The Effect of Cereal Grain Type on Production Performance and *Clostridium perfringens* Colonization in Cattle

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By

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ABSTRACT

Three experiments were conducted to investigate the effects of various cereal grain types on the production performance of dairy cattle and the colonization of *Clostridium perfringens* in the intestinal tract of cattle. The first experiment investigated the effect of feeding two different cultivars of barley (cv. Harrington and Valier) and two different cultivars oat (cv. Derby and AC Assiniboia) grain on feed intake, milk yield and milk constituents. It was found that barley or oat may both be successfully incorporated into the concentrate portion of western Canadian dairy rations. NRC Dairy 2001 predictions for both oat and barley diets very closely predicted dry matter intake and milk production. AC Assiniboia oat fed cows produced the lowest percentage of fat ($P<0.05$) compared to the other dietary treatments. Oat fed cows produced milk with lower total solids ($P<0.05$) than barley fed cows. Experiment two investigated the effect of replacing barley with corn in the concentrate of a dairy ration on performance parameters as well as fecal shedding of *C. perfringens.* Corn can successfully replace a portion of the cereal grain in a typical western Canadian dairy ration without adverse effects on milk production, feed intake and feed efficiency. Corn fed cows produced significantly higher milk protein than barley fed cows ($P<0.05$). Dairy NRC 2001 milk production predictions were similar to observed milk yields. Mean (±SEM) level of colonization (log cfu/g) of *C. perfringens* in the feces was lower ($P<0.05$) in barley-fed (1.30±0.27) compared with corn-fed (2.47±0.27) cows. Experiment three was a survey to determine the level of shedding of *C. perfringens* in feces of dairy cattle at different ages and stages of lactation and in feedlot cattle through the backgrounding and finishing phases. No relationship was found between heifer age

and level of shedding or between days in lactation and level of shedding. Sixteen percent of the sampled cows exhibited high levels of shedding (3.02±.46) log cfu/g feces) through all stages of lactation without exhibiting adverse effects on health. No relationship was found between age of heifer and level of *C. perfringens* shedding in the feces. Steers shed significantly higher ($P<0.05$) levels of *C. perfringens* during the last phase of finishing compared with other feeding phases. Further research investigating *C. perfringens* colonization in cattle is required.

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LIST OF ABBREVIATIONS

ADF	Acid Detergent Fibre
ADIN	Acid Detergent Insoluble Nitrogen
ADL	Acid Detergent Lignin
BW	Body Weight
CFU	Colony Forming Units
CHO	Carbohydrate
CNCPS	Cornell Net Carbohydrate and Protein System
CP	Crude Protein
CV	Cultivar
DM	Dry Matter
DIM	Days in Milk
EE	Ether Extract
HBS	Hemorrhagic Bowel Syndrome
HCl	Hydrochloride
MP	Metabolizable Protein
NDF	Neutral Detergent Fibre
NDIN	Neutral Detergent Insoluble Nitrogen
NEl	Net Energy for Lactation
NPN	Non-protein Nitrogen
NRC	National Research Council
NSC	Non-structural Carbohydrate
PCR	Polymerase Chain Reaction
SCP	Soluble Crude Protein
SEM	Standard Error of the Mean
SOLS	Stage of Lactation
TMR	Total Mixed Ration
W/V	Weight (g) per Volume (ml

1.0 INTRODUCTION

The dairy industry in western Canada operates in a highly efficient and competitive market. Producers strive to improve economic efficiency while maintaining high levels of production and animal health. Saskatchewan has a unique advantage in terms of feed availability as a variety of cereal grains are produced in the province. Barley is the primary cereal grain utilized for feeding cattle; however other grains such as oat and corn may be used. Depending on feed pricing and availability; corn may be imported for use in the cattle industry. As such, there are many options and a high degree of flexibility for feed formulation in dairy rations. Feed quality in conjunction with economics will affect the inclusion of feeds in the ration. Therefore, it is important to study feed grains as well as grain targeted for human food consumption that may be shunted into the livestock feed industry.

Hemorrhagic bowel syndrome (HBS) is an emerging disease in dairy cattle. HBS is a segmental hemorrhagic jejunitis causing sudden death commonly noted in high producing dairy cattle. HBS is associated with a sudden overgrowth of *Clostridium perfringens* Type A in the intestine, an organism normally present in intestine of cattle. Subsequent production of α toxin is thought to induce acute jejunal hemorrhage and intestinal obstruction (Godden 2001). It is hypothesized that 1-2 percent of the morbidity in the mature cow population in North America is associated with HBS (Seglar 2001). Godden (2001) reported that most of the HBS cases occurred in the first 100 days of lactation. Little research has been conducted on *C. perfringens* colonization patterns and associated enteritis in feedlot cattle, but similar physiology and diet formulation suggests *C. perfringens* enteritis may also occur in feedlot cattle.

The objective of this thesis was to determine the effect of various type and varieties of cereal grain on dairy production and intestinal microbiology. Barley and oat are readily available in Saskatchewan and their feeding value in dairy rations was investigated. Due to the common utilization of corn in dairy rations, an investigation of the effect of replacing barley with corn in the dairy diet was be studied. Moreover, a survey of *C. perfringens* colonization in dairy and feedlot cattle was performed and the effect of diet supplementation on *C. perfringens* colonization evaluated.

