Highlights

in Canadian Dairy Cattle Research

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Dairy Farmers of Canada

Les Producteurs laitiers du Canada

Réseau laitier canadien

Canadian Dairy Network

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Introduction

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 2005 to September 2006. 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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1 Stocking density and feed barrier design affect the feeding and social behavior of dairy cattle

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Journal of Dairy Science (2006) Vol. 89 p. 126-133.

One of the primary objectives in managing lactating dairy cows is to maximize dry matter intake, particularly in the first few months of lactation when animals often experience negative energy balance. In free-stall herds, access to feed and competition at the feed bunk are two factors that can limit dry matter intake. This study was designed to assess the effects of stocking density and feed barrier design on feeding behaviour and competitive interactions at the feed bunk. Four levels of stocking density provided 0.81, 0.61, 0.41 or 0.21 metres of feed bunk space per cow. Feed barriers were either of a post-and-rail design or individual headlocks on 0.61 metre (24 inch) centres. Time-lapse video was used to assess behaviour at the feed bunk. Cows spent more time feeding and less time standing idly in the feeding area when the post-and-rail barrier was used. With both types of barrier, as stocking density was increased, feeding time decreased while inactive standing increased. Cows were more often displaced from the feeding area by other cows at the higher stocking densities, particularly at the post-and-rail barrier. The headlock barrier provided some protection from displacement for cows ranking low in the herd's social hierarchy, especially at the higher stocking densities.

2 Effect of hoof pathologies on subjective assessment of dairy cow gait

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Journal of Dairy Science (2006) Vol. 89 p. 139-146.

It is often difficult to identify lame cows until the condition is well advanced, by which time treatment costs are high and recovery rates are low. Several locomotion scoring systems have been developed in attempts to identify cows experiencing early stages of lameness. The authors of this study developed a locomotion scoring system based on six gait attributes: back arch, head bob, tracking-up (placing back hoof in the footprint of the front), joint flexion, asymmetric gait, and reluctance to bear weight. These attributes and overall gait were scored from video recordings using a 1 to 5 rating system (1 = sound, 5 = severely lame) as well as a 100-unit visual scale. This study examined the effects of sole hemorrhages and sole ulcers on these gait measurements. When compared with healthy cows, those with sole ulcers walked with greater back arch, more head bobbing, shorter strides (incomplete tracking-up), and less symmetric gait. The numerical gait assessment system correctly identified 92% of cows with sole ulcers although it was not possible to discriminate cows with sole hemorrhages. For most attributes, results from this scoring system were reproducible between different observers and for individual observers scoring the same cows multiple times.

3 Stall dimensions and the prevalence of lameness, injury, and cleanliness on 317 tie-stall dairy farms in Ontario

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Canadian Veterinary Journal (2005) Vol. 46 p. 902-909.

Approximately 80% of Ontario dairy cows are housed in tie-stalls and some are provided periods of exercise outside of the barn. Tie-stall design can, therefore, have a significant impact on animal health, welfare, productivity and longevity. The purpose of this study was to establish benchmarks for tie-stall dimensions as well as indicators of lameness and animal welfare. Characteristics of tie-stalls on 317 Ontario dairy farms were examined, including stall length, width, tie-chain length and tie rail height. Ninety percent of the farms surveyed had stalls with dimensions that were less than those currently recommended. Lameness incidence was assessed using several previously recommended animal-based parameters: 3.2% of cows exhibited arched backs while standing; 23% had severe hind limb rotation; 44% suffered hock lesions. Among other indicators of poor stall design, 3.8% had neck lesions, 3% had broken tails, 23% had dirty hind limbs and 4.6% had dirty udders. The authors of the study suggest that veterinarians and producers could use this information as benchmarks for comparison with individual herds as they strive for improvement in animal welfare.

4 Effects of mixing on drinking and competitive behavior of dairy calves

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Journal of Dairy Science (2006) Vol. 89 p. 229-233.

Group rearing reduces labour costs and provides welfare benefits for dairy calves, including more space and opportunities for social interaction. However, the system can result in competition and other negative responses that reduce performance. Group rearing requires that new calves are introduced to the group soon after birth while older calves are removed at weaning. These dynamics can create problems related to social integration, particularly for the younger animals. This study examined calf behaviour and milk consumption when younger calves were mixed into an established group of older calves. Milk was provided in a computer controlled milk feeder with a feeding stall which limited aggressive displacements of calves while feeding. Group average milk consumption dropped from 9.7 kg/day before mixing to 8.6 kg/day on the day of mixing but subsequently increased over the next 3 days to 11.1 kg/day. Visits to the feeder decreased on the day of mixing when compared with visits during the 2 days before or 4 days after mixing. Conversely, the duration of visits and milk consumption during those visits increased on the day of mixing. In conclusion, although feeding behaviour was altered on the day when new calves were introduced, the effects were transient and milk intake recovered quickly.

5 Plasma melatonin and insulin-like growth factor-1 responses to dim light at night in dairy heifers

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Journal of Pineal Research (2006) Vol. 40 p. 225-229.

Melatonin is a hormone secreted by the pineal gland in the brain in response to information received from the retina about the daily pattern of light and dark. Normally higher at night, melatonin secretion is inhibited by light. It is assumed that the dark period is required in order to maintain normal physiological function. The objective of this study was to determine the level of light that would interfere with the normal diurnal cycle of melatonin in order to establish a safe light level that could be used in dairy barns at times when darkness is recommended. Twelve Holstein heifers approximately 8 months of age were alternately subjected to light levels of 5, 10 or 50 lux. Blood plasma levels of melatonin and insulin-like growth factor-1 (IGF-1) were determined before light levels were decreased and at intervals during the eight-hour low light treatments. Exposure to 5 or 10 lux did not decrease normal night-time melatonin levels and had no effect on IGF-1 concentrations. When heifers were exposed to 50 lux, melatonin levels were lower during the first 2 hours of treatment but not thereafter; IGF-1 concentrations were not affected. A light intensity of 10 lux or less appears safe in situations where darkness is recommended.

6 Flooring in front of the feed bunk affects feeding behavior and use of freestalls by dairy cows

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Journal of Dairy Science (2006) Vol. 89 p. 2065-2071.

Although most research on cow comfort has focused on stall design, for cows housed in free-stall barns other elements of barn design may also be important. This research examined the effects of flooring surface in the feeding area on the resting, standing and feeding behaviour of cows. In one trial, individually penned dry cows were offered either concrete or sawdust-bedded standing areas in front of their feed bunks. In a second trial, non-lactating cows were offered either concrete or rubber flooring. In both trials, cows spent more time standing on the softer surfaces, exhibiting more frequent bouts of inactive standing or eating. The higher frequency of these activities indicated that the animals were more willing to move on the softer floors. In the first trial, feed intake was higher when the surface was sawdust; in the second, total time spent eating and lying in the feeding area was higher for cows on rubber flooring. The results indicate that cows prefer soft surfaces over concrete for standing and walking. Increased feed intake and time spent eating may also have positive effects on production.

7 Effects of milking on dairy cow gait

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Journal of Dairy Science (2006) Vol. 89 p. 2084-2089.

Several locomotion scoring systems have been developed in attempts to identify cows experiencing early stage lameness. However, in applying these systems, it has become clear that lameness is not the only cause of variation in cow locomotion. In this study, the authors examined gait characteristics of cows before and after milking to determine the effects of full udders on locomotion. They also speculated that walking with a full udder might exaggerate the effects of lameness on gait characteristics. Gait scoring was based on four gait attributes: back arch, head bob, tracking-up (placing back hoof in the footprint of the front), and reluctance to bear weight. These attributes and overall gait were scored from video recordings using a 1 to 5 rating system (1 = sound, 5 = severely lame) as well as a 100-unit visual scale. After milking, all cows, with or without sole ulcers, exhibited significantly longer and higher strides, shorter stride durations, shorter periods with 3 hooves in ground contact and faster walking. Tracking-up and reluctance to bear weight improved but back arch did not change. For all gait characteristics except swing duration, cows with sole ulcers walked differently than those without, both before and after milking. The authors suggest that gait scoring should be done after milking when differences between cows with and without sole ulcers is most evident.

8 Effects of continuous versus periodic milk availability on behavior and performance of dairy calves

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For the first 1 to 3 months of life, calves are typically fed a volume of milk or milk replacer equivalent to a total daily intake of approximately 10% of their body weight. This amount of milk is normally divided into 2 feedings and fed either from a bucket or a nipple. Earlier research showed that calves offered unlimited (ad libitum) access to milk through a nipple 24 hours per day consumed more milk and grew faster than conventionally-fed calves. However, making milk available 24-hours per day poses several challenges, particularly in warm weather. This trial was designed to determine if offering milk ad libitum through a nipple for each of two 2-hour periods daily could be an alternative to 24-hour access. A secondary objective was to test the effect of offering water through the nipple during the 20 hours in which milk was not available. When offered 24 hours per day, milk intake was higher (average 11.2 kg/day) than when it was available for only 4 hours per day (average 10.0 kg/day), whether water was available or not. However, despite only have access to milk for 4 hours per day average daily gain was similar for calves in all three treatment groups. Although calves offered milk over 24 hours spent more total time sucking, those offered milk for only 4 hours sucked more frequently during the 4 hours when milk was available than did the 24-hour calves during the same 4 hour time period.

9 Hoof discomfort changes how dairy cattle distribute their body weight

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A number of locomotion scoring systems have been proposed for the early detection of lameness. However, most rely on the subjective assessment of several criteria of cow posture and gait. More recently, it has been proposed that an objective system might be based on the automated measurement of weight distribution on each hoof. This proposal is based on the observation that some animals reduce the weight borne on a painful limb, transferring the load to other limbs. The objective of this study was to determine how non-lame cows would change the weight distribution on their four limbs in response to discomfort in one or more hooves. Discomfort was provoked by replacing a soft rubber surface with concrete. When the concrete was placed under either back hoof, cows shifted their weight to the back hoof on the opposite side of the body. When a front hoof was placed on the uncomfortable surface, weight was shifted to the opposite front hoof and the back hoof on the same side as the uncomfortable front hoof. Cows shifted weight to their back hooves when both front hooves were placed on concrete but a shift forward did not occur when concrete was under both back hooves. An understanding of these weight shifting responses will help to identify limbs affected by lameness in such automated weight distribution systems.

10 Brisket boards reduce free stall use

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Brisket boards are designed to prevent cows from defecating and urinating in the stall by preventing them from lying too far forward. The objectives of this study were to evaluate cow preferences for stalls with or without brisket boards and to assess the effects of brisket boards on stall use. When offered the choice between stalls with or without brisket boards, one group of 15 non-lactating cows spent 32% of their lying time in the stalls with boards. The same cows spent 68% of their time lying in stalls where brisket boards were absent. Another group of 13 cows spent an average of 1.2 hours more time lying in the stalls without brisket boards. In the absence of brisket boards, cows placed themselves relatively forward in stalls during 98% of their lying bouts compared with 67% when boards were present; this was particularly true for longer cows. Lying bouts were, on average, 0.2 hours longer in stalls without brisket boards. The authors conclude that, although brisket boards may encourage cows to defecate and urinate in the alley behind their stalls, they do so at a cost to cow comfort.

11 Effects of roughness and compressibility of flooring on cow locomotion

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In many of Canada's typical dairy cattle management systems, cows walk and stand most often on hard concrete floors. Since smooth concrete floors covered in slurry can result in injury due to slipping, most concrete flooring is grooved to increase the friction between cows' hooves and concrete. However, the hardness of concrete can increase the incidence of hoof lesions and research has shown that, given a choice, cows will avoid concrete. The objective of this study was to assess cow locomotion on an alternative type of soft rubber flooring and to determine the relative importance of the friction of the floor and its degree of compressibility. Cows were observed as they navigated a narrow corridor incorporating a gutter crossing and a 90 degree turn. Covering the floors with slurry increased the amount of slipping and reduced the cows' walking speed, showing the importance of keeping floors dry and clean. Compared with concrete, a commercial rubber flooring material (Animat®) reduced slipping as well as increasing the speed that the cows walked, so that the time taken to traverse the corridor was shorter. Increasing the compressibility of the floor while keeping the degree of surface friction constant increased the walking speed of the cows and the length of their steps but did not reduce slipping. The results show that flooring for cows needs to both be softer than concrete as well as providing a higher degree of surface friction.

12 Feed stalls affect the social and feeding behavior of lactating dairy cows

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Earlier studies by researchers at UBC demonstrated that increasing feed bunk space per cow above the traditional 0.61 metres (24 inches) decreased aggressive encounters at the feed barrier and increased feeding activity. Cows fed at a headlock feeding barrier experienced fewer displacements than those fed at a post and rail barrier. These results led to the hypothesis that aggressive encounters resulting in reduced feeding activity might be further reduced by providing individual feeding stalls at the feed bunk. The present study was designed to assess both the effect of increased feeding space per cow and the value of providing feed stalls. Treatments were 0.64 metres per cow or 0.92 metres per cow provided at a post and rail barrier or 0.87 metres per cow with adjacent feeding spaces separated by fixed partitions to form stalls. Time-lapse video recorded feeding, standing and aggressive behaviour in the feeding area. In agreement with previous trials, providing 0.92 metres per cow resulted in increased total daily feeding time together with reductions in inactive standing time and the frequency of aggressive interactions when compared with an allowance of 0.64 metres/cow. The addition of feed stalls further enhanced these effects and forced aggressive cows to change their displacement strategy by butting cows from the rear rather than the side or front. Cows of lower social standing experienced the greatest decrease in displacements when feed stalls were provided.

Environment

1 An approach for measuring methane emissions from whole farms

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It has been estimated that ruminant livestock are responsible for the release of approximately 85 Gigatonnes of methane annually. However, there is considerable uncertainty in this estimate because of the difficulty inherent in estimating methane emissions from livestock facilities. The difficulty increases where there is no constraint to air exchange as is the case for cattle feedlots and 'open' dairy barns. In this research, methane emissions from a commercial dairy farm in southern Alberta were estimated by applying a mathematical model of air dispersion to downwind concentrations of gas and three-dimensional wind measurements. To test the accuracy of the model, methane and sulfur hexafluoride (SF_6) were simultaneously released in known quantities from 10 locations on the farm. Concentrations of SF_6 were measured by gas chromatography; methane was measured with open path lasers. The model accounted for 86% of the methane released and 100% of the SF_6. Although the choice of downwind locations for sampling gas concentrations is critical to the success of this technique, it is easily deployed and should be suitable for the quick evaluation of farm-scale emissions and the effects of mitigation practices.

2 Measuring greenhouse gas emissions from point sources in agriculture

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Canadian Journal of Soil Science (2006) Vol. 86 p. 355-371.

Agricultural greenhouse gas (GHG) emissions include methane from ruminant livestock digestive processes as well as methane, nitrous oxide and carbon dioxide from manure storage structures. Estimation of the quantities of GHG emissions from these sources is important for the establishment of current inventories and future targets and in determining the effects of mitigation strategies. This article reviews methods used to estimate emissions from point sources. Chamber techniques measure gas fluxes from single or a few animals in a well controlled, confined area. Micrometeorological methods are used to estimate emissions from sources such as livestock and manure storage facilities. New technologies that employ dispersion models may be suitable for direct measurement of emissions from whole farms. The author recommends the use of multiple techniques when estimating GHG emissions, as differences exist between estimates from the various methods available. Ideally, methods should be calibrated using the controlled release of a known amount of the target gas. Although many techniques are suitable for measuring relative changes in GHG emissions, the estimation of absolute quantities is more difficult.

3 A proposed approach to estimate and reduce net greenhouse gas emissions from whole farms

Canadian Journal of Soil Science (2006) Vol. 86 p. 401-418.

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There are two simple alternatives for reducing greenhouse gas (GHG) emissions from farms: reducing the release of GHG or increasing the storage of carbon in soils. However, practice changes that may reduce GHG emissions in one part of the farm enterprise may increase emissions in another. For example, growing a new crop that reduces methane emissions from cattle may require the use of increased nitrogen fertilizer, affecting nitrous oxide emissions. Or a new legume crop may result in the sequestration of more carbon dioxide but feeding such a crop to cattle may influence methane emissions from enteric fermentation. Such complex interactions require a complete understanding of GHG dynamics on the whole farm. The authors of this paper propose the development of a mathematical model which would integrate all aspects of the farm enterprise. Such a 'Virtual Farm' model would provide a repository of current knowledge and help to focus future research efforts in areas where knowledge is lacking. Once constructed, such a model would allow decision makers to evaluate the potential of mitigation strategies before investing in their application. The proposal describes a blueprint for the construction of a Virtual Farm model.

4 Methane and nitrous oxide emissions from Canadian animal agriculture: A review

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Canadian Journal of Animal Science (2006) Vol. 86 p. 135-158.

A large body of scientific evidence suggests that increasing emissions of carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) are, at least partially, responsible for changes in global climate that have become increasingly evident. These 'greenhouse gases' (GHG) trap heat reflected from the earth, resulting in atmospheric warming. CH_4 is approximately 21 times more effective than CO_2 in trapping heat; N_2O is about 300 times more effective than CO_2. Globally, agriculture accounts for about 20% of anthropogenic (caused by man) sources. GHG emissions can be expressed in terms of CO_2 equivalents (CO_2e). In 2002, agriculture contributed only 8% of Canada's total CO_2e emissions but 26% of the country's CH_4 and 65% of its N_2O emissions. The main sources of agricultural CH_4 and N_2O are livestock—CH_4 from rumen fermentation, CH_4 and N_2O from manure and grazed lands, and N_2O from soils after manure application. Livestock are directly responsible for about 32% of all Canadian agricultural GHG emissions; manure management practices are responsible for 17% and 50% are from soils, including that from manure application. The objective of the paper was to review the processes involved in generating GHG emissions from livestock, focusing on enteric fermentation in ruminants and on swine and ruminant manure management practices. Methods of measuring emissions, mathematical models used to estimate emissions and practices which can be used to mitigate emissions, such as modifying feed formulations and manure management strategies, are also discussed.

Feeding

1 Effects of alfalfa particle size and specific gravity on physical characteristics of alfalfa, ration and performance of Holstein dairy cows

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Agricultural Sciences and Technology (2005) Vol. 19 p. Pe105-Pe123.

Dairy cattle require dietary fibre to maintain digestive health. Physically effective fibre provokes chewing and salivation which helps to maintain a stable rumen environment favourable to the growth of fibre digesting micro-organisms. The first part of this research examined the relationships between alfalfa hay particle size and other physical characteristics. When compared with coarsely chopped hay, finely chopped alfalfa hay had lower water holding capacity, but higher hydration rate and bulk density. In the second part of the study, 9 mid-lactation cows were offered total mixed rations (TMRs) containing 20% alfalfa hay, 20% corn silage and 60% concentrate (dry matter basis). The TMRs offered to each of 3 groups of 3 cows varied in alfalfa hay particle size—fine, medium or coarse. As particle size decreased, intakes of dry matter and neutral detergent fibre (NDF) increased but NDF digestibility and physically effective fibre intake decreased. Retention time of particles in the rumen was lower and particulate passage rate through the rumen was higher for the more finely chopped hay. Although milk and fat-corrected milk yields were unaffected by particle size, rumen pH, total chewing activity, rumination, eating time and milk fat test decreased with reduced particle size.

2 Phosphorus status of lactating dairy cows fed total mixed rations containing 0.24% vs. 0.36% phosphorus

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When dairy cattle consume phosphorus (P) in excess of their nutritional requirements, the excess P is excreted, increasing environmental loading. The US National Research Council's (NRC) 2001 publication 'Nutrient Requirements of Dairy Cattle' suggests that the P requirement of lactating dairy cows is in the range of 0.32 – 0.42% of dietary dry matter. However, many milk producers believe that these concentrations are inadequate to maintain good reproductive performance, preferring to feed diets containing much higher levels. The present study was designed to determine whether P intakes even lower than NRC recommendations would be adequate to satisfy requirements in early lactation. Lactating cows were fed corn silage-based total mixed rations (TMR) containing either 0.24 or 0.36% P from 3 to 13 weeks after calving after which P balance was assessed. All cows had previously been offered diets containing identical P levels. P digestibility was lower in the cows fed 0.36% P (29.0 vs. 48.8%) and fecal P excretion was almost twice as high (49.1 vs. 25.7 g/day) compared with the cows fed 0.24% P. TMR dry matter intake and milk yield were not affected by dietary P concentration. Results suggest that a diet containing 26% less P than NRC recommendations is adequate to maintain short-term P balance in early lactation while reducing the amount of P excreted into the environment.

3 Milk production and milk composition of dairy cows fed Lac100 or whole flaxseed

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Fat or oil supplements are often added to diets fed to lactating cows to increase dietary energy levels. However, this practice often reduces feed intake, resulting in energy intake which is less than predicted. The reduction in feed intake is thought to be related to the biohydrogenation of polyunsaturated fatty acids by rumen micro-organisms. To reduce this phenomenon, 'protected' lipid supplements can be used. Protection is afforded either by coating fat particles with a substance that is resistant to ruminal degradation or by using calcium salts of free fatty acids. Some of these products are only partially protected. For example, earlier results showed that, although feeding calcium salts of unsaturated fatty acids did not affect feed intake, the concentration of conjugated linoleic acid (CLA) in milk increased. This observation indicated that some proportion of the linoleic acid in the supplement was metabolized in the rumen. The objective of this study was to examine the effects on production and milk composition of feeding a calcium salt of soybean oil fatty acids (Lac100). Whole flaxseed was fed for comparison since it is a rich source of alpha-linolenic acid (ALA) and has been shown to have little effect on feed intake. Flaxseed decreased milk production but increased concentrations of fat, protein and ALA. Lac100 increased milk CLA concentration.

4 Ensiling characteristics, nutrient composition, and in situ ruminal and whole tract degradability of brown midrib and leafy corn silage

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One of the limitations of traditional corn varieties used as animal feed is the low digestibility of its fibre fraction. Newer hybrids have been developed to overcome this limitation. Brown midrib (BMR) varieties have lower lignin levels in their stalks and leaves; leafy hybrids have more leaves and their stalks have thinner rinds. This study evaluated the ensiling characteristics, chemical composition and digestibility of representative varieties of these two corn hybrid types as a basis for judging their suitability in diets fed to lactating cows. Both hybrids fermented well, rapid increases in acidity resulting in pH values less than 4.0 after 45 days in mini-silos. Lactic acid concentration was highest for the leafy variety. Compositional analysis of the ensiled forage yielded the following results: BMR samples had lower neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), neutral and acid detergent insoluble proteins and crude protein (CP); samples of the leafy hybrid had lower starch and net energy of lactation values. Incubation of samples in the digestive tracts of cannulated, non-lactating cows revealed that the BMR variety had greater ruminal dry matter (DM), NDF and CP degradability as well as greater ruminal and total tract DM and NDF digestibilities. Although BMR hybrids are generally lower yielding than conventional varieties, the characteristics of the BMR variety examined in this study suggest that it would be a good choice for feeding to lactating cows.

