

2015/16 Results Report

Submitted to Canadian Beef Cattle Research, Market Development and Promotion Agency

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I. Executive Summary

The Beef Cattle Research Council (BCRC) administers the research allocation of the National check-off collected by the provinces. This report presents the results of research activities during the period April I, 2015 to March 31, 2016.

In addition to National check-off dollars, the majority of BCRC's research and extension programming in 2015/16 was funded through the Beef Cattle Industry Science Cluster under Growing Forward 2. This is the second Science Cluster and runs for the period April 1, 2013 to March 31, 2018. It is a \$20 million program, with \$5 million from industry including the National check-off, \$1 million from provincial government, and \$14 million from AAFC. The National check-off dollars are leveraged with federal government funding under the Beef Cattle Industry Science Cluster on a 1:3 industry:government ratio. BCRC also collaborated with other funding agencies to identify the industry's research priorities, maximize the value of research investment, and influence public sector investment in beef cattle research.

During 2015/16, 26 projects were funded under the Beef Science Cluster, each aligning with one of the following research priority areas:

- Forage and Grasslands Productivity
- Feed Efficiency
- Animal Health and Production Limiting Diseases
- Food Safety
- Beef Quality
- Environment
- Technology and Knowledge Dissemination.

Section III of this report includes a list of Cluster research projects funded by National check-off dollars and other industry investments. Many research findings reported are preliminary as most Cluster projects are on-going and will be completed by 2018. Although the research continues, several success stories are included under the various project reports in section III.

In addition to sponsoring research and knowledge and technology transfer programs in support of the Canadian beef industry, BCRC oversees the Verified Beef Production[™] Program(VBP). Throughout 15/16 VBP has moved forward with the development of additional modules covering animal care, biosecurity, and environmental stewardship. Pilot audits of the new modules were initiated in 2015/16 and planned to be released under VBP+ in 2016/17 when producers can become trained and registered under all modules.

The fiscal year for BCRC is July 1 to June 30, therefore BCRC audited financial statements are not included in this report and are available upon request after August 2016. National check-off funding allocated to research programming in 2015/16 is outlined in various sections of this report and is estimated at \$1.13 million.

II. Background

The Beef Cattle Research Council (BCRC) funds leading-edge research to advance the competitiveness and sustainability of the Canadian beef cattle industry. The BCRC administers the research allocation of the National check-off and currently receives on average \$0.15 of every \$1.00 of National check-off collected by the provinces. The BCRC leverages federal government funding under Growing Forward 2 with industry National check-off dollars on a 1:3 (industry:government) basis through Canada's Beef Cattle Industry Science Cluster. It also collaborates with other funding agencies to maximize the value of all investments in research within the Canadian beef cattle industry.

As the only national beef cattle industry research agency, the BCRC plays an important role in identifying the industry's research and development priorities and subsequently influencing public sector investment in beef cattle research. BCRC facilitates and encourages collaboration and coordination among researchers, other funding agencies and industry in order to maximize the benefits obtained from all investments in beef cattle research.

In addition to funding research, the BCRC plays a leading role in increasing industry uptake of relevant technologies through the delivery of its national Technology Transfer strategy. It is also responsible for the delivery of the Verified Beef Production (VBP) national on-farm food safety program as well as the incorporation of new VBP modules for animal care, biosecurity, and environment. The BCRC also leads the ongoing implementation of the National Beef Research Strategy, working in partnership with industry and government beef research funding agencies across Canada, to be more efficient with limited funding and ensure key research, capacity, and infrastructure priorities are addressed. The National Beef Strategy is available at http://www.beefresearch.ca/about/national-beef-research-strategy.cfm.

The majority of BCRC's current research and extension programming is funded through the Beef Cattle Industry Science Cluster under Growing Forward 2. This is the second Science Cluster and runs for the period April 1, 2013 to March 31, 2018. It is a \$20 million program, with \$5 million from industry including the national check-off, \$1 million from provincial government, and \$14 million from AAFC.

This report covers the period April I, 2015 to March 31, 2016. This period is the third year of the Growing Forward 2 Beef Science Cluster and research programming under the Cluster is centered around the following areas:

- I) Maintaining or improving competitiveness in the production of beef cattle
- 2) Supporting science-based policy, regulation and trade
- 3) Supporting science-based public education and advocacy
- 4) Supporting the Canadian Beef Advantage through continual advancements in beef quality and food safety, and
- 5) Accelerating the adoption of new innovations in the Canadian Beef Industry.



III. Key Highlights for the 2015/16 Activities

1. Beef Science Cluster II Projects Funded by Government, Industry and National check-off and Managed by BCRC

This section provides 2015/16 research results for the projects funded under the Beef Science Cluster. Included are the project number, title, budget, projected expenditures and preliminary results as several projects extend to 2018.

Forage and Grasslands Productivity:

I. FRG.04.13 - Innovative Swath Grazing/Increasing Forage Research Capacity

- <u>Key Highlights:</u> Two manuscripts from this project have been published in peer reviewed journals, contributing to an expanding base of knowledge regarding the cost-benefit of alternative extended grazing strategies in the Canadian prairies.
- <u>Success Story</u>: The data and agro-economic models being developed from the yield and quality data generated by this research will ultimately help beef producers determine costs of production and choose appropriate forage varieties for swath-grazing. Research was carried out in a grazing trial, which indicated that varieties could be chosen based on a decision-making spreadsheet designed to evaluate forage and swath grazing potential (See grazing experiment 2).

2. FRG.08.13 - Development of native plant material (grasses, legumes) and mixtures for forage production in the Prairie Region

<u>Key highlights:</u> All aspects of project are on track with new studies for 2015 to 2018 established with data collection to start in 2016. Selections for Northern wheatgrass, white prairie clove, purple prairie clover, slender milkvetch, hybrid brome, meadow brome, crested wheatgrass, blue bunch wheatgrass, and alfalfa have been made and polycross nurseries initiated. Linkage mapping and field data collection for 2 full-sib families with 10 crested wheatgrass accessions is well underway. Next generation sequencing work with crested wheatgrass has generated first set of genomic resources. Results from mixture studies are yielding results in drought adaptation and allelopathic potential. Forage quality samples were collected in 2015 and are being analyzed for nutritional quality. Potential legumes (Purple prairie clover, white prairie clover, Kura clover, slender milkvetch and Canadian milkvetch) are in place and data will be collected regarding grazability and forage quality. The project is training one Post-Doc (breeding), 2 PhD students and a MSc student.

3. FRG.09.13 - Nutritional Evaluation of Barley Forage Varieties for Silage and Swathgrazing

<u>Key highlights:</u> This cooperative project between Agriculture and Agri-Food Canada and the University of Saskatchewan was designed to provide beef producers with the information required to select which barley variety to grow for both quantity and quality of forage harvested as silage.

Three trials were conducted at the University of Saskatchewan:

<u>Trial 1:</u> a survey of barley silage varieties grown by beef and dairy producers across western Canada. Of 135 silage samples collected over two years (2012 and 2013), 80 samples harvested at the mid dough stage of maturity representing seven varieties (Xena, CDC Copeland, CDC Cowboy, Falcon, Legacy, AC Metcalfe and onlon) were selected for analysis.



<u>Trial 2:</u> this trial utilized the results of the first trial to identify three varieties for a feedlot growing and finishing study. Three barley varieties (CDC Cowboy, CDC Copeland and Xena) fed at two inclusion levels were utilized in a 2×3 factorial feedlot trial at the UofS Beef Cattle Research Unit.

<u>Trial 3:</u> involved two metabolism studies to evaluate the effect on dry matter intake, rumen fermentation, and total tract digestibility characteristics of growing/finishing heifers fed two barley silage varieties (CDC Cowboy and Xena) that vary in neutral detergent fiber (NDF) content and potential NDF digestibility when fed at two inclusion levels. Results indicated that barley varieties grown for silage by beef and dairy producers vary in nutrient content and digestibility of structural carbohydrates including the NDF fraction. This has potential impacts on dry matter intake, rumen function and performance of growing and finishing beef cattle. In the growing and finishing trial, little effect of silage variety was observed over the entire feeding period, however during the backgrounding phase cattle fed CDC Cowboy exhibited poorer performance. This likely resulted from its higher NDF content leading to lower dietary energy content or to the fact that differences in NDFD in the silages grown at the UofS in year two were not as great as found in the survey conducted in Year I. The higher NDF content of CDC Cowboy did however minimize issues with digestive distrubances in the backgrounding phase by minimizing pH reduction.

Research at AAFC Lethbridge focused on ensiling characteristics of barley silage varieties differing in NDF content and potential NDF digestibility, as well as on metabolic and growth studies involving lambs. CDC Cowboy as grown as the high NDFD (H-NDFD) variety, CDC Copeland as intermediate NDFD (I-NDFD), and Xena as the low NDFD (L-NDFD).

<u>Trial 4</u>: evaluated the ensiling characteristics of the three varieties grown at the same location and harvested at same stage of maturity and ensiled using both mini-silo and bunker-silo ensiling techniques. No differences in NDFD were identified among the three varieties, suggesting that barley variety may have less of an impact on NDFD than growing and ensiling conditions. However, differences observed among varieties were noted in terms of VFA concentrations such as higher butyrate and acetate that could impact aerobic stability of the silage. A continuation of research in this field would need to take into account the differences in growing seasons. However, first and foremost, producers must ensure they have the best ensiling practices possible; attempting to improve silage digestibility is wasted if the technique is not conducive to quality fermentation.

<u>Trial 5:</u> assessed the selected silage varieties for their effect on total-tract digestibility, animal intake, and rumen environment using nine cannulated wethers.

<u>Trial 6:</u> evaluated the effect of barley silage variety on intake, growth performance and lamb carcass characteristics. There was no difference in digestibility of the barley silage varieties selected based on *in vitro* NDFD of field silage samples, though the Xena silage fed during the feeding trial did have numerically lower NDFD than the other two varieties. The performance differences observed in the feeding trial with Xena fed lambs out-performing the other lambs could be a result of higher DM of the silage resulting in a greater proportion of the as fed diet consisting of concentrates. The trial did not support that selection for *in vitro* NDFD of field samples of barley silage would improve total tract digestibility or lamb performance as the silage varieties with improved NDFD based on analysis of silage could prove difficult owing to the myriad of factors that can influence the nutrient composition and digestibility of silage. Lastly, as a result of this project, two graduate students were trained and are in the last phases of their program with Natallie Preston completing a Master of Science degree and Jayakrishnan Nair is completing a Ph.D.

<u>Success story</u>: The results of this work highlight several important findings for ruminant producers in regard to selection of barley varieties for silage. First, our survey results show that nutritional characteristics



such as neutral detergent fiber (NDF) content and digestibility varies between varieties and thus consideration to nutritional as well as agronomic characteristics is important when selecting varieties for silage production. This finding is supported by the results of the feedlot performance trial where cattle fed CDC Cowboy (a variety with high NDF content) had poorer performance in the backgrounding period that those fed Xena or CDC Copeland. Silage variety was not as critical when finishing steers or lambs due to the reduced silage content of finishing diets. Nor was there any marked effect of variety on total tract nutrient digestibility of either lambs or heifers. Finally, there were minimal differences between varieties in silage quality other than poorer aerobic stability for CDC Cowboy.

From this research we can conclude that NDFD is difficult to maintain from one year to the next, particularly when environment, location and soil type vary. While agronomic factors and ensiling technique remain critical factors affecting silage quality, our research shows that there are differences in nutritional parameters such as NDF content that can affect animal performance and thus future research should evaluate desirable chemical and nutritive characteristics of barley forage varieties for silage, particularly from the standpoint of providing plant breeders with information upon which they can make breeding decisions.

4. FRG.13.13 - Pasture mixtures and forage legumes for the long-term sustainability of beef production

<u>Key highlights:</u> Forages are a major feed component for the cow-calf and backgrounding sectors. There is considerable room for improving beef production from pastures and stored forages. Appropriately managed pasture with a significant legume component is one of the most sustainable feed sources for cattle. Forage species have different yield potential and nutritional quality, which can influence the productivity of beef cattle in pasture. Little research has been done to determine which forage species combinations have the greatest potential to improve beef production from forages. The objective of this research is to identify forage species mixtures that provide the best opportunity to enhance beef productivity on pasture. This research will evaluate how the forage species in the seed mixtures established under the first Beef Cluster (FRG.07.10) stabilize in the pasture over several years of grazing pressure.