2.0 LITERATURE REVIEW

The cattle industry in western Canada consumes a huge quantity of cereal grain. Traditionally, the most common feed grain for cattle has been barley, however, due to changes in grain commodity prices; a trend toward using other grains that may be more economical for production has occurred. Recently, there has been an increase in the amount of corn grain used in the cattle industry. Other grains, such as oat may enter feeding systems depending on the grain quality and price, however, commodity prices and animal performance must be considered. A common method of predicting animal performance for cattle is the use of model systems. Many different types of systems available; the two most common models for dairy cattle depend on specific detailed analysis of ingredient compositions and production parameters. These are the NRC Dairy, and the Cornell Net Carbohydrate and Protein System.

As feeding systems in the dairy and beef industry become intensified, one must look at all aspects of feed and effect on animal performance. One major point of interest should be the effect of feeds on production. Many factors affect performance including nutrition, environmental conditions, animal health, as well as anti-nutritional factors such as mycotoxins and chelating agents. Another important factor is the gastro-intestinal microflora of the host. Hemorrhagic bowel syndrome is an emerging disease in the dairy industry linked to an overgrowth of *Clostridium perfringens* in the intestine of the host. The exact etiology of the disease is currently unknown, however, one hypothesis is that diet composition, including the source, type and amount of specific

nutrients, such as starch and protein, may allow for opportunistic growth and colonization of *C. perfringens* in the cow intestine and result in disease (Seglar 2001).

2.1 Barley

Barley is second to wheat as the most commonly produced crop in Canada. Barley is primarily grown for malting or as a livestock feed grain. Human consumption of barley is minor in North America compared to brewing and livestock feed. There are three main marked classes of barley; two-rowed hulled, six-rowed hulled and two or six rowed hull-less barley. Two-rowed barley tends to be lower yielding and more commonly used for malting. Six-row barley tends to have a higher yield but lower malt quality than two-row cultivars and is commonly feed grade. Hull-less barley is usually used as a feed in swine diets. Since the grain doesn't have a hull it has lower fiber levels, increased digestible energy, and is better suited for monogastric animal feeding (CGC 2001).

Currently barley cultivars are classified as either malting-type or feed-type barley. Malting-type barley standards are defined by the American Malting Barley Association. Malting barley is selected for rapid fermentation of starch granules and low protein ranging 11.3-13.3% CP as a percentage of dry matter (AMBAINC 2001). Malting barley requires a high germination capacity, low moisture content (13.0-13.5%), plump kernels, and a minimum 1000 kernel weight of 40 g (CGC 2001).

Feed grade barley is rarely classified according to nutritional value for livestock. More commonly, any barley which isn't acceptable for malting standards is shunted into the feed market. Commonly, feeding malt-type barley will result in good

performance when fed to livestock. For example, when Steptoe, a high yielding feed variety, was tested for feedlot performance, it resulted in a reduced rate of gain when compared to malting-type barley (Gibson et al. 1994). Feed-type barley cultivars should be selected based upon *in situ* dry matter and starch digestibility as well as particle size (Gibson et al. 1994, Bowman et al. 1996)

2.1.1 Composition

Barley commonly contains 13.1 % CP, 12.9% NDF, 58.1 % starch (Yang et. al. 1996). Barley contains more total protein and higher levels as a percentage of dry matter of lysine, methionine, tryptophan, and cystein than corn (Pond et al. 1995).

Barley starch composition is an important aspect of the grain. Types of barley starch may be divided into three main categories: normal, waxy, and high amylose types. Normal barley contains approximately 75% amylopectin and 25% amylose (Kasha et al. 1993). Waxy barley contains 0 to 5% amylose in the starch while high amylose varieties of barley contain approximately 60% amylopectin. The proportion and type of starch is important in terms of digestibility and enzymatic degradation of the starchy endosperm. Amylose is a long chained linear polymer of α 1-4 linked glucose molecules. Amylose tends to form a helical structure (Figure 2.1) decreasing the ability of amylase and other enzymes to degrade the molecule. Amylopectin is a branched polymer of α 1-4 linked glucose molecules with α 1-6 linked branch points (Figure 2.1). The molecule consists of approximately 60,000 glucose units and about 3,000 constituent chains (Enevoldsen 1994).