5 Frequency of feed delivery affects the behavior of lactating dairy cows

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It is important to manage the feeding of lactating dairy cows to meet their nutrient requirements. Although diet formulation is the primary means by which managers attempt to satisfy nutrient requirements, strategies for stimulating feeding behaviour can be equally important. The primary objective of this study was to evaluate how feeding frequency affects feeding behaviour in cows offered a group-fed TMR in a free-stall facility. In one trial, fresh feed was distributed in the feed alley once (1x) or twice (2x) per day. In a second trial, feed was distributed twice or 4 times (4x) per day. Feed was pushed up zero, twice or three times per day in the 4x, 2x and 1x treatments, respectively. Stocking density allowed each cow her own free stall and 0.6 metres (2 feet) of feed bunk space. Behaviour was recorded by time-lapse video. Increasing feeding frequency resulted in more even distribution of eating times and fewer displacements of subordinate cows from the feeding area, resulting in more equal access to feed for all cows throughout the day. Feeding frequency had no effect on daily lying times or total number of aggressive interactions at the feed bunk. For all feeding frequencies, the neutral detergent fibre content of feed increased progressively after feeding, indicating that cows were sorting to access dietary concentrates. Sorting was decreased by increasing feed delivery from 1x to 2x.

6 Protein secondary structures (α-helix and β-sheet) at a cellular level and protein fractions in relation to rumen degradation behaviours of protein: A new approach

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British Journal of Nutrition (2005) Vol. 94 p. 655-665.

Proteins are composed of linear chains of amino acids, often thousands of amino acids in length, linked through peptide bonds. Primary structure describes the sequence of amino acids that characterize a unique protein. Secondary structure describes one aspect of the three dimensional shape of a protein. The main protein secondary structures are referred to as the alpha-helix (α-helix) and the beta-sheet (β-sheet), both of which can be present in a single protein. Proteins having a high proportion of the β-sheet structure tend to be less digestible because this structure restricts access to the peptide bonds by digestive enzymes. Heating proteins affects their secondary structure and, thus, their digestibility. For example, soybeans are often roasted before feeding to dairy cattle to decrease the rumen degradability (increase the bypass value) of the protein fraction. The objective of this research was to determine whether protein secondary structure could be correlated with digestibility. The secondary structures of proteins in raw and roasted flaxseeds were examined using advanced synchrotron-based technology. Digestibility was calculated from rumen incubation of seed samples contained in polyester bags. Roasting increased the percentage of β-sheet structure from 37.2% to 49.8%, decreasing the proportion of α-helix from 47.1% to 36.1%. The digestibility trial confirmed that, as predicted by the observed structural changes, roasting decreased the ruminal degradability as well as the intestinal digestibility of the flaxseeds.

7 Folic acid and vitamin B12 requirements of dairy cows: A concept to be revisited

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Until recently, it had been generally assumed that the ruminant animal's requirements for folic acid and vitamin B12 were satisfied by the rumen microbial synthesis of these compounds, combined with dietary sources that escape rumen degradation. As a result, few studies have been undertaken to define the requirements of the vitamins at high levels of production. Studies summarized in this review demonstrate that supplementation can improve milk production and metabolic efficiency, particularly in early lactation. These results imply that microbial synthesis and basal dietary levels of folic acid and vitamin B12 may not always be adequate to support the increased productivity of today's lactating cows. However, supplementation of unprotected sources is not very efficient because of their rapid breakdown in the rumen. Identification of a benefit from providing supplemental amounts of these 2 vitamins may lead to the development of products in which they are protected from rumen degradation.

8 Modeling nutrient supply to dairy cattle from a feedstuff using NRC-2001 (a TDN-based model) with inputs based on in situ and mobile bag technique measurements

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Canadian Journal of Animal Science (2005) Vol. 85 p. 513-519.

The 2001 edition of the US National Research Council's (NRC) publication, 'Nutrient Requirements of Dairy Cattle' introduced the concept of metabolizable protein (MP) in its nutritional model. MP is defined as true protein absorbed in the intestine, derived from microbial protein, undegraded feed protein, gastro-intestinal secretions and protein released from the normal turnover of digestive tract tissue. Protein synthesis by rumen microbes may be limited by the supply of either degradable protein or energy-yielding components in the diet. When degradable protein is available in excess of energy, attempts are often made to rebalance the diet, increasing the contribution of undegraded feed protein to MP. To this end, one strategy often applied to oilseeds and pulse crops is to heat the seeds, decreasing the rumen degradability of their constituent proteins. The objective of this study was to assess the value of the 2001 NRC model in predicting the MP contributions of raw or toasted whole lupin seeds. Ground seed samples were incubated in the digestive tract to estimate protein degradability and digestibility. These estimates were used as inputs into the 2001 NRC model, in place of values derived from the model's feed database. This approach significantly improved the prediction of microbial protein synthesis and MP supply.

9 Dietary forage and nonfiber carbohydrate contents influence b-vitamin intake, duodenal flow, and apparent ruminal synthesis in lactating dairy cows

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Until recently, it had been assumed that basal dietary supply plus rumen microbial synthesis were sufficient to satisfy the B-vitamin requirements of ruminant livestock. However, studies have demonstrated that supplementation of the B-vitamins thiamine, biotin, niacin and folic acid to lactating dairy cows could improve production. In contrast, other studies failed to show such responses when similar amounts of these vitamins were added to diets. Possible reasons for these inconsistent results are many but one possibility is that the varying diets fed in these trials influenced the synthesis of vitamins by rumen micro-organisms. The objective of this study was to examine this possibility by estimating microbial B-vitamin synthesis in cows fed diets containing varying proportions of forage and non-fibre carbohydrates (NFC). Holstein cows fitted with ruminal and duodenal cannulae were offered each of 4 different total mixed rations (TMRs) during sequential 21 day periods. TMRs contained 35 or 60% forage and 30 or 40% NFC. No supplemental B-vitamins were fed. Rumen synthesis of pyridoxine, folic acid and B12 was lower when cows were fed the higher forage diets. Higher dietary NFC levels increased the synthesis of nicotinic acid, nicotinamide, niacin, pyridoxine, B6 and folic acid by decreased B12. These results support the hypothesis that rumen microbial B-vitamin synthesis is influenced by diet.

10 Effects of physically effective fiber on chewing activity and ruminal pH of dairy cows fed diets based on barley silage

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The high grain diets typically fed to dairy cows in early lactation often produce subacute ruminal acidosis (SARA), characterized by depressed rumen pH. Ideally, the acids produced by the fermentation of carbohydrates are neutralized by salivary buffers or absorbed through the rumen wall. SARA results when the rate of acid production exceeds the rate of absorption and/or the buffer supply. Physically effective (pe) fibre provokes chewing which stimulates saliva production so the question becomes: What is the optimal dietary balance between fermentable carbohydrates and pe fibre which will maximize production and minimze the incidence of SARA? The objective of this study was to determine this optimal balance for diets based on barley silage and barley grain. Cows were sequentially offered 3 diets containing barley silage chopped long, medium or fine with physically effective neutral detergent fibre (peNDF) levels of 13.8, 11.8 and 10.5%, respectively. Total chewing time increased in proportion to dietary peNDF but measures of rumen pH status were not affected by peNDF. These results suggest that increased chewing and subsequent saliva production were not sufficient to fully overcome the rumen pH lowering effect of the high concentrations of acids produced through fermentation of dietary carbohydrates.

11 Milk from forage as affected by carbohydrate source and degradability with alfalfa silage-based diets

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Milk from forage (MF) is a theoretical estimate of the amount of milk produced from the forage portion of a complete ration. It is calculated by subtracting from total milk yield the production theoretically supported by the concentrate portion of the diet, assuming that maintenance requirements are covered by forage. Having been used in Québec for many years as an estimate of the efficiency of feed use, MF has been shown to have economic value, since forages are generally the least costly part of the diet. However, the specific combination of forage and concentrates in the diet can influence MF—when the nutrients provided by concentrates are more complementary to those in the forage, MF is likely to be higher. In this study, 4 mixed concentrates containing non-fibre carbohydrate (NFC) with varying rumen degradability were fed in alfalfa silage-based diets to evaluate effects on MF. NFC sources in the 4 concentrates were cracked corn (control), ground corn (GC), GC plus wheat starch (GC+S) and GC plus dried whey permeate (GC+W). Diets containing GC or GC+S increased both MF and dry matter intake when compared with the control. This was likely due to the higher degradability of those NFC sources which complemented the highly degradable protein in the alfalfa silage.

12 Impact of lowering dietary cation-anion difference in non-lactating cows: A meta-analysis

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Reduction of dietary cation-anion difference (DCAD) in the late dry period is recommended as a strategy to reduce the incidence of milk fever. Over the past 30 years, a large number of studies have evaluated the efficacy of this strategy; several have suggested modifications to its application or to the method of calculating DCAD. This article presents a review of 22 of those previous studies. Trial data was combined and subjected to re-analysis to build a statistical model relating DCAD to indicators of metabolic status. Lower DCAD levels were associated with reductions in clinical milk fever, dry matter (DM) intake, urinary pH, blood bicarbonate, and blood carbon dioxide concentrations; blood pH was also slightly reduced. Blood ionized calcium was increased before and at calving. Five different equations used to calculate DCAD were compared for their ability to account for milk fever incidence and urinary pH reduction in trial groups. The equation (Na + K) – (Cl + 0.6 x S) was the most highly associated with both clinical milk fever and urinary pH. The model developed from this analysis predicted that a reduction in DCAD from +300 to 0 ionic milliequivalents per kg of dietary DM would reduce the risk for clinical milk fever from 16.4 to 3.2%, decrease urinary pH from 8.1 to 7.0 and reduce DM intake by 11.3%.

13 Effects of tannic acid and quebracho tannins on in vitro ruminal fermentation of wheat and corn grain

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In the past, tannins were considered antinutritive components of feeds because they inhibit digestion of proteins and other feed fractions. However, a number of studies have demonstrated that tannins may be beneficial in ruminant diets by reducing the rumen degradability of plant proteins and improving the efficiency of dietary nitrogen utilization. Given this effect on reducing rumen protein degradability, the objective of the present study was to determine whether tannins might have a similar effect on the rate of rumen starch degradation. If so, the addition of tannins to high starch cattle diets might help to alleviate the acidosis associated with feeding these diets. To test this hypothesis, tannins were added to laboratory fermenters in which ground corn or wheat were incubated in rumen fluid containing mixed rumen micro-organisms. Tannins inhibited the fermentation of starch in both grains but the effect was more pronounced with wheat, particularly during the early stages of fermentation. Examination of starch granules by electron microscopy revealed that tannins did not directly inhibit microbial degradation of starch. Their inhibitory effect was due to a reduction in the rate of degradation of the protein matrix surrounding the starch granules. Since the protein matrix in wheat is more degradable than that in corn, the tannins had a greater inhibitory effect on starch degradation in wheat.

14 Effect of casein and propionate supply on whole body protein metabolism in lactating dairy cows

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Protein and energy metabolism are intimately interconnected. Increasing intake will increase both energy and protein supply and little studies have examined how individual macronutrients, particularly energy or protein, might impact on protein metabolism and animal performance. This is important because, in mechanistic terms, either protein/amino acids or energy may act differently. The present study was designed to evaluate the separate and interconnected consequences of providing supplemental energy or protein to lactating cows. Propionic acid, an energy source and the primary precursor of glucose in the lactating cow, was infused directly into the rumen. Casein, the principle protein in milk, was infused into the duodenum, the first segment of the small intestine. Both were infused separately and in combination. Casein infusions resulted in higher milk yield as well as increased milk protein concentration and yield. Increased oxidation of amino acid, protein synthesis, and milk protein output indicated that casein infusions provoked a general response in protein turnover. Propionate infusions increased milk protein concentration and yield and whole body protein synthesis to a lesser extent. There was a tendency to decrease amino acid oxidation at the highest level of energy. This suggested that the impact of energy on protein metabolism depends on the energy status of the animal.

15 Acidogenic value of feeds. I. The relationship between the acidogenic value of feeds and in vitro ruminal pH changes

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Rumen pH is determined by the balance between rates of fermentation acid production, absorption of those acids across the rumen wall, and neutralization by salivary buffers. Several methods have been proposed for predicting the effects that individual feeds or complete diets might have on rumen pH. For example, the concept of physically effective neutral detergent fibre (peNDF) is based on the idea that this measure correlates with chewing activity and, therefore, salivary buffer production. The primary objective of the present study was to test the use of 'acidogenic value' (AV) to predict the impact of feeds on rumen pH. A secondary objective was to examine the relationships between AV and chemical composition. AV was determined by measuring the amount of calcium released when calcium carbonate was incubated with a feed sample in buffered rumen fluid. Change in pH after 24 hours of incubation was highly correlated with AV for all feeds tested. Among measures of feed composition, non-fibre carbohydrates (NFC) and acid detergent fibre (ADF) were the best predictors of AV for energy feeds; neutral detergent fibre was best for forages; crude protein for protein sources. Across all feed types, AV was best predicted by NFC and ADF.

16 Acidogenic value of feeds. II. Effects of rumen acid load from feeds on dry matter intake, ruminal pH, fibre degradability and milk production in the lactating dairy cow

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Feed factors that affect rumen pH include the rate and amount of acid produced from its fermentation by rumen micro-organisms and its ability to stimulate chewing and production of salivary buffers. Acidogenic value (AV) provides an estimate of a feed's acid production potential. Sub-acute ruminal acidosis (SARA) occurs when rumen pH falls repeatedly into the 5.2 to 5.6 range. Daily time below pH 5.6 has been suggested as a more important determinant of SARA than mean daily ruminal pH because extended periods of low pH are detrimental to the growth of fibre-digesting micro-organisms, decreasing feed intake, microbial protein synthesis and milk production. The objective of this study was to evaluate the effects of AV on ruminal pH, dry matter intake (DMI) and dietary fibre degradability as well as milk production and composition. Concentrate formulations with either low (LAV) or high AV (HAV) were fed with corn silage and alfalfa silage as total mixed rations (TMRs) to lactating cows. When compared with the cows fed the ration containing LAV, those on the HAV ration had ruminal pH values below 5.6 for longer periods of time, produced more milk (23.4 vs. 21.8 litres/day), milk protein and lactose, and had higher milk lactose concentrations. Neutral detergent fibre degradation of feed samples incubated in the rumen was lower in cows fed the HAV concentrate. Cows on both diets had similar daily mean rumen pH, DMI and 4% fat corrected milk production.

17 What is the true supply of amino acids for a dairy cow?

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The formulation of diets for any species requires accurate knowledge of nutrient requirement and supply. In the case of the ruminants, including the lactating dairy cow, the determination of the real supply to the host animal is particularly complex due to the transformations by the rumen microbial mass that affect nutrients between ingestion and digestion. This article provides an overview of the path travelled by dietary protein and its constituent amino acids (AA) from the rumen to the mammary gland. On entering the rumen, dietary proteins are largely broken down and re-synthesized into microbial protein. These proteins eventually pass into the small intestine along with any dietary protein which has escaped rumen degradation and proteins derived from tissue turnover and secretions into the digestive tract (endogenous secretions). Digestion of these proteins releases AA which are absorbed by mucosal cells lining the intestine and transferred into blood circulation via the portal vein. The proportion of absorbed AA which actually reaches the portal vein is not fixed and differs between AA. Some AA are oxidized to satisfy the energy requirements of intestinal tissues, others are used to make proteins that will be released into the lumen of the gut (endogenous secretions). Amino acids that reach the portal vein flow to the liver where they are subject to removal/transformation, which again differ between AA and seems to be related to supply. A complex and flexible coordination must exist between all these tissues to allow adequate metabolism of each of them and yet enough amino acids reaching peripheral tissues, including the mammary gland, to support milk production. Overall, intermediary metabolism of AA between the small intestine and the mammary gland biologically explains the decreased efficiency of the transfer of absorbed AA into milk protein as supply is increased. Therefore, variable, rather than fixed factors for transfer efficiencies must be incorporated into future predictive models.

18 Evaluation of net energy expenditures of dairy cows according to body weight changes over a full lactation

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With the advent of the dynamic computer model to describe biological systems, there has arisen a potential to predict the response of an animal over time to various interventions. In the course of developing a mathematical model that describes energy balance in terms of the contributions of feed intake, dietary energy level and energy expended for maintenance, growth and milk production, the authors of this paper found that body weight (BW) changes predicted by the model deviated over time from those observed in actual production trials. The objectives of the present study were to determine the magnitude of variation between predicted and observed values and to implement changes to model equations which would eliminate that variation. It was found that the variation could be significantly reduced by modifying the energy maintenance requirement equation proposed in the US National Research Council's 2001 publication 'Nutrient Requirements of Dairy Cattle' (NRC 2001). While that equation estimates a maintenance energy requirement of 0.08 megacalories (Mcal) of Net Energy for Lactation (NE_L) per kg of metabolic body weight ($MBW = BW^{0.75}$), it was found that an assumption of 0.096 Mcal NE_L/kg MBW removed much of the variation between predicted and observed BW changes. An alternative equation was derived which adjusts maintenance energy requirement by week of lactation starting at 0.08 Mcal NE_L/kg MBW at calving and reaching a plateau of approximately 0.098 Mcal NE_L/kg MBW at week 15.

19 Prediction of dry matter intake throughout lactation in a dynamic model of dairy cattle performance

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In the development of a mathematical model designed to simulate energy balance in lactating cows, the authors of this study incorporated dry matter intake (DMI) prediction equations from a number of different sources into the model. Initial simulations with observed DMI produced predictions which deviated from the results of actual production trials, requiring revision of the maintenance equation used. The maintenance energy requirement equation proposed in the US National Research Council's 2001 edition of 'Nutrient Requirements of Dairy Cattle' (NRC 2001) was revised upward to reduce deviation between predicted and observed body weight (BW) changes. The present paper describes work aimed at developing a DMI equation suitable for use in the model. Several commonly used equations predicting DMI from BW and milk production were tested for their agreement with trial data. Each DMI equation was evaluated in combination with either the original or the revised maintenance requirement equation. All equations underpredicted DMI and BW change but the DMI equation proposed in NRC 2001 produced the least cumulative error. To further minimize variation between predicted and observed data, a term was incorporated into this equation which accounted for negative feedback of body energy reserves on DMI. Inclusion of this function reduced error by 52% for BW predictions and by 41% for DMI predictions.

20 Effect of ruminally protected methionine on splanchnic metabolism of amino acids in lactating dairy cows

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Lysine and methionine (Met) are generally considered to be the 2 most limiting amino acids for milk production by the high producing dairy cow, although there is not unanimous agreement on this point. This study was designed to elucidate the metabolic effects of providing supplemental rumen protected methionine (RPM) to the lactating cow. The addition of RPM increased the flow of Met from the digestive tract into the portal vein and also increased the total amount of some other essential amino acids flowing from the gut to the liver, including leucine, isoleucine, phenylalanine and threonine. The latter result suggested that RPM triggered a homeostatic response which spared utilization of these amino acids by the digestive tract. Net mammary uptake of Met did not change in response to RPM supplementation. Although Met concentration in arterial blood increased, extraction of Met by the gland decreased proportionately. RPM had no effect on milk or milk protein yields although the true protein content of milk from first lactation cows increased in proportion to RPM intake.

21 Development of a remote method for the recording of cattle weights under field conditions

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This paper describes the development of a system for weighing cattle without having to confine them, using readily available weighing technology combined with radio frequency identification. The device was tested with grazing cattle over a 70 day period. Weights recorded remotely were compared with weights recorded every 14 days using a conventional scale in a centralized facility. Weights recorded with the remote system were in the range of 20 kg heavier than those measured conventionally. However, data from the remote and conventional methods did agree more closely with respect to rates of weight change—1.2 versus 1.25 kg/day, respectively. The authors conclude that the trial demonstrated the feasibility of the system and gave satisfactory results.

22 An emerging method for rapid characterization of feed structures and feed component matrix at a cellular level and relation to feed quality and nutritive value

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Archives of Animal Nutrition (2006) Vol. 60 p. 229-244.

For many feeds, the physical-chemical structure is as important as chemical composition in determining their nutritional value. For example, starch granules in feed grains are encapsulated in a protein matrix which may limit the rate of starch degradation by rumen micro-organisms. Likewise, lignin presents a physical barrier to fibre digestion in forages. Because feed samples must be finely ground before analysis, conventional wet chemical methods provide no information about physical structures or physical-chemical interactions in feeds. This paper reviews the application of a novel technology to the physical-chemical analysis of feeds. Synchrotron-based Fourier transform infrared (FTIR) microspectroscopy is capable of linking chemical and structural characteristics at the cellular level in intact tissue samples, simultaneously providing information on composition, structure, chemistry and environment. The review emphasizes the close relationship between the physical-chemical characteristics of feeds at the cellular level and their nutritional value to animals.

23 Dietary oil rich in polyunsaturated fatty acids for ruminants: Post-ruminal digesta characteristics and their implications on production

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Fats and oils are routinely added to early lactation diets to increase their energy density. This article reviews other benefits of adding plant-derived oils to diets fed to ruminant animals. The primary focus is on the effect that fatty acids may have on improving starch digestion in the small intestine. When ruminants are fed diets containing high levels of starch, a variable proportion of that starch escapes rumen degradation, passing into the small intestine. The proportion reaching the intestine depends on the level of starch fed, the specific ingredients used, the degree of processing of those ingredients and other factors which affect the rate at which starch is degraded in the rumen and its rate of passage through the rumen. The starch that enters the small intestine can, in combination with other dietary components, increase the viscosity of the digesta, restricting access by digestive enzymes. Although starch leaving the small intestine may be fermented in the large intestine, this provides relatively less energy to the animal. Dietary oil can reduce digesta viscosity, resulting in more efficient use of starch as an energy yielding nutrient. In addition to the benefits of dietary oils in increasing dietary energy yields, this review discusses the mechanisms by which they may reduce liver abscesses and decrease the shedding of pathogenic organisms into the environment.

24 Impact of B-vitamin supply on major metabolic pathways of lactating dairy cows

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Until recently, it had been assumed that the ruminant animal's requirements for B-vitamins could be satisfied by the basal levels found in dietary ingredients combined with synthesis by rumen micro-organisms. However, recent evidence suggests that the high levels of productivity achieved by today's lactating dairy cows may demand dietary supplementation of these metabolic cofactors. This paper reviews the evidence that supports that suggestion, focusing on the three B-vitamins biotin, folic acid and B12. Several studies have reported positive milk yield responses to supplementary biotin—most have shown marginal effects on milk composition. Milk and milk protein yields have been demonstrated in multiparous cows in response to supplemental folic acid when B12 supply was adequate. Those responses occurred in the absence of an increase in dry matter intake (DMI). They were not observed when B12 supply was inadequate but were restored by further B12 supplementation. When cows received supplements of both B12 and folic acid, tissue utilization of B12 increased. While plasma glucose increased in these cows, plasma biotin decreased. The combined supplementation of B12 and biotin also increased milk and milk protein yields in the absence of an increase in DMI. This review also discusses the metabolic pathways that are influenced by these vitamins.