Additional research was conducted to investigate stand establishment and productivity of complex mixtures under differing levels of nitrogen fertilization in sites in Nova Scotia, Quebec, and Ontario. Results so far indicate some grass species and legume combinations have high yield and high quality, although this analysis has not yet been completed for all five years. Trefoil-based mixtures performed better under the simulated grazing (frequent cutting with a mechanical harvester) than under animal grazing, while alfalfa-based mixtures performed better under animal grazing than under simulated grazing. The relative yield of each grass species differs depending on the legume it was seeded with and the harvest method. Site and management systems (grazed versus mechanical harvesting) appear to influence which mixtures give better yield, and also content of specific legumes. Seeded legume plant counts and seasonal dry matter yield are influenced by mixture type, both binary and complex. Low legume contribution to DM yield was noted in all mixtures by the fifth production year. Over time we do not see sufficient legume remaining in the stands. In the 5th production year, the legume density under grazing (Nappan site) declined in the binary mixtures (2015, average 7 legume plants m⁻²) when we compared with the 4th production year (2014, average 20 legume plants m⁻²). The same trends were observed for complex mixtures. Significant variation was present in binary and complex mixtures regarding non-seeded forage yield, suggesting some mixtures may be more resistant to allowing nonseeded species to invade. Grass cultivar choice has an influence on DM yield in binary mixtures and also affects legume content. Results indicate that complex mixtures seeded with trefoil tend to maintain the seeded grass levels for a longer time than the alfalfa mixtures, and yielded a greater animal gain per acre over the five years of research. Certain mixtures showed a greater portion of their forage yield in mid- or late-summer, in particular a mixture of reed canarygrass, tall fescue, Kentucky bluegrass, and meadow brome, which may be useful for maintaining pasture good regrowth and productivity throughout the growing season.

Initial results from the fertilization trials indicate very limited effects of N fertilization, grass mixture, and legume species. The data analysis with combined sites only shows a significant effect of N fertilization and the grass mixture on seasonal DM yield in the second year. This suggests that N did not limit growth at any of the sites and no N fertilizer was required to reach maximum yield.

5. FRG.14.13 - Building long-term capacity for resilient cow-calf production systems through creation of a forage industry chair supporting training and research in evaluation and utilization

<u>Key highlights:</u> In Canada, the acreage dedicated to forage and pasture production has diminished with increased competition from annual crops for human and animal consumption. Cattle producers have thus faced increased reliance on marginal lands to meet their forage needs. In order to remain sustainable and competitive, they continue to seek new avenues for extending the length of the grazing season. Given the reproductive challenges faced by beef cattle, illustrated by the large number of open cows reported in Manitoba and Saskatchewan in recent years, it is clear that research is required to identify superior strategies for extended grazing to improve animal performance under the range of soil and weather conditions observed in the prairie provinces.

Following consultation with the joint Manitoba-Saskatchewan industry-led steering committee, a variety of perennial and annual forages were selected based on potential suitability for extended grazing, accessibility and cost.

The project established experimental small plot trials at five locations across Manitoba (Parkland Crop Diversification Foundation, Roblin; Prairies East Sustainable Agriculture Initiative, Arborg, and Ian N. Morrison Research Farm, Carman) and Saskatchewan (Western Beef Development Centre, Lanigan and University of Saskatchewan, Saskatoon) to assess a variety of perennial and annual forages under a range of soil and climatic conditions.

Annual crops chosen were oats (Haymaker), barley (Maverick), corn (Fusion), soybeans (Mammoth), foxtail millet (Golden German), fall rye (Hazlet) and annual rye (Westerwold).

Perennial forages were seeded in pure and mixed stands with five grasses: tall fescue (Courtney), orchardgrass (Killarney), meadowbrome (Fleet, Success, Armada) and three legumes: alfalfa (Algonquin and Yellowhead) and cicer milkvetch (Oxley II) providing 23 perennial treatments for comparison. Perennial stands were also managed for early or late forage stockpiling.

Preliminary conclusions:

<u>Manitoba data</u>: Stockpiled annual forages demonstrated good potential for extending the grazing season for beef cows in Manitoba. Corn offers the highest potential based on yield, TDN and RFV; although protein supplementation may be required.

<u>Saskatchewan data</u>: Stockpiling perennial grass and legume species can produce greater than 3.0 Mg/ha of forage from July to mid-October. Meadow bromegrass produced the highest stockpiled and seasonal yields in both pure stands and mixtures with legumes.

Feed Efficiency

6. FDE.04.13 - Germplasm and variety development of barley and triticale for animal feed with a focus on feed quality, yield and disease resistance of both grain and annual forage production

<u>Key highlights:</u> The aim of this project is to create barley and triticale varieties that exceed the productivity and quality attributes of currently available varieties. With constant research and breeding, utilizing the latest tools available, the programs of this project are able to increase yield on a constant basis and obtain varieties that have better quality and disease resistance. That is possible due to the work with collaborative programs in which Canadian international breeders (International Maize and Wheat Improvement Center; CIMMYT and the International Center for Agricultural Research in the Dry Areas; ICARDA) join forces to develop superior cultivars. In the last two years, the programs have exchanged germplasm and grown advanced yield trials, screened for commercially relevant diseases, carried out feed and forage quality tests and released varieties that exceed the thresholds required for registration by the Prairie Grain Development Committee (PGDC). In addition to the released varieties, a collection of elite germplasm has been created that contains traits necessary for new commercial cultivars, allowing continuous genetic progress in the short, medium and long term.

Plant breeding is a long-term endeavor which reaches far beyond the timeline of the current project. The activities of this project allow us to better identify traits within previously-developed varieties, and will also help create new superior varieties that will be commercially released beyond the duration of this project. The collaborators in this proposal have all played a role in the development of more than 90% of the barley and triticale varieties grown in western Canada over the last 40 years.

- <u>Success Story</u>: The breeding programs at FCDC have in the last three years been through a deep review of results, methodologies, objectives, and more. A comprehensive Cereals Industry Review by a consultancy firm helped gather the information needed to make further decisions about the program, and a strategic planning process is nearing completion. Meanwhile, several changes have already been implemented to the breeding programs:
 - During the duration of this project two two-row hulled malting or dual purpose varieties, have been released (TR13609) and received interim registration (TR13606) in 2016, and a six-row hulled (BT598) and a six-row hulless (HB542) with superior yield and quality were approved for registration in 2015. This follows the approval for registration of two other varieties in 2014, BT596 and HB623, and three varieties in 2013, TR11698, 'Amisk' and 'Canmore'. For triticale, progress has been achieved in yield and other characteristics, and new varieties are two years away from release.
 - 2. The size of the breeding programs have been increased by making more crosses and having more lines in yield testing. Approximately 200 crosses have been completed in 2015.
 - 3. The breeding process has been accelerated. Growth rooms, growth chambers and the winter nursery at El Centro, California were at maximum capacity in order to optimize their use and accelerate the inbreeding process to its maximum. That included single seed descent, winter nursery in California, crosses in the growth rooms in the fall and winter, crossing in the growth chambers, etc.
 - 4. Decreased the years of variety yield testing prior to entering the Co-op network from 5 years to 3-4 years – lines are advanced to the co-op stage after Yield 4 trials instead of Yield 5; if enough consistent favorable data is available they will be advanced from Yield 3.
 - 5. Targeted introductions of germplasm sources continued from international centers (International Maize and Wheat Improvement Center, CIMMYT and the International Center for Agricultural Research in the Dry Areas, ICARDA) and other successful programs around the world.
 - 6. Continued the research supporting the breeding programs (Nitrogen Use Efficiency; NUE) and selected lines with favorable traits were included in the variety testing network and crossing block. Two advanced lines were advanced to the 2016 co-op trials, a variety showing higher Nitrogen Use Efficiency (NUE) could be released in 2018.



- 7. Testing of all advanced germplasm for multiple disease resistance in Brandon, Ottawa, Charlottetown, Morden, Mexico, Uruguay, Ecuador, Hermiston (Washington State), Olds, Lacombe, Edmonton.
- 8. Testing germplasm for quality traits in wet labs as well as using near-infrared spectroscopy (NIRS).

7. FDE.07.13 - The impact of genomic selection for feed efficiency on the cow-calf sector, performance parameters and underlying biology

<u>Key highlights:</u> Advances were made in the area of assessing and monitoring biological responses in cattle with divergent feed efficiency. Improving feed efficiency is crucial to the sustainability of the beef industry. However, the direct assessment of feed efficiency in the bovine is not practical for commercial operations due to the duration of the assessment and equipment and labor costs. Moreover, single trait selection for feed efficiency may lead to deleterious correlated genetic selection responses. Efforts were made to identify novel parameters that could be used to indirectly identify feed efficiency, and to monitor responses to improved feed efficiency in beef heifers and bulls. More specifically, four streams of research were advanced, including: blood hematology and metabolomics in replacement beef heifers, and heart function, rumen physiology and fertility related traits in young beef bulls. The hematological study in heifers also contributed to the understanding of estrus biology and detection.

Experiment I evaluated blood cell parameters, immunoglobulins and metabolites in relation to feed efficiency in heifers; Experiment 2 assessed metabolites to serve as proxies for estrus. Efficient heifers had greater lymphocytes, immunoglobulin M response, and lower alkaline phosphatase (ALP) concentrations. Efficient pregnant heifers had lower concentrations of cholesterol and globulin. Estrus was strongly associated with fluctuations of ALP, aspartate aminotransferase, beta-hydroxybutyric acid, creatine kinase and triiodothyronine concentrations. However, age, body size and composition may influence such associations. There is potential for hematological parameters to serve as proxies of metabolic shifts related to feed efficiency and estrus state.

Proxies have the potential to accelerate feed efficiency improvement, assisting with reduction of beef cattle feed costs and environmental impact. Heart rate (HR; BPM) is associated with feed efficiency and influenced by autonomic activity and peripheral metabolism, suggesting that HR could be used as a proxy for feed efficiency. Feed efficient heifer calves have a lower overnight heart rate and an increased heart rate upon acute stress. Contrasting acute stress results in yearling heifers suggest that coping styles vary across categories of cattle. Overall, results indicate that overnight and acute stress heart rates are potential proxies for feed efficiency in heifer calves. Overnight HR and acute stress HR are potential indicators of RFI in heifer calves. However, acute stress HR results varied in yearling heifers, suggesting previous handling experience and maturity alter acute stress response. Pending further development (predictive ability), the acute stress assessment could have potential for on-farm application as a feed efficiency proxy in young heifers.

Investigation of rumen physiological characteristics and their association with feed efficiency can provide biological markers for selecting more feed efficient cattle. The rumen microbial community produces nutrients metabolized by the rumen epithelium which maintains metabolic efficiency. More feed efficient cattle spend more time in a desirable pH range, have greater papillae thickness, higher bacterial concentration, and lower methanogen concentration compared to less feed efficient cattle. Feed efficient animals therefore have improved feed energy utilization and reduced methane emission. Using biological markers to develop selection programs can reduce production costs and improve environmental conservation of beef production.

Feed efficiency and bull fertility are two major factors affecting profitability of the beef industry. However, there is concern of an antagonistic relationship between these two factors, highlighting the need to clarify the relationship between age, feed efficiency and sexual development. Two



experiments were undertaken to evaluate associations of blood parameters and fertility-related measures with age and feed efficiency in yearling bulls. Younger and efficient bulls exhibited lower testosterone and triiodothyronine levels, respectively. Younger bulls had smaller scrotal circumference, higher scrotal radiant heat loss and fewer normal sperm. Efficient bulls had lower scrotal circumference, scrotal radiant heat loss, and a trend towards lower testicular echogenicity and higher sperm head defects. Metabolic differences associated with variation in feed efficiency may impact reproductive function as illustrated by features of delayed sexual development in efficient bulls.

<u>Success Story</u>: The Maritime Beef Test Station in Nappan, NS is normally not used during the summer. The research team enrolled 20 producers from the Maritimes who brought 144 heifers to the facility to be tested for feed efficiency and reproductive development. Besides helping with the heifers, producers also were responsible for transporting the heifers and assisting with a yardage fee. Heifers were tested for feed efficiency and performance for a total of 140 days. During this period the heifers served to promote training and education for six students (1 PhD, 3 MSc, 1 vet and 1 international undergraduate). Heifers also were part of an open-house and used to demonstrate handling practices for 4H kids. The samples and data collected from the heifers also are serving as the basis for 2 MSc thesis. The comprehensive assessment made on these heifers were compiled in a user-friendly report that was shared with beef producers, assisting them to make decisions in the breeding herd.