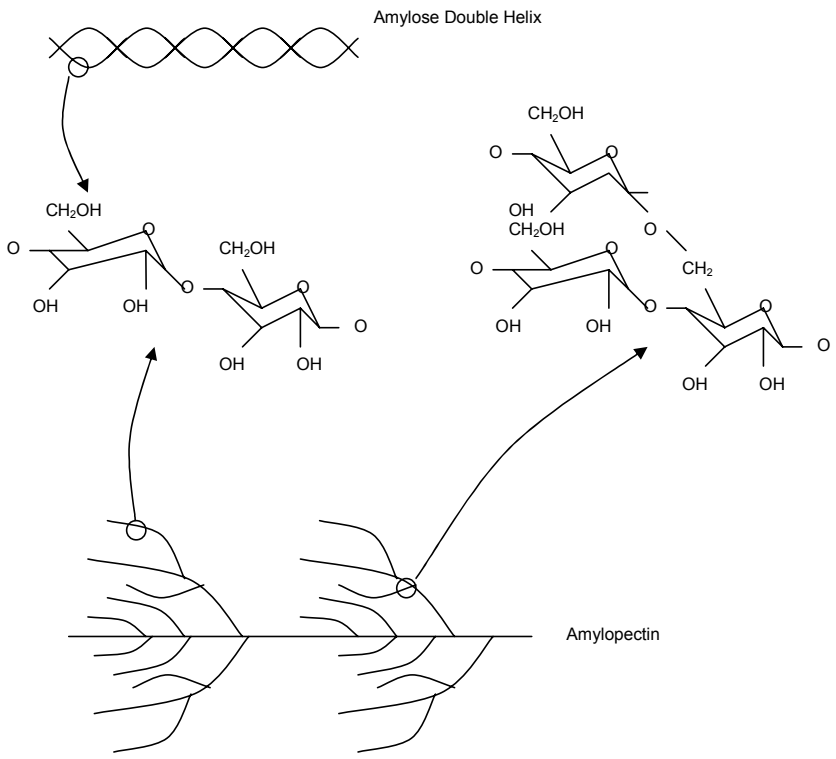

Figure 2.1 Schematic representation of amylose and amylopectin molecules as they may exist in starch granules. Amylose molecules are represented as a double helix of the two molecules. Single helixes probably exist as well. Amylopectin is represented as a part of a growth right of a starch granule. Individual branch chains of amylopectin molecules may also be found as double helixes. The center diagrams show the α (1-4) and α (1-6) linkages of the amylose and amylopectin molecules. Adapted from: Boyer and Shannon 1987.

Since amylopectin has multiple branches it is more susceptible to enzymatic degradation, allowing for more rapid degradation of the molecule into glucose units. Consequently, the proportion of amylose and amylopectin in barley starch may affect the rate and extent of digestibility of barley grain.

The amylose and amylopectin in barley starch may be arranged in various patterns, which can be viewed using scanning electron microscopy (Figure 2.2). Barley as well as most common cereal grains, has an A-type X-ray pattern (Robyt 1998). Tubers generally exhibit B-type patterns. Peas, beans and tapioca give C-type patterns intermediate between A and B patterns. These patterns are affected by environmental growing conditions and as such, each sample of barley will have a slightly different crystal structure, possibly causing differences in extent and rate of the starch degradation. Patterns of starch granule distribution are dependent upon the type of granules and number of each fraction existing in the starch.

Starch naturally exists in granules ranging in size and shape. Barley starch is composed of large granules 15-25 μm in diameter and small granules <6 μm in diameter (MacGregor and Fincher 1993). Figure 2.2 is a scanning electron micrograph showing large and small starch granules within the endosperm of barley grain. Starch granules are divided into two categories based upon size. A-type granules consist of larger granules ranging from 10 to 20 μm in size. These commonly constitute a small proportion (10-20%) of the total number of granules, but a high proportion (80-90%) of the total weight of starch (MacGregor and Fincher 1993). B-type starch granules are classified to be less than 6 μm in diameter

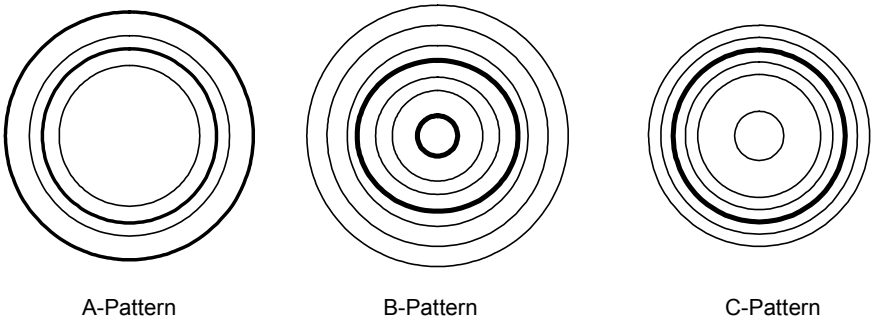

Figure 2.2 X-ray diffraction patterns of starch granules and amylose complexes. Adapted from: Robyt 1998.

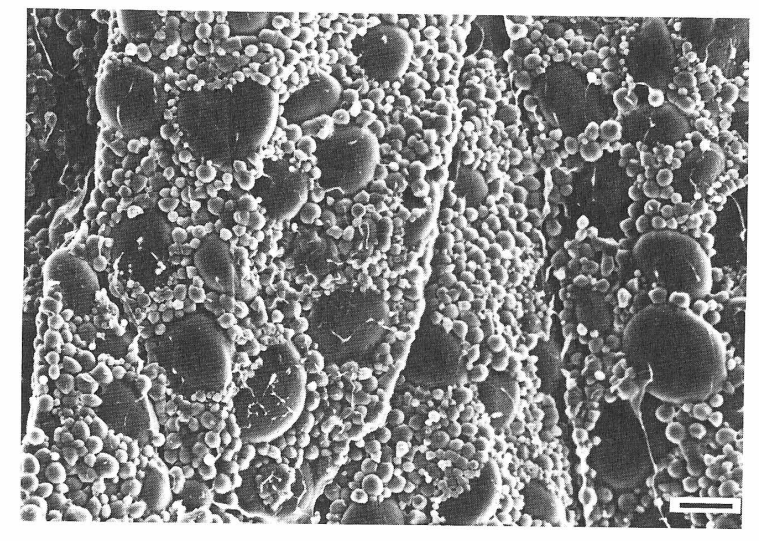

Figure 2.3 Scanning electron micrograph of large (A type) and small (B type) starch granules in barley endosperm. Adapted from: MacGregor and Fincher 1993.