25 In situ degradation of timothy conserved as restrictively or extensively fermented silage or as hay

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The objective of this study was to evaluate the effects of preservation method on the rumen degradability of timothy forage. After cutting, timothy was preserved as hay, as formic acid-treated silage or as bacterial inoculant-treated silage. Representative samples of each preserved forage were sealed in multiple porous nylon bags and incubated in the rumens of cows previously adapted to the respective forages. Individual bags were withdrawn at regular time intervals for assessment of the disappearance of forage fractions: dry matter (DM), organic matter, crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF). The solubility, degradation rate and degradability of CP in hay was lower than in either silage. CP degradability was similar in both silages but formic acid treatment reduced solubilization of CP, compared to bacterial inoculation. No significant differences in degradability of DM, NDF or ADF were found between forages preserved by the three methods.

26 Effect of maceration of bromegrass-alfalfa silage on ruminal degradability and performance of lactating dairy cows

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Maceration is an intensive mechanical conditioning technology that crushes and shears forage stems and leaves immediately after cutting. The main motivation for macerating forage is shorter drying time, reducing the risk of weather damage to the crop before it can be stored. Maceration may also improve the ensiling process by increasing the exposure of carbohydrates to acid-producing micro-organisms. The objective of this study was to compare the effects of maceration on the rumen degradability of bromegrass-alfalfa silage and on the productive performance of lactating cows. Macerated silage had higher dry matter, neutral detergent fibre, and gross energy degradability, but lower protein degradability than non-macerated silage. Substitution of conventionally harvested silage by the macerated silage in an early lactation diet had little effect on performance although it did, at times, increase dry matter intake.

27 An evaluation of the accuracy and precision of a stand-alone submersible continuous ruminal pH measurement system

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This paper describes the development and use of the Lethbridge Research Centre ruminal pH measurement system (LRCpH), a novel device for measuring rumen pH in rumen cannulated cattle. Unlike the continuous indwelling (CI) system used extensively in previous work, the new device does not require subjects to be confined. The LRCpH consists of a data logger, battery and pH electrode cable housed in a watertight polyvinyl chloride (PVC) capsule. The pH electrode itself is external to the capsule but covered by a PVC shroud, protecting it from contact with the rumen wall. A pair of weights attached to the assembly anchor its position in the ventral sac of the rumen; a tether, attached to the cannula plug, aids in locating the device and holds the electrode in a vertical position. Performance of the new system was validated by measuring rumen pH in dairy and beef heifers and lactating dairy cows. Results were compared with data collected simultaneously using the CI system and with bench top pH measurements of rumen samples withdrawn manually. The LRCpH system proved to be as accurate and precise as the CI system, providing more opportunity for researchers to monitor rumen pH in unconstrained animals such as those housed in free stall barns.

28 Responses to amino acid imbalances and deficiencies in lactating dairy cows

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The formulation of diets which satisfy the protein requirements of lactating cows has eluded even the most knowledgeable nutritionists due the complexity of the paths taken by the 20 unique amino acids (AA) from which protein is formed. To elucidate production responses to variations in amino acid supply, the authors of this study infused balanced or unbalanced solutions of AA into the abomasum of lactating cows fed a basal diet containing only 9% crude protein. Saline was infused into control cows. Infusion of a complete, balanced mix of AA increased blood levels of essential AA; milk protein production rose by 19% over controls. Unbalanced AA mixtures were similar to the balanced mix except for the absence of methionine, lysine, histidine or all 3 branched-chain AA (leucine, isoleucine and valine). Infusion of any of those solutions raised blood essential AA levels except for those omitted from the infusate, which fell 2-fold. Milk protein yield remained within 12% of that produced by cows infused with saline. Mixtures devoid of lysine or histidine provoked large increases in milk fat yields. Those devoid of the branched-chain AA reduced blood leucine levels to 58% of its basal value but raised milk protein and fat yields to levels similar to those produced by the complete AA mixture. It was concluded that hormonal mechanisms must compensate for deficiencies to maintain milk component production at higher levels than anticipated.

29 Physically effective fiber: Method of determination and effects on chewing, ruminal acidosis, and digestion by dairy cows

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Journal of Dairy Science (2006) Vol. 89 p. 2618-2633.

Lactating dairy cattle fed large quantities of highly fermentable feeds are subject to ruminal acidosis due to the rapid production of volatile fatty acids by rumen microbes. To counteract this effect, cows must consume sufficient physically effective (pe) fibre to provoke adequate chewing and salivary buffer production. However, in order to make recommendations on amounts required, a method for determining the pe fibre content of diets is required. This study examined the ability of 3 alternative methods to detect differences in the pe neutral detergent fibre (peNDF) content of 3 corn silages of varying particle size as well as total mixed rations based on those silages (csTMRs). The method of choice calculates a pe factor from the proportion of particles retained on the 2 screens of the original Penn State Particle Separator (having 2 screens above the pan). This pe factor is multiplied by feed NDF concentration to arrive at peNDF. The validity of the peNDF values measured was tested by assessing animal responses to feeding the csTMRs to lactating cows. Total chewing and ruminating times increased in proportion to dietary peNDF, although rumen pH values were only minimally affected. The digestibility of protein and fibre rose with increased peNDF intake; milk yield also increased but milk composition did not change.

30 Increasing the physically effective fiber content of dairy cow diets may lower efficiency of feed use

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Journal of Dairy Science (2006) Vol. 89 p. 2694-2704.

Rapid production of volatile fatty acids from the microbial degradation of highly fermentable feed puts the early lactation cow at risk of subacute ruminal acidosis. To counteract the effect of these acids on ruminal pH, sufficient 'physically effective' (pe) fibre must be fed to stimulate active chewing which, in turn, provokes the secretion of saliva containing acid-neutralizing buffers. However, the optimal level of pe fibre in lactation diets is uncertain. While some studies have demonstrated its benefits, others have shown that increasing dietary pe fibre concentration may reduce nutrient digestibility. The present study examined the effects of varying dietary levels of pe fibre on intake, digestion and production in mid to late lactation dairy cows. The measure of pe fibre used was pe neutral detergent fibre (peNDF), calculated by multiplying the proportion of particles retained on the screens of the original 2-screen Penn State Particle Separator by the ration NDF concentration. Cows were fed one of three different total mixed rations (TMRs) containing 10.5, 11.8 or 13.8% peNDF, achieved by including barley silage of varying chop lengths. TMR dry matter intake was highest for cows offered the 11.8% peNDF diet. Whole digestive tract fibre and organic matter digestibility decreased as peNDF increased but starch and crude protein digestibility were not affected. The efficiency of rumen microbial digestion also declined as peNDF increased. Milk production and composition were similar on all 3 diets.

31 Lower pregnancy losses in lactating dairy cows fed a diet enriched in α-linolenic acid

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In early lactation, dairy cows are frequently unable to consume enough feed to supply the energy demanded by high levels of milk production. The resulting loss of body weight has been shown to inhibit the resumption of normal reproductive function. In an attempt to limit weight loss, supplementary fats and oils (lipids) are often fed at this time to increase dietary energy intake. It has also been demonstrated that certain dietary lipids can have positive effects on reproduction, independent of their contribution to energy balance. In particular, long chain, polyunsaturated fatty acids found primarily in fish oils have been shown to be effective in delaying luteolysis and improving pregnancy rates. These observations have raised the possibility that feeding flaxseed might have similar effects since flaxseed contains high concentrations of alpha-linolenic acid (αLA), a metabolic precursor of the beneficial fatty acids found in fish oils. In the present study, reproductive parameters in early lactation cows fed diets containing rolled flaxseed (56.7% αLA) or rolled sunflower seed (0.1% αLA) were compared. The average diameter of ovulatory follicles, determined by ultrasound, was larger in the cows fed flaxseed although follicle numbers, corpus luteum size, and plasma progesterone concentrations were not affected by lipid source. At 32 days after artificial insemination, 48.4% of the cows fed flaxseed were confirmed pregnant versus 32.2% of the cows fed sunflower seed. Pregnancy losses (between 32 d of gestation and calving) were 9.8% in cows fed flaxseed; 27.3% in cows fed sunflower seed.

32 Evaluation of models to describe ruminal degradation kinetics from in situ ruminal incubation of whole soybeans

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The most commonly used method for estimating the rumen degradability of feeds and feed fractions is the 'in situ polyester bag technique'. Samples of finely ground feed, sealed in multiple small polyester or nylon bags are suspended in the rumen of a rumen-cannulated animal. At predetermined time intervals during the incubation, individual bags are withdrawn, rinsed and dried. Analysis of bag contents determines the amount of feed or feed fraction that has disappeared. For most feeds, disappearance is very rapid in the first few hours, slowing as the incubation progresses. When disappearance at each withdrawal time is plotted on a graph, a curvilinear relationship is seen. Degradation rates and degradability estimates are calculated from this data by fitting mathematical equations to the curve. In this study, the authors evaluated several different equations to determine which proved the best fit to data derived from incubations of ground samples of raw and roasted whole soybeans. Although estimates of the extent of degradability were similar for all equations, there were differences in calculated values of undegradable feed fractions.

33 Effect of postruminal glutamine supplementation on immune response and milk production in dairy cows

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Journal of Dairy Science (2006) Vol. 89 p. 3107-3121.

Among the 20 common amino acids found in proteins, 10 are considered 'essential' (EAA); the remainder are considered 'non-essential' (NEAA) because they can be synthesized by mammalian tissues. However, under some physiological conditions, an animal's capacity to synthesize certain NEAA may not satisfy its metabolic requirements. In the high-producing dairy cow, metabolic demand for the NEAA glutamine (Gln) may be especially high partly due to the fact that about 20% of the amino acids in the milk protein, casein are either Gln or the closely related NEAA glutamic acid (Glu). At the same time, Gln is in high demand by intestinal tissue as an energy source and is important in the maintenance of immune function. The objective of this study was to examine the effect of supplementary Gln on milk production and immune function in early lactation cows. Gln, infused directly into the small intestine, increased plasma Gln and urea concentrations and decreased plasma EAA. Plasma concentrations of NEAA, glucose, non-esterified fatty acids and beta-hydroxy butyrate were not affected. Slight increases in milk and milk protein yields were not statistically significant. Supplementary Gln had no significant effects on most of the indicators of immune function examined. Over the first three weeks following calving, Gln supplementation had limited effects on milk production, metabolic parameters, and immune function.

34 Propionate is not an important regulator of plasma leptin concentration in dairy cattle

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Domestic Animal Endocrinology (2006) Vol. 30 p. 65-75.

Leptin is a peptide hormone mainly produced by body fat storage cells (adipocytes). Early research focussed on establishing leptin as an agent that reduced appetite in response to expanding fat stores. Later studies demonstrated that leptin is also involved in the regulation of metabolism, growth and reproduction. More recent evidence suggests that the short-chain (volatile) fatty acids produced in the rumen may enhance leptin secretion from adipocytes and the pituitary gland. Infusion of propionate into the jugular vein of sheep increased the activity of the leptin-production mechanism in adipocytes. Oral administration of propionate to mice increased plasma leptin concentration. The objective of the present study was to determine if intravenous administration of propionate to lactating dairy cattle would provoke a response in plasma leptin concentration. In an initial trial, a large bolus of sodium propionate was administered intravenously to each of 31 lactating cows. Plasma leptin decreased significantly immediately after injection but concentrations were no different from controls 20, 50 or 100 minutes later. In a second trial, 12 cows received continuous intravenous infusions of sodium propionate at 6 different rates of infusion. Although plasma concentrations of propionate and insulin increased significantly in proportion to infusion rate, leptin levels increased by no more than 8% above control levels.

Genetics

1 The value of genetic information in selecting dairy replacements

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The primary goal of dairy herd replacement management is to raise enough heifers to replace culled cows. Ideally, when a culling decision is made, the replacement will provide more profit than the cow she replaces. What information can be used to select replacements and how valuable is the information? It is often suggested that genetic estimates, based on the heifer's pedigree should be used. The objective of this paper was to determine the actual dollar value of that information, based on the records of 115 Holstein herds in Michigan. Data for each of 1,982 potential replacements included estimated breeding value for milk (EBV) at the beginning and end of the rearing period and her estimated lifetime profit. Using an economic model of herds selecting 70 or 80% of potential replacements, herd profit was calculated for 3 selection methods. 'Ex poste' selection assumes that selection could be made with perfect knowledge of a heifer's future performance and the profit she will contribute to the herd. Random selection is based on using none of the available information to predict her future profitability. Genetic selection is based on each heifer's EBV. The value of random or genetic selection was calculated by comparing average herd profit per replacement with the value that would have been achieved using ex poste selection. Using EBV to select replacements increased herd profit by $22 to $30 per replacement, compared with random selection.

2 An iterative procedure for deriving selection indexes with constant restrictions

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Journal of Animal Science (2005) Vol. 83 p. 2313-2318.

The objective of this study was to present an iterative procedure for deriving selection indexes with constant restrictions. Constant restriction means that the genetic responses of the restricted traits are preset to actual amounts for a given selection intensity ($\bar{i}$). This study shows that an index with constant restriction alone or in combination with other types of restrictions possesses three distinctive characteristics: 1) the coefficient matrix of the index equations is non-symmetric and nonlinear; 2) the coefficient matrix contains unknown $\bar{i}$, indicating that the index coefficients (b) to be derived depends upon the value of $\bar{i}$ predetermined before selection; and 3) the coefficient matrix contains unknown b, thus requiring iterative methods to solve the index equations. As a result of these unique characteristics, the index coefficients, genetic responses of the index traits, and overall genetic gain in net merit change nonlinearly with varying levels of $\bar{i}$ in sharp contrast to both unrestricted and restricted indexes reported in the literature. The construction of constant restricted index requires predetermining the value of $\bar{i}$ intended for a selection program in order to derive the corresponding b. An index with constant restrictions has no meaning unless it is associated with a specific value of $\bar{i}$. Numerical examples are given to illustrate the construction of the index with constant restrictions and to validate the theoretical development proposed. The derived equations have yielded an index that maximized the total merit and fulfilled constant restriction at the same time.

3 Power of QTL detection by either fixed or random models in half-sib designs

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Genetics Selection Evolution (2005) Vol. 37 p. 601-614.

Characteristics determined by a single gene are sometimes referred to as qualitative traits. For example, the genetic anomaly, BLAD (Bovine Leukocyte Adhesion Deficiency) is the result of a single gene variant that codes for a defective protein on the surface of white blood cells. Conversely, most production traits are quantitative in nature and are influenced by multiple genes. Quantitative trait loci (QTLs) are locations on the genome where these genes are found. This paper describes alternative methods for mapping the locations of QTLs.

4 Comparison of protocols to estimate twenty-four-hour fat and protein percentages for herds with a robotic milking system

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Journal of Dairy Science (2006) Vol. 89 p. 1723-1726.

The milk production and composition data collected by Dairy Herd Improvement (DHI) agencies are used for both individual herd management and genetic evaluation. The results of these tests must be accurate to assure their reliability. For herds milked twice or 3 times per day, samples are taken at each milking for analysis of fat and protein concentrations, yielding accurate and reliable estimates of 24 hour component yields. But for herds milked with robotic systems, cows may be milked many more times per day with significant variability from day to day and between individual cows. Although it is possible, using specialized equipment, to sample each of these milkings, analysis of each sample becomes very expensive. In order to reduce this expense, several modified sampling and analysis protocols have been used in various countries. The objective of this study was to assess the accuracy of each of 4 alternative protocols, compared with testing all samples collected in a 24 hour period. The analysis concluded that collecting all samples during a sampling period of at least 16 hours duration gave the most accurate results.

5 Transmission disequilibrium test for quantitative trait loci detection in livestock populations

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Journal of Animal Breeding and Genetics (2006) Vol. 123 p. 191-197.

Characteristics determined by a single gene are sometimes referred to as qualitative traits. For example, the genetic anomaly, BLAD (Bovine Leukocyte Adhesion Deficiency) is the result of a single gene variant that codes for a defective protein on the surface of white blood cells. Conversely, most production traits are quantitative in nature and are influenced by multiple genes. Quantitative trait loci (QTLs) are locations on the genome where these genes are found. The objective of this study was to examine the application of several methods used to test the association and linkage between QTLs and closely associated genetic markers in cattle populations of various sizes.

6 Analysis of inbreeding and its relationship with functional longevity in canadian dairy cattle

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Journal of Dairy Science (2006) Vol. 89 p. 2210-2216.

Inbreeding refers to the mating of animals that are genetically more closely related than the average members of a population. Inbreeding increases the probability that the genes determining a given characteristic are identical in both mates. The inbreeding coefficient (IC) is an estimate of that probability. Currently, the average IC for Canadian Holsteins is in the 5.5-6.0% range; for other Canadian dairy breeds the figure is between 2.4 and 7.1%. Inbreeding decreases genetic variance in the population, reducing the potential for genetic improvement. Many studies have demonstrated that inbreeding decreases both productive and reproductive performance but few have examined its effect on cow longevity, defined as the number of days from first calving to permanent removal from recording due to death, culling or other reasons. This study examined the effect of inbreeding on risk of being culled for cows in the Canadian dairy herd. For all breeds, there was a trend toward reduced longevity as IC increased. Although little effect on longevity was observed when IC was less than 12.5%, risk of earlier culling increased when IC was greater than 12.5%.

7 Optimal random regression models for milk production in dairy cattle

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Journal of Dairy Science (2006) Vol. 89 p. 2233-2235.

Production traits are influenced by both genetics and environment. To quantify these separate influences, statistical techniques are used to find mathematical equations that account for variation in raw production data in terms of these and other factors. The choice of equations can affect the resulting estimates of genetic and environmental effects. This paper describes the methods and results of a search for an equation that would facilitate a 'best-fit' to lactation data from first lactation Canadian Holsteins. The authors conclude that the equation currently used in Canada was the most satisfactory of those tested.

8 Strategy for applying genome-wide selection in dairy cattle

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In Canada, bull proofs are expressed as Estimated Breeding Values (EBVs) which represent the genetic potential of the sire to influence the performance of his offspring. Worldwide, EBVs are currently calculated from performance data collected by milk recording agencies (DHI in North America). However, with the advent of new technologies for examining an individual's DNA, it may be possible to evaluate an animal's EBV by direct examination of its entire genetic makeup (genome). For example, it is now possible to determine genetic differences between animals for 10,000 unique locations on their DNA in a single automated test costing $400. This article describes the potential application of a 'genome-wide' EBV (GEBV) where these technologies are used to directly determine an animal's genotype. In a simulation study, correlations between GEBV and true breeding values were in the 0.78 to 0.85 range and GEBV values could be obtained at birth with an accuracy of 0.8. Using current methods, females seldom ever reach this level of accuracy and 6 or more years are generally required to achieve this accuracy in bull EBVs. Comparison with the current progeny testing strategies used in Canada suggested that the GEBV approach might result in a 92% reduction in the cost of proving bulls and a doubling of the rate of genetic change.

9 Analysis of the relationship between somatic cell score and functional longevity in canadian dairy cattle

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Journal of Dairy Science (2006) Vol. 89 p. 3609-3614.

Several studies have reported that mastitis is one of the main reasons for culling dairy cows. Previous work by the present authors showed that udder traits were the second most important traits influencing the culling of Canadian cows. In the Scandinavian countries, udder health traits are included in their national breeding objectives and there is interest in doing the same in Canada. Therefore, this study was designed to examine the relationship between somatic cell count (SCC) and longevity, defined as the number of days from first calving to permanent removal from recording due to death, culling or other reasons. SCC and longevity data for almost 2 million cows were used in the analysis. SCC data were converted to somatic cell linear scores (SCS) and test day SCS were averaged within each individual lactation. Culling rates were calculated from longevity data for cows in each of 10 SCS classes. The overall average SCC for Holsteins was 167,000 cells/ml (SCS = 3.7), for Ayrshires it was 155,000 (SCS = 3.6) and for Jerseys it was 212,000 (SCS = 4.1). Across all breeds, there were no significant differences in risk of culling when SCS was less than 5.0 (SCC = 400,000 cells/ml). However, cows in the highest SCS classes for their breeds were at 4.95, 6.73 and 6.62 times greater risk of culling than the average for Holsteins, Ayrshires and Jerseys, respectively.

10 Association of toll-like receptor 4 polymorphisms with somatic cell score and lactation persistency in Holstein bulls

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When mastitis-causing bacteria (pathogens) enter the mammary gland, the unique pattern of molecules associated with the pathogen are recognized by specific 'toll-like' receptors (TLR) on the surface of cells lining the gland. Binding of the pathogen molecules to these TLR initiates the immune response. At least 13 unique TLR have been identified, each recognizing different pathogens; 10 of these have been identified in cattle. It has been found that TLR4 is particularly responsive to endotoxins produced by gram-negative pathogens. In humans and mice, defects in the synthesis of TLR4 lead to increased susceptibility to infection by these pathogens. The objective of this study was to determine if variations in the sequence of bovine TLR4 gene might be linked to the variable susceptibility to mastitis infection observed in dairy cows. DNA samples were collected from animals with low or high estimated breeding values (EBVs) for somatic cell score (SCS). Samples from each group were examined for variations (polymorphisms) in the genes that code the TLR4 receptor. Three single nucleotide polymorphisms (SNPs) were detected, two of which were associated with higher SCS. When the presence of these SNPs was examined in 388 bulls, it was found that they were associated with lower EBVs for SCS and lactation persistency. Therefore, selection against sires carrying these SNPs may be effective in improving these traits in Canadian cows.

11 Detection and characterization of amplified fragment length polymorphism markers for clinical mastitis in Canadian Holsteins

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Journal of Dairy Science (2006) Vol. 89 p. 3653-3663.

Complex genetic traits such as resistance to mastitis are influenced by several genes that individually contribute modest variation to observed performance (phenotype). Such traits are referred to as quantitative traits and the locations on the genome (the sum of all DNA in the cell) where these genes are found are called quantitative trait loci (QTLs). To determine the genetic sources of variation for mastitis resistance, these QTLs must be located and variations (polymorphisms) in the genes at those loci must be characterized. This paper describes a combination of techniques that can be used for the genome-wide screening of large numbers of loci for polymorphisms. Pooled DNA from two groups of cows that were rated either highly resistant or highly susceptible to mastitis was subjected to analysis using amplified fragment length polymorphism (AFLP) technique. The most promising AFLP was characterized into a single nucleotide polymorphism (SNP), which differentiated the two pools and was significantly associated with somatic cell score, clinical mastitis and production traits. Selection of animals carrying the resistant version of this SNP will improve genetic progress toward lower mastitis rates in dairy cattle.

Health

1 Bacterial meningitis and encephalitis in ruminants

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Veterinary Clinics of North America Food Animal Practice (2004) Vol. 20 p. 363-377.

The meninges are the 3 membranous layers that envelop the brain and spinal cord. The outside membrane is called the dura mater; the center layer is the arachnoid membrane and the thin innermost layer is the pia mater. These membranes support, protect and contribute to the irrigation and nourishment of the central nervous system (CNS) by cerebrospinal fluid. Meningitis refers to inflammation of one or more of these three layers. The main focus of this article is the description of bacterial meningitis in newborn calves resulting from infection by *Escherichia coli* and other gram-negative bacteria. Also discussed are other specific types of meningeal infections in calves, including Mycoplasma meningitis and meningoencephalitis (inflammation of meninges and brain) caused by *Histophilus somni*. Frontal sinusitis as well as brain and pituitary abscesses are related infections affecting adult cattle.