8. FDE.09.13 - Increased Use of High Energy Forages in Conventional Feedlot Beef Production

<u>Key highlights:</u> Ruminants can digest roughages, but the limitation of higher fiber feeds is their lower energy content which lowers feed conversion efficiency in growing cattle. It may be possible for starch-containing forages such as corn and cereal silages to play a greater role in beef cattle production, if the energy content of such forages can be enhanced. High digestibility in these forages is dependent on maximum kernel development and low plant lignification at harvest, both affected by maturity and hybrid selection. Increasing numbers of short season corn hybrids are available that fit within Prairie maturity zones, but kernel maturity before frost occurrence is an issue. Thus, digestible energy content of corn silage is highly variable. If corn silage is to be used in beef cattle diets at higher inclusion rates to supply both energy and effective fiber, information is required to achieve high energy content of corn silage. The relationships among maturity, starch content, fiber content, and digestible energy for corn silage hybrids grown in Canada is required to optimize their use. Furthermore, there is a need to know the optimum inclusion rate of corn silage in beef cattle diets.

9. FDE.15.13 - Prebiotic, probiotic, and synbiotic technologies for targeted applications in food safety and ruminant productivity

Key highlights: Some of the key highlights from 2015/16 include:

I. Establishing a reproducible methodology for characterizing the carbohydrate metabolism of the rumen ecosystem;

2. Forming of a collaboration with a group at the Max Planck Institute for Marine Microbiology in Bremen, Germany, which will provide access to validated fluorescent techniques and expertise in the generation of new probes (MOS and POS), and will enable the high resolution visualization of rumen microorganisms in complex with fluorescent carbohydrates;

3. Demonstrating that fluorescently labeled carbohydrates (e.g. MOS) can be modified by bacterial enzymes (two publications in preparation);

4. Isolation of the first mannan degrading microorganisms from a ruminant (including several new genera);



- 5. Hiring and training of Adam Smith MSc as a new technician dedicated to this project;
- 6. MSc. Defense of Anu Anele (April 2016).
- <u>Success Story</u>: An international collaboration with a research group with complementary research goals and techniques has been established at the Max Planck Institute for Marine Microbiology in Bremen, Germany. This group includes Drs. Rudi Amann (Director of the MPI), Carol Arnosti (University of North Carolina), and Jan-Hendrik Hehemann (Assistant Professor MPI). This group of researchers has been studying the interactions of oligosaccharides and marine microorganisms, and has repeatedly demonstrated high-resolution binding of their probes using fluorescence microscopy. The basis of visualizing the interactions lies in fluorescently labelling oligosaccharides, and observing the oligosaccharide using a fluorescent microscope. This method shares many similarities with the techniques planned for the probiotic, prebiotic, and synbiotic technologies project. However, the team at MPI-Bremen uses a fluorophore which has an emission spectrum that is more suitable for our available resources. The team at MPI-Bremen will provide fluorescently-labelled oligosaccharides for use in binding studies, visualizing the interactions (via super-resolution microscopy) between oligosaccharides and bacterial cells prepared at AAFC-Lethbridge, and assistance in development of the fluorescent labelling protocol successfully used at MPI-Bremen.

10. FDE.17.13 - Improvement of cow feed efficiency and the production of consistent quality beef using molecular breeding values for RFI and carcass traits

- <u>Key highlights:</u> The Kinsella Project is on-target with the timeline included in the original proposal. The third year of the project has continued successfully with the groups of animals: Kinsella Composite (KC) Efficient and Control, Angus (AN) and Charolais (CH) being managed and sorted according to plan. The following have been achieved:
 - Measured RFI (GrowSafe) of potential breeding heifers for 2015
 - Measured RFI (GrowSafe) of bulls for 2015 breeding season
 - Measured RFI (GrowSafe) of all steers for 2015 feed efficiency and carcass data collection
 - Creation and refinement of selection indexes and select 2015 breeding stock
 - Finalized MBV-steer sort groups
 - Calving 2015 Genotyping C-calves and prediction of MBVs
 - Artificial insemination of AN/CH females, set up breeding groups for KC. Measured rib and rump fat depths and weights.
 - Weaning in 2015, measured rib and rump fat depths and weights.
 - Tested if carcass outcome groups produced the desired result
 - Preparations for Kinsella Day initiated including "save the date" flyers- July 2016

755 animals were tested for feed efficiency last year. Dr. Li is leading a team responsible for RFI calculation and compilation of all the phenotype and genotype data. He is also responsible for predicting genomic breeding values for selecting efficient bulls and efficient replacement heifers using multiple trait selection indexes, and for sorting steers into quality groups based on predicted DNA marker breeding values. So far we have collected feed efficiency data on 1421 Angus, 1191 Charolais, and 1673 Kinsella crossbred animals at the ranch.

<u>Success Story</u>: Selection in the KC herd has resulted in the expected differences in feed efficiency. For heifers, average RFI is 0.0406 (n=73) for the control vs -0.0377 (n=67) for the efficient group and for steers average RFI is 0.1378 (n=82) for the control group vs -0.14237 (n=75) for the efficient group. Phenotypically the efficient steers grew faster, ate less, had similar birth weights, but had smaller weaning and metabolic mid-weights.

A preliminary analysis of RFI data collected from calves produced from high vs low RFI assortative

matings within the Kinsella purebred Angus population showed that the calves' genetic potential for RFI, based upon the high vs low RFI mating, displayed the expected trend for the calves' actual RFI as measured during their own GrowSafe test (p = 0.07, LSMeans: High RFI = +0.217, Low RFI = -0.026).

An initial economic evaluation of the Kinsella "efficient" and "control" lines was performed by simulation based on within-line estimates of means for the traits using records that were collected in 2013 and 2014. The results support the expected difference, although the size of the difference after such a short period is (as would be expected) small. This difference arises primarily from lesser expenses debited from the efficient line. The reduction in expenses results from the efficient line cows being lighter in weight and the steer progeny spending fewer days in the feedlot than their counterparts in the control line.

11. FDE. 19.13 - Understanding the physiology behind changes in feed efficiency throughout the finishing period

<u>Key highlights:</u> Historically, cattle feeding management practices have not considered how the type of energy substrate influences cattle productivity at different stages of production. This research has demonstrated that the type of energy source provided and the timing for when the specific energy sources are provided influences (positively or negatively, depending on the timing and duration) feed efficiency and carcass characteristics for finishing steers. This research is providing initial proof of concept for new practices that can be used to improve carcass yield and minimize the negative impact of using low-cost feed products, such as HLP, on feed efficiency.

Animal Health and Production Limiting Diseases

12. ANH.01.13 - Identifying Mycobacterium avium subsp. parathberculosis (MAP) exproteome components recognized early during infection to develop diagnostic and vaccine targets

<u>Key highlights:</u> Novel secreted antigens of *Mycobacterium paratuberculosis* as serodiagnostic biomarkers for Johne's disease in cattle. **Clin Vaccine Immunol**. 2013. Antonio Facciuolo, DF Kelton, LM Mutharia.

Johne's disease is a chronic, gastroenteritis of cattle caused by Mycobacterium avium subspecies paratuberculosis (MAP), and afflicts 40% of dairy herds worldwide. MAP-infected cattle can remain asymptomatic for years while transmitting the pathogen via fecal and milk contamination. Current serodiagnosis by Enzyme Linked ImmunoSorbent Assay (ELISA) fails to detect asymptomatic MAPinfected cattle due to the use of poorly-defined antigens, and knowledge gaps in our understanding of MAP components eliciting pathogen-specific immune responses. These studies set out to define a subset of proteins that contain potential antigenic targets and screen these antigen pools for immunogens relevant in detecting infection. To accomplish our first objective, MAP-secreted proteins were captured and resolved using a 2-step fractionation method and reversed-phase liquid chromatography to identify 162 unique proteins, of which 66 had not been previously described as secreted in MAP CF. Subsequent screening of MAP secreted proteins showed four antigens, of which one or more reacted on immunoblotting with individual sera from 35 MAP-infected cows. Moreover, these antigens reacted with sera from 6 low-MAP shedders, and 3 fecal-culture positive cows labeled as ELISA seronegative. The specificity of these antigens was demonstrated using negative control sera from uninfected calves (n = 5) and uninfected cows (n = 5), which did not react to any of these antigens by immunoblotting. As three of the four proteins have not been previously reported as antigens, their characterization and incorporation into an ELISA-based format will aid in detecting asymptomatic cattle in early or subclinical stages of disease.

<u>Success Story:</u> The proteins we have identified both contribute to the growing list of potential diagnostic reagents for Johne's disease, and provide a basis for future investigation into the use of



these components as better tools for the control of this significant veterinary pathogen. A patent has been applied for "Biomarkers for *Mycobacterium avium paratuberculosis* (MAP)" U.S. Provisional Application No. 61/881,756

13. ANH.12.13 - Geographic variation in abundance and genetics of Dermacentor andersoni, a vector of bovine anaplasmosis

<u>Key highlights:</u> The Rocky Mountain wood tick (*Dermacentor andersoni*) and the American dog tick (*Dermacentor variabilis*) affect the health of livestock through the transmission of pathogens, such as *Anaplasma marginale*, which causes bovine anaplasmosis. The wood tick also causes paralysis in livestock within south-central British Columbia, resulting in a significant loss of productivity. In Canada, the wood tick occurs east of the coastal Rocky Mountains to western Saskatchewan, while the dog tick occurs in southern Saskatchewan, Manitoba and northwestern Ontario. The geographic range of the dog tick has expanded at its northwestern distributional limits in Saskatchewan over the last few decades. Despite the importance of both species of tick to animal health, we do not know how the abundance of tick varies, or how tick populations differ genetically throughout their distributions. These are key factors that determine the risk of tick exposure and the potential for pathogen transmission. The purpose of this study is to acquire information on the current distribution, abundance, and genetic diversity of *D. andersoni* and *D. variabilis* in western Canada.

During the second year of this project, we sampled for host-seeking ticks at 128 sites throughout British Columbia, Alberta, Saskatchewan and Manitoba from April 20 to July 23, 2015. *D. andersoni* was collected from 21 sites (BC, AB & SK), *D. variabilis* was collected from 66 sites (SK & MB), and both species were found together at 6 sites (SK). In general, the density of *D. variabilis* (1 to 1331 ticks / 2 km²) was greater than *D. andersoni* (1 to 91 ticks / 2 km²). Thus, the greatest density of dog ticks was 14.6 times greater than the greatest density of wood ticks. The distributional overlap of these two species is primarily a result of the westward migration of the dog tick over the last few decades. The distribution of *D. variabilis* has also expanded to the north in Saskatchewan and Manitoba, where it was collected at least 200 km north of the historical geographic limits. The geographic range of *D. andersoni* in Alberta appears to be consistent with reports from the 1940s, but has likely expanded at the northeastern limits by more than 100 km in Saskatchewan and results this year suggest the wood tick is now found further in southeastern SK than previously known. A comparison of the sampling results from 2014 and 2015 shows that the density of ticks can vary greatly among different locations and between years at the same location. We will get a better idea of the variation in tick abundance by sampling for one more year (spring/summer 2016).

The second major objective of this project was to estimate the genetic diversity within and among tick populations of the wood tick and dog tick throughout western Canada. This can be a useful indicator of variation in biological and behavioural characteristics that affect the ability of ticks to transmit pathogens. We continued to compare the DNA sequences of the 16S ribosomal RNA gene for an additional 238 D. andersoni. There were 15 different genetic types from populations from BC, AB and SK. All of these types were identified in the first year of this study. Approximately 95% of the ticks comprised only 5 of the 15 haplotypes. The same gene was sequenced for 252 D. variabilis from populations in SK and MB. 13 different genetic types were identified, all of which have been described in western Canada by previous studies. In addition, approximately 100 D. variabilis 16S rDNA sequences were determined to be identical by an electrophoresis technique that can detect single nucleotide changes. Approximately 90% of the D. variabilis comprised a single 16S genetic type. Therefore, we examined a second genetic marker (Cytochrome Oxidase I; COI) to estimate the genetic variation among tick populations of both tick species. There was much greater sequence variability for this gene, with 25 genetic types from 123 D. andersoni (16 populations) and 86 genetic types from 1409 D. variabilis (42 populations). In depth analysis will soon be completed to examine the extent of genetic variation



within tick populations and the patterns of genetic similarity among tick populations using the 16S rRNA and COI genes. This may provide clues to the patterns of tick dispersal and range expansion, and indicate potential biological differences among ticks from different regions.

<u>Success Story</u>: Shaun Dergousoff, a PhD student and post-doctoral fellow trained under the first and second Beef Science Clusters and hired as an AAFC Research Scientist is participating in the BCRC's Beef Researcher Mentorship program for 2015-16.

