\$49/cow and \$409/MAP-positive cow), assuming an average within-herd prevalence of 12.7%. Increased culling, decreased milk production, mortality and reproductive losses accounted for 46%, 9%, 16%, and 29% of the losses, respectively.



27

Relative Expression of Matrix Metalloproteinase-2 and -9 in Synovial Fluid from Healthy Calves and Calves with Experimentally Induced Septic Arthritis

American Journal of Veterinary Research, August 2008, Volume 69, Number 8, pages 1022-1028.

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Young calves are subject to joint infection, leading to inflammation (arthritis) and lameness, often resulting from colibacillosis, a systemic infection by *E. coli* (EC). EC infection of the joint (synovial) fluid results in a rapid influx of substances that promote inflammation. Among these are the matrix metalloproteinases (MMPs), enzymes that play a role in the turnover of cartilage by breaking down denatured collagen. Other authors have proposed that 2 of these enzymes, MMP-2 and MMP-9, might serve as markers of arthritic disease. The objective of this study was to follow changes in the expression of these MMPs resulting from EC joint infection and recovery in young (14-20 days of age) Holstein calves. On day 1 of the study, EC was injected into the right tarsal joints of 7 calves; 5 control calves were not infected. On day 2, joints of all calves were washed out and infected calves were treated daily for 20 days with the antibiotic, ceftiofur. Synovial fluid samples were drawn from both groups of calves before and at regular intervals after EC infection. On day 1 before EC infection, MMP-2 was found in joint samples from all calves; MMP-9 was not detected. Compared with control calves, synovial fluid MMP-9 levels in infected calves rose significantly from days 2 to 24; MMP-2 was significantly increased from day 3 until day 20. The authors suggest that the persistence of elevated levels of these enzymes beyond recovery from infection indicates that they might be targets for the therapeutic treatment of infectious arthritis.



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

Journal of Dairy Science, September 2008, Volume 91, Number 9, pages 3554-3567.

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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. Recovery is usually spontaneous but in rare, severe cases, metabolic acidosis can ensue. The objective of this study was to determine whether cows that are already experiencing marginally low ruminal pH (HR, high risk cows) are at more risk of experiencing SARA than cows with higher rumen pH levels (LR, low risk cows). Eight HR cows were in early lactation, being fed a total mixed ration (TMR) containing 45% forage, 55% concentrate. Their daily mean rumen pH, measured with continuous recording indwelling pH system, was 5.81. Eight mid-lactation LR cows, fed a 60% forage, 40% concentrate TMR, had a daily mean rumen pH of 6.21. All cows were subjected to 3 acidosis challenges at 14 day intervals where they were offered 50% of their normal TMR allotment for 1 day followed by an offer of 4 kg of a ground barley-wheat blend for 1 hour. Although the challenges provoked similar, immediate rumen pH declines in both groups, the HR cows experienced more severe SARA because their initial rumen pH levels were lower. Each subsequent challenge caused a greater decline in rumen pH in spite of the fact that fewer cows consumed their entire allotment at each subsequent challenge.



29

Milking-to-Milking Variability for Milk Yield, Fat and Protein Percentage, and Somatic Cell Count

Journal of Dairy Science, September 2008, Volume 91, Number 9, pages 3412-3423.

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This study examined milking-to-milking variability in milk, fat and protein yields, and somatic cell counts (SCC). Additional objectives were to investigate the factors that affect variation in milk fat yield and to study the seasonal variations in milk, fat, and protein yields and SCC. A total of 16 farms in Alberta and Ontario participated in a 5 day milk-sampling study, with 27,328 milk samples collected and analyzed for fat, protein and SCC. Cows were milked 2 times per day (2x) on 14 of the farms and 3 times per day (3x) on the other 2. The 2x herds had higher average milk yields in the morning (~17 versus ~14 kg), whereas the 3x herds had the lowest milk yields in the morning with yields peaking at the night (NT) milking (~9 vs ~11.2 kg). Fat and protein tests were highest for PM milkings in the 2x herds and for night milkings in the 3x herds. Milking times had a much greater effect on fat test than on protein test for both 2x and 3x herds. SCC results (corrected for milk yield) showed no consistent diurnal variation; however, morning milkings of the 3x herds usually had the lowest value each day. Within herds, diurnal variation was not always consistent for milk yield, fat and protein tests, or SCC. Seasonal differences in milk, fat, and protein yields were investigated in 910 cows on 3 farms. The 3x herds had results opposite those of the 2x herds, with milk fat and protein yields being slightly higher in the winter vs summer months in the 3x herds.



30

Milk Losses Associated with Somatic Cell Counts per Breed, Parity and Stage of Lactation in Canadian Dairy Cattle

Livestock Science, November 2008, Volume 117, Number 2-3, pages 225-232.

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The somatic cells (SC) found in milk are made up of both white blood cells and epithelial cells that line the inside of the mammary gland. Both of these types of cells are shed into the milk as a result of mastitis—inflammation caused by intramammary infection (IMI). Therefore, the concentration of SC in milk is used as an indicator of IMI status. It is recognized that IMI reduces milk yield but the actual amount of associated milk production loss is uncertain. This study examined the Dairy Herd Improvement (DHI) records of 313,000 Ayrshire and 1.9 million Holstein cows in the province of Québec. The objective was to estimate relationships between SC scores and milk loss, including the effects of herd, year and season of DHI test, days in milk and age at calving. Relationships were computed separately for each breed, parity and stage of lactation. SC scores were expressed as the natural logarithm of SC count. Overall, it was found that milk yield starts to be reduced when SC score rises above 2 (about 7,400 SC/ml). Milk losses varied from 0.55 to 0.84 kg/day per unit increase in SC score in first lactation Ayrshires and from 0.33 to 0.55 kg/day in first lactation Holsteins. In multiparous cows, losses varied from 0.74 to 2.45 kg/day per unit SC score in Ayrshires and from 0.77 to 1.78 kg/day in Holsteins. The authors conclude that breed, parity and stage of lactation are important variables that need to be considered when estimating milk losses due to IMI.

Milk Production





1

Agreement of Predicted 305-Day Milk Yields Relative to Actual 305-Day Milk Weight Yields

Journal of Dairy Science, October 2007, Volume 90, Number 10, pages 4684-4692.

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At each DHI test day, predicted 305-day milk yields are calculated for most cows on test using a multiple trait prediction (MTP) model. In this study, predicted 305-day yields were compared with 305-day production records of cows on 20 Canadian farms enrolled in DHI and using automated parlour milking recording systems with electronic ID. A total of 10,175 DHI test days from 1,103 cows with complete 305-day lactation yields were entered into the MTP model, and lactation yields were predicted. Test days were grouped into first, second, and third and greater lactations. Within each parity group, days in milk (DIM) were categorized in 3 stages (5 to 60, 61 to 120, and 120 to 305 DIM) for a total of 9 classes. Agreement analysis was used to compare the 305-day sums of daily milk from the automated systems to the MTP model 305-day lactation yield predictions by using inputs from test days throughout the lactations. Results indicated that the MTP model overestimated lactation yields across all parity groups, ranging from 310 to 1,552 kg in parity 1, 640 to 2,000 kg in parity 2, and 567 to 1,476 kg in parity 3 and greater. Overestimation was greatest in early lactation when only a few test results are available, but improved in later lactation when more test day data was available. The results of this study indicate that further refinement of the MTP model estimates in early lactation may be needed and may require adjustment to produce a more accurate lactation yield.



2

Evaluation of the Dairy Comp 305 Module “Cow Value” in Two Ontario Dairy Herds

Journal of Dairy Science, December 2007, Volume 90, Number 12, pages 5784-5797.

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DairyComp 305 is herd management software used by a high proportion of North American dairy farmers. The DairyComp 305 Cow Value module (COWVAL) calculates a farm-specific net present value for each cow in the milking and dry herds, relative to an average replacement heifer having a COWVAL of \$0. This allows a ranking of the cows on the farm compared with replacing them with a typical replacement heifer on the same farm. Retaining a cow with a negative COWVAL is projected to be less profitable than replacing that cow with a new heifer. The objectives of this study were to explore trends in COWVAL over and during multiple lactations for the same cows; to describe factors that influence changes in COWVAL from one monthly DHI test to the next; and to evaluate the behaviour of COWVAL after it drops below a baseline of \$0 during the lifetime of a cow. Over 20,000 monthly test records of 1,463 cows in 2 Ontario herds were analyzed. Within the first 60 days in milk (DIM), COWVAL was unstable and showed large fluctuations over a range of several thousand dollars (\$CA). After 60 DIM, COWVAL becomes more stable for the remainder of the lactation. Fresh, open, and pregnant cows had a greater COWVAL than cows declared ‘do not breed’. Month to month variability declined as the lactation progressed and the risk of a change in reproductive status decreased. As parity increased, there was a tendency toward lower COWVAL and smaller monthly changes. The authors concluded that farm managers should not rely exclusively on COWVAL for culling decisions, particularly for cows that have not had at least 3 milk tests.