2 Otitis media in dairy calves: A retrospective study of 15 cases (1987 to 2002)

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Otitis media (OM) is an inflammation of the middle ear, usually due to the extension of an infection from the oropharyngeal cavity by the auditory tube.The signs of OM may include ear and eyelid drop, and head tilt toward the affected side. Purulent ear discharge may be seen in the ear canal. Concurrent pneumonia is usually observed. Because the facial pass through the middle ear, facial nerve paralysis can occur. The incidence of OM in individual dairy herds may ranges from 1% to as high as 80% in individually housed calves. This article reviews the signs, diagnosis, treatment and outcomes of 15 cases of OM presented to University of Montréal veterinary hospital between 1987 and 2002. Results suggest that *Mycoplasma bovis* was responsible for many of the OM infections and that prolonged antibiotic therapy is the preferred treatment.

3 Management of peritonitis in cattle

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Veterinary Clinics of North America Food Animal Practice (2005) Vol. 21 p. 155-171.

The peritoneum is the membrane that covers the inside surfaces of the abdominal and pelvic cavities of the body and the organs contained within. Peritonitis refers to an inflammation involving this membrane and the peritoneal cavity which it encloses. In cattle, peritonitis is usually due to a bacterial infection following trauma, surgery, intestinal obstruction, interruption of blood supply to the intestines or a gastrointestinal ulcer. This article describes the gross anatomy of the peritoneal cavity in relation to the visceral organs, the microscopic anatomy of the peritoneum, and the physiological events that occur in the development of peritonitis. Clinical signs range from evidence of intermittent, mild discomfort caused by abscesses to severe systemic toxemia. Distension, rigidity or tenderness of the abdomen, fever, loss of appetite and reduced milk production are common. Recommended diagnostic procedures include analysis of peritoneal fluid for bacteria, abdominal examination using radiography (x-ray) or ultrasound, exploratory surgery and blood tests. Antibiotic, surgical and supportive approaches to therapy are described as well as prognosis and other conditions related to peritonitis.

4 Synovial fluid changes in induced infectious arthritis in calves

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Arthritis (inflammation of joints) may have infectious or non-infectious causes. It is often difficult to determine the cause of arthritis in cattle because bacteria are isolated in only about 60% of cases where an initial infection was the underlying cause. Therefore, differentiation of infectious and non-infectious cases requires knowledge of the specific kinds of tissue damage caused by each. The objective of this study was to characterize changes in synovial (joint) fluid (SF) after provoking infectious arthritis in the lower leg (tarsal) joint of healthy calves by inoculation of *Escherichia coli* bacteria. Antibiotic treatment commenced 2 days later. SF white blood cell counts were significantly increased on days 2-4 and total protein increased significantly for the full 24-day length of the study. Although all bacterial cultures were negative by day 8, inflammation persisted until day 20. Results confirmed that infusion of *E. coli* into the tarsal joint successfully replicated the typical course of infectious arthritis in calves and demonstrated that rapid recovery is possible when antibiotic treatment is started early.

5 Synthetic peptide vaccination in cattle: Induction of strong cellular immune responses against peptides derived from the *Mycobacterium bovis* antigen Rv3019c

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Many vaccines are effective at increasing the level of antibodies to infective organisms (pathogens). However, for organisms that produce intracellular infections, antibodies are ineffective and vaccines must provoke cell-mediated immunity – the release of signalling chemicals (cytokines) and the activation of cells that attack the pathogen. Several strategies have been attempted to develop such vaccines but the use of synthetic peptides (SP) is particularly attractive for a number of reasons. SP can be produced to strict standards in large scale at low cost. They also eliminate the risk inherent in using live vaccines and they avoid consumer concerns about genetically modified organisms. SP vaccines require both selection of an appropriate peptide and the development of an appropriate adjuvant which will amplify the induction of cellular immunity. The objective of this study was to evaluate the efficacy of a synthetic peptide vaccine/adjuvant preparation against *Mycobacterium bovis*, the cause of bovine tuberculosis. The adjuvant chosen proved particularly valuable in amplifying cell-mediated immune responses to the synthetic peptide.

6 Characterization of the nuclear localization and nuclear export signals of bovine herpesvirus 1 VP22

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Bovine herpesvirus 1 (BHV-1) is associated with several diseases in cattle, including infectious bovine rhinotracheitis (IBR), conjunctivitis, abortion and encephalomyelitis. The virus particle is composed of 4 concentric compartments—the core which surrounds the viral DNA, the capsid, the tegument and the envelope. When BHV-1 infects a cell, it releases proteins from the tegument which exert effects on the host cell in preparation for the subjugation of its machinery to replicate the virus. This paper describes research designed to elucidate the way that one of those proteins (VP22) exerts its effects on the host cell. Although VP22 is not essential to viral replication, a mutant form of the virus lacking this protein fails to produce the symptoms typically seen in BHV-1 infections. Previous studies found VP22 in the nuclei of infected cells, suggesting that it has a role in regulating DNA transcription. But how does VP22 get into the host cell's nucleus? This study identified specific amino acid sequences in VP22 that may contribute to the transport of the protein in and out of the nucleus.

7 Specificity dependence between serological tests for diagnosing bovine brucellosis in Brucella-free farms showing false positive serological reactions due to *Yersinia enterocolitica* O:9

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Brucellosis is a disease with worldwide distribution affecting humans and many animal species, both domestic and wild. Caused by bacteria of the genus Brucella, the disease in female cattle is characterized by abortion, stillborn or weak calves, retained placentas, and reduced milk yield. Eradication of brucellosis is based on culling animals that have positive blood tests for antibodies to Brucella species. Although several tests can be used, some can yield false positive results. Therefore, to minimize the chance of culling animals giving false positive test results, the use of at least 2 independent tests is usually recommended. However, the choice of tests to use is critical. If the specificity of one test is independent of the other, then the combined specificity of the 2 tests should be higher than either alone. If the specificities of the 2 tests are not independent, then their combined specificity will be lower than expected. This study demonstrates that the latter is the case for the currently most widely used test combination—the rose bengal and complement fixation tests. Use of that test combination yielded false positives in Brucellosis-free herds infected with *Yersinia enterocolitica* O:9. The authors suggest that the indirect enzyme-linked immunosorbent assay (iELISA) for Brucella antibodies, used alone, might be a better choice.

8 Amended recombinant cells (ARCsTM): An economical and surprisingly effective production and delivery vehicle for recombinant bovine IFN-γ

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Interferon-gamma (IFN-γ) is one of a number of biochemicals called cytokines which play important roles in initiating and controlling the immune response. Although initially recognized for its antiviral activity, IFN-γ is now known to regulate the synthesis of a large number of important immune system proteins. These activities created interest in the therapeutic potential of IFN-γ. Once the genetic code for IFN-γ was identified, it was possible to splice this code segment into the bacterial genome to manufacture recombinant IFN-γ (rIFN-γ). The therapeutic potential of purified rIFN-γ was subsequently tested in a number of human and cattle trials. Although several of these trials demonstrated that rIFN-γ could enhance the immune response, its routine use was hampered by its high cost and rapid breakdown. A number of approaches to achieving sustained release preparations of rIFN-γ have been attempted. The most successful have been those where the cytokine was delivered in live bacterial cells. However, administration of live organisms raises concerns about patient safety and it is difficult to assure consistent delivery. This paper describes a novel method for the production of rIFN-γ by recombinant *Pseudomonas fluorescens* cells which are subsequently treated to sterilize the bacteria and 'amend' their cell walls. These amended, recombinant cells (ARCs) can be produced at low cost and have high immunostimulatory activity with little risk of side effects.

9 Bovine toll-like receptor 9: A comparative analysis of molecular structure, function and expression

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Toll-like receptors (TLR), located on cell surfaces, are among the important molecules that alert the immune system to the presence of infection. First discovered about 10 years ago, 11 mammalian TLR have now been identified. Each is capable of recognizing unique molecular patterns associated with foreign organisms. Binding of these molecular sequences to the TLR initiates the immune response through the release of cytokines and the activation of immune cells. One of the TLR (TLR9) has been found to recognize and bind short DNA segments referred to as CpG, commonly present in bacteria and viruses. Numerous studies of TLR9-CpG binding in mice and humans have demonstrated that synthetic DNA fragments containing CpG can be used to stimulate the immune system in a variety of clinical conditions. The objective of this study was to compare bovine TLR9 with that found in mice and humans, to assess whether the beneficial effects of TLR9-CpG binding in other species might be applied to cattle. Analysis of the bovine gene coding for TLR9 synthesis revealed close similarity to that in humans. Binding to similar CpG-containing DNA fragments provoked optimal immune cell activation in both species. The characteristics of bovine TLR9 had less in common with that found in mice.

10 Evaluation of the stability of *Ostertagia ostertagi* ELISA microtitre plates over time using cow milk samples

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Veterinary Parasitology (2005) Vol. 133 p. 329-337.

Ostertagia ostertagi is a stomach worm, a gastrointestinal parasite of cattle. Signs of infection include watery diarrhea, progressive weight loss, weakness, rough coat, and reduced appetite. Although fecal egg counts have been used to estimate worm burdens, this method has proven unreliable. A better measure is based on an enzyme-linked immunosorbent assay (ELISA) capable of detecting *O. ostertagi* antibodies in blood serum and milk. However, one of the reagents commonly used in the ELISA procedure (bovine serum albumin: BSA) requires that the microtitre plates used in the assay must be kept frozen before use. This is an inconvenience when plates need to be transported. Therefore, the objective of this study was to examine alternatives to the use of BSA in combination with either monoclonal or polyclonal anti-bovine antibody reagents. Two alternatives to BSA were identified which are stable for prolonged periods at ambient temperature. Use of one of these, in combination with the monoclonal anti-bovine antibody reagent, was found to yield the best results.

11 Multiattribute evaluation of two simple tests for the detection of *Cryptosporidium parvum* in calf faeces

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Veterinary Parasitology (2005) Vol. 134 p. 15-23.

Cryptosporidium parvum is a gastrointestinal protozoan parasite and the cause of Cryptosporidiosis in young calves. Signs are mild to moderate diarrhea with feces that are yellow or pale, watery, and contain mucus. Cryptosporidiosis can also be contracted by humans who contact infected calves or ingest contaminated food or water. The authors of this paper identify a need for a simple and inexpensive diagnostic and screening test for *C. parvum* infection suitable for use in the field by practising veterinarians. Current diagnostic tests depend on the detection of fecal oocysts by microscopy. Samples must be stained and/or concentrated before examination, both procedures requiring substantial time to complete. The objective of this study was to compare the performance of a commercial test-stick method with a microscopy method developed at the Ontario Veterinary College. Results from both of these diagnostic test alternatives were compared with a highly accurate test for *C. parvum* DNA which is normally only used in a research context. Both diagnostic tests were judged sufficiently accurate, inexpensive and easy to use. The test-stick method had advantages in terms of speed, simplicity and user-friendliness.

12 Assessing the agreement between *Ostertagia ostertagi* ELISA tests performed using the crude adult antigen and the adult and larval stage 4 excretory/secretory antigens

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Veterinary Parasitology (2005) Vol. 134 p. 147-152.

Ostertagia ostertagi is one of the most common gastrointestinal nematode parasites (stomach worms) of cattle. Signs of infection include watery diarrhea, progressive weight loss, weakness, rough coat, and reduced appetite affecting milk production and reproduction. In the past, fecal egg counts (FEC) have been used to estimate the parasite burden but this method has been shown to be inaccurate in adult cattle. More recently, an enzyme-linked immunosorbent assay (ELISA) has been proposed, using a crude *O. ostertagi* antigen from adult parasites. This assay has yielded more accurate results than the FEC method and has shown promise as an predictor of production and reproduction responses to wormer medications (endectocides) in dairy cows. However, the use of the crude adult antigen may not permit a true estimate of the current worm burden because the parasitized animal may not be exposed to these antigens until the worm dies. If this is the case, antibody production may not occur until after a period of active infection and may persist after parasite death. This study compares results from an ELISA using the crude adult antigen preparation with assays using antigens that are excreted and secreted during active parasitisation (ES antigens). Two ES antigen preparations were evaluated, one from adult worms, the other from larval stage 4. Results suggest that the parasitized animal's antibody response is primarily directed toward the ES antigens rather than those in the crude adult preparation.

13 Immunization with plasmid DNA encoding a truncated, secreted form of the bovine viral diarrhea virus E2 protein elicits strong humoral and cellular immune responses

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Vaccine (2005) Vol. 23 p. 5252-5262.

Bovine viral diarrhea virus (BVDV) infections are common in cattle. Signs include fever, increased respiratory rate, diarrhea and reduced white blood cell counts. Suppression of the immune system can result in secondary bacterial pneumonia. Currently, a number of different vaccines against BVDV are used but many of these have limited effectiveness and/or produce adverse reactions. The objective of this research was to develop a new DNA vaccine for BVDV. DNA vaccines are designed to deliver a piece of DNA which codes for the synthesis of an antigen associated with the infecting organism. In this study, genes coding for several modified forms of one of the proteins found in BVDV (the E2 protein) were tested. E2 plays an important role in the attachment and entry of BVDV into host cells and, in this role, it remains anchored to the cell membrane. Removal of the anchor region of the protein's peptide chain resulted in its release. This modification to the E2 gene was combined with the addition of sequences coding for peptides known to mediate protein secretion. Together, these changes resulted in significant increases in antibody response when tested in cell culture. For administration to live animals, the modified E2 genes were inserted into different plasmids (circular strands of DNA) which serve as vehicles (vectors) for intradermal delivery of the vaccine. One of the E2-gene-encoding plasmids that proved particularly effective in eliciting immune responses in cattle could be a candidate DNA vaccine for BVDV.

14 Analysis of the contribution of Salmonella pathogenicity islands 1 and 2 to enteric disease progression using a novel bovine ileal loop model and a murine model of infectious enterocolitis

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Infection and Immunity (2005) Vol. 73 p. 7161-7169.

This paper describes the use of a novel live animal preparation which enabled the authors to better study the time course of enteric infections by Salmonella species. A short loop of the small intestine (ileal section) of a calf is exteriorized for observation with the intestine remaining open to digesta passage. Preparations used previously required occlusion of the intestine which limited the length of time over which observations could be made. Depending on both the host and bacterial species (serotype) involved, Salmonella infections may be confined to the intestines or may spread to systemic organs. The area of the bacterial genome which codes for proteins whose primary role is to colonize the intestine is called Salmonella Pathogenicity Island 1 (SPI-1). Coding for virulence proteins primarily responsible for systemic attack is in SPI-2. Although SPI-1 appears to have little or no role in systemic infection, the role of SPI-2 in enteric disease is unclear. Use of the calf ileal loop preparation allowed the authors to further delineate the contributions of SPI-1 and SPI-2 to the development of intestinal infection over a 5 day period. The discovery of previously unidentified SPI-1 and SPI-2 variants is described and confirmed using a mouse preparation.

15 Antioxidants to prevent bovine neutrophil-induced mammary epithelial cell damage

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Journal of Dairy Science (2005) Vol. 88 p. 4295-4303.

Mastitis is an inflammation of the mammary gland, most often following infection by pathogenic (disease-causing) bacteria. Inflammation is the result of the activation of neutrophils, immune cells that release toxic chemicals in response to infection. Among these chemicals are reactive oxygen species (ROS), including the superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), the hydroxyl radical (OH) and hypochlorous acid (HOCl). While toxic to bacteria, these ROS can also damage mammary cells. Host cell defence against damage revolves around glutathione, the primary substrate for the enzyme glutathione peroxidise which inactivates ROS. The authors of this study speculate that ROS production in severe infections may overwhelm this defence system, resulting in extensive tissue damage. Therefore, they conducted studies in cell culture to determine whether the addition of antioxidants could reduce cell damage by ROS. Mammary gland cells were cultured together with neutrophils which were activated by *E. coli* endotoxin. Cell damage was assessed by measuring the release of lactate dehydrogenase. Several antioxidants had positive effects. Since iron participates in the generation of ROS, the authors also tested the addition of deferoxamine, a chemical that removes iron from the cell. Although deferoxamine inhibited bacterial growth, it did not interfere with their destruction by immune cells.

16 Ecology of *Escherichia coli* O157:H7 in commercial dairies in southern Alberta

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Escherichia coli O157:H7 (EC) is a normal inhabitant of the large intestine in cattle. Although the bacteria are not pathogenic (disease-causing) in the animals themselves, upon ingestion by humans EC releases a potent toxin which causes severe gastrointestinal distress including vomiting, stomach cramps and diarrhea. Common sources of infection are contaminated meat, unpasteurized milk and the environment. The objectives of this study were to elucidate factors influencing EC populations in dairy cattle and to compare two methods of monitoring EC shedding on dairy farms. Shedding was monitored monthly for a year on 5 dairy farms in southern Alberta by identifying EC in pooled fecal pats. The second collection method tested involved providing access to manila ropes which the cattle would chew on, leaving saliva samples on the rope. EC was isolated from fecal samples taken from individual cows on 4 of the 5 farms. Over the one year sampling period, EC was isolated from 13.5% of pooled fecal pats and from 1.1% of rope samples. The likelihood of finding EC-positive samples was 15 times higher from June through September compared with other months of the year. Samples from calf and heifer pens were 2.6 times more likely to contain EC than those from cow pens. The manila rope method of collecting samples was judged unsuccessful.

17 Progress in the development of mastitis vaccines

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Livestock Production Science (2005) Vol. 98 p. 101-113.

Mastitis is the most costly disease of lactating dairy cattle. Efforts aimed at controlling intramammary infections include improved hygiene, antibiotic treatment and vaccination. Mastitis vaccines based on the use of bacterial extracts or purified antigens continue to be developed. However, their use has been limited because of their limited efficiency, high production costs and stability. Although these types of vaccines may produce acceptable responses in terms of antibody production (humoral immunity) their effectiveness against intracellular infections is often limited because they do not provoke activation of cellular immunity. Recent research has focussed on the development of DNA vaccines which use the animal's own synthetic machinery to produce a modified version of a specific bacterial virulence protein (MVP). 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 MVP. The immune system responds by producing antibodies to the MVP and activating the mechanisms responsible for cellular immunity. This paper describes research aimed at developing a DNA vaccine against *Staphylococcus aureu*s, one of the most difficult mastitis organisms to control.

18 The prevalence of milk and serum antibodies to *Mycobacterium avium* subspecies *paratuberculosis* in dairy herds in Ontario

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Canadian Veterinary Journal (2005) Vol. 46 p. 1126-1129.

Mycobacterium avium subspecies *paratuberculosis* (MAP) is the bacterium responsible for Johne's Disease, a production-limiting disease of dairy cattle and other ruminants. Several studies have evaluated the prevalence of MAP in Canadian dairy cattle. A previous Ontario survey found that 10% of the herds tested had 2 or more positive cows, based on a serum enzyme-linked immunosorbent assay (ELISA). Among all cows tested, 2.2% gave positive results. However, the survey sample in that study was not representative of all herds in the province, being biased towards those with above average management. The primary objective of the present study was to assess MAP prevalence in a more representative sample of Ontario herds. A secondary objective was to compare serum ELISA results with those obtained from milk samples. In contrast to the results from the previous survey, in 18% of the 50 herds tested, 2 or more cows had positive serum ELISA tests; 30% had 2 or more cows with positive milk ELISA tests. Of the 2,122 cows tested, serum ELISA results were positive for 2.6%; milk ELISA results were positive for 1.7%. There was a moderate level of correlation between serum and milk ELISA results.

19 Effects of noncytopathic type 2 Bovine viral diarrhea virus on the proliferation of bone marrow progenitor cells

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Canadian Journal of Veterinary Research (2006) Vol. 70 p. 20-27.

Bovine viral diarrhea virus (BVDV) causes significant economic loss to the cattle industry. Although the virus usually causes only mild or subclinical disease, a severe acute form of BVD infection may produce unusually high morbidity (sickness) and mortality (death) rates in both calves and adults. Clinical signs include fever, loss of appetite, diarrhea and severe depression of both red and white blood cell (WBC) counts which may predispose animals to secondary viral and bacterial infections. The objective of this study was to determine whether the low WBC count (neutropenia) was due to a reduction of WBC proliferation in bone marrow. Groups of calves were infected with one of 2 strains of BVDV of high (HV) or low virulence (LV). WBC proliferation was measured by culturing samples of bone marrow drawn from the calves before and each day for 5 days after being infected. BVDV was found in bone marrow cells as soon as 2 or 3 days after infection and neutropenia developed in both calf groups. However, WBC counts rebounded earlier in calves infected with the LV than in those given the HV strain. WBC proliferation decreased significantly in calves infected with HV while, in those given LV, it was not different from control calves. The results of this study support the hypothesis that the neutropenia experienced by calves infected with the severe acute form of BVDV is due, at least in part, to reduced WBC proliferation in bone marrow.

20 Clopidogrel induced suppression of bovine platelet activation in vitro and a preliminary study of its effect on the development of *Mannheimia haemolytica* induced pneumonia

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Shipping fever (SF) causes significant economic loss to the cattle industry. SF is a result of stress, viral infection or other challenge leading to bacterial pneumonia, primarily caused by *Mannheimia* (formerly *Pasteurella*) *haemolytica*, a normal inhabitant of cattle nasal passages. The host responds to the initial challenge with an inflammatory response resulting in congestion of the lung alveoli due to an accumulation of white blood cells, platelets and fibrin, the protein which forms blood clots. Reduced lung function resulting from this congestion favours the proliferation of *M. haemolytica* in the alveoli. The objective of this study was to clarify the role of platelets in the inflammatory response. Platelet function was examined in blood samples drawn from calves treated with clopidogrel, a platelet antagonist. Clopidogrel treatment produced consistent reduction in normal platelet function. When clopidogrel-treated calves were challenged with an intra-tracheal injection of *M. haemolytica*, there was an inhibition of platelet function in affected areas of the lungs. Compared with control calves, clopidogrel-treated calves had similar amounts of fibrin deposition and enhanced fibrous tissue formation in their lungs.

21 Immune response to *Staphylococcus aureus* GapC/B chimera and its potential use as a component of a vaccine for *S. aureus* mastitis

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Veterinary Immunology and Immunopathology (2006) Vol. 109 p. 85-97.

Mastitis due to *Staphylococcus aureus* infection is one of the most costly and challenging health problems facing the dairy industry worldwide. Antibiotic treatment of infected animals meets with variable, and often minimal, success. Vaccination with killed bacterial cells or bacterial extracts has often failed to provide protection against new infections, probably because of variation between antigens present in the vaccine and those of the new strain (serotype). Given the wide variety of antigens associated with the multiple serotypes of *S. aureus*, production of a vaccine containing all possible antigens would be impossible to produce. This paper describes research aimed at developing a DNA vaccine using a gene that codes for a protein having characteristics of 2 surface antigens which are common to all *S. aureus* serotypes (GapC/B chimera). The vaccine is comprised of the GapC/B gene linked to a DNA loop (plasmid) which serves as a vehicle (vector) for delivering the gene to host cells. Once in the cell, the gene is decoded to produce the GapC/B protein which elicits an immune response. When injected into mice, the GapC/B protein elicited strong humoral (antibody) and cellular (activation of immune cells and signalling mechanisms) immune responses. The results suggest that the GapC/B protein has potential in the development of a DNA vaccine against *S. aureus*.