This is the most comprehensive study conducted to directly determine the abundance of *Dermacentor* andersoni and *Dermacentor variabilis* in western Canada, a region that includes a large portion of the geographic range of these ticks and beef cattle production in Canada. This type of study is necessary to determine the presence of established tick populations and to estimate and compare the risk of exposure to potential animal disease vectors. The results will also allow us to associate the presence and relative abundance of ticks with specific habitat variables to help predict the distribution and the relative density of ticks throughout western Canada.

The results from the second year of sampling confirmed that *D. variabilis* has undergone a northward range expansion in Saskatchewan and Manitoba since the 1960s. Likewise, the distributional limit of *D. variabilis* has expanded westward, as indicated by the collection of both tick species at sites in southwestern Saskatchewan. We also detected *D. andersoni* further east in southern SK, indicating possible range expansion that wasn't detected in previous years. The geographic range of *D. variabilis* now overlaps the range of *D. andersoni*, which has the potential to affect the survival of one or both tick species through competitive interactions and may allow for the transmission of tick-borne pathogens from one species of tick to the other (i.e. host-switching) when they feed on the same host.

A comparison of the sampling data from 2014 and 2015 shows that there is significant variability in tick density from year to year. This has a few important implications for the actual and estimated risk associated with these ticks. First, the chance of encountering ticks and for tickborne pathogen transmission varies from year to year at a particular location. Secondly, there is less chance of detecting ticks where they occur at very low densities and repeated sampling within and between years may be needed to determine if ticks are present at a location.

14. ANH.13.13 - Development of a fully-automated DNA microarray chip for multiplex detection of bovine pathogens

<u>Key highlights:</u> Bovine respiratory and enteric diseases are often associated with multiple pathogens. However, current diagnostic tests for these diseases are primarily single pathogen tests and are thus inefficient. Addressing these limitations, the main objective of this project was to develop two fully-automated DNA microarray chips for multiplex detection of BRD and BED pathogens.

In the present project, four multiplex PCR assays, namely 1) respiratory virus, 2) respiratory bacteria, 3) enteric virus, and 4) enteric bacteria/parasite were designed in order to detect target genes of pathogens that are most commonly associated with BRD and BED. The specificity of each assay was confirmed *in silico* using publically available sequence databases and by PCR using the targeted pathogens, relevant non-target pathogens and clinical samples from healthy and sick animals. The BRD viral and bacterial multiplex PCR assays were capable of detecting five BRD associated viruses and four bacterial pathogens, respectively. Similarly, BED viral multiplex PCR assays successfully identified four enteric viral pathogens and the BED bacterial/protozoal multiplex assay specifically detected five bacterial and three protozoan pathogens. In order to differentiate multiplex PCR products using a low density microarray, a panel of capture probes specific for each BRD and BED pathogen was designed. The specificity of those probes was successfully demonstrated on the NanoChip400 electronic microarray platform using target specific multiplex PCR products from laboratory pathogen strains and clinical samples. Sensitivity limits of these probes were demonstrated using titrated target pathogen strains, if available, or



using *in vitro* synthesized nucleic acids. Based on the electronic microarray data, a panel of 61 probes were selected to develop two fully-automated DNA microarray chips, one for BRD pathogens and the other for BED pathogens. The chips were successfully validated using a panel of clinical samples on the Rheonix Encompass MDx^{TM} platform where sample extraction, multiplex PCR, and microarray detection are fully-automated. Once fully-validated, these DNA microarray chips may provide us with a rapid, efficient, and cost effective method for detecting multiple pathogens associated with respiratory and enteric diseases in cattle.

<u>Success Story:</u> We have successfully completed development and initial validation of two microarray chips (i.e. BRD chip and BED chip) to be used on a user-friendly fully-automated microarray platform that enable us to simultaneously detect multiple respiratory and enteric pathogens of beef cattle. The BRD chip is capable of multiplex detection of 4 bacteria and 5 viruses that are mainly associated with bovine respiratory diseases, and the BED chip can simultaneously detect 4 bacteria, 5 viruses and 3 parasites that are responsible for neonatal calf *diarrhea*. Specificities of the detection probes used in each chip were successfully validated using a panel of laboratory amplified targeted pathogens and a limited number of clinical samples from healthy (clinical negatives) and infected animals. The sensitivity of the assay was determined at the level of PCR amplification and detection. For initial validation, diagnostic samples were obtained from provincial veterinary diagnostic laboratories in Canada, where they had tested positive for at least one of the pathogens of interest.

These two microarray chips, which are cost-effective and capable of rapid and early detection of multiple etiological agents, would empower routine diagnostic capabilities at the veterinary diagnostic laboratories in Canada to detect etiological agents associated with these two high-impact infectious disease complexes in beef cattle. This tool also allows efficient use of samples and significantly reduces labor, potential cross-contamination during sample handling and time required for disease diagnosis. Early identification of pathogens in samples will allow rapid targeted application of treatment and control strategies essential for successful control of these highly prevalent and costly diseases.

15. ANH.21.13 - Effect of age and handling on pain assessment and mitigation of common painful routine management procedures

Key highlights: As of 2018, a requirement of the Canadian Codes of Practice for the management of beef cattle is that Canadian beef calves older than 6 months of age must be castrated using pain control. However, at this time, an optimal pain mitigation technique for castration has not been determined. The aim of this study was to assess the effects of a single dose of subcutaneous meloxicam (Metacam®) on pain mitigation during and after castration in 1 wk old beef calves. Seventy-two 1 wk old Angus bull calves were randomly assigned to one of 6 treatments (n = 12); sham (C), band (B), or surgical castration (S) without meloxicam; and sham (CM), band (BM) or surgical castration (SM) receiving a single s.c. injection of meloxicam (0.5 mg/kg BW) immediately before castration. Samples were collected on d -1, immediately before castration, 60, 90, 120 min and 1, 2, 3, and 7 d after castration, except for a visual score only obtained during castration. Physiological measures included salivary cortisol (SC), haptoglobin (HP), substance P (SP) and scrotal area temperature (SAT). Behavioral measures consisted of a visual analog score (VAS), hind limb stride length (SL) and daily lying and standing durations. As expected, knife and band castrated calves exhibited more signs of acute pain compared to non-castrated controls, however results suggest that subcutaneous Meloxicam administered immediately prior to band or knife castration did not eliminate behavioral or physiological indicators of acute pain or discomfort in 1 wk old calves.

Assessing of time of administration of subcutaneous Meloxicam on indicators of pain during and after knife castration of 7-8 month old beef calves: The aim of this study was to identify the optimal time of administration for the analgesic meloxicam (Metacam®). Thirty four Angus bull calves (282.23 ± 4.80



kg BW, 7-8 mo old) were randomly 11) before castration. Calves were surgically castrated using a assigned to one of three treatments receiving a single s.c. injection of meloxicam (0.5 mg/kg BW): 6 h (6H; n = 11); 3 h (3H; n = 12); or immediately (0H; n = Newberry knife and emasculator. Behavioural and physiological measures included visual analog score (VAS), head movement (HM), hind stride length (SL), lying and standing behaviour, salivary cortisol (CL), haptoglobin (HP), serum amyloid A (SAA), substance P (SP), body temperature (BT), and scrotal area temperature (SAT). Samples were collected on d -7, -5, -2, -1, and immediately before castration; and 30, 60, 120, and 240 min, 1, 2, 5, 7, 14, 21 and 28 d after castration, except for VAS and HM only obtained during castration. Overall results varied across parameters; however, 0H and 3H group presents fewer indicators of pain and inflammation compared to 6H in 7-8 mo old knife castrated calves.

Effect of topical healing agents on scrotal lesions after surgical castration in 4-5 month old beef calves: Beef cattle are castrated in North America to reduce aggressive and sexual behaviour, and to improve carcass quality. However, surgical castration results in lesions that require several weeks to heal. The objective of the present study was to determine the efficacy of commercially available topical healing agents (HA) to improve wound healing and reduce inflammation and secondary infection. Forty-eight Angus bulls (187 \pm 4.9 kg BW and 4-5 mo of age) were randomly assigned to control (CT, castrated without the application of a post-operative HA), or surgical castration followed by either the application of a topical germicide (GR), aluminium powder spray (AL), or liquid bandage (LB). At the time of castration, all calves were administered an anesthetic (xylazine at a dose of 0.07 mg/kg via epidural). Wound healing was assessed in all calves over a 77 d period post castration. Indicators of wound healing assessed included scrotal area temperature (ST; maximum; °C) using infrared thermography, scrotal circumference (SC; cm), visual evaluation of swelling (SW, 5 point-scale) and healing rate (HR, 5-point scale) collected on d -1 and immediately before castration, d1, d2, and d7 post-castration, and weekly thereafter until the end of the study. Pain sensitivity of the wound and surrounding skin to manual pressure was evaluated using a digital algometer (DA) on d -1, 0, 1 and 2 and a Von Frey anesthesiometer (VA; kg) weekly for the first 42 d post-castration. Animal BW and rectal temperature (RT) were also recorded weekly. Although no statistical differences were observed in HR between the treatments calves treated with HA had numerically greater HR scores on d 35 and 42 than CT calves. The HA used in this study did not improve indictors of healing such as swelling and healing rate scores or indicators of inflammation including scrotal temperature and circumference of surgical castration lesions.

<u>Success Story</u>: Experiment I of this project was completed successfully and the first important milestone, which was determining the optimal age of castration, was achieved. The results clearly indicate that calves at I wk of age exhibit very few physiological or behavioural responses indicative of reduced welfare. The identification of this age is also a key mile stone for the successful completion of Experiment 2 in which the age determined in Experiment I will be used as the only age group to access the effects of pain mitigation.

16. ANH.23.13 - Implementation of a longitudinal disease surveillance network for cowcalf operations in Western Canada

<u>Key highlights:</u> Three surveys were carried out in the past year within the network of cow-calf producers. Surveys included:

- management associated with painful procedures in cow-calf herds and producer's attitudes towards animal welfare,
- an annual production survey
- a survey (currently in progress) on parasite management and control.

These surveys are in varying stages of being returned, analyzed and entered into data bases.



A secondary study was carried out in conjunction with Dr. Claire Windeyer, Dr. Ed Pajor and Dr. Melissa Moggy from the University of Calgary in which a subset of network producers participated in a face to face interview with Dr. Moggy to gain more qualitative data on producer attitudes towards animal welfare and the code of practice.

The screening of preputial samples from bulls within the network herds have been analyzed and the prevalence of *Tritrichomonas foetus* and *Campylobacter foetus* subsp *venerealis* has been estimated for Western Canada. The individual animal prevalence and herd prevalence of Johne's disease has been estimated with samples obtained from the network herds. Analysis has been completed on the antimicrobial use survey and antimicrobial resistance analysis is also close to completion.

Svanovir ELISA kits for Ostertagia ostertagi antibodies have been utilized on all cow blood samples obtained from network herds and available in the serum biobank. This is a semi-quantitative ELISA test that enables the detection and determination of the exposure levels of Ostertagia ostertagi in grazing cattle. This will provide an estimate of the internal parasite burden on all of these herds and will be linked to data on grazing management, parasite control management and local weather and geographic data. This along with data on the fecal egg counts from the pooled fecal samples will provide a novel assessment of the parasite load on western Canadian cow-calf herds. These results are currently being analyzed. In addition, laboratory analysis is in progress for serological testing for *Neospora caninum* antibody levels which is an important cause of abortion in cow-calf herds. This will provide current evidence of the level of infection of this parasite in Western Canadian cow-calf herds.

<u>Success Story</u>: A major success story has been the development of the analysis on antimicrobial use and antimicrobial resistance in cow-calf herds. This data is the most comprehensive study on antimicrobial use in cow-calf herds and will provide valuable information to researchers and industry when considering issues around antimicrobial resistance and antimicrobial stewardship with respect to the cow-calf industry.

The results of the trace mineral studies, the Johne's prevalence study, the *Campylobacter foetus* subsp venerealis and *Tritrichomonas foetus* prevalence study have been analyzed and initial efforts on extending this information to Western Canadian veterinarians have begun. These provide current baseline levels of disease prevalence in Western Canadian cow-calf herds which is important information for veterinarians and producers alike.