The distribution of starch granules is related to the ambient temperature at which the cultivar was grown (MacGregor and Fincher 1993). Barley grown at lower ambient temperatures (10 ^{o}C) contains more small (B-type) granules and fewer large (A-type) starch granules than samples grown at higher ambient environmental temperatures (20 ^{o}C). The smaller B-type granules are commonly lost during extraction and purification. Since Saskatchewan's ambient temperature tends to be lower, it is probable that barley grown here will have more B-type granules.

2.1.2 Effect of processing on feeding value

Mathison (1996) found that feeding whole rather than rolled grain in a 33% concentrate diet resulted in a 6% decrease in the digestibility of organic matter. Furthermore, Mathison (1996) found that the extent of reduction in digestibility of whole barley is dependent upon the degree of kernel disruption. This is directly affected by the extent of chewing by ruminants. In general it is recommended that for cattle rations barley is coarsely ground to allow for microbes and enzyme to access the starchy endosperm of the kernel. Khalili et al. (2001) found no difference between dry ground and steam rolled barley in terms of milk, yield, milk protein, milk fat, or organic matter and protein digestibility.

In commercial situations, barley is often coarsely rolled to ensure a reduced rate of ruminal digestion compared to ground barley compared to grinding. However, the process must be more critically evaluated, as the size of the particle produced by the rolling process influences the rate of degradation within the rumen. Currently there is little information on the optimal degree of processing for dairy production rations.

2.1.3 Harrington Barley

Harrington barley has been the most common malting-type barley grown in Saskatchewan. It is the benchmark for two-row barley breeding for malting characteristics according to the American Malting Barley Association Incorporated (AMBAINC 2001). Harrington has characteristics unique to its cultivar. According to AMBAINC (2001), the crease is narrow, shallow, flaring toward the beard end. Its hull is smooth to slightly wrinkled. Barbs on lateral veins are absent. The kernel of the grain is broad, diamond shaped, and dehulls more readily than Klages barley, which may be of importance for processing. Harrington exhibits an extremely rapid rate of fermentation, which is optimal for the malting industry. When Harrington does not meet the criterion for the malting industry, it commonly enters the feed market. Ramsey et al. (2001) reported the chemical composition of Harrington barley as 97.5% organic matter, 60.0 % starch, 12.5% protein, and 6.3% ADF on a dry matter basis,

2.1.4 Valier Barley

Valier was selected for improved feed quality. It is a two-rowed, white kernelled, midseason spring barley (Montana State University 2001). It was developed by the Montana Agricultural Experiment Station and was released for commercial production in the United States in February of 1999. Selection was based on positive agronomic performance, large dry rolled particle size and slow *in situ* rate of dry matter disappearance (Boss et al. 1999). Valier was developed from a Lewis-Baronesse cross. Analysis by Boss et al. (1999) for nutritional characteristics showed 89% DM, 14.6% CP, 4.6% ADF and 60% starch as a percentage of dry matter.

2.5.1 Chemical and nutritive characteristics of Harrington and Valier barley

A comparison of Valier and Harrington barley chemical characteristics and ruminal degradation characteristics was conducted by Yu et al (2003) and Meier (2002). The Harrington and Valier samples were hammer milled and roller milled to compare the effect of particle size. Subsequently, the four samples were incubated in ruminally cannulated animals using the stepwise addition/all out schedule (Yu et al. 2003). Residues from the rumen incubations were analyzed for dry matter, starch and protein. Chemical analysis, particle size analysis and scanning electron microscopy were conducted on the hammer milled and roller milled samples.

The chemical composition: dry matter (DM), ash, crude protein (CP), carbohydrates (CHO), neutral detergent fiber (NDF) and soluble crude protein (SCP) were similar for Valier and Harrington barley samples (Yu et al. 2003). Valier was lower in non-structural carbohydrates (NSC), non-protein nitrogen (NPN), and acid insoluble crude protein (ADICP) than Harrington barley. Both samples had similar chemical composition to values reported by NRC (2001).

Table 2.1 shows a particle size analysis of the rolled Valier and Harrington at a gap size of 0.533 mm. Significant difference in the two cultivars was noted for all levels of screenings (Meier 2002). Valier was more evenly processed with 84.4% of the rolled grain on the top two screens. Comparatively, Harrington had its particles more evenly distributed between the second, third and fourth screens (32.17%, 34.40 and 13.0% respectively), as well as 6.92% of the rolled material showing as fines. These results

Table 2.1 Particle size distribution of Harrington and Valier barley. Adapted from Meier (2002).

	Barley Cultivar		
Screen Size	Harrington	Valier	SEM
4 mm	1.27[a]	7.32[b]	0.5014
2.362 mm	32.14[a]	77.08[b]	1.9758
1.70 mm	34.40[a]	9.23[b]	0.4196
1.40 mm	13.41[a]	2.40[b]	0.4709
1.19 mm	5.22[a]	1.36[b]	0.2241
0.840 mm	4.54[a]	0.93[b]	0.3327
Pan	6.92[a]	1.53[b]	0.7598

suggested that Valier barley was much more resistant to shattering since a lower proportion of material was recovered on the smaller screen sizes.