3

Supplemental Algal Meal Alters the Ruminal Trans-18:1 Fatty Acid and Conjugated Linoleic Acid Composition in Cattle

Journal of Animal Science, January 2008, Volume 86, Number 1, pages 187-196.

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Unsaturated fatty acids (FA) in diets fed to ruminant animals are subject to stepwise biohydrogenation (BH) by rumen microbes. For example, linoleic acid, an 18-carbon FA with 2 double bonds (18:2) can be reduced to oleic acid (18:1) then to stearic acid (18:0) in a series of reactions that may be carried out by different microbial populations. One of the unique intermediates produced in the BH of 18:2 is cis-9, trans-11 conjugated linoleic acid (c9, t11 CLA) which can be reduced to trans-11 18:1 (t11 18:1) before conversion to stearic acid. A parallel pathway is the conversion of 18:2 to trans-10, cis-12 CLA (t10, c12 CLA) then to trans-10 18:1 (t10 18:1) and, finally, stearic acid again. The objective of this study was to determine the effects of supplemental algal meal (AM) on rumen FA metabolism. Three different levels of AM (1.1, 2.1 and 4.2 kg of dry matter (DM)/day), a rich source of docosahexaenoic acid (DHA, 22:6) and eicosapentaenoic acid (EPA, 20:5), were included in lactation cow diets containing 61% corn silage and 34% high moisture corn (DM basis). A control diet contained no AM. Rumen stearic acid concentrations decreased linearly with increasing levels of AM supplementation, from 32.9% down to 6.0% of total FA. Total trans 18:1 (t11 18:1 + t10 18:1) increased from 19% of total FA on the control diet to 43% on the highest level of supplemental AM, mostly due to t10 18:1. While rumen concentrations of t11 18:1 and c9, t11 CLA peaked at the intermediate level of AM supplementation, those of t10 18:1 and t10, c12 CLA were highest at the highest AM level. The authors conclude that AM supplementation of these diets inhibited the BH of trans 18:1 FA to stearic acid.



4

Milk Synthetic Response of the Bovine Mammary Gland to an Increase in the Local Concentration of Amino Acids and Acetate

Journal of Dairy Science, January 2008, Volume 91, Number 1, pages 218-228.

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Synthesis and secretion of milk components (fat, protein, lactose) are controlled by both substrate supply and mechanisms that regulate the activities of synthetic enzymes. The objective of this study was to determine the effect of increasing the supply of substrates on milk component production in lactating cows. Two different mixtures of amino acids (AA) were infused into an artery (external iliac) supplying nutrients to one side of the mammary gland. One mixture had the AA profile of mixed rumen microbes (MicAA); the other had the same profile as milk proteins (MilkAA). AA infusions produced a 60% increase in arterial AA concentration. Over the course of the 10 hour infusion, 49% of the MicAA were absorbed by the mammary gland; 42% were absorbed in the first pass of blood through the gland. Similarly, 44% of the MilkAA were absorbed over 10 hours; 50% in the first pass. Compared with production from the non-infused side of the mammary gland, milk protein yield increased by 8% with infusion of either AA mixture, representing only 7% of the total AA absorbed. To determine whether energy supply might limit responses, AA were infused with or without a supply of acetate or acetate was infused alone. Acetate infusions increased mammary acetate uptake by an average of 128%. Although acetate infused alone had no effect on milk component production, when infused with AA, acetate provoked decreases in milk protein and lactose yields. The authors conclude that energy supply did not limit the response to infused AA. They also speculate that the additional acetate absorbed by the mammary gland during infusion may have been deposited in mammary gland adipose tissue.



5

Effects of Supplementing Fish Oil in the Drinking Water of Dairy Cows on Production Performance and Milk Fatty Acid Composition

Journal of Animal Science, March 2008, Volume 86, Number 3, pages 720-729.

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The objective of this study was to compare methods of delivery of a fatty acid supplement to lactating dairy cows. Each of 2 groups of 8 lactating dairy cows were fed a total mixed ration (TMR) containing 52% forage, 48% concentrate. The TMR of one group (FOT group) was top-dressed with menhaden fish oil (FO) at a rate of 10 grams per kg of TMR dry matter (DM). For the second group (FOW group), the FO was provided at a rate of 2 grams per litre in their water. Responses were measured over a 4 week period after a 1 week adjustment period. Dietary DM intake, water intake and milk yield were unaffected by FO source. Milk fat percentage and yield declined significantly over the 4 week trial period for both groups. Although there were differences between the 2 groups in terms of the concentrations of some minor fatty acids in milk and blood, none of the differences were of practical significance. The authors conclude that drinking water may be a practical alternative for the supplementation of FO to lactating cows, particularly during the transition period when DM intake may be reduced.



6

Effects of Twice-Daily Nursing on Milk Ejection and Milk Yield During Nursing and Milking in Dairy Cows

Journal of Dairy Science, April 2008, Volume 91, Number 4, pages 1416-1422.

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Increasing public interest in animal welfare and 'natural' food has led to research into alternative production systems. Among these, allowing dairy calves to nurse from their dams is a departure from the more common practice in North American dairy production systems where calves are removed from their dams shortly after birth. Some studies have suggested that a combination of machine milking and nursing could increase total milk production, perhaps due to changes in hormone secretion patterns. The present study was designed to examine the effects of combined milking and nursing on production and hormone levels in the context of a typical Canadian production system. Ten cows were machine-milked twice per day and allowed to nurse their calves 2 hours after each milking for 9 weeks. Calves were removed from another group of 8 control cows, matched with the nursing cows for parity, production potential, sex of calf and date of calving. Control cows were machine-milked on the same schedule as the nursing cows. Average calf milk consumption increased from 6.5 kg/day in week 1 to 12.5 kg/day in week 9. Although average daily milk yields at milking were lower for the nursing cows over the 9-week period (26.1 versus 35.5 kg/day), when calf milk consumption was included, average daily milk production was exactly the same for both groups of cows. Likewise, for the 6 weeks after weaning the calves, average daily milk yields were the same for both groups. During milking, blood levels of the milk ejection hormone oxytocin were higher for the control cows but equivalent to levels in the blood of nursing cows during nursing. The authors speculate that the lack of difference in oxytocin levels accounted for the similarity in milk production between the 2 groups.



7

Effect of Conjugated Linoleic Acid on Bovine Mammary Cell Growth, Apoptosis and Stearoyl Co-A Desaturase Gene Expression

Domestic Animal Endocrinology, April 2008, Volume 34, Number 3, pages 284-292.

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In the lactating dairy cow, conjugated linoleic acids (CLA) are produced in 2 locations, the rumen and the mammary gland. In the rumen, the biohydrogenation (BH) of linoleic acid (cis-9, cis-12 18:2) leads to the production of cis-9, trans-11 18:2 (9,11 CLA), trans-11 18:1 (vaccenic acid), trans-10, cis-12 18:2 (10,12 CLA) and other intermediates. Upon absorption into the bloodstream and uptake by the mammary gland, vaccenic acid may be converted to 9,11 CLA by the enzyme stearoyl Co-A desaturase (Scd, also known as delta-9 desaturase). In previous experiments, feeding (rumen-protected) or abomasal infusion of low doses of CLAs have been shown to reduce mammary milk fat synthesis. The CLA primarily responsible for this effect is thought to be 10,12 CLA. At higher doses, CLAs have caused severe milk fat depression as well as decreased milk yield. In the present study, cultured mammary epithelial cells were exposed to graded concentrations of 9,11 CLA and 10,12 CLA to test for effects on cell growth and viability. At higher concentrations, both CLAs reduced cell growth and increased programmed cell death (apoptosis). CLAs did not affect the activity of the promoter that controls the rate of Scd synthesis suggesting that the enzyme did not play a role in the observed effects of CLA. The authors conclude that the negative effects of CLAs on cell growth and viability support the idea that high doses of CLA may cause cell death in the mammary glands of lactating cows.



8

Use of Metabolic Control Analysis in Lactation Biology

Journal of Agricultural Science, June 2008, Volume 146, Number 3, pages 267-273.

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Metabolic pathways, such as those leading to the synthesis of particular long-chain fatty acids in milk (e.g., conjugated linoleic acids), involve the stepwise conversion of substrates and intermediates into the final product. At each step, the activity of a specific enzyme regulates the rate at which the process progresses, assuming that the concentration of enzyme substrate is adequate. Although it has often been assumed that only one particular step in a multi-step pathway is responsible for regulating the flow rate (flux) through the entire pathway, this is rarely the case. In most pathways, there are multiple control points, regulated by the activities of several enzymes. The rate of conversion of substrate to product by the components of a pathway may be controlled by a number of factors, including the concentration of substrate, the abundance and activity of the enzymes and the levels of cofactors, inhibitors and modulators of enzyme activity. An understanding of the specific steps and control points involved in metabolic pathways is essential to efforts aimed at, for example, altering milk composition. This paper describes the application of metabolic control analysis (MCA), a method for assessing the sensitivity of flux through a metabolic process to changes in the activities of the individual enzymes involved in each step. An example of the application of MCA to lactation biology is described.