17. ANH.33.13 - Improving the barrier function of the gut: an approach to minimize production limiting disease

The cells lining the digestive tract have critical role in promoting selective permeability. Despite limited research evaluating barrier function in ruminants, the rumen is often implicated as the region where antigens or pathogens gain access to arterial circulation. Several studies were conducted to evaluate barrier function among regions of the ruminant gastrointestinal tract, to evaluate whether nutritional challenges affect gastrointestinal barrier function, and whether feeding management strategies can be used to accelerate recovery of gastrointestinal barrier function.

The objective of the first study was to characterize the barrier function across the gastrointestinal tract. Six Holstein steer calves (6 mo of age) fed a common diet were used. Calves were killed by captive bolt stunning and pithing, and tissues were collected from the rumen (caudal dorsal blind sac), omasum (laminae from the central region), duodenum (proximal to the duodenal colic fold), jejunum (middle point), ileum (proximal to ileocecal junction), cecum, proximal colon (end of centripetal turns), and distal colon (mid-point between duodenal colic fold and rectum). Tissues were carefully washed using a pre-heated (38.5° C) buffer solution (pH 7.4) saturated with oxygen and then transported to the laboratory. The mucosa was prepared by hand stripping and mounted between two halves of an Ussing chamber (n = 3/region with an exposed surface area of 3.14 cm² for rumen and omasum and 1 cm² for

all other tissues). All tissues were incubated under short-circuit conditions and exposed to a similar buffer solution except for the energy source; rumen, omasum, cecum, and colon tissues were incubated with buffer containing short-chain fatty acids while tissues from the small intestine were bathed in buffer containing glucose. Tissue conductance and the serosal-to-mucosal flux of ¹⁴C-inulin and ³H-mannitol were measured as indicators of barrier function. The correlation between inulin or mannitol and tissue conductance was dependent on region and in all cases but the rumen and omasum there was a positive correlation between mannitol and inulin flux. This data indicates that the translocation of a large molecule (inulin) across the omasum and rumen is greatest despite having apparently tight epithelium based on tissue conductance and mannitol flux.

The objective of the second study was to identify whether ruminal acidosis (RA) or feed restriction (FR) differentially affect permeability of the GIT. Twenty-one Holstein steers were randomly assigned to I of 3 treatments: control (CON); ruminal acidosis (ACID), and low feed intake (LFI). Steers were fed a common diet with a 50:50 F:C ratio once daily at 0800 h for a 5-d baseline period followed by a challenge period. Rumen acidosis was induced by restricting feed to 25% DMI for I d and then offering pelleted barley (30% DMI:BW) the next day. Steers on the LFI treatment were restricted to 25% DMI for 5 d. Steers were killed and tissues were collected from the rumen, omasum, duodenum, jejunum, ileum, cecum, and proximal and distal colon for measurement of ¹⁴C-mannitol and ³H-inulin flux in Ussing chambers as markers for gut permeability. Data were analyzed as a randomized complete block design using Proc Mixed. Rumen pH was recorded throughout the study. Data indicate that feed restriction and ruminal acidosis do not appear to differentially affect permeability of the GIT. The duodenum and rumen are likely regions with greatest permeability.

In the second study we studied whether ruminal acidosis (ACID) or low feed intake (LFI), affect genes influencing barrier function [Claudin (CLDN); Occludin (OCLN); Tight-junction protein-I (ZO-I); Tight-junction protein-2 (ZO-2)] and immune response [Toll-like receptor -2 (TLR2); Toll-like receptor -4 (TLR4); Fc fragment of IgA receptor (FCAR)]. Twenty-one Holstein steers were randomly blocked and assigned to 1 of 3 treatments: control (CON), ACID, and LFI. Steers were fed a common diet with a 50:50 F:C ratio once daily at 0800 h for a 5-d baseline period followed by a challenge period. Rumen acidosis was induced by restricting feed to 25% DMI for 1 d and then offering pelleted barley (30% DMI:BW) the following day. Steers on the LFI treatment were restricted to 25% DMI for 5 d. Steers were killed and tissues were collected from the rumen (RUM), jejunum (JEJ), and distal colon (DC) for measurement of mRNA expression using real-time PCR. Relative fold change was calculated by the $\Delta\Delta$ Ct method, using pairs of endogenous controls (glyceraldehyde 3-phosphate dehydrogenase, large ribosomal protein P0, or β -actin) and then normalized to the mean of the CON. Data were analyzed as a randomized complete block design using treatment as a fixed effect and block as a random effect. Results indicate that mRNA expression of genes relating to barrier function and immune response in the gastrointestinal tract are differentially affected by nutritional stressors. Nutritional challenges affect the expression of genes related to barrier function in immune response in the gastrointestinal tract. This may have implications in identifying how cattle adapt to nutritional challenges and to identify strategies to improve gut barrier function.

The objective of the third study was to determine the effect of low feed intake on ruminal fermentation and gastrointestinal barrier function when fed a high-grain diet and 2 potential strategies to improve recovery to the challenge. In this study, lambs (n = 32) were used as a model for cattle. Lambs were exposed to a 5-d period to measure DMI (BASE) and then were restricted to 50% of that measured during BASE for a period of 3 d (CHAL), followed by a 5-d recovery period (RECOV) where lambs were provided feed for *ad libitum* intake. This model was used to represent exposure to adverse environmental conditions for feedlot cattle. All lambs were adapted to a common high-grain diet (9% silage on a DM basis) and assigned to I of 4 treatments: a control (CON) where lambs were adapted to the high-grain diet but were not challenged (i.e. they were fed *ad libitum* throughout the study); a high-grain recovery treatment



(HG), where the lambs were restricted to 50% during the RECOV and provided the high-grain diet during RECOV; a storm diet approach (STORM), where lambs were restricted to 50% during CHAL but were provided a diet containing 20% silage during RECOV; or a storm diet approach with additives (STORM+), where lambs were restricted to 50% during CHAL but were provided a diet containing 20% silage with additives designed to improve recovery of the gastrointestinal tract during RECOV. The additives included butyrate (0.21% of the diet), rumen protected betaine (0.7% of the diet), and antioxidants (0.01% of the diet). Ruminal pH was measured during the study and samples of ruminal digesta were collected at killing. In addition, an oral dose of lactulose was administered to evaluate total tract barrier function in vivo. On the 5th d of RECOV, lambs were killed and tissues from the rumen, jejunum, and distal colon were used to assess permeability and nutrient absorption. Results indicate that feeding a diet with a lower proportion of concentrate after a period of low feed intake may help lambs recover with respect to DMI and ruminal pH. Moreover, low feed intake appears to result in a compensatory increase for genes related to barrier function. Compounds that stimulate gastrointestinal development (butyrate, betaine, and antioxidants) may enhance absorptive capacity following low feed intake and reduce the permeability of the gastrointestinal tract.

<u>Success Story</u>: This was the first study to evaluate selective permeability among regions of the gastrointestinal tract for ruminants and identified that while the rumen and omasum have relatively low paracellular permeability, the relative permeability for large molecules is greater than for intestinal regions. The proximal regions of the small intestine have greatest permeability for small molecules. These new findings present a challenge to the current understanding of paracellular permeability and a unique feature for ruminants as the regional permeability differs from monogastric animals.

Applying the knowledge of regional permeability, we evaluated whether ruminal acidosis and low feed intake affect permeability of the gastrointestinal tract. We observed that the gastrointestinal tract attempts to prevent an increase in permeability by upregulating the expression of genes for tight cell junction. This finding provides a new insight into the adaptive mechanisms of the gastrointestinal tract and provided evidence to suggest that the main effects (based on changes in gene expression) likely occur in the rumen, omasum, duodenum, and jejunum. Knowing the regions affected allows for the development and evaluation of strategies to mitigate the effect or accelerate recovery.

We also found that we can help accelerate recovery of the gastrointestinal tract by reducing the proportion of concentrate in a diet after a period of low feed intake and that provision of betaine, butyrate, and an antioxidant blend may help support ruminal epithelial function.

Food Safety

18. FOS.01.13 - Prevalence, Persistence and Control of Non-O157 Shiga Toxin Producing Escherichia coli

<u>Key highlights:</u> The primary objective of the study was to determine the prevalence of and characterize the Top 6 STEC isolated from beef processing operations. The study compared three RT-PCR screening methods all of which were AOAC approved for Top 6 STEC screening. Samples were collected from the holding area, stun floor, hides and de-hided carcasses. The GDS-Biocontrol RT-PCR returned a presumptive prevalence of 54% Top 6 non-O157 STEC with BAX and PALLgene recording a prevalence >88%. However, attempts to recover Top 6 non-O157 STEC from presumptive positive cultures proved unsuccessful, with only 114 of the 2400 isolates screened possessing the full complement of virulence factors (stx, eae). The virulence factors of all but two of the 114 isolates were unstable and readily lost during sub-culturing. The only virulent isolates harboring stable virulence factors were identified as O76 and O187:H52, neither of which belong to the Top 6 non-O157 STEC. These results indicate that the prevalence of Top 6 non-O157 STEC is very low in Canada, although



cattle harbor a diverse range of *E coli* serotypes. However, significant genetic exchange during enrichment can lead an over-estimation of prevalence and problems when attempting to undertake culture confirmation of presumptive positive samples. The clinical significance of temporary acquisition of virulence factors by *E. coli* needs to be elucidated.

Success Story:

- Virulence genes (principally *stx* encoding for shiga toxin) that are required to make *E. coli* toxigenic, are widely distributed in processing and associated with cattle. However, strains harboring all the virulence factors associated with cattle and their environment are relatively rare.
- Rapid screening genetic tests based on detection of virulence genes and genes encoding for O serogroups return a high level of false-positive results. The false-positive results were not the result of mis-identification of genes, but because the targets were present in different cells. There was also evidence that stx genes are mobile during enrichment, leading to the observed false-positive results.
- No Top 6 colonies were recovered from any of the samples screened. A total of 74 isolates harbored virulence genes along with a toxin producing phenotype, but only two were EHEC. Shiga toxin production in the other 72 isolates was unstable and readily lost.
- Although no Top 6 serotypes were recovered from samples of Enteropathogenic *E. coli*, the EPEC could be converted to Top 6 by acquisition of *stx*. However, acquired toxin producing ability was unstable and readily lost.
- In Canada, the food safety risks associated with the Top 6 STEC associated with beef appear to be low and likely less than for the *E. coli* O157:H7.

19. FOS.04.13 - Identification and validation of commercially practicable practices and procedures for improving the microbiological safety stability of beef

<u>Key highlights:</u> Recent work at Lacombe has shown that, in commercial practice, *Escherichia coli*, an indicator organism for the presence of enteric pathogens including verotoxigenic *E. coli* (VTEC) and *Salmonella*, can essentially be eliminated from dressed beef carcasses. However, this achievement is not always maintained as sporadic contamination does happen. Also, beef can be re-contaminated during the fabrication process. The purpose of this project is to characterize which practices at beef packing plants are effective for controlling pathogenic bacteria. Actions to control *E. coli* on product can also be extended to enhance control over spoilage bacteria. That is desirable to facilitate trading of chilled beef to overseas markets. A number of research projects have been conducted.

The efficacy of an on-line hide-on carcass wash, a practice currently being used at some large commercial packing plants, was tested in winter, spring and fall. The populations of *E. coli* on hide before the hide-on wash and during the dressing process were also analyzed. Results show that the hide-on wash is effective for reducing the numbers of bacteria on carcasses and *E. coli* is susceptible to the treatment. However, *E. coli* is transferred from the hide to the carcass during skinning as well as from sources other than the hide during the dressing process.

Dry chilling is commonly used in small beef abattoirs in many countries. However, information on the rate and extent of inactivation of bacteria on carcasses during commercial dry chilling was largely lacking. This research studied how dry chilling affects the microflora on beef carcasses at a Canadian beef packing plant. Carcasses selected at random at the beginning of and various points during a commercial chilling process were sampled for determination of counts of total aerobes, coliforms and *E. coli*. The numbers of aerobes were reduced by I log unit after the first hour of cooling, and by a further log unit during the subsequent 23 hour of chilling. Coliforms (predominantly *E. coli*) were recovered from carcasses before chilling at numbers about 2 log cfu 4000 cm⁻². Very few coliforms or *E. coli* were recovered after 24 h, and no such organisms were recovered after 67 h. Thus, the dry chilling process at the packing plant involved in the study was very effective for improving the



microbiological safety and quality of beef carcasses.

The microbiological effect of a common process used to clean and sanitize commercial fabrication facilities and equipment was determined. The process was largely ineffective for reducing the numbers of indicator organisms. Thus, the cleaning process was modified and the microbiological effects of the modified procedures were determined. The findings show that the modified procedures can effectively reduce the numbers of all three indicators organisms on conveying equipment.