22 Rationale and perspectives on the success of vaccination against bovine herpesvirus-1

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Veterinary Microbiology (2006) Vol. 113 p. 275-282.

Bovine herpesvirus-1 (BHV-1) is the cause of a variety of clinical symptoms, including rhinotracheitis, conjunctivitis and genital infections. Although BHV-1 commonly causes only mild clinical signs or reduced milk production in adults, it can also reduce fertility and cause abortions. BHV-1 causes more severe problems in young calves and is one of the viruses involved in the initiation of shipping fever. In spite of its potential to cause disease, BHV-1 can be controlled to a certain extent with conventional vaccines. This paper describes the reasons for this success and suggests potential future improvements to BHV-1 vaccines. Most current vaccines use either attenuated or inactivated whole organisms as their antigenic components. Although these vaccines are relatively ineffective against chronic and intracellular pathogens, they have been quite successful in controlling pathogens that circulate in the blood because they provoke a strong humoral (antibody-based) immune response. BHV-1 is one of those organisms whose ability to cause disease is dependent upon its distribution by the circulatory system. Recent developments include the use of marker (DIVA) vaccines which can differentiate vaccinated from infected animals. These are particularly valuable where they are used to test animals destined for import into countries that have eradicated BHV-1. Since conventional vaccines are less effective in protecting neonates from BHV-1, the development of new protein or DNA vaccines would be valuable. Needle-free delivery methods for beef cattle would reduce the tissue damage caused by the intramuscular injection of current BHV-1 vaccine preparations.

23 Epidemiology: A foundation for dairy production medicine

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Veterinary Clinics of North America Food Animal Practice (2006) Vol. 22 p. 21-33.

Epidemiology refers to the study of factors that influence the frequency and distribution of disease in a population. Its objective is to provide a basis for the development of programs to control their development and spread. This article describes the application of epidemiology to the practice of dairy production medicine. Three aspects of the science of epidemiology are particularly applicable to dairy practice: (1) Quantitative epidemiology is based on measuring rates of disease occurrence and includes the development of simulation models that assist in prescribing appropriate responses to disease outbreaks; (2) The evaluation and interpretation of tests used to diagnose and monitor the health status of individuals and the population; (3) Evaluation of the strengths and weaknesses of currently published evidence in support of specific health management decisions. Each of these aspects is discussed, with emphasis on the collection, interpretation and use of herd-level data and its relevance to diagnosis and intervention decisions.

24 Genotype and subtype analyses of Cryptosporidium isolates from dairy calves and humans in Ontario

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Parasitology Research (2006) Vol. 99 p. 346-352.

Cryptosporidia are protozoan parasites that can infect and cause diarrhea in humans and many wild and domestic animals, including cattle. Although effects are usually mild and short-lived, death may occur in newborn animals and people with compromised immune systems. Treatment options are limited. Hosts excrete large numbers of Cryptosporidium oocysts in their feces. Infections can spread rapidly when others come into direct contact with feces or consume contaminated water. Several species of Cryptosporidium can be passed between humans and animals. *Cryptosporidium parvum* is the most common species in domestic ruminants and infections are common among dairy calves. One study reported that 59% of dairy calves under 24 weeks of age in British Columbia were shedding oocysts. Another found infected calves on 89% of 505 Quebec dairy farms. In southern Ontario, work carried out on 51 dairy farms found that 40.6% of 500 calves aged 7 to 21 days were shedding oocysts. This high prevalence of Cryptosporidium infections on dairy farms suggested that dairy calves might be a potential source of infections in humans. The objective of the present study was to determine whether human cases of infection in Ontario were due to the same species of Cryptosporidium that were found on dairy farms in that province. Of 44 isolates from infected dairy calves, all were of the same species of *Cryptosporidium parvum*. Isolates from 11 human cases were of a variety of species; 3 were the same as that found in calves.

25 Characterization of cytokine expression in milk somatic cells during intramammary infections with *Escherichia coli* or *Staphylococcus aureus* by real-time PCR

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Escherichia coli (EC) and *Staphylococcus aureus* (SA) are the 2 most prevalent bacteria responsible for bovine mastitis. EC is an environmental gram-negative organism that causes acute mastitis which normally resolves spontaneously. SA is a contagious gram-positive bacterium that induces a chronic mastitis which may persist for the lifetime of the cow. These contrasts in duration and severity of infection suggest that there are differences in the way that the cow's immune system responds to these 2 pathogens. The objective of this study was to examine the patterns of immune system activation signals (cytokines) that are produced in response to mammary gland challenges by EC or SA. One quarter in each of 8 cows was injected with EC; quarters in a second group of 8 were challenged with SA. In each of the 16 cows, a second quarter was used as a control. At time intervals after injection, somatic cells from each quarter were examined for the expression of genes coding for various cytokines. With the exception of interferon-γ in SA-challenged quarters, the average level of expression of all cytokine-coding genes increased in response to both pathogens. However, responses were higher and faster in cells from quarters challenged with EC. The lower responses in SA cows and the absence of interferon-γ gene expression may partly explain why SA infections tend to become chronic.

26 DNA immunization of dairy cows with the clumping factor A of *Staphylococcus aureus*

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Staphylococcus aureus (SA) is one of the most common and costly causes of chronic mastitis in dairy cattle. One of the first events in intramammary infection by SA is attachment to the cells lining the inside of the gland. Clumping factor A (ClfA) and fibronectin-binding protein (FBP) are SA surface proteins that facilitate attachment. In a previous study, the authors of this paper showed that a DNA vaccine targeting ClfA and FBP provided partial protection to cows challenged with an intramammary dose of SA. The level of protection provided by the vaccine was directly proportional to the level of antibody response it provoked. The objective of the current study was to investigate ways to improve the effectiveness of the vaccine through the addition of agents designed to amplify the immune response. Although most of the agents tested did not augment the antibody response, they did decrease the number of cows that failed to respond to the vaccine. Administration of a dose (boost) of recombinant ClfA 3 months after vaccination further reduced the ability of SA to adhere to mammary cells.

27 Activation of the hypothalamic-pituitary-adrenal axis and autonomic nervous system during inflammation and altered programming of the neuroendocrine-immune axis during fetal and neonatal development

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Brain, Behavior and Immunity (2006) Vol. 20 p. 144-158.

When a pathogenic (disease-causing) organism enters the body, molecules associated with the pathogen are recognized by receptors on cells that are part of the body's innate immune system. Binding of pathogen molecules by these receptors initiates a local inflammatory response, including the release of signals that activate immune cells and the influx of white blood cells, whose primary function is to destroy the invading pathogen. Although such a response typically remains localized, if it becomes excessive or prolonged it may result in permanent tissue damage and/or contribute to the progression of other diseases. The scale of the 'acute-phase' response is normally limited by anti-inflammatory hormones such as cortisol whose release from the adrenal gland is controlled by the pituitary gland and hypothalamus at the base of the brain. The autonomic nervous system (ANS) also plays a role in controlling the inflammatory response through the release of acetyl-choline, epinephrine and norepinephrine.

28 Ventricular septal defects in cattle: A retrospective study of 25 cases

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Congenital defects of the heart are relatively rare in cattle. Examination of 50,742 bovine hearts in a slaughterhouse study revealed only 86 defects. In a study of 2,293 calves with congenital anomalies, malformations of the heart occurred in only 2.7% of the cases. Ventricular septal defect (VSD) is the most common abnormality of the heart in newborn cattle. In 2 separate studies, VSD was found in 11 of 36 and 7 of 10 calves diagnosed with heart defects. VSD allows blood to flow directly from the left ventricle to the right ventricle and pulmonary (lung) arteries which can lead to high blood pressure in those vessels. The left atrium and left ventricle become enlarged due the higher pressure and increased volumes of blood flowing through them. This study reviews clinical findings and outcomes of 25 heifers diagnosed with VSD and ranging in age from 36 hours to 21 months. The most common reasons for veterinary consultation were chronic respiratory problems (11 heifers) or detection of a heart murmur (5 heifers). Pneumonia was found in 15 calves. Only 10 were discharged from the clinic and none of the 6 heifers for which follow-up information was available had a productive life in the herd, most often attributed to poor reproductive performance.

29 Characterization of antimicrobial resistance of *Salmonella* Newport isolated from animals, the environment, and animal food products in Canada

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Canadian Journal of Veterinary Research (2006) Vol. 70 p. 105-114.

Salmonella is found in the intestines of animal populations, and human illness is usually linked to foods of animal origin. Salmonella usually causes an intestinal infection in both animals and humans and less commonly can cause localized infections and even septicaemia. Over the past decade, infections of the North American human population by *Salmonella* Newport have increased. As well, the number of strains of *S.* Newport and other Salmonella species that are resistant to antibiotic treatment has risen. They are often referred to as multidrug-resistant (MDR) isolates. The purpose of this study was to determine the occurrence of *S.* Newport in Canada among domestic animals, food sources of animal origin and the environment. Also, *S.* Newport isolates recovered from these sources were examined for the presence of genes known to code for antimicrobial resistance. Of 36,841 Salmonella isolates examined between 1993 and 2002, 119 were identified as *S.* Newport. Among the 70 isolates of *S.* Newport recovered between January 2000 and December 2002, 35 were resistant to at least 11 antimicrobials and many of these were isolated from cattle. In contrast, before 2000, none of the 49 *S.* Newport isolates found in Canada was resistant to more than 3 antimicrobials. Known antimicrobial resistance genes (blaCMY-2, flost, strA, strB, sulII, int1, aphA-1 and tetA) were identified in many of the more recently recovered isolates and these were located on mobile elements called plasmids. The increased occurrence of MDR isolates of *S.* Newport recovered from cattle suggests an increased risk of human infection via the food chain.

30 Influence of the genotype of *Staphylococcus aureus*, determined by pulsed-field gel electrophoresis, on dry-period elimination of subclinical mastitis in Canadian dairy herds

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Canadian Journal of Veterinary Research (2006) Vol. 70 p. 115-120.

Intramammary administration of antibiotics to cows at dry-off (dry cow therapy: DCT) is a recommended and widely accepted practice. DCT, properly applied, can eliminate environmental pathogens that cause mastitis and prevent 50 to 80% of new intramammary infections, compared to not using DCT. Of the infections that DCT does not prevent, many are caused by *Staphlococcus aureus*. The objectives of this study were to compare the efficacy of 2 different DCT antibiotics in reducing new *S. aureus* infections and to determine if different strains of *S. aureus* were more or less susceptible to DCT. Three strains of *S. aureus* were classified by pulsed-field gel electrophoresis and identified as lineage groups A, D and F. Cows infected by group D had higher somatic cell scores at last DHI test day before dry-off than those infected by groups A or F. Of the 2 antibiotics tested, tilmicosin phosphate cured significantly more cases (74%) than did benzathine cloxacillin (53%). Among the 3 lineage groups, significantly more of the infections by group D (87%) were cured than those of group A (46%) or F (33%) when benzathine cloxacillin was used. There were no differences in cure rates between groups when tilmicosin phosphate was used. The results demonstrate differences in antibiotic efficacy and susceptibility to treatment of different *S. aureus* lineage groups.

31 Ventral laparoscopic abomasopexy on adult cows

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Canadian Veterinary Journal (2006) Vol. 47 p. 343-348.

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. Although the success rate of these procedures is in the 90% range, all require that an incision be made in the side or bottom of the body cavity. Complications include infection of the wound, herniation through the wound and generalized infection inside the body cavity (peritonitis). An alternative is blind-stitch fixation and, although its success rate is similar to the surgical procedures, this technique can precipitate additional, more severe complications such as partial obstruction of the stomach and inadvertent fixation of another part of the digestive tract. This paper describes a procedure for abomasal fixation (abomasopexy) using a laparoscope, a 42 cm long rigid fibre optic device which allows internal organs to be viewed. The laparoscope is used to guide the placement of 4 sutures through very small incisions in the abdominal wall. The procedure was performed on 10 cows with no major complications and its success was evaluated 3 months later using the laparoscope. The authors suggest that the technique could be routinely applied to the correction of LDA.

32 Major advances in disease prevention in dairy cattle

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Journal of Dairy Science (2006) Vol. 89 p. 1267-1279.

Dairy herd health management can be defined as the promotion of health, improvement of productivity and prevention of disease within the economic framework of the owner and industry. Given this definition, any factor that limits animal performance can be considered a component of poor health, including poor nutrition, uncomfortable housing, aversive handling and sub-clinical diseases whose signs may not be readily apparent. This way of thinking about dairy herd health represents an evolution from a focus on treating disease in individual animals to the prevention of disease and maintenance of good health across the group, herd and population. The role of the veterinarian has evolved from that of a task-oriented therapy provider to an advice-oriented consultant on herd management. Veterinarians have increasing applied the principles of epidemiology to the maintenance of herd health and the development of programs to limit disease occurrence and spread within and between herds. These changes in approach have resulted in decreases in the incidence of milk fever, clinical respiratory disease in adult cattle, contagious mastitis and clinical parasitism. They have also facilitated rapid expansion in herd sizes and individual productivity without concurrent increases in disease occurrence.

33 Intercellular trafficking of the major tegument protein VP22 of bovine herpesvirus-1 and its application to improve a DNA vaccine

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Archives of Virology (2006) Vol. 151 p. 985-993.

Bovine herpesvirus 1 (BHV-1) is associated with several diseases in cattle, including infectious bovine rhinotracheitis, conjunctivitis, abortion, encephalomyelitis. The virus particle is composed of 4 concentric compartments—the core which surrounds the viral DNA, the capsid, the tegument and the envelope. When BHV-1 infects a cell, it releases proteins from the tegument which exert effects on the host cell in preparation for the subjugation of its machinery to replicate the virus. This study demonstrates the role of one those tegumental proteins (VP22) in being able to spread (trafficking) from cell to cell after initial infection of the host. VP22 is a sequence of 258 amino acids. It was determined that the 138 amino acids at one end of the sequence (the C-terminal end) were responsible for the trafficking role of VP22. DNA vaccines carrying the code for another BHV-1 protein (glycoprotein D), fused to the code for the complete VP22 protein or to that for either end (C-terminal or N-terminal) of the protein, were administered to mice. The vaccine encoding only the N-terminal end of VP22 produced a weaker immune response than either of those that included the C-terminal end, verifying the potential of VP22 or its C-terminal portion to enhance DNA vaccine efficacy presumably due to the spread of the expressed antigen to a larger number of host cells—possibly antigen-presenting cells.

34 Agreement between three ELISAs for *Mycobacterium avium* subsp *paratuberculosis* in dairy cattle

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Veterinary Microbiology (2006) Vol. 114 p. 285-291.

The control and eradication of Johne's Disease requires testing of individual cows for the presence of the causative bacterium, *Mycobacterium avium* subspecies *paratuberculosis* (MAP). Two types of tests are currently available: fecal culture and enzyme-linked immunosorbent assay (ELISA). ELISA is the preferred method due to its convenience, lower cost and immediate availability of results. However, the sensitivity (ability to correctly identify a positive animal) of ELISA is low (about 15%) for animals shedding few bacteria in their feces; sensitivity is in the 85-90% range for clinical cases. The objective of this study was to evaluate and compare results from 3 alternative ELISA test kits, 2 that are licensed for use in North America and a third licensed for use in Europe. All 3 ELISAs were used to assay serum samples collected from 994 dairy cows at slaughter. The cows were from herds in the 4 Canadian Maritime provinces and Maine. Overall, there was poor agreement between results from the 3 assays and the number of positive tests were significantly different between tests. Although these ELISA kits are primarily recommended for use in assessing herd-level prevalence of MAP infection, they are routinely used to test individual animals. The results of this study raise concern about using ELISA in this way. The poor agreement between the 3 test kits, combined with their low sensitivity for pre-clinical infection, means that a pre-clinical cow testing positive with one ELISA test kit has a high likelihood of testing negative with an alternative kit.

35 Effect of different nuclear localization sequences on the immune responses induced by a MIDGE vector encoding bovine herpesvirus-1 glycoprotein D

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Vaccine (2006) Vol. 24 p. 4625-4629.

Typical DNA vaccines consist of a segment of DNA which codes for a specific antigen, spliced into a bacterial plasmid which serves as a delivery vehicle (vector). To be effective, the vaccine needs to enter the host cell and be transferred to the nucleus where the cell's machinery will decode the vaccine's DNA and initiate synthesis of antigen proteins. DNA vaccines developed to date have not achieved commercial success because their uptake by host cell nuclei has been limited and/or the level of antigen protein synthesis by the host cell is inadequate. This paper describes research into the use of a minimalistic immunogenically defined gene expression (MIDGE) vector containing a minimal portion of bacterial DNA. Antigens delivered using a MIDGE vector were linked to synthetic peptides containing nuclear localization sequences (NLSs). NLSs responsible for transport across the nuclear membrane, facilitating transport of MIDGE DNA into the nucleus. The combination of MIDGE vector and NLS-linked antigen improved the immune response to the antigen when injected into mice.

36 *Neospora caninum* and *Leptospira* serovar serostatus in dairy cattle in Ontario

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Canadian Veterinary Journal (2006) Vol. 47 p. 467-470.

Neosporosis is currently the most common cause of abortion in Ontario dairy cattle. A previous survey of 56 Ontario herds indicated that approximately 10% of adult cows were seropositive to *Neospora caninum*, indicating that they were likely infected with the parasite. Within individual herds, from 0 to 68% of cows were seropositive, with a median value of 7%. Work elsewhere has indicated that, overall, the risk of abortion is increased in animals that are seropositive for *N. caninum*. Furthermore, research done in France has indicated that exposure of cattle to surface water was associated with *N. caninum* seropositivity. Since horizontal transmission is not the primary route of *N. caninum* infection in cattle, we hypothesized that the association of *N. caninum* seropositivity with surface water was due to exposure to *Leptospira* in water, and that infection with this bacteria increased the susceptibility of cattle to congenital *N. caninum* infection. If so, cows that are seropositive for *N. caninum* should also be seropositive for *Leptospira*. However, testing of cows from 78 farms in Ontario revealed no such association. Among herds not vaccinated against *Leptospira*, the proportion of herds with one or more cows seropositive for one of 3 individual species (serovars) was 45% (*L. hardjo*), 42% (*L. icterohaemorrhagiae*) and 58% (*L. pomona*).

37 Efficacy of using an internal teat sealer to prevent new intramammary infections in nonlactating dairy cattle

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Journal of the American Veterinary Medical Association (2006) Vol. 228 p. 1565-1573.

Treatment of all cows at dry-off with a single intramammary dose of antibiotic is a practice currently recommended by the National Mastitis Council. A potential adjunct or alternative to this practice has recently become available. This is an inert (bismuth subnitrate) internal teat sealant (ITS) with a gel-like consistency which is infused into the teat cistern either alone or after a dose of intramammary antibiotic. This study tested the efficacy of alternative protocols in 2 groups of cows, based on their intramammary infection (IMI) status 14 days before dry-off. Cows in group 1 had no IMI; those in group 2 had an IMI in at least 1 quarter. Quarters in group 1 cows were infused with either ITS or the antibiotic, cloxacillin. All quarters in group 2 cows were treated with cloxacillin; half were also infused with ITS. Milk samples were drawn from all quarters 8 days after calving. For group 1 cows, there were no differences in IMI rates between treatments—ITS and cloxacillin were equally effective. In group 2 cows, quarters treated with both cloxacillin and ITS had fewer new IMI than those treated with cloxacillin alone.

38 Prevention of fatty liver in transition dairy cows by subcutaneous injections of glucagon

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At or around calving, dairy cows are vulnerable to a number of metabolic disorders, often related to accumulation of fat in the liver. Fatty liver (FL) is the result of negative energy balance in early lactation—the energy demand for milk production exceeds the animal's ability to consume energy-yielding nutrients. To satisfy energy demand, non-esterified fatty acids (NEFA) are mobilized from adipose (fat) tissues and are taken up by the liver where they may be oxidized, stored or exported. When storage exceeds oxidation or export, FL develops and ketosis may ensue. Glucagon is a protein hormone that promotes an increase in blood glucose by increasing both the rate of glycogen breakdown and the rate of glucose synthesis in the liver. Previous work by these authors demonstrated that continuous 14-day intravenous infusion of glucagon could be used to treat fatty liver in early lactation cows. The objective of the present trial was to determine if subcutaneous injections of glucagon could prevent FL. Cows were fed high energy diets in the last month of the dry period to increase their fat stores. At calving, they were assigned to one of 3 treatments: 7.5 mg/day of glucagon in saline, 15 mg/day of glucagon in saline or saline alone (control). Injections were administered 3 times per day for 14 days starting at 2 days after calving. The 15 mg/day glucagon treatment increased blood glucose and insulin, decreased blood NEFA and prevented FL. Feed intake, milk production and milk composition were not affected.

39 *Lactobacillus* GG does not affect D-lactic acidosis in diarrheic calves, in a clinical setting

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Diarrhea, caused by digestive tract infection, is the most common cause of illness and death in young calves. Systemic effects include loss of body fluids, electrolyte imbalances and acidosis. In the past, it was thought that the acidosis resulted from metabolic changes in response to infection. However, it has recently been found that it is due to the over-production of lactic acid by aberrant bacterial populations inhabiting the lower digestive tract. The objective of this study was to determine whether probiotic bacteria could be of value in treating diarrheic calves by helping to re-establish normal bacterial populations. Probiotics are living organisms that confer health benefits when ingested—the bacteria in yogurt are classic examples. Studies with human subjects have demonstrated the value of probiotics in treating cases of infectious diarrhea. One of the probiotics that has yielded the best results is *Lactobacillus rhamnosus* strain GG (LGG). In the present study, LGG was given to 24 diarrheic calves in either milk or an oral electrolyte solution; 24 other diarrheic calves did not receive LGG. LGG was recovered in the feces of 13 of the treated calves, demonstrating that it did survive passage through the digestive tract. Although 37 of the 48 calves initially had acidosis, only one of the LGG-treated calves had acidosis 48 hours after treatment. In spite of these favourable observations, results failed to clearly demonstrate decreased mortality or increased fecal dry matter content in calves treated with LGG.

40 Ventral laparoscopic abomasopexy in 18 cattle with displaced abomasum

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Veterinary Surgery (2006) Vol. 35 p. 347-355.

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 torsion and anchor the abomasum into its normal position. Although the success rate of these procedures is in the 90% range, all require that an incision be made in the side or bottom of the body cavity. Complications include infection of the wound, herniation through the wound and generalized infection inside the body cavity (peritonitis). An alternative is blind-stitch fixation and, although its success rate is similar to the surgical procedures, this technique can precipitate additional, more severe complications such as partial obstruction of the stomach and inadvertent fixation of another part of the digestive tract. This paper describes the application of a novel procedure for abomasal fixation (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 a very small incision in the abdominal wall. The procedure was performed on 17 cows with LDA and 1 with right displacement. Follow-up including wound complications, displacement recurrence, return to normal feed consumption, owner satisfaction and long-term survival demonstrated the feasibility and efficacy of the technique.