9

Onset of Lactation in the Bovine Mammary Gland: Gene Expression Profiling Indicates a Strong Inhibition of Gene Expression in Cell Proliferation

Functional and Integrative Genomics, August 2008, Volume 8, Number 3, pages 251-264.

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During the dairy cow's transition from pregnancy to lactation, the mammary gland undergoes dramatic changes in preparation for high levels of milk production. In late pregnancy, changing blood levels of estrogen, progesterone and prolactin initiate development of the gland's duct system and the formation of alveoli. These changes are followed by the initiation of milk component synthesis. Just before parturition, the final stages in the development of alveoli are completed with the commencement of colostrum and milk secretion. In the early stages of lactation, the number of milk secretory cells increases dramatically along with their metabolic and nutrient transport activity. Earlier studies demonstrated a 9-fold increase in the mammary uptake of glucose between 7-9 days pre-partum and 1 day postpartum. The objective of the present study was to characterize the changing expression patterns of genes that play important roles in the rapid increase in milk secretion that occurs immediately after parturition. Of 23,000 genes examined in mammary tissue extracts, the expression profiles of 389 were significantly altered between 5 days pre-partum and 10 days postpartum—105 were up-regulated (i.e., their expression was increased); 284 were down-regulated. The major up-regulated genes were those associated with amino acid, glucose, and ion transport; lipid and carbohydrate metabolism; and cell signalling functions. The main down-regulated genes were associated with cell proliferation, differentiation and growth; DNA replication and chromosome organization; microtubule-based processes; and protein and RNA degradation. The observed increase in expression of the gene coding for the glucose transport protein, GLUT1, was supported by the increased abundance of its messenger RNA transcript and by a 2-fold increase in the concentration of GLUT1 itself.



10

Effects of Intravenous Infusion of Conjugated Diene 18:3 Isomers on Milk Fat Synthesis in Lactating Dairy Cows

Journal of Dairy Science, September 2008, Volume 91, Number 9, pages 3568-3578.

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Fatty acids (FA) are composed of linear chains of carbon (C) atoms. Saturated FA have 2 atoms of hydrogen (H) attached to all but the first C atom in the chain, which carries the acid group. Unsaturated FA are characterized by the presence of double bonds between adjacent carbon atoms, due to a missing H atom on each. When the remaining H atoms on adjacent C are on the same side of the chain, the double bond is referred to as *cis*; if on opposite sides, the double bond is *trans*. For example, *trans*-11 refers to a *trans* double bond between carbons 11 and 12 in the chain, counting from the acid end. Biohydrogenation (BH), a process that takes place in the rumen, adds H atoms to these C atoms to reduce the degree of unsaturation of the FA. For example, alpha linolenic acid (α LA) has an 18 carbon chain with 3 double bonds, all in the *cis* conformation (*cis*-9, *cis*-12, *cis*-15 18:3); BH adds H atoms to the 3 double bonds in linolenic acid, converting it sequentially to 18:2, 18:1 and 18:0. In the process, the placement and conformation of the double bonds may be altered. Common intermediates in the BH of α LA are *cis*-9, *trans*-11, *cis*-15 18:3 and *cis*-9, *trans*-13, *cis*-15 18:3, referred to as conjugated diene 18:3s (CD 18:3) - conjugated means that the double bonds are only 2 C apart. It has been shown that an intermediate in the BH of linoleic acid (*cis*-9, *cis*-12 18:2), *trans*-10, *cis*-12 18:2 (10, 12 CLA) enters the bloodstream and is a potent inhibitor of mammary gland milk fat production, leading to milk fat depression (MFD). The objective of this study was to determine whether the 2 CD 18:3 intermediates might also contribute to MFD. Intravenous infusions of combinations of α LA, CD 18:3 and 10,12 CLA demonstrated no change in the magnitude of MFD when other FA were infused with 10, 12 CLA.

Reproduction





1

Genomic Stability and Physiological Assessments of Live Offspring Sired by a Bull Clone, Starbuck II

Theriogenology, January 2007, Volume 67, Number 1, pages 116-126.

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Starbuck was a Holstein bull whose genetic superiority contributed significantly to the advancement of the breed. For this reason, Starbuck was cloned, using somatic cell nuclear transfer (SCNT) to produce Starbuck II. Although cloned animals often have overt phenotypic abnormalities, these characteristics are apparently not transmitted to their progeny. To verify that this was the case for the progeny of Starbuck II, his frozen/thawed semen was used to artificially inseminate a number of normally cycling Holstein cows. Blood profiles, physical exams as well as growth and reproductive parameters of 30 of the resulting calves were assessed from weaning to puberty and compared to the same criteria in a group of age-matched control calves. Growth parameters and blood profiles did not differ between the 2 groups but Starbuck II progeny had lower heart and respiratory rates as well as lower body temperatures. Reproductive assessment revealed no differences in the anatomy of external or internal genitalia. Among the female progeny, age at puberty, number of ovarian follicle waves per estrous cycle and blood progesterone concentrations during their initial estrous cycles were also similar. A characteristic often observed in SCNT clones is shorter telomere lengths. Telomeres are regions of repetitive DNA sequences and associated proteins found at the end of each chromosome. One widely accepted theory about aging holds that telomeres are shortened at each cell replication cycle and that senescence occurs when telomere length reaches a critical minimum. Shortened telomeres are thought to account for the premature aging observed in some clones. Examination of telomere lengths in sperm and blood cells from Starbuck II and from cells of his progeny revealed no differences from control animals of similar age and sex.



2

Precalving Factors Affecting Conception Risk in Holstein Dairy Cows in Tropical Conditions

Theriogenology, April 2007, Volume 68, Number 4, pages 567-581.

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This study examined risk factors for low first service conception rate (FSCR) in 21 dairy herds on Réunion, an island located in the Indian Ocean, 800 km east of Madagascar. Data included 473 lactation records for 404 Holstein cows. Average FSCR over all records was 0.27. FSCR was lower for cows whose calving to first service interval was shorter than 60 days, for those on an estrus synchronization protocol and for those whose previous 305-day milk yield was greater than 8,000 kg. Blood metabolite levels that were associated with low FSCR included low blood glucose concentrations in high-yielding cows and combined high urea and beta-hydroxybutyrate concentrations in all cows. The authors conclude that precalving energy imbalance, indicated by low prepartum glucose concentration, was a strong nutritional predictor of low FSCR in high-yielding cows.



3

Expression and Function of Fibroblast Growth Factor 10 and Its Receptor, Fibroblast Growth Factor Receptor 2B, in Bovine Follicles

Biology of Reproduction, October 2007, Volume 77, Number 4, pages 743-750.

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During each of the cow's estrous cycles, there are typically 2 or 3 waves of follicle development. Although a large number of follicles begin development in each wave, only one becomes dominant; the others undergo atresia (degeneration). The dominant follicle is the follicle that ovulates at estrus and releases an egg (oocyte). Healthy follicles contain 2 specialized cell types: thecal cells, a type of connective tissue cell (fibroblast) that surround and support the follicle and granulosa cells which surround the oocyte and produce the hormones estrogen and inhibin in response to stimulation by follicle-stimulating hormone (FSH). Follicle development is regulated by a number of signalling molecules that pass between these cells. One of these intercellular signals is fibroblast growth factor 7 (FGF7) which is produced by thecal cells and binds to a receptor (FGFR2B) in granulosa cells. Another signalling molecule, FGF10, is also known to bind to FGFR2B in other biological systems (e.g., mice). The objective of this study was to determine whether FGF10 might play a part in the regulation of bovine follicle development. The presence of FGF10 messenger RNA (mRNA) was detected in the oocytes and thecal cells of antral follicles, as well as in preantral follicles. The FGF10 protein itself was also found in the oocytes of preantral and antral follicles and in granulosa and thecal cells of antral follicles. FGF10 mRNA abundance decreased with increasing follicular estrogen levels in healthy follicles, and was lowest in follicle undergoing atresia. In culture granulosa cells, added FGF10 decreased estrogen production while FSH increased the expression of FGFR2B. Thus, FGF10 is a potential signalling molecule that regulates the health of growing follicles.



4

Diets Enriched in Unsaturated Fatty Acids Enhance Early Embryonic Development in Lactating Holstein Cows

Theriogenology, October 2007, Volume 68, Number 7, pages 949-957.

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Earlier studies, by these authors and others, had demonstrated that supplementation of diets with omega-3 fatty acids (ω -3 FA) improved reproductive success in lactating dairy cows. The present study was designed to test the effects of feeding ω -3 FA on embryonic development after synchronized ovulation. Diets fed to 3 groups of 8 lactating Holstein cows were supplemented with sources primarily providing either saturated fatty acids (SAT) or unsaturated fatty acids in the form of whole sunflower seeds (SUN) or whole flaxseeds (FLX). SUN is a rich source of linoleic acid (LA) while FLX contain a high level of alpha-linolenic acid (ALA), an ω -3 FA. All diets provided 750 grams of supplemental fat per cow per day. Cows were subjected to treatments to synchronize ovulation approximately 30 days after the beginning of fat supplementation. After a further step to synchronize ovarian status, cows were subjected to 4 days of treatment with follicle stimulating hormone to induce superovulation. Cows were then artificially inseminated and embryos collected nonsurgically, 7 days later. Neither fertilization rates nor numbers of follicles, ovulations or embryos were significantly different among the 3 treatment groups. Embryos collected from cows on the SAT diet had significantly lower numbers of undifferentiated embryonic cells (blastomeres) than those from cows on the SUN or FLX diets. However, there were no significant differences in blastomere numbers between SUN- and FLX-fed cows, indicating no advantage in supplementing diets with ALA versus LA.