A recent study at Lacombe showed that a storage life of 120-140 days at -1.5 °C was attainable for the vacuum packaged Canadian boneless and bone-in beef. To determine the factors limiting the storage life of such product, the bacteria isolated from beef were analyzed. More than 20 microbial species were present on both types of cut before storage. After storage for \geq 30 days, the microflora was dominated by carnobacteria and Enterobacteriaceae. The bone-in beef stored at 2°C were spoiled earlier, likely by species of Enterobacteriaceae. Even though long storage life is attainable, vacuum packaged beef can spoil well before the intended storage life by blown pack spoilage (BPS), characterized by copious amount of gas accumulation in the pack and primarily caused by spores of C. estertheticum. Work was carried out to determine the effects of meat pH and initial numbers of spores on BPS development. The findings show that 10-30 spores and \geq 30 spores, respectively, of C. estertheticum can pose a risk of BPS for vacuum packaged beef of normal and higher pH. To cause BPS, the spores of C. estertheticum have to germinate. Spores can resist harsh environments like the decontaminating interventions routinely applied at meat packing plants. So, to control BPS, interventions that can induce spore germination and then inactivate the germinated spores are desirable. Work was carried out to determine the conditions required to germination C. estertheticum spores. Germination rate of spores increased as pH approached neutrality and germination at pH 5.5 or lower was minimal. Spores were inactivated when co-heated with germinants. The results show that vacuum packaged beef stored at chiller temperature provides all the conditions required for the germination of spores of C. estertheticum and that BPS could be potentially controlled by thermal inactivation of germinated spores on meat.

Success Story:

1. The on-line hide on carcass microflora showed that the hide-on wash is a very effective intervention for controlling *E. coli* on beef carcasses. These findings have been communicated with the management of the large federally inspected plant involved in the study and our provincial counterpart for the potential use at smaller provincial packing plants. A new beef packing plant, with a capacity of processing 600 head cattle per day, will likely be in operation in the near future. We were invited to discuss with the plant management and owner of the plant on the design of potential interventions for controlling microbiological contamination. We suggested the hide-on wash and other control measures that have been proven to be effective for controlling contamination.

2. The cleaning practice of a beef plant was not entirely effective for reducing the numbers of bacteria on the surfaces of equipment. The root cause for this problem was investigated and a model cleaning practice was developed for the plant. The microbiological conditions of the equipment and in turn, that of the product have been greatly improved. The improved cleaning method can be adopted by other meat packing plants as well.

3. Despite the common usage of dry chilling by small/medium beef plants in North America and many meat plants in Europe, the rate of inactivation of *E. coli*, if there is any, by this mode of chilling has never been established before. CFIA requires federally-inspected meat packing plant to have at least one antimicrobial intervention in the carcass dressing process. This study showed that dry chilling can reduce the number of generic *E. coli* on dressed carcasses to an undetectable level and thus can be considered an effective intervention. This study helps the plant involved in

this study and other similar plants to demonstrate the antimicrobial efficacy of dry chilling and to be in compliance with regulations.

20. FOS.10.13 - Surveillance of E. coli, enterococci, antimicrobial resistance (AMR) and Enterococcus species distribution in beef operations-associated environments

<u>Key highlights:</u> Public concern for antimicrobial use (AMU) and resistance (AMR) in livestock is increasing, as is continuing pressure for industries and governments to address these concerns. A One Health approach is required to correlate AMU and AMR across the agriculture-environment-public continuum and effectively advise on the current state of antimicrobial resistance. This research will determine how AMU in beef cattle potentially contributes to AMR of indicator bacteria within feedlot cattle and downstream environmental reservoirs, relative to AMR in the public.

An extensive sampling network was established to represent diverse areas of the One Health continuum, focusing in and around four beef cattle feedlots within Alberta. Two years of sampling from these sites was completed in April 2016. Overall, 1,707 samples were collected representing beef pen composite fecal samples, catchment basin, surface water, soil, sewage influent and treated effluent, and beef processing sites as well as retail meat. The majority of samples were preserved and archived for future analyses. All samples were processed for isolation of the indicator bacteria, *E. coli* and enterococci using non-selective and selective (cephalosporin and macrolide antibiotics, respectively) media. A substantial collection of *E. coli* (7,325) and enterococci (8,677) were deposited.

Characterization of the bacterial isolates has begun, including identifying enterococci species using an amended DNA sequence-based approach. Initial results suggest that unique enterococci species are well-established within specific host or environmental niches, and that dissemination of enterococcus species beyond the feedlot may be minimal. Similarly, phylo-typing *E. coli* isolates will help decipher trends in the virulence and transmission of *E. coli*. Additional characterization of the isolates including genotyping and susceptibility testing against a custom panel of antibiotics is commencing.

The potential hazards of AMU in feedlots include development of resistant bacteria, and also the risk of antibiotic residue contamination in the environment, which may lead to the development of resistance down-stream from the feedlot. To assess this possibility, residue analyses has been conducted on a subset of environmental samples which are associated with two of the feedlots from this study. Several antimicrobials were detected in the samples, including those used at the feedlot. However, the levels detected diminish as distance from the feedlot increases.

To extend the analysis beyond the indicator bacteria, a large-scale metagenomic analysis will help elucidate the extent of resistance throughout the microbial population and compare the overall resistome from diverse samples. Pilot trials have already been conducted to refine the sequencing and bioinformatic methodologies. To complement the sequencing, advanced molecular techniques are also being employed to establish representative metagenomic libraries to perform functional screening of resistance and potentially reveal novel mechanisms of resistance. In both cases the resulting data will be analyzed to understand the microbial community and for mining AMR genes and their prevalence.

Concurrently with sampling, AMU data from the enrolled feedlots is being collected for the period beginning one year prior to sample collection, up until the final sample was collected. This extensive dataset including injected and in-feed antibiotic use will be evaluated against the information gathered from the bacterial isolates and metagenomic data to identify possible relationships between AMU in feedlots and the development and/or transmission of resistance to humans.

In parallel to the ongoing surveillance, an applied research experiment was designed to assess stockpiling manure compared with composting. Results demonstrated that composting is more effective than stockpiling to prevent the spread of veterinary antibiotic residues and transmission of antimicrobial resistance during land-application of manure. These results can help identify onfarm interventions to minimize the transmission of resistance.



Success Story: Enterococci are well-recognized as an indicator species predict fecal contamination of water or other environments, or as a measure of personal hygiene (Boehm & Sassoubre, 2014). However, if individual enterococci species are adapted to particular hosts or environments. and thus the use of Enterococcus only identified at a genus level may have limited utility as an indicator organism (Boehm & Sassoubre, 2014). To circumvent ambiguous conclusions about fecal contamination in this study it was proposed that the enterococci isolates detected from the various sources would be submitted to a stringent speciation procedure (Zaheer et al., 2012). Initially, due to limited financial and time resources, it was uncertain whether a sub-set of enterococci would be selected, or if all isolates could be speciated. However, early in the project it was recognized that the majority of isolates originating from the feedlot environment were Enterococcus hirae. In response to this realization, the original GroES-GroEL sequencing-based approach was modified to include a second PCR-target which was specific to E. hirae and thus samples with positive amplification of this target could be excluded from the more expensive and time-consuming process of DNA-sequencing, while those isolates which were only positive for the GroES-EL target would proceed to sequencing. This development increased the efficiency of our approach so significantly that we were able to pursue speciation of all enterococci isolates collected.

As the enterococci species data was compiled a trend began to emerge in agreement with previous reports describing certain species preference for specific hosts or niches. The data clearly illustrate how the predominant enterococci species shift from E. hirae in cattle and their immediate environment with an apparent increase in more naturalized species such as E. casseliflavus and E. durans in the environmental samples, and a preponderance of E. faecium and E. faecalis in the sewage treatment and clinical isolates. In particular, the enterococcus population profile of the surface water samples demonstrates a blending of isolates from the cattle sources with those likely to survive and proliferate in the environment. In samples collected from a wetland during the winter thaw, E. casseliflavus was the predominant species being isolated. However, as the season progressed and cattle waste was moved through the system, the relative proportion of *E. hirae* increased dramatically correlating with the observed high prevalence of this species in bovine feces. The enterococci isolated from an ephemeral creek, up- and down-stream water samples with respect to a feedlot, had very similar community compositions and, unlike feedlots, E. hirae was not found as a predominant species. Since E. hirae was most prevalent in the feedlot fecal samples and associated catch basins and not in the environmental samples, this indicates that the feedlot operations are being prudently managed. Likewise, the enterococci identified in the beef processing facility suggest a merging of human-associated enterococci with cattle-associated enterococci. For instance, E. hirae comprise 46% of isolates collected early in the processing line (after hide removal), but make up only 1% of the isolates at the end of the processing line (ground product). In the same progression (after hide removal to ground product), E. faecalis proportions go from 40% to 91%. This demonstrates the effectiveness of the disinfection measures employed by the processing plants to control microbes coming from the animals, but also suggests the potential for foodhandler contamination.

Beef Quality

21. BQU.01.13 - Effect of high pressure processing on quality, sensory attributes and microbial stability of marinated beef stead during refrigerated storage

<u>Key highlights:</u> High pressure processing is used for commercial applications in many parts of the world to extend the shelf life of ready-to-eat meat products. However, research has shown that fresh muscle foods are susceptible to pressure-induced colour change which has limited the adoption of HPP for raw meat applications. Marinating is commonly used by the meat industry to enhance moisture and



improve the texture of meat products. Colour imparted by the marinade may partially mask undesirable discoloration caused by the HPP treatment. The objective of the study was to determine the effects of high pressure treatments on quality, nutrition, sensory and shelf life extension of marinated beef steaks. The main objective of this project was to investigate the effects of high pressure processing (HPP) on the quality, sensory attributes, microbiological stability, nutritional and chemical characteristics of marinated beef steaks stored under refrigeration. This project established new processing methodologies for marinated HPP beef products. These results provided useful technical information to improve beef product quality attributes and establish the safety of HPP-treated fresh marinated beef products with extended shelf life. Product-oriented guidelines for commercial production of fresh, marinated HPP beef steaks with an extended shelf life were developed.

Beef cut from hip muscle was purchased and processed into marinated beef steaks with pilot scale industrial meat processing equipment and further processed using HPP technology at the Food Processing Development Centre (FPDC). Preliminary screenings altered product formulations and processing parameters including pressure and time of HPP to find the best potential parameters for this product.

The processing parameters with the best potential to improve shelf-life, texture, and cooking loss of products were used in a full-scale study to determine the effectiveness and benefits of these interventions on the marinated beef product. The investigations including nutritional, chemical, microbiological and sensory properties and a microbiological challenge study of marinated beef steaks were conducted at the FPDC, the Consumer Product Testing Centre (CPTC) and Agri-Food Discovery Place at the University of Alberta.

Treatment of marinated steaks at 450 MPa/3min significantly extends shelf life without adverse effects on the meat quality, nutrition, or sensory attributes measured in this study. This work demonstrates that marinating raw meats prior to HPP has the potential to expand the application of HPP to value added raw meat products.

<u>Success Story</u>: The application of HPP on four HPP products (HPP teriyaki beef steak and HPP honey garlic steak) were submitted to Health Canada for approval on June 3, 2015. This project contributes to the Health Canada's decision that "any food except fish and seafood that has been treated for HPP for the sole purpose of shelf life extension is no longer considered a novel food". A letter of non-novelty for these HPP-treated foods was issued by Health Canada on April 8, 2016. Industry clients can now start producing and selling HPP marinated raw meats in the marketplace immediately.

22. BQU.03.13 - Genetics and Proteomics of dark cutting cattle in Alberta

<u>Key highlights:</u> Recent research indicated that reduced carcass muscling and weight are associated with beef dark cutting. Existing data from a single farm (44 heifers, 136 steers) from three normal beef quality grades and the dark cutting grade (n = 35 AAA, 106 AA, 28 A and 11 B4, respectively) were used to identify relationships between sex, live animal and carcass characteristics and the incidence of dark cutting. Categorical modelling, mixed model analysis of variance and logistic regression suggested that dark cutting is more likely in cattle that are female, light weight (at weaning or slaughter), slow growing, or have low feed intake.

In terms of packing plant factors, the risk of dark cutting (both typical and atypical) was highest in cattle that were re-loaded from abattoir to temporary feedlot and then returned to abattoir following a short stay at an unfamiliar feedlot. Proteomic data indicated that differences existed in enzymes controlling metabolism of glycogen, suggesting that metabolic differences existed between normal and dark cutting muscles. Early genetic association studies indicated that four gene regions are of interest for dark cutting. Further research on data collected from industry partners will be used to test the influence of growth promotants on the incidence of dark cutting.