Scanning electron microscopy was conducted on the fractions derived from particle size analysis. For the larger particle size screenings the Harrington particles were more fractured than the Valier particles, showing higher amounts of endosperm exposed for degradation in the rumen. The Valier barley had a more structured arrangement of the starch granules within the grain, due to the complex protein matrix within the grain. The Harrington scanning electron micrographs show a higher number of the smaller, B-granules loosely adhered to the grain particle. Conversely, Valier had its B-granules tightly embedded within the grain particle. Moreover, the Valier sample continued to show more tightly embedded A and B-granules, as well as a more structured pattern of starch granule position in the rolled particles. There appears to be a trend in the scanning electron micrographs showing that Harrington barley screen fractions were more fractured than the Valier during rolling (Figure 2.4). Valier has a tighter, more structured pattern of starch granules within the grain. This may be a result of selection for soluble CP, lower intermediately degradable CP, and higher unavailable CP and slower rates of starch degradation for Valier compared to the malt-type Harrington cultivar.

Ruminally incubated samples showed no cultivar differences in DM, CP and starch degradation for hammer milled samples. Rolled samples demonstrated differences in degradation patterns. Valier had lower amounts of rapidly degradable carbohydrate, higher intermediately degradable carbohydrate and higher unavailable

carbohydrate than Harrington. Similar differences were noted for CP degradation (Meier 2002).

2.1.5 Characteristics of barley in dairy rations

Barley is a common grain in dairy concentrate in western Canada. Due to lower digestible energy compared to corn, rations must be formulated to have increased amount of concentrates compared to corn based rations (Yang et al. 1997). Due to its starch characteristics, barley is rapidly fermented in the rumen, increasing the prevalence of bloat, acidosis, laminitis, liver abscesses, and feed intake problems (McAllister et al. 1990).

Barley starch degradation is principally carried out in the rumen. Yang et al. (1997) stated that 50-94% of barley grain starch was ruminally degraded. Rapid rates of starch degradation lead to a more acidic rumen pH and reduced fiber digestion. Consequently, cattle may develop digestive disturbances such as acidosis, rumenitis, liver abscesses, and bloat (McAllister et al., 1990). Furthermore, barley is lower in digestible energy than corn because of its hull. Thus, cows fed diets based on barley receive less digestible energy, leading to decreased milk production, unless the diet contains a higher proportion of concentrate (Yang et al.1997).

Barley grain may be successfully utilized in high production dairy rations (Table 2.2). Many researchers have found barley based diets to perform as well as corn based diets in terms of milk production (Yang et al. 1997, DePeters et al.1985, Grings et al. 1992). Others have found varying results. When comparing barley based concentrates to corn based concentrates McCarthy et al. (1989) found that barley fed cows produced less milk than corn fed cows,

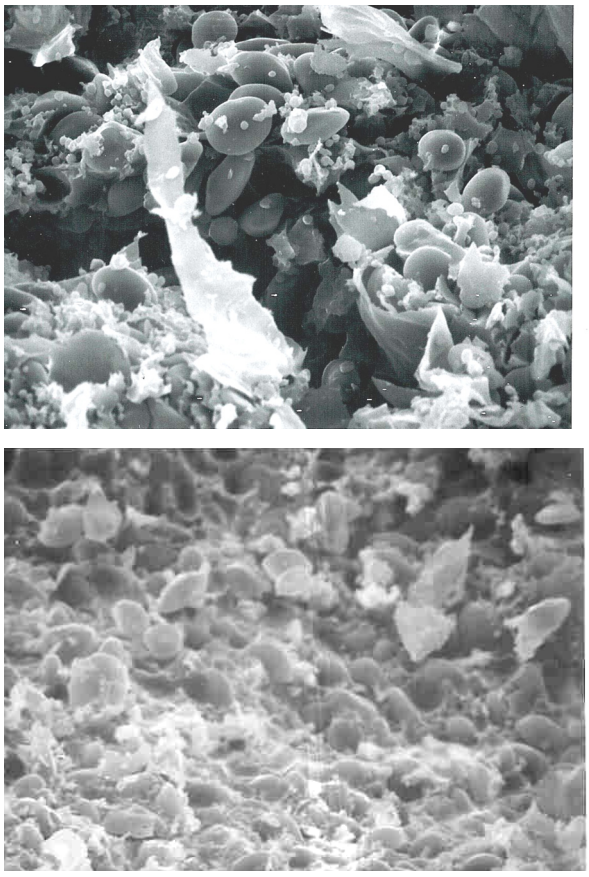

Figure 2.4 Scanning Electron Micrograph of Harrington (a) and Valier (b) barley particles from the 2.362 mm screen at a 6.25 x 10^{-2} magnification. Adapted from: Meier 2002

Table 2.2 A summary of experimental comparisons of barley, oat and corn concentrate in dairy rations.