5

The Oxidative Stress Adaptor p66Shc Is Required for Permanent Embryo Arrest In Vitro

BMC Developmental Biology, November 2007, Volume 7, Number 132, pages 1-15.

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Embryo transfer (ET) programs are routinely used to increase the number of offspring from donor dairy cows of high genetic merit. One technique used to produce embryos for transfer involves the removal of oocytes from dominant follicles of superovulated donors using a hypodermic needle. The oocytes are then subjected to in vitro (in laboratory) fertilization (IVF) and the resulting embryos are allowed to develop before being implanted in foster dams. In many cases, IVF embryos fail to develop, fewer than 50% reaching the blastocyst stage. This study investigated the possibility that this developmental failure was due to the activity of a 'lifespan determinant protein', labelled p66Shc. To reduce the activity of p66Shc, the translation of p66Shc messenger RNA (mRNA) was inhibited by microinjecting a large number (12,000-24,000) of p66Shc-specific short hairpin RNA (shRNA) molecules into oocytes before IVF. Control oocytes were either not injected or were injected with medium not containing shRNA; all were subjected to IVF. Once inside a cell, the shRNA is cleaved into small interfering RNA (siRNA) fragments that bind to and cleave p66Shc mRNA, thus reducing the number of copies of p66Shc produced. As cells divide after IVF, the p66Shc shRNA is passed on to daughter cells where the process continues. Compared with cells that were not treated, those that were treated with p66Shc shRNA had significantly lower levels of p66Shc mRNA and significantly fewer of the subsequent embryos experienced arrested development. The authors interpret these results as demonstrating that p66Shc is involved in mediating developmental arrest in early-stage embryos.



6

Progesterone Metabolism in Bovine Endometrial Cells and the Effect of Metabolites on the Responsiveness of the Cells to OT-Stimulation of PGF2alpha

Steroids, November 2007, Volume 72, Number 13, pages 843-850.

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The luteal phase of the estrous cycle begins at ovulation with the formation of the corpus luteum (CL) which secretes large quantities of progesterone (P4). CL dominance ends when prostaglandin F2alpha (PGF2α), produced by cells lining the uterus (endometrial cells), cause the CL to lyse (luteolysis). PGF2α synthesis is stimulated by oxytocin (OT) secreted by the CL, an effect that depends on the presence of endometrial OT receptors (OTR). During the early luteal phase, P4 prevents secretion of PGF2α by suppressing the expression of OTR but this effect is gradually lost as luteolysis approaches. The mechanism by which P4 suppression of OTR is lost is thought to be through reduction in the expression of an inhibitory factor, as yet unidentified. The fact that OTR expression is not suppressed when endometrial cells are grown in tissue culture suggests that a non-endometrial inhibitor is involved. The objective of this study was to determine whether the metabolism of P4 by endometrial cells might play a role in the reduced responsiveness of OTR synthesis to P4. Endometrial cells maintained in tissue culture were incubated with P4 and the metabolites produced were characterized. Results of further incubations with either P4 or its metabolites showed that only P4 was able to increase basal PGF2α synthesis but not OT-stimulated PGF2α synthesis. Neither P4 or its metabolites had an effect on OTR concentration. The authors conclude that the P4 metabolites identified play no role in the regulating OT-stimulated PGF2α synthesis during the bovine estrous cycle.



7

Milk Urea-Nitrogen Negatively Affected First-Service Breeding Success in Commercial Dairy Cows in Prince Edward Island, Canada

Preventive Veterinary Medicine, November 2007, Volume 82, Number 1-2, pages 42-50.

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This study examined the relationship between milk urea nitrogen (MUN) concentrations and first service breeding success among cows in all Prince Edward Island dairy herds enrolled in the Dairy Herd Improvement program. A successful first service breeding meant that a cow was bred for the first time after calving between June 1, 1999 and May 31, 2000 followed by calving 270-290 days later. Of the total of 5,802 first services between those dates, 48% were successful; 52% unsuccessful. A change in MUN concentration from 10 mg/dL to 20 mg/dL on the test day closest to the date of first service was associated with a 13.9% decrease in the odds of a successful first service breeding. Results were adjusted for differences in parity, production level and days in milk.



8

Investigation of MYST4 Histone Acetyltransferase and its Involvement in Mammalian Gametogenesis

BMC Developmental Biology, December 2007, Volume 7, Number 123, pages 1-14.

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Chromosome structure is often described as 'beads on a string' where the core of the beads is composed of proteins referred to as H2, H3 and H4 'core' histones. Double-stranded DNA forms the string which is wound around the core histone beads to form nucleosomes. At a secondary level of organization, H1 'linker' histones link adjacent nucleosomes to form a coil of 'chromatin'. Tight packing of chromatin restricts the transcription of DNA but modification of histones can open up (remodel) the chromatin to allow gene expression. One type of modification involves the addition of acetyl ($-\text{COCH}_3$) groups to lysine residues in core histones. Acetylation is carried out by enzymes called histone acetylases (HATs). The objective of this study was to characterize one of these HATs, labelled MYST4, and investigate its presence in various bovine tissues including reproductive tissues, ova and sperm. Although MYST4 messenger RNA was found in all tissues examined, the MYST4 protein itself was found in only a few, notably ovaries and testes. In ovaries, MYST4 was confined to oocytes, antral follicles and blood vessels. In oocytes and embryos, MYST4 protein was found in both cytoplasm and nucleus. Within testes, MYST4 was specific to only one cell type, the elongating spermatids, where it was found only in the nucleus. The authors conclude that the localization of MYST4 in these cells suggests that the enzyme may be involved in core histone acetylation that regulates gene expression during gamete and embryo development.



9

The Kinetics of Donor Cell mtDNA in Embryonic and Somatic Donor Cell-Derived Bovine Embryos

Cloning and Stem Cells, December 2007, Volume 9, Number 4, pages 618-629.

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Mitochondria are cellular organelles primarily involved in energy metabolism. The mitochondria in all cells contain multiple copies of mitochondrial DNA (mtDNA), a loop of genetic material that codes for many of the enzymes required in the synthesis of ATP, the cellular fuel. However, control over the expression of these mtDNA genes is largely controlled by enzymes and transcription factors produced in the nucleus. In the normal course of reproduction, mtDNA is derived from the oocyte (egg) due to the destruction of sperm mitochondria shortly after fertilization and mtDNA expression in the embryo is regulated by the new embryonic nucleus. However, in the production of animal clones through somatic cell nuclear transfer (SCNT), although most of the mitochondria and its mtDNA come from the recipient enucleated oocyte, the embryonic nucleus is derived entirely from the donor somatic cell and some intact mitochondria may also be transferred to the recipient. Therefore, mtDNA expression in the clone is not identical to that in the donor because, although its regulation is influenced by the donor nucleus, the mtDNA genes that are expressed are those largely inherited from the recipient oocyte. The objective of this study was to evaluate differences in mtDNA content in clones produced by 2 different techniques. The first involved transfer of nuclei from mature donor fibroblast (connective tissue) cells (NT-F). In the second, nuclei were from embryonic donor cells (NT-E). In order to distinguish between donor and recipient mtDNA, donor nuclei were from *Bos indicus* (zebu) cattle while recipient enucleated cells were from *Bos taurus* (European) cattle. Significant differences were found in mtDNA in clone embryos and mature animals constructed with the 2 techniques.



10

Comparison of 2 Enzyme Immunoassays and a Radioimmunoassay for Measurement of Progesterone Concentrations in Bovine Plasma, Skim Milk, and Whole Milk

Canadian Journal of Veterinary Research, January 2008, Volume 72, Number 1, pages 32-36.

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For purposes of assessing the reproductive status of dairy cows, progesterone (P4) concentrations in plasma are commonly measured using radioimmunoassay (RIA). Although RIA provides accurate results relatively quickly, its use of radioactive chemicals requires specialized facilities. This study was designed to evaluate the use of 2 alternative enzyme immunoassays (EIA) for the measurement of P4 in plasma as well as whole and skim milk. The 2 EIA examined were a solid-phase EIA (SPEIA) and a direct EIA (DEIA). Plasma and milk samples were drawn from cows at stages in their reproductive cycles where either high or low P4 concentrations were expected. Across all samples, P4 concentrations measured by all assays were similar. However, compared with RIA, both EIAs yielded higher P4 concentrations in plasma but lower concentrations in whole milk. In samples expected to have low P4 concentrations, all 3 assays gave similar results for whole milk but the DEIA measured higher levels in both plasma and skim milk. In samples expected to contain higher P4 concentrations, RIA gave higher results than SPEIA in all samples and RIA results for these samples were higher in whole milk than in skim milk. The authors conclude that both EIAs could be used for the determination of P4 concentrations in whole milk with a level of precision similar to RIA. However, using DEIA to measure P4 in plasma and skim milk samples expected to have low P4 levels might yield erroneous results.