Success Story: This project was conducted with industry engagement, represented a comprehensive study of dark cutting, and has brought substantial understanding as to why dark cutting occurs in Canada.

23. BQU.06.13 - Genetics of the eating quality of high connective tissue beef

Key highlights: Beef tenderness is limited by the amount of connective tissue. Collagen, the major connective tissue protein, forms cross-links over time that make cooked beef tougher. This research project is identifying variation in the mechanisms controlling collagen cross-linking. Single nucleotide polymorphisms (SNPs) associated with intramuscular concentrations of two heat-stable collagen crosslinks, pyridinoline (PYR) and Ehrlich's Chromogen (EC) are being identified and related to cooked beef toughness and collagen heat solubility. Four muscles of different collagen concentrations were sampled from steer carcasses sourced from low or high residual feed intake (RFI) purebred (Angus, Charolais) and crossbred (Kinsella composite) populations. Finished steers were slaughtered and tissues for DNA and functional genomics analyses were collected. The triceps brachii, gluteus medius, semimembranosus and longissimus lumborum muscles were removed from one side of the carcass and analyzed for intramuscular pH, fat, protein, moisture, colour, drip loss, cooking loss, Warner-Bratzler shear force, collagen content, collagen cross-links and collagen heat solubility. These measurements will be related to gene expression and genetic data from phenotypic extremes to identify genes affecting collagen contribution to meat toughness.

24. BQU.07.13 - Beef Quality Audit

- Key highlights: The 2015/16 portion of the National Beef Quality Audit benchmarked retail consumer satisfaction and obtained objective meat quality measurements, including tenderness and shelf life microbiology. 1200 interviews were conducted with Canadian consumers after they prepared steaks obtained from the national retail banners. An equal number of steaks from the same stores were sent to AAFC Lacombe for laboratory measurements and sensory panel evaluation. Information was also recorded for 21,211 packages of beef to document merchandising trends including method of production claims, grade and origin information, packaging formats, product assortment and the allocation of the retail display case allocated to beef and other proteins.
- Success Story: To the best of our knowledge the 2015 National Beef Quality Audit is the most comprehensive evaluation of retail beef ever performed in Canada. It combines information from 1,200 interviews of Canadian consumers following in-home preparation of beef steaks obtained from a representative sample of Canada's national retail banners with objective laboratory measurements. Laboratory measurements include those related to tenderness and shelf-life as well as trained sensory panel testing. In addition, merchandising information was obtained from 21,211 packages of beef in stores where consumer and laboratory samples were obtained. Given the very high retail prices in recent years the importance of understanding perceptions of beef quality and consumer expectations has never been more important to the future of Canada's beef industry. The results will be shared with participating retail banners in confidential reports as well as consolidated information for use by the primary production and processing sectors.

Environment

25. ENV.02.13 - Environmental Footprint of the Canadian Beef Industry

Key highlights: Canada produces approximately 2% of the world's beef and is the 5th largest exporter of beef in the world. Beef production also contributes an estimated \$33 billion to the Canadian economy. Traditionally, economic performance has been the primary driver of the beef industry and the subject of considerable research. More recently, however, the environmental impact of agricultural commodities has attracted public interest and debate. The footprint of the beef industry is complex

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with implications for greenhouse gas emissions, nutrient cycling, water and air quality, carbon storage, and grassland and wetland ecosystems.

The current phase of the project documented the environmental impact of improvements in the efficiency of Canadian beef production in 1981 and 2011. Producing the same amount of beef in 2011 required 29% less breeding stock, 27% fewer slaughter cattle and 24% less land, with a 15% reduction in greenhouse gases compared to 1981.

<u>Success Story:</u> A significant milestone for this reporting period is the publication of Legesse et al. entitled "Greenhouse gas emissions and resource use of Canadian beef production in 1981 as compared with 2011" in Animal Production Science. This manuscript reported a significant reduction in greenhouse gas intensity over the past three decades as a result of increased average daily gain and slaughter weight, improved reproductive efficiency, reduced time to slaughter, increased crop yields and a shift towards high-grain diets that enabled cattle to be marketed at an earlier age. The paper was an outcome of a collaborative work of researchers from different institutions mainly Agriculture and Agri-Food Canada (Lethbridge Research Centre), University of Manitoba and Environment Canada. The researchers also worked closely with the Canadian Roundtable for Sustainable Beef (CRSB) and Beef Cattle Research Council (BCRC) with regard to news release, speaking notes, interviews and other communications on the project findings. Information was picked up across the country and highlighted in the Minister of Agriculture's February news release.

Technology and Knowledge Dissemination

26. TEC.01.13 - Improving Technology Transfer and Knowledge Dissemination in the Canadian Beef Industry

<u>Key highlights:</u> Both government and industry make significant investments to continually find better and more efficient methods of producing high quality beef and beef cattle, but effective technology transfer is needed to realize the benefits of research efforts. Governments and universities used to employ many extension specialists and support field days, seminars and other initiatives but these activities have greatly declined over the past two decades. This has resulted in significant shortfalls in industry adoption of new knowledge and technology.

The purpose of this project is to improve knowledge dissemination by supporting and delivering a range of technology transfer mechanisms with a clear focus on accelerating the uptake of research results and outcomes by industry. A primary focus of the initiative is extension of results from research activities completed under the Beef Cattle Industry Science Cluster.

This project is guided by the 10-year Knowledge Dissemination and Technology Transfer Plan that was developed by the BCRC under the first Beef Cluster. A range of technology transfer activities are being used, as well as coordination of extension efforts with industry partners.

This project includes regular communication with industry through the creation and distribution of fact sheets that summarize project findings and articles that discuss research outcomes or priorities which are published on BeefResearch.ca and various other channels. New resources, such as new webpages, videos, and cost of production decision tools for producers have been created and made available through BeefResearch.ca. Engagement of researchers with industry is being improved through the Beef Researcher Mentorship program, by supporting the participation of a young researcher in the Cattlemen's Young Leaders Development Program and by recognizing highly engaged researchers with the Canadian Beef Industry Award for Outstanding Research and Innovation. An economic analysis project completed by Canfax Research Services will continually help to inform BCRC's approach to technology transfer.

While it is difficult to measure or qualify the adoption of innovative knowledge, especially in the short term, BCRC's technology transfer efforts appear to be successful. Website traffic has increased each month and analytics indicate that the audience is interested in a variety of topics. Articles and fact sheets have been regularly redistributed by trade magazines and other media, as well as by producers on social media. Views per video are increasing and social media networks of stakeholders continually grow. The number of email subscriptions also continually increases. Most significantly, follow-up with webinar participants one year later confirms that many producers make changes on their operation following the information and advice presented during the webinar.

<u>Success Story</u>: Perhaps the greatest single achievement of the activity in 2015-16 was the successful communication of results from Cluster project ENV.02.13 ("Defining the Environmental Footprint of Canadian Beef Production"). Through a collaborative effort with the research team, the Canadian Roundtable for Sustainable Beef, and the Canadian Cattlemen's Association, the BCRC led the development and execution of a comprehensive communication plan to communicate new information about the environmental footprint of Canadian beef production.

Several resources were developed including: a news release, speaking points for project spokespeople, a BCRC "factoid" (quirky image highlighting reduced GHG emissions), a 2-page fact sheet summarizing the project, an 1,100 word article about the results for industry audiences, and a PowerPoint presentation. These resources enabled widespread, consistent delivery of information to numerous audiences in numerous formats and venues among industry and the general public. Numerous industry groups and publications utilized and distributed the resources, several media outlets requested interviews with project spokespeople and reported on the project's results, and several industry events invited spokespeople to present the project results. Impacts of the comprehensive extension strategy for the interim results of ENV.02.13 are believed to be increased awareness and understanding of continuous improvement and sustainability in the beef industry both within and outside the industry, and increased motivation within industry to further understand and minimize the industry's environmental footprint.

Project #	Project description	2015-16 budget AAFC, NCO and other industry	2015-16 projected AAFC, NCO and other industry	2015-16 projected NCO funds	2013/14 to 2017/18 5-yr budget
	Forage and Grassland Productivity				
FRG.04.13	Innovative Swath Grazing/Increasing Forage Research Capacity	178,873	178,873	-	798,084
FRG.08.13	Development of native plant material (grasses, legumes) and mixtures for forage production in the Prairie Region	668,552	614,224	89,929	2,310,118
FRG.09.13	Nutritional Evaluation of Barley Forage Varieties for Silage and Swathgrazing	133,300	133,300	-	212,233
FRG.13.13	Pasture mixtures and forage legumes for the long-term sustainability of beef production	165,025	165,025	-	623,990

Summary of Beef Science Cluster Research projects – 2015-16



FRG.14.13	Building long-term capacity for resilient cow-calf production systems through creation of a forage industry chair supporting training and research in evaluation and utilization	264,765	264,690	40,704	948,512
Total		1,410,515	1,356,112	130,633	4,892,937
	Feed Efficiency	2015/16 budget	15/16 proj expenditure	15/16 proj NCO funds	2013-2018 budget
FDE.04.13	Germplasm and variety development of barley and triticale for animal feed with a focus on feed quality, yield and disease resistance of both grain and annual forage production	300,000	300,000	-	I,400,000
FDE.07.13	The impact of genomic selection for feed efficiency on the cow-calf sector, performance parameters and underlying biology	167,325	167,325	-	552,874
FDE.09.13	Increased Use of High Energy Forages in Conventional Feedlot Beef Production	125,750	120,000	-	444,091
FDE.15.13	Prebiotic, probiotic, and synbiotic technologies for targeted applications in food safety and ruminant productivity	136,356	136,356	-	499,767
FDE.17.13	Improvement of cow feed efficiency and the production of consistent quality beef using molecular breeding values for RFI and carcass traits	250,117	250,117	-	461,771
FDE.19.13	Understanding the physiology behind changes in feed efficiency throughout the finishing period	370,660	142,284	50,084	657,135
Total		1,350,208	1,116,082	50,084	4,015,638
	Animal Health and Production Limiting Diseases	2015/16 budget	15/16 proj expenditure	15/16 proj NCO funds	2013-2018 budget
ANH.01.13	Identifying Mycobacterium avium subsp. parathberculosis (MAP) exproteome components recognized early during infection to develop diagnostic and vaccine targets	67,390	67,390	-	190,325
ANH.12.13	Geographic variation in abundance and genetics of Dermacentor andersoni, a vector of bovine anaplasmosis	189,768	189,768	123,715	570,650



ANH.13.13	Development of a fully-automated DNA microarray chip for multiplex detection of bovine pathogens	105,350	105,247	-	289,501
ANH.21.13	Effect of age and handling on pain assessment and mitigation of common painful routine management procedures	424,608	424,553	-	I,364,760
ANH.23.13	Implementation of a longitudinal disease surveillance network for cow-calf operations in Western Canada	158,700	158,700	123,715	1,067,405
ANH.33.13	Improving the barrier function of the gut: an approach to minimize production limiting disease	173,685	173,685	-	385,708
Total		1,119,501	1,119,343	247,430	3,868,349
	Beef Quality and Food Safety	2015/16 budget	15/16 proj expenditure	15/16 proj NCO funds	2013-2018 budget
FOS.01.13	Prevalence, Persistence and Control of Non-0157 Shiga Toxin Producing Escherichia coli	-	-	-	48,300
FOS.04.13	Identification and validation of commercially practicable practices and procedures for improving the microbiological safety stability of beef	177,608	177,608	-	460,538
FOS.10.13	Surveillance of E. coli, enterococci, antimicrobial resistance (AMR) and Enterococcus species distribution in beef operations-associated environments	536,132	488,727	112,695	1,809,625
BQU.01.13	Effect of high pressure processing on quality, sensory attributes and microbial stability of marinated beef stead during refrigerated storage	11,500	9,872	-	34,500
BQU.03.13	Genetics and Proteomics of dark cutting cattle in Alberta	32,902	32,902	-	245,794
BQU.06.13	Genetics of the eating quality of high connective tissue beef	188,985	188,985	-	175,088
BQU.07.13	Beef Quality Audit	442,539	433,075	48,154	762,085
Total		1,389,666	1,331,170	160,849	3,535,930
	Environment	2015/16 budget	15/16 proj expenditure	15/16 proj NCO funds	2013-2018 budget
ENV.02.13	Environmental Footprint of the Canadian	69,000	69,000	-	310,788

	Technology and Knowledge Dissemination	2015/16 budget	15/16 proj expenditure	15/16 proj NCO funds	2013-2018 budget
TEC.01.13	Improving Technology Transfer and Knowledge Dissemination in the Canadian Beef Industry	273,193	235,109	79,451	931,662
Cluster Management	Management and administration of all Cluster projects	306,345	305,548	130,564	1,517,807
Total all Cluster projects		5,918,428	5,532,364	799,011	19,073,111*

Beef Industry

The 2015/16 budget for Cluster II projects was \$5,918,428 with projected expenditures of \$5,532,364.