Reference	Treatment	Effect
Khalili et al. 2001	Corn or barley grain Dry ground or steam rolled	Higher MY, MP, ML for corn fed cows Higher MUN and blood b-hydroxybutyrate levels in barley fed cows Steam rolling increased OM and N digestibility for corn but not barley
Khorsani et al. 2001	Corn or barley or 50:50 mix of corn and barley	Quadratic effect of corn on DMI, CP intake MY, 4% FCM, and MP No treatment effect on milk composition
Casper et al. 1999	Corn or barley grain Soybean meal or extruded soybean meal	MY and DMI was higher for corn fed cows No effect of protein source on MY or DMI
Yang et al. 1997	Hull-les barley, barley or corn	No effect on DMI, MY, MF, MP, or ML Higher starch intake for corn and hull-less barley fed cows
Grings et al. 1992	Corn or barley grain	No effect on DMI, MY, MF, MP or ML
Eisenbeisz et al. 1990	Corn or barley grain Recombinant bST	No effect on DMI, MY,MF, MP, ML or TS No effect on efficiency of bST
McCarthey et al. 1989	Corn or barley grain Soybean meal or fish meal	Higher MY, DMI and starch intake for corn fed cows No effect of protein source on MY or DMI Increased bypass starch and amino acids for corn fed cows
DePeters and Taylor 1985	Corn or barley grain	No effect on DMI, MY,MF, MP, ML or TS Lower fibre digestibility for barley diet

bST= Bovine somatotropin, DMI=Dry matter intake MF=Milk fat percentage, ML= Milk lactose, MP= Milk protein, MUN= Milk urea nitrogen MY= Milk yield, OM= Organic matter, TS= Total solids

It was noted the barley diet had increased ruminal degradability allowing for increased energy for microbial growth. The barley based diets tend to have lower neutral detergent fiber digestibility compared to corn, likely leading to an overall decrease in available energy for the animal (McCarthy et al. 1989; DePeters et al. 1985). Petit et al. (1999) demonstrated that feeding dairy concentrates consisting of 100% hull-less barley or a 50:50 mixture of hull-less barley and corn resulted in similar milk yield, milk composition, milk fat yield, and milk protein yields. Conversely, Beauchemin et al. (1997) determined that for diets formulated to have similar levels of non-structural carbohydrates cow fed hull-less barley had lower milk yields compared to cows fed diets containing corn or rolled barley.

2.2 Oat

The origin of oat cereal grains can be traced back to about 2000 B.C. in the Middle East, particularly the areas surrounding the Mediterranean Sea. Some of the first evidence of oat production was found in Egypt and parts of Switzerland (Can-Oat Milling 1998). Oat (*Avena sativa*) is commonly grown in the black soil zone of western Canada in areas with good drainage, sandy loam to heavy clay soil textures (Manitoba Agriculture and Food 2003). However, they require more moisture to produce a given unit of dry matter than any other cereal crop except rice, and are vulnerable to crop damage from hot dry weather, particularly from early heading through kernel production. Oat are not well suited to areas subject to high winds because of shattering (Hartman 1998).

Oat is known to have high amounts of fibre and fat. Oat tends to have lower digestible energy compared to wheat, barley and corn due to its fibrous hull. Oat also

tends to have a lower amount of starch in the kernel at 41.1% compared to other cereal grains (Crop-Livestock Interface, 2002). Oat starch has been shown to be more degradable than starch from corn but less rapidly degradable than wheat or barley starches (Mustafa 1998). Traditionally, oat has a fat content of approximately 4.7% (Pond et al. 1995). Oat has relatively high protein content and a well balanced amino acid distribution. NRC (2001) reports oat to have 13.2% CP as a percentage of dry matter. NRC reports oat to have a higher amount of arginine, isoleucine, lysine, methionone, cysteine, phenylalanine, threonine, tryptophan and valine as a percentage of crude protein compared to corn and barley grain.

2.2.1 AC Assiniboia Oat

AC Assiniboia is a high yielding, tan hulled oat cultivar developed by Agriculture and Agri-Food Canada, Cereal Research Center. AC Assiniboia is well suited for the oat growing areas of western Canada and in particular, the Black soil zone of Manitoba and Saskatchewan (Brown et al. 2001). Compared to many other commercial varieties of oat, AC Assiniboia has a thinner hull (Figure 2.5). Thompson (2001) stated that AC Assiniboia oat hulls had lower ADL and higher in vitro dry matter digestibility compared to nine other oat varieties and the highest ash content. Compared to Calibre oat, AC Assiniboia exhibited higher effective degradability of NDF and ADF. This may be attributed to the lower ADL content of the AC Assiniboia oat hulls. This decrease in hull ADL may allow for an increase in the proportion of digestible components in the grain.

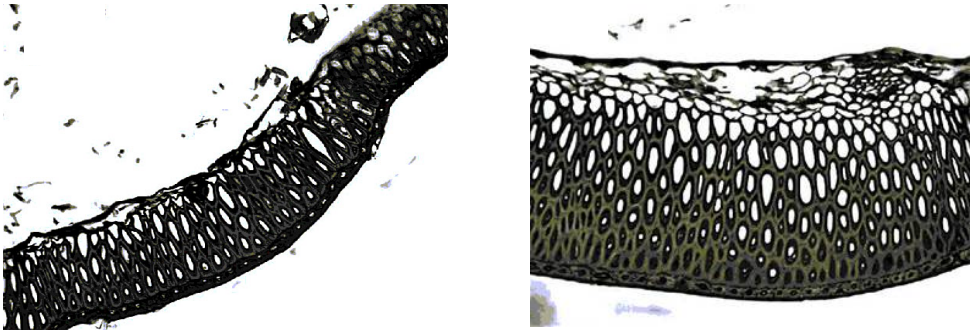

Figure 2.5 Light micrograph the thickness of the AC Assiniboia hull (a) and a commercial oat cultivar (b). Source: P. Yu

2..2.2 Derby Oat

Derby is a white spring oat (*Avena sativa)* variety developed at the Crop Development Center at the University of Saskatchewan in 1980. It was developed from crossing Calibre by Cascade. Derby was originally selected for grain quality, yield and adaptation parameters suited to western Canada (Alberta Seed Industry, 2002). Derby combines high test weight and low hull content with improved plumpness to attain excellent grain quality. Derby is similar to Calibre in height, yield and maturity and has a slightly longer straw.