11

Alternative Approaches to Setting Up Donor Cows for Superstimulation

Theriogenology, January 2008, Volume 69, Number 1, pages 81-87.

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Embryo transfer (ET) programs are routinely used to increase the number of offspring from donor dairy cows of high genetic merit. Incorporation of protocols designed to control follicular wave dynamics offer the convenience of being able to initiate superstimulatory treatments quickly and at a self-appointed time, without the necessity of estrus detection and without sacrificing results. However, estradiol, which has proven to be most useful for these purposes in the field, is being withdrawn from many veterinary markets around the world, leaving only ultrasound-guided follicle ablation as a reliable method to synchronize follicular wave emergence for superstimulation. Unfortunately, follicle ablation is difficult to utilize in the field. Although the administration of GnRH or pLH to synchronize follicular wave emergence would appear to be too variable for superstimulation, pre-synchronization may improve response. An alternative currently available may be initiating FSH treatments at the time of emergence of the first follicular wave with the inclusion of a progesterone-releasing device (on the day of ovulation), but the duration of the treatment to synchronize ovulation in groups of donors at random stages of the estrous cycle may preclude the use of this approach. An exciting alternative may be to use FSH or eCG to recruit follicles into the wave, regardless of the stage of development of the dominant follicle, and to initiate the regular superstimulatory treatment protocol at a predetermined time thereafter (e.g., 2 or 3 days).



12

Progress in Understanding Ovarian Follicular Dynamics in Cattle

Theriogenology, January 2008, Volume 69, Number 1, pages 72-80.

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Wave-like follicle development in cattle is manifest as simultaneous emergence of a group of small (i.e., 1 mm) follicles in both ovaries in response to a surge in circulating concentrations of FSH. The largest of the group at its earliest detection (1 mm) usually remains dominant (random distribution in either left or right ovary), but individual follicle growth rates are similar until the dominant follicle reaches 8 to 9 mm (i.e., 5 days after emergence at 1 mm). Acquisition of LH receptors in the granulosa of the dominant follicle results in rapid divergence in the growth rates; the dominant follicle continues to grow as the subordinates cease and begin to regress in a milieu of minimal FSH. Growing, static and regressing phases of dominant and subordinate follicles are morphologically distinct (histologic and ultrasonographic) and are reflective of functional status (steroid and protein metabolism and oocyte competence). The vast majority of estrous cycles are composed of two or three follicular waves. Two-wave cycles are consistently shorter (19-20 d) than 3-wave cycles (22-23 d). The number of follicles recruited into each wave varies greatly among individuals, but is highly repeatable within individuals. The wave pattern tends to be repeatable within individuals, and duration of dominance of the first wave is predictive of the wave pattern. Reproductive aging in cattle is characterized by an elevation in plasma concentrations of FSH, a decrease in the number of follicles recruited into each wave, a lower superovulatory response, and a lower oocyte fertilization rate.



13

Safety of a Progesterone-Releasing Intravaginal Device as Assessed from Vaginal Mucosal Integrity and Indicators of Systemic Inflammation in Postpartum Dairy Cows

Canadian Journal of Veterinary Research, January 2008, Volume 72, Number 1, pages 43-49.

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This study examined animal reactions to the placement of a progesterone-releasing intravaginal device (PRID) commonly used in timed insemination programs for cattle. The PRID is a silastic coil approximately 8 cm long, with an outside diameter of 4.5 cm, impregnated with 1.55 grams of progesterone. PRIDs were placed in the vaginas of anestrus lactating Holstein cows averaging 63 days in milk. Similar (placebo) intravaginal devices (PIDs) containing no progesterone were placed in second group of cows. A third (control) group was not treated. PRIDs and PIDs were removed 7 days after insertion. Blood samples were collected at insertion and removal. On device removal, 5% of the PRID-treated and 19% of the PID-treated cows had a copious, purulent discharge of vaginal mucous with no evidence of damage to vaginal tissue in either case. Compared with controls, both devices reduced total blood white cell counts, mainly attributable to a reduction in neutrophils. The population of these cells normally declines during the phase of the estrous cycle when progesterone secretion is elevated (the luteal phase). Analysis of blood samples for inflammatory proteins revealed no differences between the 3 groups of cows. Culture of vaginal mucous identified only normal bacterial species. The authors conclude that, despite slight changes in white blood cell numbers, there was no evidence of a negative response to the PRID.



14

Immunolocalization of the High-Mobility Group N2 Protein and Acetylated Histone H3K14 in Early Developing Parthenogenetic Bovine Embryos Derived From Oocytes of High and Low Developmental Competence

Molecular Reproduction and Development, February 2008, Volume 75, Number 2, pages 282-290.

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The development of a bovine embryo begins with the maturation of an oocyte (egg cell) inside an ovarian follicle. Upon release from the follicle at ovulation, the oocyte must undergo meiotic division in preparation for fertilization, allow the entry of a single spermatozoa and decondense the sperm head. Once the zygote is formed, it must cleave to form 2 identical embryonic cells and these cells must divide again until the 8-cell stage (the maternal-zygote transition, MZT). All of the cellular machinery necessary for development to this point is transcribed from maternal DNA during the latter stages of follicle development. After MZT, further development is determined by the genome (genetic material, DNA) of the embryo. Expression of the embryonic genome requires the 'remodelling' of chromosome structure. Chromosome structure is often described as 'beads on a string' where the core of the beads is composed of histone proteins. Double-stranded DNA forms the string which is wound around the histone beads to form nucleosomes. At a secondary level of organization, H1 'linker' histones link adjacent nucleosomes to form a coil of 'chromatin'. High-mobility group proteins associated with nucleosomes help with transcription, replication, recombination, and DNA repair. When in vitro fertilization (IVF) is used in the production of embryos for transfer to surrogate dams (ET), it has been found that zygotes that cleave earlier after IVF are more likely to yield implantable embryos than those that cleave later. The objective of this study was to compare the distributions of 2 histone-associated proteins in early- and late-cleaving zygotes as an indication of their developmental competency. Results suggested that altered chromosome remodelling at MZT played a role in the lower developmental competency of late-cleaving zygotes.



15

Exploring the Impact of Sexed Semen on the Structure of the Dairy Industry

Journal of Dairy Science, February 2008, Volume 91, Number 2, pages 847-856.

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The use of sexed semen is expected to rapidly increase within the next decade as continued improvements in the technology lead to improved sorting capacity, higher fertility rates and lower costs. This study examined the potential impact of the technology on the structure of the US dairy industry. The use of sexed semen allows producers to separate breeding decisions necessary to obtain an adequate supply of dairy replacement heifers from those needed to achieve the pregnancies required to start new lactations. Producers are able to select among their herds' potential dams and produce dairy replacement heifers from only the genetically superior animals. Because selection in dams is possible, the rate of genetic progress is expected to increase by up to 15%. The supply of dairy replacement heifers is expected to grow to meet and temporarily exceed current demand, resulting in reduced prices. Consequently, herd turnover rates are expected to increase slightly, and herd expansions may accelerate. Widespread application of sexed semen may temporarily increase the supply of milk, which will result in lower milk prices but the cost of milk production will be reduced as well. Increased specialization is expected with more dairy producers deciding not to raise their own heifers but to purchase replacements. Other dairy farms might specialize in producing genetically superior dairy replacement heifers for sale. Depending on the value of calves not raised for replacements, artificial insemination organizations might market beef conventional semen or beef male sexed semen to dairy farms. The use of sexed semen should lower the cost of progeny-testing programs and embryo transfer and enhance the value of genetic markers.



16

Low Oxygen Delays Fibroblast Senescence Despite Shorter Telomeres

Biogerontology, February 2008, Volume 9, Number 1, pages 19-31.

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A telomere is a region of repetitive DNA sequences and associated proteins found at the end of each chromosome. It is thought that telomeres protect chromosomes from the loss of genes at their terminal ends. One widely accepted theory about aging holds that telomeres are shortened at each cell replication cycle and that senescence occurs when telomere length reaches a critical minimum. This study examined the effect of reduced oxygen (O_2) concentration on the lifespan of bovine fibroblasts (connective tissue cells) grown in culture. Lowering the O_2 concentration from 20% to 2% increased the replicative lifespan of fibroblasts 5-fold, from 30 days to 150 days. While the telomeres of high O_2 fibroblasts lost 182 DNA base pairs (bp) per replication, low O_2 cells lost only 171. In addition, low O_2 fibroblasts that had replicated more than 50 times had shorter residual telomeres (average 11,135 bp) than high O_2 cells that had senesced after 25-34 replications (average 14,827 bp). Furthermore, although chromosomal abnormalities increased with continued cell division under both O_2 concentrations, low O_2 fibroblasts experienced fewer abnormalities than high O_2 cells. The authors speculate that these effects due to lower O_2 concentration may be due to increased activity of telomerase, an enzyme that replaces lost telomere DNA fragments. They also point to the possibility that replicative senescence may be regulated by the integrity of telomere structure rather than the absolute length of the residual telomere.