41 The cell-mediated immune response induced by plasmid encoding bovine herpesvirus 1 glycoprotein B is enhanced by plasmid encoding IL-12 when delivered intramuscularly or by gene gun, but not after intradermal injection

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Vaccine (2006) Vol. 24 p. 5349-5359.

Bovine herpesvirus 1 (BHV-1) is associated with several diseases in cattle, including infectious bovine rhinotracheitis, conjunctivitis, abortion, and encephalomyelitis. Although several types of vaccines have been used for the prevention of BHV-1 infections, DNA vaccines offer potential advantages over older vaccine technologies, including low cost, safety and improved efficacy. DNA vaccines typically consist of a segment of DNA that contains a gene which encodes a specific antigen. In previous work by these authors, a DNA vaccine carrying the gene for one of the BHV-1 virulence proteins (glycoprotein B: gB) was tested in mice and cattle. This vaccine provoked both antibody-mediated immunity (AMI) and cell-mediated immunity (CMI). An important element of the CMI response to the vaccine was activation of cytotoxic T lymphocytes (CTL), white blood cells produced by the thymus gland which are responsible for destroying virus-infected host cells. The objective of this study was to determine if the CTL response could be amplified by also stimulating production of interleukin-12 (IL-12), an important regulator of CMI. The BHV-1 gB vaccine was administered alone or together with a DNA vaccine encoding IL-12, either intradermally, epidermally or intramuscularly. When delivered by either of the latter 2 routes the combination of IL-12 and gB vaccines enhanced both AMI and CMI, compared with the response to the gB vaccine alone.

42 Passively acquired membrane proteins alter the functional capacity of bovine polymorphonuclear cells

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Polymorphonuclear leukocytes (PMNLs) are one of a variety of white blood cell types that play important roles in the innate immune system. The primary roles of PMNLs are the clearance of products of the immune response and the release of inflammatory mediators. The attachment of antibodies to surface antigens on bacteria cells marks those bacteria for destruction, after which they are engulfed and destroyed by PMNLs. This study examined another aspect of PMNL interaction with other cells – their ability to incorporate membrane proteins from dead and dying body cells into their own cell membranes and the effect that those acquired proteins had on PMNL function. Interactions between PMNLs and a variety of other cell types were studied using microscopy, revealing transfer of both cytoplasmic and membrane proteins to PMNL through direct cell-to-cell contact. When PMNLs were incubated with membranes from cells susceptible to adenovirus infection, the transfer of proteins from those membranes increased adenovirus infection of PMNLs. Incubation with membranes from sheep immune cells also resulted in the transfer of functionality associated with those cells to PMNLs.

43 Bovine polymorphonuclear cells passively acquire membrane lipids and integral membrane proteins from apoptotic and necrotic cells

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Journal of Leukocyte Biology (2006) Vol. 79 p. 1226-1233.

Polymorphonuclear leukocytes (PMNLs) are one of a variety of white blood cell types that play important roles in the innate immune system. Historically, the primary function of PMNLs was thought to be phagocytosis by which they engulf and destroy pathogens and damaged body cells. However, in recent years a much wider range of functions has been ascribed to PMNLs, raising the possibility that some of these may be acquired from other cell types rather than being an inherent function on the PMNLs themselves. This study demonstrated that this possibility is real. When PMNLs were cultured with other cell types, it was observed that membrane proteins from these cells were transferred to the PMNLs as the other cells died. PMNL recipients of these proteins acquired the specific activities associated with those proteins in their native cell types. Membrane protein transfer involved the fusion of cell membrane fragments from the dead and dying cells into the plasma membranes of the PMNLs by direct contact rather than by the phagocytosis of these fragments.

44 Prevalence of contagious mastitis pathogens in bulk tank milk in Prince Edward Island

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Canadian Veterinary Journal (2006) Vol. 47 p. 567-572.

Mastitis is the most common and costly disease affecting dairy cows. Inflammation of the mammary gland is primarily due to the presence of pathogenic organisms which are classified as either environmental or contagious. The principle contagious pathogens identified world-wide are *Staphylococcus aureus*, *Streptococcus agalactiae* and *Mycoplasma* species. The primary objective of this study was to estimate the prevalence of these 3 pathogens in bulk tank samples from dairy farms on Prince Edward Island (PEI). In addition, the relationship between individual herd bulk tank pathogen loads and somatic cell counts (SCC) was investigated. Three consecutive milk samples were drawn at weekly intervals from bulk tanks on each of PEI's 258 dairy farms. Among the 774 samples cultured, 74% were positive for *Staph. aureus*, 1.6% for *Strep. agalactiae* and 1.9% for *Mycoplasma*. The presence of the 2 Mycoplasma species isolated in this study (*M. bovis* and *M. alkalescens*) had not been reported in Canadian dairy herds since 1972 and had never been previously reported in PEI herds. Bulk milk SCC were higher when *Staph. aureus* was present.

45 A comparison of serum harvesting methods and type of refractometer for determining total solids to estimate failure of passive transfer in calves

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Adequate intake of colostrum is essential to the future viability of the newborn calf. In addition to providing essential nutrients, colostrum contains immunoglobulins (IgG, antibodies) which protect the calf from infection at a time when its own immune system is not fully functional. These IgG must be ingested within the first 24 hours of life because, after this time, absorption from the digestive tract becomes severely restricted. Therefore, the amount of IgG absorbed depends on its concentration in the colostrum ingested, the amount of colostrum consumed and the efficiency of absorption into the bloodstream. Although several methods are currently available for estimating blood IgG level, many require laboratory facilities and are impractical for application on-farm. The objective of this study was to evaluate the use of refractometry for estimating calf blood IgG levels. A refractometer measures the bending of light as it passes through a sample of liquid. When the sample is blood serum, the refractive index is a measure of the total dissolved solids. Since IgG are the primary variable constituent in the serum of newborn calves, the refractive index correlates with IgG concentration. Results of this study demonstrate that serum need not be separated by centrifugation before testing and that a simple, hand-held refractometer is as accurate as digital model.

46 Characterization of bacterial populations recovered from the teat canals of lactating dairy and beef cattle by 16S rRNA gene sequence analysis

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Mastitis is an inflammation of the mammary gland of which the primary cause is bacterial infection. However, a wide variety of other organisms can also infect the gland, including viruses, fungi and mycoplasmas; all of these enter the gland through the teat canal. Previous surveys of organisms inhabiting teat ends and canals have used culture methods and these have often been biased towards identifying common mastitis pathogens. The objective of this study was to investigate the impact of hygienic management differences between dairy and beef cattle on the bacterial population of teat canal. We used a genetic method to identify teat canal bacteria and to compare populations found in beef cattle with those in dairy cattle. The 16S ribosomal RNA (rRNA) gene sequence analysis method involves comparison of nucleic acid sequences of teat isolates to a database of sequences from known bacterial species. A total of 156 unique sequences were identified, representing 45 distinct taxonomic groups in the dairy sample; 53 in the beef. Clostridia and Bacilli dominated both dairy and beef samples, followed by Actinobacteria and Proteobacteria. Dairy samples also contained a variety of Staphylococci.

47 The role of cow, pathogen, and treatment regimen in the therapeutic success of bovine *Staphylococcus aureus* mastitis

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Staphylococcus aureus is the most common and problematic contagious pathogen causing intramammary inflammation (mastitis) in Canadian dairy cows. Reported cure rates for *Staph. aureus* mastitis range from 4 to 92%. This paper describes determinants of treatment success, including cow, pathogen and treatment factors. Cow factors associated with lower cure rates include increasing age, higher somatic cell score, longer duration of infection, higher pathogen load before commencing treatment and higher number of quarters infected. Treatment of infections in front quarters is generally more successful than treatment of hind quarter infections. Among pathogen factors affecting cure rates, antimicrobial resistance and other *Staph. aureus* strain-specific variables are important. Penicillin resistant strains also tend to be resistant to other antibiotics. Duration of antibiotic therapy is the most important treatment factor affecting cure rate—prolonged therapy is associated with greater success. However, extended treatment is not always economically justified due to the costs of treatment and lost milk revenue. In summary, although treatment of young animals with penicillin-sensitive *Staph. aureus* infections may be justified, treatment of older animals, chronic infections, or penicillin-resistant strains is costly and seldom successful. Control of *Staph. aureus* mastitis should focus on prevention, based on the National Mastitis Council's 10-point mastitis control program.

48 Effects of prepartum intramammary antibiotic therapy on udder health, milk production, and reproductive performance in dairy heifers

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Journal of Dairy Science (2006) Vol. 89 p. 2090-2098.

It is common practice to treat dairy cows with intramammary antibiotics after each lactation has ended. The objective of this 'dry cow treatment' is to eliminate bacteria from the mammary gland in order to minimize the risk of infectious mastitis in the next lactation. In contrast, although mastitis infections are common in heifers at first calving, prepartum intramammary antibiotic treatment is not commonly used to reduce mastitis risk in these animals. This study examined the possibility that this practice might eliminate existing intramammary infections, raise milk production, lower early lactation somatic cell scores (SCS) and improve reproductive performance. Mammary secretions were collected at 10 to 21 days before calving from each quarter of 561 heifers in 9 herds with wide geographic distribution in the US and Canada.

49 Assessment of antimicrobial transfer from treated to untreated mammary gland quarters by use of high-pressure liquid chromatography for detection of cloxacillin in milk samples from nonlactating dairy cows

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American Journal of Veterinary Research (2006) Vol. 67 p. 1140-1144.

In many trials designed to test the efficacy of antibiotic treatment of infectious intramammary infections (IMI), antibiotics are infused into one or two mammary quarters while one or more of the remaining quarters serve as untreated controls. The question arises: could transfer of antibiotic from treated to untreated quarters invalidate the results of trials using this experimental design? The objective of this study was to answer that question. Antibiotic (cloxacillin) was infused into 2 mammary quarters of 20 cows the day after their lactations were terminated. In half the cows, the 2 treated quarters were on the same side of the udder. In the other half, quarters diagonal to one another were treated. Three days later, milk samples were drawn from untreated quarters and analysed for antibiotic concentration. Trace levels of cloxacillin were found in 25% of these samples—all were below the minimum concentration required to inhibit bacterial growth. The relative positions of the untreated and treated quarters (same side or diagonal) had no effect on results. In conclusion, the use of individual quarters in the same udder in control versus treatment comparisons is a valid experimental design.

50 Test characteristics from latent-class models of the California mastitis test

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Preventive Veterinary Medicine (2006) Vol. 77 p. 96-108.

Treatment of dairy cows with intramammary antibiotics at the end of each lactation is common practice. The objective of 'dry cow treatment' (DCT) is to eliminate intramammary infections (IMI) in order to minimize the risk of infectious mastitis in the next lactation. In most cases, producers use 'blanket' DCT, treating all cows without assessing individual IMI status. Given the cost of treatment and concerns about antibiotic use in animal agriculture, a simple method of assessing IMI status at dry off would make it possible to eliminate treatment of low-risk animals. The California Mastitis Test (CMT) has been proposed for this purpose, but earlier evaluations concluded that it was not accurate enough to identify all, or even the majority, of infected cows. The authors of this study suggest that statistical methods used in those evaluations were flawed. Therefore, the objective of this study was to use a more appropriate statistical methodology to assess the value of the CMT for detecting the presence of IMI at dry-off. Using this method, the ability of the CMT to correctly identify infected cows (sensitivity) was estimated at 70%. The test's ability to detect uninfected animals correctly (specificity) was estimated at 48%. Specificity for detection of cows infected with major pathogens was 86%. Results suggest that the CMT could be used as a screening test in herds where the prevalence of IMI by major pathogens is less than 15%. Otherwise, blanket DCT is advised.

51 Bovine whey proteins inhibit the interaction of *Staphylococcus areus* and bacteriophage K

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Journal of Applied Microbiology (2006) Vol. 101 p. 377-386.

Staphylococcus aureus (SA) is the most costly and problematic pathogen causing intramammary infections (IMI) of dairy cattle. Poor cure rates and the continued emergence of multiple drug resistant strains of SA have prompted the search for alternative treatments. One such potential alternative is the use of bacteriophages (phages)—viruses that infect and kill their host bacteria. Earlier works exploring the use of phages to control SA have yielded mixed results. In this study bacteriophage K was effective in lysing (killing) SA cells in broth culture (in vitro) but, when administered into the mammary glands of cows (in vivo) experimentally infected with SA, or when raw milk or whey were used as the culture medium, phage K was much less effective. These divergent results suggested that some component of whey may inhibit attachment of the phage to SA cells. This study identified a component of whey protein as the inhibiting factor—treatment of whey with heat, protease or ultrafiltration removed the inhibition. Furthermore, suspension of SA in whey, followed by resuspension in broth also reduced phage binding due to the adhesion of whey protein to the SA cell surface. These observations explain the divergent results obtained from studies of SA-phage efficacy in broth culture compared with those from animal trials. In addition, these results highlight the importance of conducting through in vitro experiments prior to animal experiments.

52 The relationship between herd level disease incidence and return over feed index in Ontario dairy herds

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Canadian Veterinary Journal (2006) Vol. 47 p. 767-773.

The objective of this study was to examine associations between herd profitability and the herd level incidence of health disorders in 48 Ontario dairy herds. Profitability was calculated as the difference between milk income and feed cost (return over feed, ROF). No significant associations were found between ROF and incidence of displaced abomasum, retained placenta, clinical or subclinical mastitis (assessed with the California Mastitis Test), milk fever, clinical ketosis or lameness. However, a negative relationship was found between ROF and subclinical ketosis, detected using a cow-side test for betahydroxybutyrate in milk. Each percentage unit increase in herd incidence of subclinical ketosis was associated with a decrease in ROF of $0.15/cow/day.

53 Seroprevalences of antibodies against bovine leukemia virus, bovine viral diarrhea virus, *Mycobacterium avium* subspecies *paratuberculosis*, and *Neospora caninum* in beef and dairy cattle in Manitoba

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Canadian Veterinary Journal (2006) Vol. 47 p. 783-786.

Neosporosis, Johne's Disease (JD), bovine viral diarrhea (BVD) and enzootic bovine leukosis (EBL) are diseases that have significant health and/or economic impacts for the beef and dairy industries. In addition to reducing productivity and production efficiency, the presence of these diseases in Canada's livestock may also reduce consumer confidence in the safety of animal products and have negative impacts on export market opportunities. The objective of this study was to assess the past exposure of Manitoba dairy and beef cattle to the microbes that cause these 4 diseases by testing blood samples for the prevalence of antibodies to their causative organisms (seroprevalence). Antibodies to *Neospora caninum* were found in 8.3% of 1,204 dairy cow blood samples and 9.1% of samples from 1,425 beef cows. The seroprevalence of *Mycobacterium avium* subspecies *paratuberculosis* (the bacteria causing JD) was 4.5% in dairy cow samples but only 1.7% in beef cows. Bovine leukemia virus (cause of EBL) antibodies were found in 60.8% of dairy and 10.3% of beef cows. Significant levels of antibody to BVD virus were found in unvaccinated heifers and cows from 28.1% of the dairy herds tested.

54 Efficacy of monensin sodium for the reduction of fecal shedding of *Mycobacterium avium* subsp. *paratuberculosis* in infected dairy cattle

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Preventive Veterinary Medicine (2006) Vol. 75 p. 206-220.

Johne's Disease (JD) is a production limiting disease of ruminants caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP). In infected dairy herds, JD results in reduced milk production and premature culling of lactating cows. The primary route of infection is contamination of calf facilities by feces from adult animals. Therefore, prevention efforts focus on reducing such contamination. The removal of heavily shedding animals would be an effective strategy, but identification of these cows is not practical with currently available diagnostic tests. Neither vaccines nor antibiotics have been found effective against MAP infection. The objective of this study was to test the possibility that fecal shedding of MAP could be reduced by the oral administration of monensin sodium, a polyether ionophore that inhibits bacterial replication by modifying the permeability of the cell membrane. A controlled release capsule (CRC) which releases 335 mg/day of monensin was placed in the rumen of treated animals. Control animals received a placebo capsule. Although treated animals shed marginally fewer MAP cells than controls, the authors were unable to attribute any biological significance to this finding.

55 DNA-protein immunization against the GapB and GapC proteins of a mastitis isolate of *Staphylococcus aureus*

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Veterinary Immunology and Immunopathology (2006) Vol. 113 p. 125-138.

Mastitis due to *Staphylococcus aureus* infection is one of the most costly and challenging health problems facing the dairy industry worldwide. Antibiotic treatment of infected animals meets with variable, and often minimal, success. Vaccination with killed bacterial cells or bacterial extracts has often failed to provide protection against new infections, probably because of variation between antigens present in the vaccine and those of the new strain (serotype). Given the wide variety of antigens associated with the multiple serotypes of *S. aureus*, production of a vaccine containing all possible antigens would be impossible to produce. In previous work, the present authors describe the development of a DNA vaccine using a gene that codes for a protein having characteristics of 2 surface antigens which are common to all *S. aureus* serotypes (GapC/B chimera). The vaccine is comprised of the GapC/B gene linked to a DNA loop (plasmid) which serves as a vehicle (vector) for delivering the gene to host cells. Once in the cell, the gene is decoded to produce the GapC/B protein which elicits an immune response. The objective of this study was to test the efficacy of this vaccine and of vaccines coding for the individual (GapC and GapB) proteins with or without a subsequent boost with the respective proteins. Although the DNA vaccines alone were unable to elicit a significant immune response, boosting with the respective proteins did produce a detectable response.

56 Efficacy of Two Hydrogen Peroxide Teat Disinfectants Against *Staphylococcus aureus* and *Streptococcus agalactiae*

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Journal of Dairy Science (2006) Vol. 89 p. 3696-3701.

Post-milking teat disinfection is a widely accepted component of a successful mastitis control program. Judged on the basis of bactericidal activity and promotion of teat skin health, many effective teat disinfectants are available, most being iodophor-based. Ongoing concern about milk iodine concentrations have led to the development of alternative, hydrogen peroxide (H_2O_2)-based alternatives but few, if any, of these have been tested under conditions of challenge exposure to mastitis pathogens. The objective of this study was to compare the performance of a new 0.5% H_2O_2-based disinfectant (DeLaval) with that of an 0.5% H_2O_2-based disinfectant that was currently available (Oxy-Gard, EcoLab, St. Paul, MN). During a 10 week trial period, all teats of 45 cows were challenged after milking by immersion in a teat dip-cup containing a suspension of *Streptococcus agalactiae* and *Staphylococcus aureus* bacteria. The bacterial suspension was left to air-dry on the teats for 2 to 4 minutes after which the right front and left hind teats were dipped in the new DeLaval product while the right hind and left front teats were dipped in the Oxy-Gard product. There were no differences between products in the number of new infections caused by either pathogen. However, the DeLaval disinfectant resulted in significant improvement in teat skin condition with no adverse effects on teat end condition.

57 Efficacy and pharmacokinetics of bacteriophage therapy in treatment of subclinical *Staphylococcus aureus* mastitis in lactating dairy cattle

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Antimicrobial Agents and Chemotherapy (2006) Vol. 50 p. 2912-2918.

Staphylococcus aureus (SA) is the most costly and problematic pathogen involved in intramammary infections (IMI) causing mastitis in dairy cattle. Poor cure rates and the continued emergence of multiple drug resistant strains of SA have prompted the search for alternative treatments. One such potential alternative is the use of bacteriophages (phages) – viruses that infect bacteria. The objective of this study was to test the efficacy of phage therapy in the treatment of lactating cows infected with SA. Thirteen cows (18 quarters) received an intramammary infusion of phage K, which has been shown to lyse (kill) SA in broth culture. Eleven control cows (20 quarters) were infused with saline. All infusions were administered daily for 5 days after which milk samples were drawn for assessment of efficacy. In the phage-treated group, the cure rate was 3 of 18 quarters (16.7%); none of the saline-treated quarters were cured. The difference in cure rates was not statistically significant. Intramammary infusion of phage in non-infected cows provoked a large increase in milk somatic cell counts, a response that was not observed in phage-treated SA-infected cows. The decline of phage concentrations in milk overtime suggested that the mammary gland was able to inactivate and/or degrade phage particles

58 Network analysis of Danish cattle industry trade patterns as an evaluation of risk potential for disease spread

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Preventive Veterinary Medicine (2006) Vol. 76 p. 11-39.

This paper describes the construction and analysis of a network of cattle premises in Denmark. A premise is defined as a farm, an abattoir or a market. The objective was to characterize cattle movements between premises as an aid to understanding potential disease transmission through animal contact during movement between premises. A path for potential transfer of pathogens is created when cattle move from premise to premise. A total of 29,999 premises were included in the network model with 130,265 transfers between those premises in a 6-month period. Although network analysis could also be used to analyze within-premise (i.e., herd) disease transmission, this was not attempted in this study.

59 Formulation with CpG oligodeoxynucleotides increases cellular immunity and protection induced by vaccination of calves with formalin-inactivated bovine respiratory syncytial virus

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Virology (2006) Vol. 353 p. 316-323.

Bovine respiratory syncytial virus (BRSV) is one of the four viruses known to be associated with shipping fever in calves. Protection from BRSV has been attempted with the use of a formalin-inactivated (FI) BRSV vaccine but with little success. In some cases, calves exposed to the virus after vaccination with FI-BRSV exhibit a more severe reaction than those who are not vaccinated. The failure of FI-BRSV vaccines to provide protection is thought to be due to their inadequate induction of cell-mediated immunity. This study examines the potential to improve the cell-mediated immune response by formulating a FI-BRSV vaccine with a CpG oligodeoxynucleotide (ODN) – a short DNA sequence previously shown to promote the release of mediators (cytokines) which stimulate cellular immunity. Newborn calves were vaccinated twice with FI-BRSV alone or with a formulation that included CpG ODN and were subsequently challenged with BRSV. When compared with calves vaccinated with FI-BRSV alone, those immunized with FI-BRSV/CpG-ODN formulation demonstrated improved cell-mediated immune responses as well as higher BRSV-specific serum antibody (IgG2) levels. The amount of BRSV in lung tissue also decreased in calves that received the FI-BRSV/CpG-ODN formulation.

60 Monensin might protect Ontario, Canada cows from paratuberculosis milk-ELISA positivity

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Preventive Veterinary Medicine (2006) Vol. 76 p. 237-248.

Johne's Disease (JD) is a production limiting disease of ruminants caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP). In infected dairy herds, JD results in reduced milk production and premature culling of lactating cows. In a clinical trial with naturally MAP-infected cattle, the severity of gastrointestinal lesions caused by MAP was reduced by feeding monensin, a polyether ionophore that inhibits bacterial replication by modifying the permeability of the cell membrane. Fewer lesions and reduced fecal shedding of MAP were also observed in a study where monensin was fed to young calves. Dairy cattle diets are routinely supplemented with monensin. It is fed in calf starters to control coccidiosis, in heifer supplements to increase feed efficiency and in lactation diets to minimize ketosis. Farm managers may use monensin in all, none or any combination of these diets and practices may vary depending on the JD status of the herd. The objective of this study was to evaluate associations of these and other management practices with herd JD-status assessment using a milk enzyme-linked immunosorbent assay (ELISA). Milk samples from 4,933 dairy cows in 94 Ontario dairy herds were tested. In 48 herds where JD had not previously been diagnosed, cows were less likely to be found ELISA-positive if calf hutches were used and cows received monensin, either as a premix or controlled release capsule (CRC). Feeding monensin to breeding age heifers reduced the likelihood that cows would be ELISA-positive in the 46 herds in which JD had previously been diagnosed.