2015/16 National Check-off funding to Cluster II projects is projected at \$799,011.

Unspent National check-off funding of \$386,064 in 2015/16 will be deferred to subsequent cluster years (2016/17 and 2017/18) to enhance cluster research relating to feed efficiency, feed production and technology transfer and extension. * additional government funding towards the Beef Science Cluster projects totals 1,156,707.

2. Projects Funded by National check-off and Managed by BCRC

In addition to Beef Cluster II projects funded with national check-off dollars, BCRC and industry partners also fund projects outside of the cluster based on identification of specific needs and opportunities. The projects identified below are all managed through BCRC, with funding from various sources.

The non-Cluster projects funded through National check-off revenues in 2015/16 are highlighted below.

- ANH.05.11- Determining the incidence, prevalence, and severity of ruminal acidosis in feedlot cattle Ruminal acidosis is a digestive disorder affecting cattle and other ruminants. Traditionally, ruminal acidosis is thought to be most common and severe as cattle transition onto the finishing diet. However, no previous studies have actually determined the prevalence or severity of ruminal acidosis in feedlot cattle. This study demonstrated that: 1) the prevalence and severity of ruminal acidosis is much lower during finishing than was suggested, with the greatest risk occurring in the latter part of the finishing phase; 2) that pen conditions (i.e. mud in pens) affect feeding behaviour and may influence risk for ruminal acidosis; and 3) that the severity for ruminal acidosis during dietary adaptation is lower than had been suggested in the literature. That said, with respect to the dietary transition phase, the greatest risk for ruminal acidosis appears to be on the second day relative to the dietary change. The results of this project provide needed information to assess how current feeding practices in the feedlot industry affect the health and productivity of beef cattle.
- MISC.03.12 Enhancing Barley Straw Digestibility
 On a pound-for-pound basis, there is as much energy in barley straw as there is in the grain. The problem is that cattle can't access the energy in barley straw because even rumen microbes have a



hard time digesting it. This research is studying a wide range of microorganisms from ruminants that are said to thrive on rely on higher fiber diets (e.g. bison) as well as fungi that decompose wood. This research aims to identify genes coding for enzymes capable of breaking down complex fibers, potentially leading to feed treatment or dietary additives enabling cattle to cost-effectively extract more energy from high fiber feeds.

• Canadian Global Food Animal Residue Avoidance Database – CgFarad

The Canadian global Food Animal Residue Avoidance Database (CgFARAD) plays an important role in the prevention of drug and chemical residues in foods of animal origin. Based at the Western College of Veterinary Medicine, University of Saskatchewan and the Ontario Veterinary College, University of Guelph, the CgFARAD service provides technical information and advice to Canadian veterinarians and government regulators on withdrawal issues relating to extra-label drug use and exposure to toxic chemicals in food animals. The clinical pharmacologists responsible for the CgFARAD are uniquely positioned to provide expertise to meet industry needs. BCRC contributed \$7,500 to the annual budget of \$195,500.

• Beef Research Capacity – Development of a New Research Chair Position

With recognition of a significant gap in research capacity in the areas of forage breeding, agronomy, and utilization the BCRC has allocated funding (\$500,000) to establish a new forage management and utilization chair at the University of Saskatchewan. In collaboration with the University and the Saskatchewan Forage Network, efforts are being made to establish an endowed chair, which requires a total investment of \$5 million but ensures a chair position is maintained over the long-term. The BCRC has obtained a commitment in principal by Saskatchewan Cattlemen's Association proposing to match the BCRC's commitment. Discussions are also underway with the Dairy Farmers of Canada and the Saskatchewan Ministry of Agriculture who are both supportive of the initiative and are looking at options to provide funding. Efforts are also underway to engage private investments into the initiative. It is the goal of the BCRC to have a funding plan finalized in 2016/17 such that a chair can be hired to begin their research program shortly thereafter.

Project description	2015/16 budget	2015/16 Projected Check-off dollars**
MISC.03.12 - Enhancing Barley Straw Digestibility	20,000	20,000
ANH.05.11- Determining the incidence, prevalence, and severity of ruminal acidosis in feedlot cattle	4,484	4,484
Canadian Global Food Animal Residue Avoidance Database-CgFarad	7,500	7,500
Beef Research Capacity	500,000	0

2015/16 check-off and industry funding to non-Cluster projects is projected at \$31,984.

** These projects are aligned with the BCRC fiscal year, July 1 to June 30. Consequently the 2015/16 actual expenditures are to be finalized subject to the close of the year end on June 30th.



3. Projects funded by Industry (not check-off dollars) and Managed by BCRC

The following projects, outside of Beef Cluster II, were funded by industry partners and other funding organizations and managed by BCRC. National check-off dollars were not allocated to these projects. Reports on these projects are available upon request.

Project description	2015/16 budget	2015/16 projected ***
Misc. 01.13 - Trim Sampling	213,650	197,650
Misc.04.13 - Offal Quality	30,000	24,000
Misc.01.14 – Enhancing traceability solutions for the Alberta cattle industry using mobile device technology	59,750	59,750
Misc.02.14 – CRSB Sustainability Assessment	167,668	167,668
Misc.03.14 - E.coli O157 Research and Education Strategy Phase II	68,000	54,326
Misc.01.15 Retail Meat Benchmark Study	81,643	74,513
Misc.03.15 Remote Sensing Applications to Insure Individual Farm Forage Production	58,995	1 58,995
Misc.01.16 Enhancing traceability and management Solutions for the Alberta cattle industry using mobile device technology : Phase II	177,689	10,000
VBP Plus Program Development (GF 2)	232,133	232,133
Enhanced VBP+ Database & Communications	275,320	275,320
Total	I,464,848	1,254,355

*** Funding for some projects is deferred to the next fiscal year aligning with project completion date.

The budget for 2015/16 partner contributions (not check-off) to research projects = \$1,464,848 and the projected expenditures are \$1,254,355.

4. Verified Beef Production[™]

In addition to sponsoring research and technology development in support of the Canadian beef industry, BCRC oversees the Verified Beef ProductionTM (VBP). The VBP program grew from its roots in the Quality Starts Here $\checkmark^{®}$ (QSH) program, an educational initiative started to help the beef industry move toward the highest beef quality in the world. The VBP program further supports the industry's vision to have high quality Canadian beef products recognized as the most outstanding by Canadian and world customers.





VBP's on-farm food safety program identifies practical, industry-sanctioned practices to enhance confidence in Canadian beef. Throughout 15/16 VBP has moved forward with the development of additional modules covering animal care, biosecurity, and environmental stewardship. Pilot audits of the new modules were initiated in 2015/16 and will be completed in 2016/17. The modules are then planned to be released under VBP+ in 2016/17 when producers can become trained and registered under all four modules.

End-users are increasingly looking for means to verify production practices related to sustainability and specific production practices and how they relate to animal care and environment. With the addition of the new modules VBP+ is being well positioned to meet the indicators established under the Canadian Roundtable for Sustainable Beef and provide a credible, cost-effective, producer-led option for verifying responsible production practices through training, simple record keeping and on-farm validation audits.

It is recognized that VBP must prepare for a reduced federal/provincial funding structure once modules are fully developed in the years to come. Consequently, in 2015/16 a new business plan and strategy was developed for VBP+ that sets out a long-term sustainable funding and delivery model for VBP+. The objective of this process is to ensure the VBP program is appropriately structured and resourced to meet the expectations of end-users and have the capacity to train and audit a large volume of producers across all four VBP modules. Significant consultations have occurred with industry stakeholders as to the proposed business plan and strategy and a Transition Management Committee was formed, whose membership includes provincial and national producer representatives, who are now tasked with moving forward with its implementation.

The VBP program is expected to grow in importance, as it begins to deliver on all four modules and becomes a core pillar in verifying sustainable beef production in alignment with the Canadian Roundtable for Sustainable Beef and end-users looking for options to verify on-farm production practices.

The VBP project budget is aligned with the BCRC fiscal year, July 1 to June 30. Consequently the 2015/6 actual program expenditure will be finalized subject to the close of the year end on June 30th. The 2015/16 check-off and industry budget for VBP Plus was \$133,950, with actual expenditures projected at \$133,950.

5. BCRC Administration and Management

The BCRC is overseen by an operating committee of 12 committee members which proportionally represent provincial allocation of the National check-off to research. Lead by an Executive Director, the BCRC oversees research program development and implementation, playing a key role in establishing and refining industry research priorities in consultation with other stakeholders. The Executive Director acts as a liaison and facilitation link among the BCRC committee and BCRC staff, CCA, Canada Beef, Canadian Beef Cattle Research, Market Development and Promotion Agency, technical advisors, and national and provincial interest groups with similar research objectives. The Executive Director encourages coordination of priorities and funding allocations between agencies in alignment with the national beef research strategy.

A Science Advisory Panel supports the research program development process within the Cluster to ensure the delivery of research plans that are directed towards industry's research objectives and achieve the outcomes desired by industry. The Panel also assists with the technology transfer and knowledge dissemination process and identification of commercialization opportunities. In 2012, a five year (2013-18) National Beef Research Strategy was developed following extensive consultation with a very broad group of value chain stakeholders - producers, researchers, government, service providers and funding agencies.

The 2012 National Beef Research Strategy has been instrumental in guiding industry and government research investments at both a national and provincial level across multiple funding agencies. Given the benefits and results realized through the first Strategy and the need for the Canadian beef industry to remain innovative and competitive in the world market, BCRC and Agriculture and Agri-food Canada's (AAFC) national Beef Value Chain Roundtable (BVCRT) are working to develop the next five year (2018-23) National Beef Research Strategy.

To ensure the strategy development process is truly collaborative and highly focused to target future research priorities and funding, industry input is being sought through various means including direct stakeholder consultation, an online survey, and most importantly a research workshop in Calgary on June 22-23, 2016, and an extension workshop in September 2016. The online survey was launched in March 2016, and the results will be tabulated following the May 31, 2016 survey close date. Planning is well underway for the two workshops where participants will look at the progress on research outcomes of the current Strategy, assess and define where continued research is required and evaluate knowledge and technology transfer programs on both provincial and national levels. Focus will also be placed on identifying new and emerging research priorities that should be included in the next five year Strategy.

BCRC general administration and management expenses, covered by National check-off funding, is projected at \$166,881 for 2015/16.

IV. Ongoing Research Performance Reporting and Evaluation

BCRC has taken a leadership role in communicating the value of investments, including the National checkoff, made in beef, cattle and forage research. The BCRC partnered with Canfax Research Services to develop and monitor a series of research indicators that aid in assessing the economic returns to beef research in Canada, developing BCRC research priorities, and tracking the economic benefit of BCRC funded research over the long term. An inaugural results report was developed and released in February 2014. The report outlines how dollars were invested between 2009 and 2013, and how that research is contributing to advancements in production efficiencies, quality and demand for Canadian beef. In many cases the financial impacts of deliverables to the industry were calculated; some impacts may not be fully apparent for several years. The intent is to complete a similar evaluation in 2018 upon the completion of the current 5-year research plan through the Beef Science Cluster, which ends on March 31, 2018.

The 2014 report reveals that the largest financial improvements to industry over the past five years were in the priority areas of 'animal health and welfare' and 'feed grains and feed efficiency,' as research in these areas allow for almost immediate adoption of new technology and have a high level of private investment. View the full report at: <u>http://www.beefresearch.ca/files/pdf/BCRC_results_report_jan2014.pdf</u>.

V. Financial Note

The fiscal year for BCRC is July I to June 30, therefore BCRC audited financial statements are not included in this report. In most instances, the projected expenditures in this report reflect the July to June fiscal period. Consequently the 2015/16 actual expenditures are to be finalized subject to the close of the year end on June 30th. The 2015/16 financial summary for BCRC will be available upon request after August 2016.

Projected National check-off funding allocated to research programming in 2015/16 is outlined in various sections of this report and includes the following:

Beef Science Cluster research projects - \$799,011 Non Cluster research projects - \$31,984 VBP+ - \$133,950 BCRC general program management and administration - \$166,881