17

Epigenetic Reprogramming in Embryonic and Foetal Development upon Somatic Cell Nuclear Transfer Cloning

Reproduction, February 2008, Volume 135, Number 2, pages 151-163.

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Somatic cell nuclear transfer (SCNT) is a technique used to produce animal clones, typically from a genetically superior individual (donor). The technique involves removing the nucleus from an oocyte and replacing it with the nucleus of a fully differentiated somatic cell from the donor animal. The success rate of SCNT has been very limited—only 1-5% of transfer have given rise to live clones and many of those have died at birth or have been malformed. One of the proposed explanations for these poor results relates to incomplete de-differentiation of the implanted donor cell DNA resulting in abnormal expression of genes essential for proper embryonic development. In the development of normal embryos, all cells are capable of forming a complete individual up until the blastocyst stage when differentiation begins. For a SCNT embryo to develop normally, the expression program of the somatic cell genome must be completely erased before the normal sequence of embryonic cell cleavage and differentiation can be initiated. This paper reviews current knowledge about the mechanisms that are involved in reprogramming the somatic cell genome. Epigenetic programming (EP) refers to heritable changes in gene expression that are caused by mechanisms other than changes in genomic DNA sequences. In the context of this review, EP refers to mechanisms such as the addition of methyl (CH₃) groups to DNA and protein molecules which inhibits their expression. Other mechanisms, including imprinting, X-chromosome inactivation and telomere length restoration are also discussed.



18

Molecular and Subcellular Characterisation of Oocytes Screened for their Developmental Competence Based on Glucose-6-Phosphate Dehydrogenase Activity

Reproduction, February 2008, Volume 135, Number 2, pages 197-212.

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Although milk production per cow has increased significantly over the past decades, reproductive efficiency has declined. One of the causes of this decline is thought to be abnormal development of the pre-implantation embryo, at least in part due to low oocyte competence. As the oocyte develops in the maturing dominant follicle, it acquires the ability to successfully partition its complement of DNA, to cleave following fertilisation and to contribute to the development and implantation of the embryo. In earlier studies, these authors demonstrated that the activity of the enzyme glucose-6-phosphate dehydrogenase (G6PDH) was lower in oocytes that had achieved competence than in those that had not. When incubated with the dye brilliant cresyl blue (BCB), immature cumulus cell-enveloped oocytes that have not achieved competence appear colourless due to the complete degradation of BCB by G6PDH. Competent oocytes appear blue because their lower G6PDH activity does not as efficiently degrade the dye. The objective of the present study was to compare developmental, metabolic and gene expression profiles in oocysts identified as competent (BCB+) or incompetent (BCB-). While only 12.1% of embryos from BCB- oocysts reached the blastocyst (beginning of differentiation) stage of development, 33.1% of those from BCB+ oocysts reached this stage. BHB+ oocysts had higher activities of enzymes (kinases) involved in activating other proteins and in the expression of genes regulating DNA transcription, the cell division cycle and protein synthesis. BCB- oocytes, had higher mitochondrial activity and increased expression of genes involved in energy metabolism, calcium ion binding and growth factor activity.



19

Opportunities and Challenges in Applying Genomics to the Study of Oogenesis and Folliculogenesis in Farm Animals

Reproduction, February 2008, Volume 135, Number 2, pages 119-128.

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Many current studies in the field of genomics are focussed on characterizing variations in gene expression associated with species, phenotypes within species or different physiological states. These studies rely on the evaluation of the population of messenger RNA (mRNA) transcripts present in particular cells (the cell's transcriptome) using microarrays that contain tens of thousands of known gene sequences (DNA) to which the mRNA transcripts may adhere (hybridize). The use of this technique would be very valuable in studying the dynamics of gene expression during the early development of oocytes and follicles. However, many of the genes that may be involved in these developmental processes have yet to be identified. This is but one of a number of challenges to the application of current genomics techniques to the study of gene expression in early development. Another challenge is the availability of tissue samples for analysis. The amounts of material available from the various components of the follicle are limited by the size of the primordial follicle itself. Assuming that these limitations can be overcome, the characterization of the mRNA transcriptome is only a first step in understanding the roles of specific genes. Regulation of expression of specific mRNA transcripts and as well as regulation of their translation into proteins must be elucidated before their roles are completely understood.



20

Postcalving Factors Affecting Conception Risk in Holstein Dairy Cows in Tropical and Sub-Tropical Conditions

Theriogenology, March 2008, Volume 69, Number 4, pages 443-457.

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This study examined risk factors for low first service conception rate (FSCR) in 21 dairy herds on Réunion, an island located in the Indian Ocean, 800 km east of Madagascar. Data included 1,309 lactation records for 1,007 multiparous Holstein cows. Subsets of the complete data set were subjected to 2 different analyses. Average FSCR over all records was 0.27. In both analyses, FSCR was decreased when the interval from calving to first service was less than 61 days and when estrus was hormonally induced. In the first analysis, FSCR was lower for cows whose average milk yield in the first 100 days of lactation was less than 24 kg/day or greater than 27 kg/day. Cows who lost more than 1.5 condition score units, or who experienced retained placenta also had lower FSCR. In the second analysis, FSCR was lower for cows who were inseminated during the wet season, were raised off-farm or had a low blood magnesium level. Among cows with average 100-day milk yields greater than 27 kg/day, a high blood glutamate dehydrogenase concentration was also a significant risk factor for lower FSCR. The authors conclude that high body lipid mobilization during the first month of lactation was a strong nutritional predictor of both low FSCR and liver damage in high-yielding cows.



21

Spermatozoa Modulate Epididymal Cell Proliferation and Protein Secretion In Vitro

Molecular Reproduction and Development, March 2008, Volume 75, Number 3, pages 512-520.

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After their initial development in the testes, sperm cells (spermatozoa) travel into the epididymis where they gradually mature as they travel through this long, tubular organ. The first part of the epididymis, the caput (head), is a tightly convoluted segment at the top end of the testis. At the base of the testis is the similarly convoluted cauda (tail). Joining these segments is the corpus (body). Sperm maturation is regulated by androgenic hormones released by the testes and by tissue temperature, both of which affect the secretion of maturation proteins by epididymal epithelial cells into the lumen of the organ. Secretions in the caput and corpus are responsible for increasing sperm motility and their ability to successfully interact with an oocyte (egg). Mature sperm are stored in the cauda until ejaculated; secretions in this segment are responsible for maintaining sperm viability. This study was designed to determine whether the release of these secretions is influenced by the spermatozoa themselves, as they travel through the 3 segments of the epididymis. Cells extracted from each of the segments were cultured in the presence of androgenic hormones at either 37°C (body temperature) or 32°C (testis temperature). Spermatozoa were added to the cultures along with radioactive methionine to identify new proteins synthesized in response to the presence of the sperm cells. The complete pattern of proteins synthesized were profiled using electrophoresis. At an incubation temperature of 37°C, the presence of spermatozoa depressed total protein synthesis in cells from all segments of epididymis; at 32°C, the opposite effect was observed. At both incubation temperatures, spermatozoa stimulated the synthesis of new proteins by caput cells but not by corpus or cauda cells. The authors conclude that spermatozoa do affect the pattern of epididymal protein secretion.



22

A Comparison of Diagnostic Techniques for Postpartum Endometritis in Dairy Cattle

Theriogenology, April 2008, Volume 69, Number 6, pages 714-723.

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Endometritis (EM) is one of several postpartum disorders that impair the dairy cow's ability to conceive in a timely manner. While metritis has been defined as inflammation of the uterus resulting in systemic signs of sickness, EM is inflammation limited to the endometrium (lining of the uterus) occurring at least 21 days after calving and not associated with systemic illness. In the past, the lack of a clear definition and effective methods of diagnosis have impaired progress in understanding the causes and incidence of EM. The primary objective of this study was to compare 5 different methods for diagnosing EM in order to make recommendations to practising veterinarians. All 5 methods were used to examine 221 Holstein cows on 8 Saskatchewan farms between 28 and 41 days postpartum. Cytobrush cytology (CC) was found to be the most reliable method for diagnosing EM. This technique involves the insertion of a rod-shaped brush into the uterus to abrade cells from the endometrium, followed by microscopic examination of the sample to quantify inflammatory cells (polymorphonuclear cells, PMNs). Of the 189 cows from whom complete data were available, the CC samples from 21 cows (11.1%) had more than 8% PMNs, indicating EM. These cows had a 17.9% lower first service conception rate and a 24-day increase in median days open compared with their non-EM herdmates. The EM cows were also 1.9 times more likely to be non-pregnant at 150 days postpartum than cows who did not experience EM.



23

Alternative Splicing and Expression Analysis of Bovine DNA Methyltransferase 1

Developmental Dynamics, April 2008, Volume 237, Number 4, pages 1051-1059.