61 Johne's disease in Canada Part I: Clinical symptoms, pathophysiology, diagnosis, and prevalence in dairy herds

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Canadian Veterinary Journal (2006) Vol. 47 p. 874-882.

Johne's Disease (JD) is an infectious disease caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP). Although the natural hosts for MAP are wild and domestic ruminants, other wildlife including fox, weasel, crow, rat, mouse, rabbit, hare and badger can also harbour the bacteria and may contribute to its spread. In cattle, the primary route of infection is ingestion by calves of MAP from teats, milk, feed and water contaminated with faeces. This paper reviews current knowledge about the clinical stages, pathology and diagnosis of JD as well as its prevalence in Canadian cattle populations. MAP infects the gastrointestinal tract and associated lymph nodes, limiting nutrient absorption, ultimately resulting in chronic diarrhea, emaciation, debilitation and death. Subclinical infections cause weight loss and reduced milk production leading to premature culling and decreased slaughter value. Fertility and udder health may also be negatively affected. Currently available diagnostic tests yield many false-negative and false-positive results, particularly in blood, milk and fecal samples from animals with subclinical infections. Studies of JD prevalence among dairy herds in 7 Canadian provinces estimated that 37-74% of herds had at least one animal that tested MAP-positive; 9.8-43.1% had at least 2 MAP-positive animals.

Milk Production

1 Effects of insulin, recombinant bovine somatotropin (rbST) and their interaction on DMI and milk fat production in dairy cows

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Livestock Production Science (2005) Vol. 97 p. 173-182.

It is currently thought that the depression of milk fat concentration seen in cows fed high concentrate diets is due to the synthesis of conjugated linoleic acid by rumen micro-organisms. An older theory held that milk fat depression (MFD) under these conditions was due to an increase in blood insulin resulting in greater uptake of nutrients by adipose tissue, reducing the availability of precursors for milk fat synthesis. However, studies aimed at validating this theory have failed to demonstrate increases in blood insulin levels in cows experiencing significant MFD. The objective of this study was to further investigate the possible role of insulin in MFD and the possibility of an interaction between the effects of insulin and somatotropin. Cows were subjected to 4 treatments: (1) intravenous (IV) infusion of saline (controls); (2) IV saline plus subcutaneous (SC) recombinant bovine somatotropin (rbST); (3) IV insulin plus glucose; (4) IV insulin plus glucose plus SC rbST. Insulin, with or without rbST, decreased plasma triglycerides and milk fat yield, the latter primarily through decreases in long chain fatty acids (LCFA). In contrast, milk fat yield and LCFA concentrations were higher with rbST treatment, with or without insulin. Plasma non-esterified fatty acids were higher with rbST treatment but this effect was prevented when insulin was also infused. Overall, these results clearly indicated that insulin decrease milk fat yield but this decrease, unlike during classical milk fat depression, is associated only with a decrease in long chain fatty acids in milk.

2 Interactive visualization of multi-dimensional data in dairy production

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Large amounts of data are available to milk producers who subscribe to Dairy Herd Improvement Services or who have automated on-farm milk recording systems. Profitable management decisions require the analysis and correct interpretation of this data. Current dairy herd management programs typically allow data to be visualized in 2 dimensions as histograms and scatter plots. However, the visualization of some relationships would benefit from 3 dimensional (3-D) visualization and an ability to interactively manipulate those views. This paper describes the potential to use current computer technologies for this purpose. Interactive techniques are discussed including chart zooming, translation, brushing of individual data points to display details, rapid attribute selection and dynamic data filtering. Application of these techniques to analysis of dairy herd data is demonstrated.

3 17β-estradiol reduces milk synthesis and increases stanniocalcin gene expression in the mammary gland of lactating cows

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Livestock Production Science (2005) Vol. 98 p. 57-66.

After cows become pregnant in early lactation, blood estrogen levels rise slowly as a result of increasing estrogen synthesis by the fetus and placenta. At the same time, milk production declines steadily due to progressive loss of milk secreting cells and involution of the mammary gland. It has been suggested that these 2 processes are related—that increasing blood estrogen may be partly responsible for decreasing production. The objective of this study was to test the validity of this suggestion and to investigate the effects of estrogen on the expression of genes in milk secretory cells. Non-pregnant, mid to late lactation cows were treated with daily injections of 17β-estradiol in ethanol. Controls received ethanol alone. Dry matter intake and milk production declined in treated cows. Expression of the gene coding for β-casein decreased but that of the gene coding for stanniocalcin (STC) increased. STC is a hormone that regulates calcium and phosphate transport across cell membranes. Changes in its activity in response to estrogen suggest a possible role of STC in mammary gland involution.

4 HS-SPME gas chromatographic characterization of volatile compounds in milk tainted with off-flavour

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Various ingredients included in diets fed to lactating cows are capable of imparting undesirable flavours to milk. Fermented forages (silages) are the feeds most often implicated. Until recently, it has not been possible to identify the specific chemical compounds responsible for these off-flavours because of limitations in analytical technology and the large number of compounds involved. However, with the development of new instrumentation and methods, this has now become possible. The objectives of this study were to devise a reliable method of producing off-flavoured milk and to determine whether 'headspace solid-phase micro-extraction' (HS-SPME) gas chromatography (GC) could be used to characterize the chemical components responsible for the undesirable flavour. Milk samples were collected 30 minutes, 3 hours and 12 hours after cows were fed baled grass silage. Although samples collected at 12 hours were judged by a taste panel to be of good flavour quality, 89% of those collected earlier were judged to have a 'feed' flavour. HS-SPME GC analysis identified approximately 75 compounds that contributed to flavour, most being found in both good- and off-flavoured samples. Results suggested that the undesirable flavour was due to concentration differences in these flavour components rather than the presence or absence of specific compounds.

5 Suppression of estrous cycles in lactating cows has no effect on milk production

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Journal of Dairy Science (2006) Vol. 89 p. 636-639.

Milk production in dairy cows typically reaches a peak between one and two months after calving, after which it declines due to the programmed destruction (apoptosis) of mammary gland milk-secreting cells. Since cows normally resume estrous cycle activity at around this same time, it has been considered possible that higher blood levels of estrous cycle hormones (estrogen and progesterone) may be involved in this process. This hypothesis has received support from studies where administration of estrogen, with or without progesterone, resulted in mammary gland involution. However, other research has failed to provide support. The objective of this study was to compare the milk production of cows experiencing normal estrous cycles (control cows) with that of non-pregnant herdmates treated with deslorelin, an agent which suppresses estrous activity by blocking the release of estrogen and progesterone. Treated cows received deslorelin ear implants at 10 and 100 days in milk. Production effects were monitored until 210 days in lactation. There were no differences in feed intake, milk production or milk fat or protein concentrations between control and deslorelin-treated cows. Results suggest that fluctuating blood levels of estrogen and progesterone associated with normal estrous cycles do not influence the milk production capacity of the mammary gland.

6 Effect of safflower oil, flaxseed oil, monensin, and vitamin e on concentration of conjugated linoleic acid in bovine milk fat

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Journal of Dairy Science (2006) Vol. 89 p. 733-748.

Conjugated linoleic acid (CLA) refers to a number of 18 carbon fatty acid isomers having 2 double bonds with a single intervening single bond. In cow's milk, the cis-9, trans-11 isomer (9,11 CLA) accounts for more than 82% of the total CLA content. Studies using experimental animals have revealed several health benefits associated with dietary intake of 9,11 CLA. Attention has focused on its anti-carcinogenic properties. Previous research has revealed that milk 9,11 CLA concentration is largely dependent on the amount of trans-vaccenic acid (TVA) synthesized in the rumen and released into the bloodstream. On reaching the mammary gland, TVA is converted to 9,11 CLA. The objective of this study was to identify dietary ingredients that might provoke higher levels of TVA synthesis in the rumen, leading to higher 9,11 CLA concentrations in milk. The dietary ingredients tested were safflower oil (SO), flaxseed oil (FO), monensin (M) and vitamin E (E). In an initial trial, inclusion of SO at 6% of dietary dry matter (DDM) produced a 7.5-fold increase in milk 9,11 CLA; SO at 6% plus M at 24 mg/kg of DDM resulted in an 11.4-fold increase in milk 9,11 CLA relative to milk from cows fed an unsupplemented diet. In a second trial, supplementation of SO and SO plus M at the same levels yielded 6.1- and 6.7-fold increases in milk 9,11 CLA concentrations, respectively. Supplementation of E had no effect on increases in milk 9,11 CLA produced by feeding either SO or FO.

7 Characterization and regulation of the bovine stearoyl-CoA desaturase gene promoter

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Biochemical and Biophysical Research Communications (2006) Vol. 344 p. 233-240.

Conjugated linoleic acid (CLA) refers to a number of 18 carbon fatty acid isomers having 2 double bonds with a single intervening single bond. Because CLAs and their precursors are produced by rumen microbes, CLA levels in ruminant tissues are higher than those found in the tissues of monogastric animals. In cow's milk, the cis-9, trans-11 isomer (9,11 CLA) predominates. Studies using experimental animals have revealed several health benefits associated with dietary intake of 9,11 CLA. For this reason, there is interest in understanding the mechanisms controlling milk 9,11 CLA content with the goal of increasing it to therapeutic levels. The majority of 9,11 CLA in milk is produced in the mammary gland from trans vaccenic acid which is synthesized in the rumen. Stearoyl-CoA desaturase (Scd, sometimes referred to as delta-9 desaturase) is the enzyme responsible for this transformation and the abundance of this enzyme affects the rate at which the transformation occurs. The objective of this study was to characterize the genetic code responsible for controlling the synthesis of Scd. A 'promoter' region, designated as the Scd transcription enhancer element (STE), was found to have a critical role in activating the transcription of the Scd gene to produce the Scd enzyme. Using mammary cells in tissue culture, it was found that the addition of CLA reduced the rate of enzyme synthesis while insulin and oleic acid increased it. Both effects were mediated through the activity of the STE.

8 Geographic and temporal aspects of an outbreak of off-flavours in bulk-tank milk in Prince Edward Island, Canada

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Preventive Veterinary Medicine (2006) Vol. 74 p. 154-161.

An outbreak of off-flavoured milk occurred in Prince Edward Island dairy herds between September 2000 and June 2002. An earlier study by these authors examined the risk factors associated with this outbreak. In the present study, a novel statistical analysis (cluster-detection test) was applied to data collected during the outbreak in order to assess associations between off-flavour incidents, farm location, time of outbreak and off-flavour characteristics. The analysis resulted in the discrimination of several discrete time-location-flavour clusters within the overall 21-month outbreak.

9 Acetyl CoA carboxylase shares control of fatty acid synthesis with fatty acid synthase in bovine mammary homogenate

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Journal of Dairy Science (2006) Vol. 89 p. 2552-2558.

Better understanding of the nutritional roles of various fatty acids (FA) has provoked attempts to alter the FA composition of milk fat, primarily through changing the cow's diet. Many of these attempts have been successful in changing the concentrations of specific FA with chain lengths of 16 carbons or more due to the fact that about half of the 16 carbon FA and all of the longer chain FA in milk are absorbed directly from the bloodstream. However, the remaining FA in milk are synthesized in the mammary gland, limiting the effects that dietary changes can have on their concentrations. To effect changes in these FA, an understanding of the mechanisms by which they are synthesized is required. The objective of this study was to determine the relative influence of the 2 primary enzymes involved in synthesizing these FA in determining their rate of synthesis. Although it had been thought that acetyl CoA carboxylase (ACC) was the enzyme that was rate-limiting, results demonstrated that ACC and fatty acid synthase shared control over the rate of mammary short-chain FA synthesis.

10 Clinical trial to determine the productivity impact of milk urea nitrogen reports

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Bovine Practitioner (2006) Vol. 40 p. 86-94.

Many Dairy Herd Improvement organizations provide milk urea nitrogen (MUN) testing as part of their regular service offerings. High MUN concentrations are an indication of dietary imbalance. The objective of this study was to determine whether MUN test results were of value to milk producers. Based on results of previous testing, 50 dairy farms with high MUN results and 30 farms with low MUN results in Prince Edward Island were randomly divided into 2 groups (40 per group). For an 11-month period, one group (the intervention group) received monthly MUN test results and were provided with assistance in interpreting results and identifying possible reasons for abnormal results when they occurred. The second group (the control group) received their monthly MUN test results but were provided no assistance in their interpretation or application. Results, assessed during the last 3 months of the trial, revealed no differences in average MUN levels or in standardized milk production between the 2 groups. However, herds in the intervention group that made feed changes in response to interpretation of MUN results produced an average of 1.1 kg/day more milk in the month after the feed change than herds that did not make a feed change. Most producers in the intervention group felt that MUN testing was at least somewhat useful as a nutritional management tool.

Reproduction

1 Protein kinases influence bovine oocyte competence during short-term treatment with recombinant human follicle stimulating hormone

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Reproduction (2005) Vol. 130 p. 303-310.

Embryo transfer (ET) in cattle requires the extraction of oocytes (egg cells) from ovarian follicles. Once they are harvested, these oocytes are expected to continue their development in vitro (cultured in an artificial environment outside the cow) in preparation for fertilization and subsequent implantation of a mature blastocyst in the uterus of a surrogate dam. Culture conditions can have significant effects on the success of this maturation process and on the likelihood that implantation will lead to the birth of a live calf. In this study, oocytes were exposed to recombinant human follicle-stimulating hormone (rhFSH) to determine its effects on the competence of oocytes to form mature blastocysts. It is known that FSH exerts its effects through the stimulation of cyclic adenosine monophosphate (cAMP) synthesis which, in turn, stimulates the activity of the enzymes protein kinase A and C (PKA and PKC). Therefore, this study also examined the effects of an activator of cAMP synthesis and of activators and inhibitors of PKA and PKC. A highly significant improvement in blastocyst development was seen when oocytes were exposed to rhFSH for 6 hours, compared to exposure for 2 or 24 hours. Exposure to the cAMP activator for 6 hours also increased the rate of blastocyst formation as did activation of PKC. PKA inhibitors did not affect the stimulatory effect of rhFSH but PKC inhibitors did.

2 Progesterone (CIDR)-based timed AI protocols using GnRH, porcine LH or estradiol cypionate for dairy heifers: Ovarian and endocrine responses and pregnancy rates

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Theriogenology (2005) Vol. 64 p. 1457-1474.

Hormone treatment protocols eliminate the requirement for heat detection, facilitating fixed-time artificial insemination (FTAI) of dairy females. For example, in the Ovsynch protocol, two doses of gonadotropin-releasing hormone (GnRH) are administered 9 days apart with a single dose of prostaglandin $F_{2\alpha}$ (PGF) followed by FTAI 7 days after the first dose of GnRH. Although Ovsynch yields acceptable conception rates (CR) when used with cows, CR results with heifers are unacceptably low. The objective of this study was to devise an alternative FTAI strategy for use in dairy heifers. The reason for the poor response to Ovsynch in heifers is thought to be the failure of the first dose of GnRH to provoke ovulation, likely due to estrus having occurred shortly before the beginning of treatment. Therefore, the first component of the alternative strategy was the use of an intravaginal progesterone-releasing controlled internal drug release (CIDR) device which effectively suppresses normal estrous activity. Alternatives that were tested after CIDR withdrawal included the use of porcine luteinizing hormone (pLH) or estradiol cypionate (ECP) to replace GnRH. Three experiments examined pregnancy rate responses to various combinations of GnRH, pLH and ECP after CIDR treatment. Protocols using 2 doses of GnRH or ECP resulted in pregnancy rates that were 20 percentage points higher than those typically reported when Ovsynch without CIDR was used in FTAI programs for heifers.

3 Follicular fluid concentration of transforming growth factor-β1 is negatively correlated with estradiol and follicular size at the early stage of development of the first-wave cohort of bovine ovarian follicles

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Domestic Animal Endocrinology (2005) Vol. 29 p. 623-633.

In a cow experiencing regular estrous cycles, ovulation occurs when an oocyte (egg) is released from a dominant ovarian follicle. This dominant follicle is one of several that began to develop from a so-called wave of growing follicles that emerge from the ovary at regular intervals throughout the estrous cycle. Within about 3 days of the beginning of this 'wave' of development, one of the follicles will begin to become dominant and, under the influence of follicle stimulating hormone (FSH) and other factors, will start to produce more estradiol than the others. The amount of estradiol produced by each follicle is influenced by signalling molecules which affect the responsiveness of its estradiol-secreting cells to FSH. Transforming growth factor beta (TGF-β) is a class of signalling molecules which includes 3 unique variations: β1, β2 and β3. The objective of this study was to determine whether TGF-β1 is involved in determining which follicle will become dominant by modulating the effect of FSH on estradiol production. In one experiment, estradiol and TGF-β1 concentrations were measured in follicular fluid from 3 or 4 of the largest follicles at several stages of development of the follicle wave. Early in the wave, as follicles grew and estradiol levels rose, TGF-β1 concentrations declined. In a second experiment, the addition of TGF-β1 to follicle cells maintained in tissue culture caused a dose-dependent decrease in FSH-stimulated estradiol secretion. These findings underline the importance of inhibitory mechanisms that control ovarian follicle growth and suggest that a reduction of TGF-β1 inhibition of estradiol production may play a role in the mechanism by which the dominant follicle is selected.

4 Effect of hormonal stimulation on bovine follicular responses and oocyte developmental competence in a commercial operation

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Theriogenology (2006) Vol. 65 p. 102-115.

Multiple ovulation embryo transfer (MOET) programs are widely used in dairy cattle genetic improvement programs to increase the number of offspring from cows with high genetic potential. One type of MOET program involves stimulating the development of multiple primary follicles with follicle stimulating hormone followed by the collection of oocytes (egg cells) under the guidance of transvaginal ultrasound (TVU). After in vitro (in an artificial medium outside the cow) fertilization (IVF) of the oocytes, the embryos are allowed to mature to the blastocyst stage and are then implanted into the uteri of foster cows. The success of these programs is highly variable. For example, a summary of the results of more than 2000 MOET attempts in beef cattle reported that 24% produced no viable embryos while 30% yielded over 70% of all of the viable embryos produced. The present study examined data collected over a 5-year period at a commercial ET unit in Quebec to determine criteria for success of MOET programs. It was determined that the number of small antral follicles (follicles with a fluid-filled cavity), visualized by TVU at the time of oocyte collection, was a good predictor of the number of oocytes produced and of the number of viable embryos subsequently available for transfer. FSH treatment protocol variations had no effect on oocyte production or rates of embryo development.

5 Contribution of the oocyte to embryo quality

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Theriogenology (2006) Vol. 65 p. 126-136.

The successful application of embryo transplant (ET) programs to dairy cattle genetic improvement requires the development of pre-implantation embryos (blastocysts) that have the ability to implant in the endometrium of the surrogate dam, signal the establishment of pregnancy, and continue developing to a healthy full-term fetus. Two techniques are commonly used to obtain such blastocysts. The first involves the natural (in vivo) development of ovarian follicles and oocysts (egg cells) followed by intrauterine insemination and harvesting of embryos from the uterine horns. The alternative is to harvest oocytes from ovarian follicles followed by fertilization and development in vitro (in artificial media outside of the cow). In either case, the quality of the resulting blastocysts depends on the ability of the oocyte to resume meiotic division and the capacity of the zygote to divide after fertilization. This paper discusses each of the critical steps in the development of the oocyte, zygote, embryo and fetus leading to a live birth, with emphasis on the importantance of oocyte maturation processes.

6 The impact of chromosomal alteration on embryo development

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Theriogenology (2006) Vol. 65 p. 166-177.

Throughout life, the chromosomes in body cells divide and replicate while those in germ cells replicate several times before they unite to initiate the development of a new individual. Chromosomal alterations can occur during each replication cycle. Some of these alterations are predictable, others occur at random. Although spontaneous chromosomal change is an important source of genetic variation, it can also result in the uncontrolled growth of tumour cells and other anomalies. In the developing embryo, chromosomal alterations can reduce viability and impair normal development. In vitro (in artificial media outside of the dam) manipulation of embryos has been shown to cause an increased incidence of chromosome alterations affecting early development. This paper presents a summary of recently published reports dealing with chromosomal alterations resulting from in vitro embryo production, highlighting information on controlling alterations and requirements for dealing with remaining challenges.

7 Quantitative expression of candidate genes for developmental competence in bovine two-cell embryos

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Molecular Reproduction and Development (2006) Vol. 73 p. 288-297.

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 was 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. 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 identify differences in maternal gene expression between two-cell embryos from early (24-32 hours post IVF) versus late (36-44 hours post IVF) cleaving zygotes. Of 16 genes examined, 3 were identified as having significantly different levels of expression between the two classes of zygotes.

8 15-Hydroxyprostaglandin dehydrogenase in the bovine endometrium during the oestous cycle and early pregnancy

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Reproduction (2006) Vol. 131 p. 573-582.

Prostaglandins (PG) are locally produced hormone-like regulators that play important roles in reproduction. In the dairy cow, the relative activities of prostaglandin E2 (PGE_2) and prostaglandin F2alpha ($PGF_{2\alpha}$) determine whether a new estrous cycle will begin or whether pregnancy will be established. The effects of each PG are determined by the rates of their synthesis and destruction, transport to target tissues and binding to receptors. All of these factors can change, depending on reproductive status. This study examines the role and activity of the enzyme, 15-hydroxy prostaglandin dehydrogenase (15-PGDH) in regulating the effects of PG during the bovine estrous cycle. After cloning the 15-PGDH gene, a recombinant version of the enzyme was produced. This was used to generate a specific antibody to 15-PGDH which allowed for an assessment of localization and enzyme activity in the endometrium. It was found that the expression of 15-PGDH varied during the estrous cycle, reaching a peak in the uterine glandular epithelium on days 16-18 with another transient peak in the luminal epithelium and in the outer cell layer of the developing embryo on day 21. These observations suggested that 15-PGDH plays an important role in the regulation of PG activity, especially at critical times of the estrous cycle.

9 Peroxisome proliferator-activated receptor (PPAR) expression in cultured bovine endometrial cells and response to omega-3 fatty acid, growth hormone, and agonist stimulation in relation to series 2 prostaglandin production

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Domestic Animal Endocrinology (2006) Vol. 30 p. 155-169.