2.2.3 Characteristics of oat grain in dairy rations

Feeding oat to dairy cattle commonly results in decreased performance due to the high lignin content of its hulls compared to barley or corn. The decrease in digestible energy of oat compared with other cereal grains may decrease milk production. However, Moran (1986) reported that feeding dairy cows oat based diets had improved organic matter digestibility, increased milk yield and milk fat percentage compared to wheat or barley based diets. Traditionally oat has been utilized in breeding animal rations for beef cattle and starter grower concentrates for young ruminants due the high fibre content. However, advances in oat breeding has developed hull-less and hulled oat varieties conducive to incorporation in high production dairy rations.

Little research has been conducted on the incorporation of oat grain in dairy rations. Moran (1986) noted that for cows fed *ad libitum* complete diets containing rolled barley, wheat or oat, those fed oat produced the most milk and milk fat but produced the lowest amount of milk protein. There was no significant difference

between voluntary dry matter intake. Organic matter digestibility was greatest for wheat, intermediate for barley and lowest for oat. Fecal starch concentration was greatest for barley, intermediate for wheat and lowest for oat (Moran 1986).

2.3 Corn

Corn (*Zea mays*) originated approximately 7000 years ago in Mexico and spread northward across the America to Canada and southward to Argentina (Benson and Pearce 1987). There are many different types of corn grown in North America for human food and animal production. The most common type grown comes from crossing inbred lines of yellow dent corn.

Corn is the primary grain utilized in the United States and eastern Canada for ruminant feeds. Globally, over 90% of corn produced is utilized for animal feed (Watson 1977). In the past, feeding corn has been increasingly popular in western Canada due to its economic competitiveness. Due to the relative stability of dairy producer income and the growing popularity of corn utilization in cattle, corn based concentrates paired with barley silage rations are becoming increasingly common in western Canada.

Corn grain is a warm season crop that requires about 130-140 days to mature. It shows little growth at temperatures below 10 ^{o}C and above 45 ^{o}C. Corn requires abundant sunlight and moisture for optimal yield due to its long growing season (Benson and Pearce 1987). Varieties include popcorn, sweet, flint corn, floury corn, dent corn and specialty varieties such as high lysine corn.

2.3.1 Composition

Starch is the primary carbohydrate in corn. Carbohydrate constitutes 72-73% of the kernel on a dry matter basis. NRC (2001) reports that dry ground corn has a higher amount of starch as a percentage of dry matter, compared to barley (63% starch) and oat (41 %) grain (Watson 1987; MacGregor and Fincher 1993; Racz and Heck 2002). Starch is located primarily in the horny endosperm of the kernel and is embedded in complex protein matrix (Figure 2.6). Starch granules tend to be larger in the inner portion of the endosperm and smaller closer to the pericarp (Watson 1987). Corn starch is composed of about 25-30% amylose and 70-75% amylopectin. Corn amylose has a degree of poymerization of 100-1,000 glucose units (Boyer and Shannon 1987). Amylopectin unit chains are 12-20 or 40-60 glucose units long. Corn starch granules vary in size up to 25 μm in size and are round in shape, but may take on polygonal shapes as the endosperm cells become packed with expanding starch granules (Boyer and Shannon 1987). The starch granules have a crystalline structure and exhibit a characteristic A-type X-ray diffraction pattern.

Corn contains protein and lipid at approximately 9.4% CP and 4.2% EE (NRC 2001). The protein matrix of the endosperm is an amorphous protein material embedded with discrete protein bodies. These protein bodies are almost entirely zein, a storage protein fraction extremely low in lysine and tryptophan, moderately low in threonine, valine and sulphur amino acids (Wilson 1987). Zein contains an excess amount of leucince which may antagonize the utilization of isoleucine. Zein is insoluble in water but is soluble in aqueous alcohol solutions and considered a sub-class of glutelin protein. Zein protein forms approximately half of the total protein in the kernel.