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Until the developing bovine embryo reaches the 8-cell stage, all cells are capable of developing into a complete individual. Beyond this stage, cells begin to take on specific functions (differentiate) in the process of forming specific tissues. Differentiation requires that the expression of some genes be inhibited while others are activated. One mechanism by which gene expression is inhibited involves the addition of methyl (CH_3) groups to specific cytosine residues in the gene's DNA sequence. Methylation is carried out by enzymes called methyltransferases, of which several different forms have been identified in some species. In the bovine, only a single form has been identified: DNA methyltransferase 1 (Dnmt1). In fetal mice, a deficiency of Dnmt1, results in death at mid-gestation. The objective of this study was to determine whether Dnmt1 is expressed in the bovine embryo and to identify other Dnmt forms that might also be involved in embryonic or fetal development. Dnmt1 was found to be widely distributed in both embryos and fetal tissues as was a variant of Dnmt1 closely related to a Dnmt1a form identified in other species. The newly-identified form was labelled Dnmt1b.



24

A Study of 55 Field Cases of Uterine Torsion in Dairy Cattle

Canadian Veterinary Journal, April 2008, Volume 49, Number 4, pages 366-372.

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In dairy cattle, uterine torsions (UT) have been reported to occur in 3 to 10.7% of difficult calvings (dystocias) attended by veterinarians and in 19% of 918 obstetrical cases referred to a veterinary hospital. UT usually occur between the initiation of labour and the expulsion of the fetus but they can also occur prepartum. Although the possible causes of UT are unclear, the authors of this paper speculate that predisposing factors may include the way that the uterus is attached to the body wall, the way in which the cow lies down and rises again and the increased fetal movements that occur in the early stages of labour. The objective of this study was to evaluate the incidence of UT in dystocias attended by veterinarians working out of the veterinary teaching hospitals at Cornell University and the Université de Montréal. Fourteen veterinarians attended 273 dystocias in New York State and Québec over a 13-month period. UT occurred in 55 (20%) of the cases with cows being 5.22 times as likely to be affected as first-calf heifers. Heifers calving alone in a pen had a lower risk of UT than those calving in a tie stall. No effect of season on UT was detected.

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Gonadotropin Releasing Hormone Receptor Gene and Protein Expression and Immunohistochemical Localization in Bovine Uterus and Oviducts

Domestic Animal Endocrinology, April 2008, Volume 34, Number 3, pages 319-326.

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It is generally accepted that the primary site of action of gonadotropin-releasing hormone (GnRH) is the pituitary gland where it stimulates the secretion of follicle stimulating hormone (FSH) and luteinizing hormone (LH). In turn FSH and LH regulate the secretion of hormones from gonadal tissues. GnRH exerts its action by binding to GnRH receptors (GnRH-R) in target tissues. Recent studies have identified GnRH-R in various reproductive tissues other than the pituitary, including ovaries, oviducts, endometria, placentae, testes, prostates, embryos, oocytes and spermatocytes in species ranging from rodents to humans. It is speculated that GnRH acts in these tissues as a local regulator of processes such as hormone production, programmed cell death and cellular proliferation. The present study describes experiments which demonstrate the presence of both GnRH-R and GnRH-R messenger RNA (mRNA) in bovine uteri and oviducts in all phases of the estrous cycle. Receptors were found to be localized in cells lining the uterus (endometrial cells) and the oviducts (epithelial cells). GnRH densities in these tissues were similar to those found in the pituitary. The authors suggest that, since GnRH is widely used in bovine reproductive programs, further investigation into its direct, local activity in reproductive tissues might result in improvements in the application of these programs.



26

Postpartum Uterine Disease and Dairy Herd Reproductive Performance: A Review

The Veterinary Journal, April 2008, Volume 176, Number 1, pages 102-114.

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Reproductive failure is the most common reason given by Canadian milk producers for culling cows from the herd. Failure to get cows bred has many potential causes. Among these, postpartum uterine disease is an important, but by no means the most significant, contributor. This paper reviews current knowledge about the incidence, causes, prevention, treatment and impacts of retained placenta (RP), metritis (MT) and endometritis (EM) in postpartum dairy cows. Incidences of all 3 of these diseases are influenced by immune competence which is typically suppressed in the period of transition from pregnancy to lactation. Defining RP as failure to expel the placenta within 24 hours after calving, this disease occurs after 5-10% of calvings with typical cases lasting 7 days. Causes are uncertain but risk factors frequently cited include twins, difficult calving, stillborn calf, induced labour, abortion, milk fever, and increasing age. RP itself is a risk factor for MT and EM, 2 diseases that are often not differentiated. MT has been defined as inflammation of the uterus resulting in systemic signs of sickness, including fever, red-brown watery foul-smelling uterine discharge, dullness, inappetance and elevated heart rate. EM is inflammation of the uterus without systemic illness, characterized by muco-purulent or purulent uterine discharge associated with chronic bacterial infection of the uterus, occurring later than 3 weeks postpartum. It is estimated that clinical EM affects 15–20% of cows at 4–6 weeks postpartum with an additional 30–35% experiencing subclinical EM between 4-9 weeks postpartum.



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Prepartal Concentration of Estradiol-17 β in Heifers with Stillborn Calves

Journal of Dairy Science, April 2008, Volume 91, Number 4, pages 1433-1437.

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The objective of this study was to determine whether abnormal blood levels of estrogen (estradiol-17 β , EB) and/or progesterone (P4) were related to stillbirths in dairy heifers. The study involved 433 heifers on a German dairy farm. Weekly blood samples were drawn starting from 3 weeks before calving as well as on the day of calving. Variables recorded at calving included calving ease, sex, birth weight and vitality status (stillborn or alive). Lower blood EB and higher P4 concentrations were observed as early as 2 weeks pre-partum in heifers who gave birth to stillborn calves. Blood EB concentrations at calving were higher with bull calves, heavier calves and when calving difficulty was increased. P4 concentrations were not associated with calf weight or sex or by degree of calving difficulty. The authors speculate that the lower blood EB concentrations of the heifers with stillborn calves could indicate an abnormality of the placenta or of hormonal signals from the calf to the placenta in the weeks before the calving.



Expression of Fibroblast Growth Factor 10 and Its Receptor, Fibroblast Growth Factor Receptor 2B, in the Bovine Corpus Luteum

Molecular Reproduction and Development, May 2008, Volume 75, Number 5, pages 940-945.

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Fibroblast growth factors (FGF) are signalling molecules that are known to play a role in the regulation of follicular development. The objective of this study was to determine whether one of these, FGF10, and its receptor, FGFR2B are involved in the development and regression of the bovine corpus luteum (CL). CLs were collected from an abattoir at 4 stages of development: corpus hemorrhagica (just ovulated), developing, developed and regressed. The abundance of messenger RNA (mRNA) transcripts coding for FGF10 and FGFR2B was measured in CLs at each stage. Both transcripts were found at all stages. There were no differences in FGF10 mRNA abundance between stages but FGFR2B abundance was 3-fold higher in regressed CLs in comparison with the other stages. To determine if FGF10 and FGFR2B expression changed during luteolysis, a dose of prostaglandin F₂α (PGF₂α) was administered to cows in the mid-luteal phase of their estrous cycles followed by collection of CLs at several time points. No differences in FGF10 mRNA abundance were found among CLs collected from 0 to 64 hours after PGF₂α but FGFR2B mRNA abundance decreased significantly at 2, 4, and 12 hours post-injection, returning to pre-injection levels between 24 and 64 hours. The authors suggest that the results indicate a role for FGF signalling during luteolysis in the cow.



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Quality of Embryos Produced From Dairy Cows Fed Whole Flaxseed and the Success of Embryo Transfer

Journal of Dairy Science, May 2008, Volume 91, Number 5, pages 1786-1790.

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The establishment of pregnancy is a major challenge in high-producing dairy cattle. Even when conception occurs, an unacceptable number of embryos are lost 15 to 17 days later, a time when elevated secretion of prostaglandin F₂α (PGF₂α) by the uterus results in lysis of the corpus luteum (luteolysis). It is thought that embryonic loss may be due to the inability of the developing embryo to sufficiently inhibit PGF₂α synthesis. Prior studies by these researchers and others showed that supplementing lactation diets with fat sources providing high levels of omega-3 fatty acids (ω3FA) decreased the secretion of a PGF₂α metabolite. This observation suggested that ω3FA feeding might lower PGF₂α synthesis and improve pregnancy rate. In the present study, whole flaxseed, a source of ω3FA, was fed to embryo donors and recipients to test its effect on donor embryo quality and recipient embryo viability. Calcium salts of palm oil, a fat source low in ω3FA, was fed to control donors and recipients. Fat source did not affect the number of viable embryos, the number of degenerated embryos, or the number of unfertilized oocytes per donor cow. However, contrary to expectations, donors fed flaxseed had lower fertilization rate (64.3 versus 78.4%) and a lower proportion of good quality embryos (56.5 vs. 74.1%) as well as a higher rate of embryo degeneration (27.4 vs. 18.2%) compared with control cows. Pregnancy rates after implantation of high quality embryos into recipient heifers were unaffected by fat source fed to either donors or recipients themselves.



Ultrasonographic and Histological Characterization of the Placenta of Somatic Nuclear Transfer-Derived Pregnancies in Dairy Cattle

Theriogenology, May 2008, Volume 69, Number 2, pages 218-230.