Trials in which dairy cow diets are supplemented with flaxseed or fish oils have demonstrated positive effects of embryo survival. These effects are thought to be due to the presence of long chain polyunsaturated omega-3 fatty acids (omega-3 PUFA) in these oils. Although the mechanism is unclear, these fatty acids appear to suppress the synthesis of prostaglandin F2-alpha ($PGF_{2\alpha}$) while increasing production of other prostaglandins. Since $PGF_{2\alpha}$ is the signal that initiates a new estrous cycle by causing regression of the corpus luteum, reducing its activity may prevent the termination of a latent pregnancy. The purpose of this study was to elucidate the mechanism by which omega-3 PUFA suppresses $PGF_{2\alpha}$ synthesis. Peroxisome proliferator-activated receptors (PPAR) are thought to play an important role in this mechanism since they are found in the promoter region of the gene that codes for $PGF_{2\alpha}$ and they are able to bind PUFA. Cultured bovine endometrial cells were exposed to omega-3 PUFA and other substances known to stimulate or inhibit $PGF_{2\alpha}$ synthesis. Results revealed that that 2 particular PPAR were possibly involved in the regulation of $PGF_{2\alpha}$ secretion in association with both growth hormone and omega-3 PUFA.

10 Spontaneous uptake of exogenous DNA by bull spermatozoa

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Theriogenology (2006) Vol. 65 p. 683-690.

Transgenesis has been used to improve productivity traits in farm animals, to synthesize large quantities of pharmaceuticals and other medically important chemicals and to produce tissues that can be grafted into humans. Although transgenic animals are typically produced by transferring foreign (exogenous) DNA into early embryonic host cells, this method is laborious, expensive and has had a very low success rate. An alternative technique is to use sperm cells (spermatozoa) to carry the exogenous DNA into an oocyte, thus producing a transgenic embryo. The spontaneous uptake of exogenous DNA by spermatozoa of several species has been previously demonstrated but uptake efficiencies have been quite low. The objective of this study was to examine factors affecting the uptake of exogenous DNA by bull spermatozoa. Fresh or frozen-thawed spermatozoa from each of 4 bulls were incubated at 37° C with exogenous DNA in the form of plasmids (DNA loops) coding for green fluorescent protein or chloramphenicol acetyl transferase. Uptake was significantly higher into the frozen-thawed versus the fresh spermatozoa but was not affected by either the individual bull or the particular exogenous DNA used. All spermatozoa which assimilated the exogenous DNA were immotile, whether they were dead or alive. Although uptake of exogenous DNA into bull spermatozoa was clearly demonstrated, further research aimed at maintaining motility is required before this technique can be used to produce transgenic cattle.

11 Cross-species hybridization on a multi-species cDNA microarray to identify evolutionarily conserved genes expressed in oocytes

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BMC Genomics (2006) Vol. 7 p. 113-126.

Although evolution has resulted in the development of many different species of animals, many animals share much the same complement of DNA. Many features of early embryonic development are also shared among evolutionarily distant animals. For example, in many species, development of the oocyte (egg) stops after the first division of chromosomes (meiosis). At this point the oocyte contains a large number of messenger RNA (mRNA) molecules (transcripts) and proteins that will play a role in subsequent development after fertilization. This study describes the construction of a DNA microarray that was used to compare active genes in cattle with those from the mouse and the African clawed frog. Of 3,456 transcripts examined, there were 1,541 that were expressed in body cells from all three species. Comparison of transcripts in arrested oocytes revealed 268 that were shared by the three species.

12 Short- and long-term skin graft survival in cattle clones with different mitochondrial haplotypes

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Theriogenology (2006) Vol. 65 p. 1465-1479.

Animal clones can be produced by transfer of nuclei from body cells of a donor animal into enucleated oocytes of a recipient. Although the resulting clones should have exactly the same complement of nuclear DNA as the donor, it has been shown that gene expression among first-generation clone siblings is often not identical. This may simply be due to variations in the programming of nuclear DNA transcription. Another possibility is that, along with transfer of the nucleus, some cytoplasm from the donor cell is transferred to the recipient. Mitochondrial DNA (mRNA) contained in that cytoplasm may be responsible for the variation observed among clones since it is known that mRNA can affect many traits through its role in generating cellular ATP. In addition, variation may be due to incompatibilities between mRNA from the donor and that remaining in the recipient oocyte. The objective of this study was to determine whether incompatibility between donor and recipient mRNA would affect immunological compatibility of cloned adult siblings. Three bovine clones were produced by transplanting the nuclei of skin cells (dermal fibroblasts) from a fetus into bovine oocytes collected from an abattoir. Amounts of donor cell mRNA in the skin cells of adult clones varied from 1 to 2.6% and sequencing confirmed that the mRNA from the 3 clones was derived from unrelated sources. In spite of these differences, skin grafts between clones demonstrated immunological compatibility.

13 Defining postpartum uterine disease in cattle

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Theriogenology (2006) Vol. 65 p. 1516-1530.

Bacterial contamination of the uterus after calving is common in dairy cattle. Although most cows will eliminate these bacteria in the first few weeks after calving, in 10-17% of cases pathogenic bacteria will persist, causing inflammation of and damage to the lining of the uterus. Systemic effects of infection can result in lower conception rates, longer intervals to first service and conception and increased rates of culling for reproductive failure. Many studies have been conducted on the causes, incidence and treatment of uterine infection but, in many cases, descriptions of the disease have been inadequately defined, making results often difficult to interpret. The objective of this paper was to provide clear clinical definitions of five distinct conditions resulting from uterine infection: puerperal metritis, clinical metritis, clinical endometritis, subclinical endometritis and pyometra. It is suggested that these diseases be distinguished on the basis of uterine enlargement, systemic illness, uterine content and discharge, body temperature and time after calving.

14 Characterization of linker histone H1FOO during bovine in vitro embryo development

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Molecular Reproduction and Development (2006) Vol. 73 p. 692-699.

Chromosome structure is often described as 'beads on a string' where the core of the beads is composed of H2, H3 and H4 histone proteins. Double-stranded DNA forms the string and is also wound around the histone beads to form a nucleosomes. At a secondary level of organization, H1 'linker' histones link adjacent nucleosomes to form a coil of 'chromatin'. In addition to their role in chromatin organization, H1 histones can repress or activate transcription of DNA into messenger RNA (mRNA) which carries the genetic code to be translated into proteins. One of a number of specialized H1 linker histones (H1FOO) is found in the bovine oocyte (egg cell) but its function has not been clearly defined. The objective of this study was to clarify the role of H1FOO by defining the abundance of its mRNA and the localization of the protein itself at several stages in oocyte and embryo development. The highest level of H1FOO mRNA was found in the primary oocyte near the end of its first meiotic division. Levels decreased steadily from this point until, in the 16-cell embryo, they were 200 times lower and, in the blastocyst, 2,000 times lower. In the oocyte as well as in 1-, 2- and 4- cell embryo, H1FOO was found both uniformly distributed in the cytoplasm and closely associated with chromatin. In 8- and 16-cell embryos, cytoplasmic levels of H1FOO had diminished but nuclear levels were still high. H1FOO was not detected in either morula or blastocyst stages.

15 Identification and characterization of a novel bovine oocyte-specific secreted protein gene

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Gene (2006) Vol. 375 p. 44-53.

All of the cellular machinery necessary for development of the bovine embryo up to the 8-cell stage is transcribed from maternal DNA during the latter (germinal vesicle) stage of the oocyte's development within the Graafian follicle. The specific functions of most of the many messenger RNA (mRNA) transcripts and proteins contained in the primary oocyte remain unknown. The objective of this study was to characterize one particular maternal gene thought to play important roles in early embryonic development. The OOSP1 gene was localized to chromosome 15 and found to transcribe 2 distinct mRNA transcripts, OOSP1-v1 and OOSP1-v2. OOSP1-v1 encodes a protein of 163 amino acids in length which is identical to a protein found in mouse oocytes, spleen and liver. OOSP1-v2 codes for a 35 amino acid protein. In contrast with the mouse, the bovine OOSP1 gene is expressed exclusively in oocytes. Abundance analysis demonstrated high levels of both OOSP1 transcripts in primary oocytes near the end of first meiotic division (germinal vesicle stage), gradually decreasing as development progressed until they were undetectable in the blastocyst.

16 Bovine SNRPN methylation imprint in oocytes and day 17 in vitro-produced and somatic cell nuclear transfer embryos

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Biology of Reproduction (2006) Vol. 75 p. 531-538.

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

17 Expression of phospholipase A2 group IVA (PLA2G4A) is upregulated by human chorionic gonadotropin in bovine granulosa cells of ovulatory follicles

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Biology of Reproduction (2006) Vol. 74 p. 1096-1103.

Ovulation in the cow is preceded by the growth and maturation of a primary ovarian follicle. Ovulation is initiated by the release of luteinizing hormone (LH) which provokes the production of prostaglandin F2alpha, the agent responsible for cleavage of the follicle and release of the oocyte (egg). The first step in the synthesis of prostaglandins is the release of their precursor, arachidonic acid (AA), from cell membrane phospholipids, facilitated by 'phospholipase' enzymes. Earlier work demonstrated increased expression of a fragment of DNA associated with phospholipase A2 group IVA (PLA2G4A) enzymes in cultured granulosa cells (GC) from bovine ovulatory follicles that had been treated with human chorionic gonadotropin (hCG, possessing LH activity). The objectives of this study were to determine the location of increased PLA2G4A synthesis and to follow the time course of changes in PLA2G4A messenger RNA (mRNA) following hCG stimulation. PLA2G4A mRNA levels were 14-fold higher in ovulatory follicles treated with hCG than in dominant follicles at an earlier stage of development. Examination of the walls of ovulatory follicles at 0, 6, 12, 18 and 24 hours after hCG stimulation revealed a 16-fold increase in PLA2G4A mRNA at 12 hours, followed by a 45-fold increase at 24 hours, compared to 0 hour levels. PLA2G4A protein levels reached an initial surge at 18 hours with a peak at 24 hours. PLA2G4A mRNA levels were higher in GC near the periphery of the follicle than in those lining its antral cavity.

18 Maternal housekeeping proteins translated during bovine oocyte maturation and early embryo development

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Proteomics (2006) Vol. 6 p. 3811-3820.

Virtually all of the cellular machinery necessary for development of the bovine embryo up to the 8- to 16-cell stage is transcribed from maternal DNA to messenger RNA (mRNA) during the latter (germinal vesicle) stage of the oocyte's development within the Graafian follicle. Although some transcription of the embryo's own DNA (genome) occurs as early as the 2-cell stage, major transcription activation of the embryonic genome begins at the transition from 8 to 16 cells—the maternal-embryonic transition (MET). Translation of the mature oocyte's complement of mRNA transcripts (its transcriptome) as well as activation of the embryo's genome is managed by proteins encoded by 'housekeeping genes' on the oocyte genome and expressed throughout oocyte and embryo maturation. The objectives of this study were to characterize patterns of expression of proteins from the oocyte transcriptome and to determine which of these were maternal housekeeping proteins (MHKP). The expression of most proteins varied as the embryo matured from 2-cell to 8-cell stage. Only 46 proteins, present throughout all stages of early embryo development, could be categorized as MHKP. Of these, only 2 had previous been identified as such.

19 Lower Pregnancy Losses in Lactating Dairy Cows Fed a Diet Enriched in α-Linolenic Acid

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Journal of Dairy Science (2006) Vol. 89 p. 3066-3074.

When added to diets fed to lactating cows, long chain omega-3 polyunsaturated fatty acids (ω3 PUFA) derived from fish oil have been shown to improve pregnancy rates. It has been suggested that this effect is due to a reduction in prostaglandin F2-alpha ($PGF_{2\alpha}$) synthesis. $PGF_{2\alpha}$ is the local mediator responsible for signalling pregnancy failure and the beginning of a new estrous cycle by initiating luteolysis (breakdown of the corpus luteum, CL). Lower utero-ovarian $PGF_{2\alpha}$ levels might result in delayed luteolysis, preventing the termination of what might have been a successful pregnancy. Alpha linolenic acid (ALA), itself an ω3 fatty acid, accounts for about 57% of the fatty acid content of flaxseed oil and it is a precursor to even longer chain ω3 PUFA. A preliminary study demonstrated an improvement in conception rates of cows fed a diet containing formaldehyde-treated flaxseed. The objective of the present study was to observe the effects of flaxseed feeding on ovarian function, conception rates, early embryo survival and pregnancy losses in lactating dairy cows. Rolled flaxseed was included in the diets of early lactation cows to provide a minimum daily intake of 410 grams of ALA. Control cows received a similar amount of oil in the form of rolled sunflower seeds containing negligible ALA. The average diameter of ovulatory follicles was larger (16.9 vs 14.1 mm), conception rates to first insemination (estimated using plasma progesterone concentrations) were higher (72.6 vs. 47.5%) and pregnancy losses were lower (9.8 vs 27.3%) in cows fed flaxseed.

20 Effect of eprinomectin pour-on treatment around calving on reproduction parameters in adult dairy cows with limited outdoor exposure

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Preventive Veterinary Medicine (2006) Vol. 75 p. 267-279.

Dairy cattle and other grazing ruminants are subject to parasitisation by several species of lung and stomach worms (nematodes). Previous studies have demonstrated improvements in production and reproduction when grazing dairy cows were treated with anthelmintics (dewormer drugs). Although the normal route of infection by these parasites is the consumption of forages on pasture, there is some evidence of high parasite loads in cattle not exposed to pasture. It has also been suggested that nematodes may be transmitted between cows in confinement. The objective of this study was to determine if treatment with the anthelmintic eprinomectin (Eprinex™) around calving time might improve reproductive performance of cows housed in either complete confinement or in a facility where they were allowed limited access to a small outdoor paddock. A total of 2,381 cows in 35 herds were enrolled in the trial—half were randomly chosen for anthelmintic treatment; the other half received a placebo. Anthelmintic treatment had no effect on the interval from calving to first breeding, calving to conception interval or number of services per conception in these cows which had very limited outdoor exposure.

21 Regulation of serine protease inhibitor-E2 and plasminogen activation expression and secretion by follicle stimulating hormone and growth factors in non-luteinizing bovine granulosa cells in vitro

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Matrix Biology (2006) Vol. 25 p. 342-354.

The development of ovarian follicles during the estrous cycle is influenced by follicle stimulating hormone (FSH) and a number of other growth factors, including insulin-like growth factor-1 (IGF-I), bone morphogenetic protein-7 (BMP-7), fibroblast growth factor 2 (FGF-2) and epidermal growth factor (EGF). The objective of this study was to provide evidence that the actions of these growth factors are regulated by the interaction of serine protease inhibitor-E2 (SERPINE2) with plasminogen activators (PA). SERPINE2 inhibits PA which, in turn, promotes the conversion of plasminogen to plasmin, an enzyme that plays an important role in the changes that occur in the outer layers of the follicle as it matures. When incubated with follicular granulosa cells in culture, all of the growth factors affected the activities of both SERPINE2 and PA. SERPINE2 and estradiol levels were positively correlated but incubation of cells with estradiol did not affect SERPINE2 expression. Since high estradiol levels are an indicator of follicle health and SERPINE2 is known to inhibit programmed cell death (apoptosis), this observation suggests that SERPINE2 may be involved in preventing the regression (atresia) of the primary follicle as it develops toward ovulation.

22 Somatic cell nuclear transfer alters peri-implantation trophoblast differentiation in bovine embryos

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Somatic cell nuclear transfer (SCNT) has been used to create clones (identical individuals) of several animal species. Unfortunately, the success rate of SCNT has been very low—only about 6% of the reconstructed bovine embryos are born alive; 80% of SCNT pregnancies are lost by day 90 of pregnancy. One of the causes of these high rates of loss appears to be poor placental development due to the failure of cells in the outer layer (trophoblast) of the developing embryo to properly differentiate into trophoblast giant cells (TGC). TGC, which normally represent about 20% of all trophoblast cells, are responsible for forming the junction between the embryo and the uterus. In this study, the activities of genes that code for factors known to play a role in the TGC differentiation were compared in trophoblast cells derived from intrauterine artificial insemination (AI), in vitro fertilization (IVF) and SCNT. The Mash2 gene codes for a protein that stimulates cell proliferation but inhibits TGC differentiation. The Hand1 gene encodes a promoter of TGC formation. Prior to implantation, Mash2 expression was higher in SCNT than in AI embryos; Hand1 expression was higher in AI and IVF compared with SCNT embryos. After implantation (40 days pregnant), SCNT fetal cotyledons (placenta elements) had higher expression levels of both genes but fewer TGC than did cotyledons of either AI or IVF embryos. Results demonstrate altered expression in bovine SCNT embryos of genes critical to normal placental development.

23 Conception rate and reproductive function of dairy cows fed different fat sources

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Theriogenology (2006) Vol. 65 p. 1316-1324.

During the bovine estrous cycle, if implantation of an embryo into the uterine endometrium has not occurred by about 16 days after ovulation, prostaglandin F2-alpha ($PGF_{2\alpha}$) synthesis by ovarian and uterine tissues rises, resulting in luteolysis (regression of the corpus luteum) and the initiation of a new cycle. The precursor of $PGF_{2\alpha}$ synthesis is arachidonic acid while other prostaglandins that are antagonistic to $PGF_{2\alpha}$ are derived from eicosapentaenoic acid (EPA) a long chain polyunsaturated omega-3 fatty acid predominantly found in fish oils. Earlier trials demonstrated that fish oil supplementation of diets fed to lactating cows had beneficial effects on reproduction and it was speculated that this might be due to a reduction in $PGF_{2\alpha}$ activity. Since alpha linolenic acid (ALA) is a precursor of EPA and ALA is found in high concentration in flaxseeds (about 57% of total fatty acids), it was hypothesized that feeding flaxseed might also improve reproduction. A preliminary trial demonstrated this effect. The present study was designed to further test this hypothesis. Three groups of lactating dairy cows were offered diets supplemented with either whole flaxseed (FLAX), Megalac™ (MEG—calcium salts of palm oil fatty acids) or micronized soybeans (SOY). Embryo mortality was lower in cows fed FLAX (0%) compared with those fed MEG (15.4%) or SOY (8.9%). Average corpus luteum diameter was larger in cows fed FLAX (19.1 mm) or MEG (18.3 mm) than in those fed SOY (16.3 mm). These results confirmed the beneficial reproductive effects of feeding FLAX.

24 Inhibition of prostaglandin F2α synthesis and oxytocin receptor by progesterone antagonists in bovine endometrial cells in vitro

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The luteal phase of the bovine estrous cycle begins at ovulation with the formation of the corpus luteum (CL) and ends about 16-18 days later with its regression. Throughout this phase, ovarian progesterone levels are high; estrogen levels are very low. As the CL develops, it produces increasing amounts of oxytocin. However, oxytocin has little effect during the early luteal phase because of the low levels of oxytocin receptors present in its target tissue, the uterine endometrium, due to progesterone suppression. Although progesterone levels remain high, after about 10-12 days uterine oxytocin receptor levels begin to rise and, in response to the binding of oxytocin to these receptors, prostaglandin F2-alpha (PGF2α) synthesis is stimulated which, in turn, stimulates the release of more oxytocin from the CL. Rapidly rising PGF2α levels resulting from this positive feedback loop eventually cause regression of the CL and the initiation of another cycle. The reason for the apparently spontaneous increase in oxytocin receptor levels prior to luteolysis, at a time when progesterone levels are high, is not well understood. The objective of this study was to elucidate the mechanism involved in that increase. Results demonstrated that a decrease in endometrial progesterone receptor activity may result in a reduction in progesterone suppression of oxytocin receptor activity.

25 Na^+/K^+ ATPase as a signaling molecule during bovine sperm capacitation

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After entering the female reproductive tract, spermatozoa undergo a short period of 'capacitation' in preparation for inseminating an ovum. The contributions to this process of the various enzymes found in sperm are not well understood. The objective of this study was to elucidate the role in capacitation of an enzyme commonly found in the plasma membranes of many body cells. Of the 4 forms of the enzyme Na^+/K^+ ATPase that have been identified, two (ATP1A1 and ATP1A4) have been found in spermatozoa and inhibition of ATP1A4 has been shown to eliminate sperm motility. In other tissues, Na^+/K^+ ATPase is involved in the maintenance of ionic gradients across cell membranes. In heart muscle, the enzyme is important for the restoration of calcium ion concentrations after contraction. It has recently been shown that Na^+/K^+ ATPase acts in cell signalling, independent of its action on ionic gradients. When cardiac muscle cells are treated with ouabain, a drug that blocks the activity of Na^+/K^+ ATPase, cellular responses are similar to those associated with sperm capacitation. In the present study, ouabain inhibited motility and induced sperm capacitation through the actions of Na^+/K^+ ATPase on depolarization of the cell membrane and cell signalling without provoking an appreciable increase in intracellular calcium.

26 Pregnancy, bovine somatotropin, and dietary n-3 fatty acids in lactating dairy cows

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At about 17-18 days after the cow ovulates, either another estrous cycle will be set into motion by luteolysis (regression of the corpus luteum, CL) or the CL will survive to support a latent pregnancy. Although the agent that ultimately provokes luteolysis is prostaglandin F2-alpha ($PGF_{2\alpha}$), a cascade of signals and enzyme activity changes is required to increase the $PGF_{2\alpha}$ concentration adequately. If pregnancy has been initiated, the conceptus will secrete a level of interferon-tau (IFN-τ) sufficient to block $PGF_{2\alpha}$ synthesis. IFN-τ achieves this by inhibiting the synthesis of both estrogen and oxytocin receptors (OTR) in the uterine endometrium. Lowered OTR activity reduces oxytocin induced $PGF_{2\alpha}$ synthesis by reducing the activities of key synthetic enzymes. The objective of this study was to characterize the effects of recombinant bovine somatotropin (rbST), pregnancy and dietary omega-3 polyunsaturated long chain fatty acids (ω3 PUFA) on the events controlling $PGF_{2\alpha}$ synthesis at the time when luteolysis would normally occur in non-pregnant cows. Both rbST and pregnancy changed expression patterns of the genes controlling the cascade of events influencing $PGF_{2\alpha}$ synthesis compared with patterns observed in cyclic cows. Supplementation of diets with ω3 PUFA also reduced factors regulating $PGF_{2\alpha}$synthesis in cyclic cows that would favour the establishment and maintenance of pregnancy.

27 Impact of a progesterone-releasing intravaginal device on plasma progesterone levels in lactating dairy cows

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The administration of progesterone (P4) to cows experiencing normal heat cycles suppresses estrous activity. When the P4 level falls, the cycle resumes and many studies have demonstrated higher fertility levels after P4 treatment. The progesterone-releasing intravaginal device (PRID) is particularly effective in this regard as its withdrawal results in a rapid drop in P4 activity. Although it has been assumed that the effectiveness of the PRID is mediated by changes in circulating P4, actual measurement of peripheral blood P4 concentrations have not been reported. In this study, PRIDs were administered to lactating cows on day 25 or 31 after first observed estrus and withdrawn 14 days later. Peripheral blood was collected daily from first estrus until 56 hours after PRID withdrawal. Blood of untreated control cows was sampled every second day from first observed estrus until a second estrus was observed. P4 response was assessed as the area under the curve (AUC) of plasma P4 concentration plotted against time. AUC was similar in control and treated cows before PRID insertion but was significantly greater for treated cows after insertion. In cows receiving a PRID 31 days post-estrus, maximum plasma P4 concentration was higher after than before insertion.

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