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Somatic cell nuclear transfer (SCNT) is a technique used to produce animal clones, typically from a genetically superior individual (donor). The technique involves removing the nucleus from an oocyte and replacing it with the nucleus of a fully differentiated somatic cell from the donor animal. To date, the efficacy of the technique in terms of producing live progeny has been very low, in the 1-5% range. Many SCNT embryos are lost before reaching the blastocyst stage and, of those that are eventually transferred to a recipient dam, many are lost during gestation, the majority between days 30 and 90 of pregnancy. The objective of this study was to compare changes in placental structure in normal pregnancies with those in SCNT pregnancies. Viable embryos derived from 2 different variations of the SCNT technique were implanted into estrus-synchronized Holstein heifers. Normal pregnancies were initiated by artificial insemination. Placental structure was assessed using ultrasonography. Of 20 SCNT pregnancies, only 8 reached term; 7 were born alive. Throughout gestation, placentome lengths and umbilical cord diameters were larger in clones than in normal fetuses. Numerous amniotic membrane abnormalities were detected, including irregularities around the umbilical cord. Microscopic examination of tissues revealed degenerate inflammatory cells, edematous placental membranes, and decreased thickness of surface membranes. The authors concluded these structural anomalies compromised fetal development. Ultrasonographic monitoring should be used to assess fetal welfare in future attempts to improve the efficacy of SCNT.



31

Meiotic Recombination in Normal and Clone Bulls and their Offspring

Cytogenetic and Genome Research, May 2008, Volume 120, Number 1-2, pages 97-101.

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Each somatic cell in the body contains 2 copies of each chromosome (except the sex chromosomes). In the formation of sperm cells (spermatocytes) and egg cells (oocytes) during meiosis, the 2 copies of each chromosome pair up and exchange segments of DNA before dividing to produce sex cells containing only one copy of each chromosome. This process ensures genetic diversity in progeny created through the fertilization of an oocyte by a spermatocyte. Several male clones have been produced from genetically superior dairy sires using somatic cell nuclear transfer (SCNT). SCNT involves the removal of the nucleus of an oocyte and its replacement by a nucleus from a somatic (body) cell of the superior sire, bypassing the cell division and chromosome recombination events that take place during the normal reproductive process. The objective of this study was to determine whether circumventing these events in producing a clone could affect chromosome recombination (crossing-over) in the clone's progeny. Recombination frequencies during spermatogenesis were compared between 5 bulls of proven fertility, 2 SCNT-derived bulls and 4 mature male progeny of SCNT bulls. No differences were found suggesting that SCNT does not influence meiosis in clones or their offspring.



S-adenosylhomocysteine Treatment of Adult Female Fibroblasts Alters X-chromosome Inactivation and Improves In Vitro Embryo Development after Somatic Cell Nuclear Transfer

Reproduction, June 2008, Volume 135, Number 6, pages 815-828.

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One of the ways by which the expression of particular genes is inhibited is the addition of methyl (CH_3) groups to certain cytosine residues in the DNA code and to other chromosomal components that regulate gene expression. Upon fertilization, the highly-methylated genomes of spermatocytes and oocytes are demethylated before DNA replication and cell division commence. When the embryo reaches the blastocyst stage, re-methylation of specific genes is one of the mechanisms that regulate cellular differentiation. Somatic cell nuclear transfer (SCNT) is a technique used to produce animal clones by implanting the nucleus of a fully differentiated somatic cell of the animal to be cloned into an enucleated oocyte. The success rate of SCNT has been very limited—only 1-5% of transfer have given rise to live clones and many of those have died at birth or have been malformed. One of the proposed explanations for these poor results relates to incomplete de-differentiation of the implanted donor cell DNA resulting in abnormal expression of genes essential for proper embryonic development. The objective of this study was to determine whether demethylation of the donor genome would improve the success of SCNT. Bovine female fibroblast (connective tissue) cells were treated with a DNA demethylation agent before their nuclei were removed and used for SCNT. Compared with embryos produced from untreated donor cells, those arising from treated cells had similar cell division frequencies but more of the treated-cell embryos advanced to the hatched blastocyst stage. Increased activities of other marker enzymes led the authors to the conclusion that demethylation of the donor genome might improve the success of SCNT by facilitating the process of reprogramming the embryonic genome.



33

Pregnancy Rates to Timed Artificial Insemination in Holstein Heifers Given Prostaglandin F2 α Twenty-Four Hours Before or Concurrent with Removal of an Intravaginal Progesterone-Releasing Insert

Journal of Dairy Science, July 2008, Volume 91, Number 7, pages 2678-2683.

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Timed artificial insemination (TAI) protocols eliminate the need to conduct visual heat detection, potentially increasing pregnancy rates (PR). The objective of this study was to compare PR responses to 3 different TAI protocols applied to heifers that had not previously calved (nulliparous). Heifers (299 total) in 2 herds were assigned to one of 3 TAI treatment groups. To commence treatment (day 0), all heifers were given a dose of gonadotropin-releasing hormone (GnRH) and a controlled internal drug release device (CIDR) that suppresses estrous activity through the constant release of progesterone. One treatment group (PG7/P8) was given a dose of prostaglandin F2- α (PGF2 α) on day 7, followed by removal of the CIDR on day 8. In a second group, PGF2 α was administered on day 7 at the same time that the CIDR was removed (PG7/P7). In a third group, PGF2 α was given and the CIDR was removed on day 8 (PG8/P8). Heifers in all 3 groups received a second GnRH dose 48 hours after the PGF2 α and TAI followed 16-20 hours later. Average PR, assessed 32 days after TAI, were 61.8, 55.5 and 54.1% for PG7/P8, PG7/P7 and PG8/P8 groups, respectively. PR was highest for heifers that were started on their protocol when they were in diestrus, the estrous cycle stage where progesterone levels are highest - 57.0% versus 34.8% for heifers in other stages. Based on these results, the authors recommend giving PGF2 α 24 hours before CIDR removal rather than concurrent with CIDR removal.



Identification of Potential Markers of Oocyte Competence Expressed in Bovine Cumulus Cells Matured with Follicle-Stimulating Hormone and/or Phorbol Myristate Acetate In Vitro

Biology of Reproduction, August 2008, Volume 79, Number 2, pages 209-222.

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In the follicular phase of the cow's estrous cycle, leading up to ovulation, a single dominant follicle begins to develop, eventually becoming a mature Graafian follicle. The mature follicle contains an oocyte surrounded by several layers of granulosa cells including a layer of corona radiata cells, a layer of mural cells, and 'bridge' of cumulus cells connecting the corona-ovum complex to the mural cells. Cumulus cells are essential to the development of oocyte competence, the ability of the oocyte to complete maturation, be successfully fertilized and reach the embryonic stage where cell differentiation begins (the blastocyst stage). Denuded oocytes in culture do not undergo normal fertilization and development, leading to embryos with low viability when transferred to recipient females. The objective of this study was to identify genes whose expression in cumulus cells might serve as indicators of oocyte competence. Previous work by these authors demonstrated that treatment of cultured cumulus-oocyte complexes with follicle stimulating hormone (FSH) or phorbol myristate acetate (PMA) could markedly increase oocyte competence. In the present study, therefore, the authors looked for genes whose expression was increased in the presence of FSH and/or PMA. Eight genes were identified that were considered potential candidates to predict oocyte competence. Doing so will improve our ability to select cultured embryos with higher probabilities of surviving transfer to recipient dams.



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Permanent Embryo Arrest: Molecular and Cellular Concepts

Molecular Human Reproduction, August 2008, Volume 14, Number 8, pages 445-453.

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Embryo transfer (ET) programs are routinely used in both human and bovine reproductive programs. One technique used to produce embryos for transfer involves the removal of an oocyte from the dominant follicle of a donor using a hypodermic needle. The oocyte is then subjected to in vitro (in laboratory) fertilization (IVF) and the resulting embryo is allowed to develop before being implanted in a foster mother. In many cases, IVF embryos fail to develop, fewer than 50% reaching the blastocyst stage. This paper reviews the possible causes of permanent embryo arrest, focussing on the roles of telomere shortening, cellular damage from reactive oxygen species (ROS) and the activity of a 'lifespan determinant protein', labelled p66Shc. A telomere is a region of repetitive DNA sequences and associated proteins found at the end of each chromosome. It is thought that telomeres protect chromosomes from the loss of genes at their terminal ends. One widely accepted theory about cellular death holds that telomeres are shortened at each cell replication cycle and that senescence occurs when telomere length reaches a critical minimum. The generation of ROS is related to the activity of p66Shc through its regulation of mitochondrial metabolism. Increased p66Shc activation induces ROS generation which may have a central role in both direct cell damage and telomere shortening, leading to permanent embryo arrest or programmed cell death (apoptosis).

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Ali, A.	Reproduction	34	207		Feeding	3	53
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	Feeding	40	88	Beauregard, G.	Health	16	147
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	Feeding	52	100	Charbonneau, E.	Feeding	37	85
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	Reproduction	16	190		Feeding	5	55
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