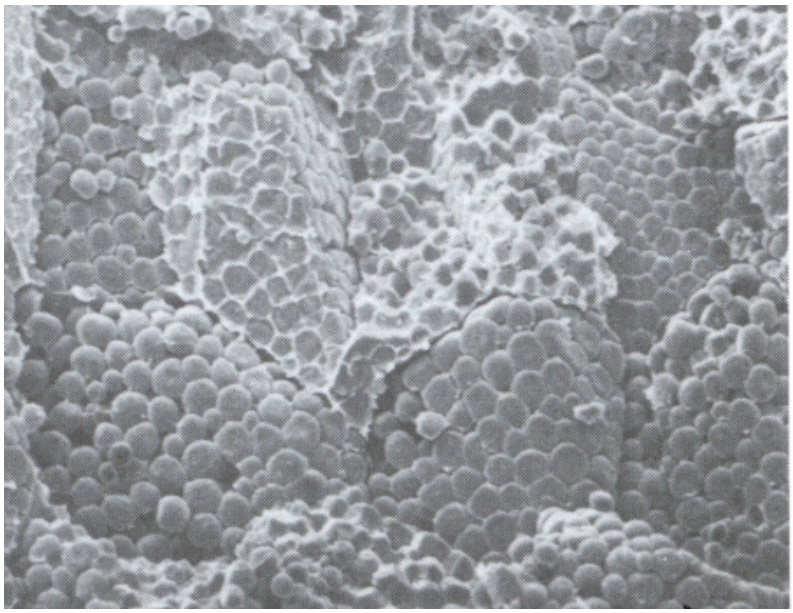

Figure 2.6 Scanning electron micrograph of endosperm of corn. Adapted from: Watson 1987.

Lipid in corn grain is primarily concentrated in the germ embryo. The increased amount of lipid and high starch content of corn makes it the benchmark cereal for comparison among different species of grain especially in terms of digestible energy.

2.3.2 Formulating dairy rations utilizing corn grain

Feeding corn grain may allow for up to 30% of the starch in the crushed endosperm to escape ruminal fermentation (Ørskov 1986). Comparatively, barley starch may be up to 90% fermented in the rumen. However, the decrease in ruminal fermentation may be compensated through intestinal digestion of corn starch and cecal fermentation. Starch granules in the corn endosperm are 5-30 μm in size and are embedded in a continuous protein matrix (Watson 1987). Khalili et al. (2001) noted that corn fed cows show higher milk production, milk protein and lactose production than cows fed barley when the grains were either ground or steam rolled. Corn-fed cows had lower milk urea concentrations and lower blood β-hydroxybutyrate concentrations than barley-fed cows. McCarthy et al. (1989) reported that when cows are fed corn based rations they have higher dry matter intake and starch intake compared to barley fed cows. In turn, cows have higher amounts of starch and amino acid reaching the duodenum for intestinal absorption, allowing for more glucose to be available for lactose synthesis.

2.4 Modelling Systems

Modelling systems are integral to the modern dairy system. They allow nutritionist and researchers to predict animal performance from rations prior to feeding

the diet. This allows one to have target production goals as well as avoid feeding inadequate diets. These model systems are primarily designed to evaluate the protein and carbohydrate fractions of rations in order to predict the amount of energy available from ruminal and post-ruminal digestion. The two most common model systems are the National Research Council model systems, such as NRC Dairy, and the Cornell Net Carbohydrate and Protein System.

2.4.1 National Research Council Dairy 2001

The National Research Council has developed a revised model system for the Nutrient Requirements of Dairy Cattle. This system applies new information and technology to current issues in the field of dairy cattle production (NRC 2001). Prediction equations utilize animal type, diet and management conditions as a basis to estimate the nutrient requirements. The feed energy equations are based on the model developed by Weiss et al. (1992). NRC Dairy contains equations that have been developed to predict dry matter intake, energy, digestibility of nutrients, as well as protein and amino acid utilization of the dairy cow. NRC Dairy allows a producer to examine a ration for the energy and protein requirements for lactation, however it lacks to ability to perform least cost analysis.. The software is used commercially to evaluate rations and as a problem-solving tool.

2.4.2 Cornell Net Carbohydrate and Protein System

The objective of the Cornell Net Carbohydrate and Protein System (CNCPS) is to predict the performance of cattle and to identify factors limiting animal performance. It is a computer based model used to evaluate feeding systems for beef and dairy cattle.

Parameters considered in the model include animal breed type, animal frame size, environment, feeding system, anabolic agents and the animal's previous plane of nutrition. The focus of the feed and ration characteristics is fractionation of the carbohydrate and protein into degradable and undegradable fractions, as well as rates of digestion and efficiencies of nutrient absorption. A description of the model and validation are described by Russel et al. (1992), Sniffen et al. (1992), and Fox et al. (1992).

The carbohydrate component of the CNCPS models is partitioned into four fractions. The A fraction is considered to be very rapidly available carbohydrate (primarily sugars) and was fermented within minutes of ingestion. Fraction B1 is similar to Fraction A but is composed of soluble carbohydrates. Fraction B1 includes starches and pectins which are fermented at rates of 5-60% per hour (Christensen et al. 1995). Fraction B2 consists of available cell wall carbohydrates fermented at a rate of 2-10% per hour. Fraction C is the unavailable or indigestible cell wall carbohydrate. Carbohydrate fractions are estimated from the feed content of non-structural carbohydrates (NSC), soluble carbohydrates and indigestible fibre (Sniffen et al. 1992). Fraction C is estimated to be 2.4 times the percent lignin in material after 72 hr of ruminal incubation. Fraction B2 may be estimated by subtracting Fraction C from the amount of ash-free NDF in a feed sample has been corrected for associated protein. NSC contains Fractions A and B1 and may be calculated by 100 minus protein, NDF corrected for protein, lipids, and ash in a feedstuff (Sniffen et al. 1992).

The amount of Fraction A carbohydrate tends to be low in ruminant diets unless lush grass or sugar refining by-products are fed. Fraction B1 carbohydrate is very

common in cereal grains because starch is a major component of these feeds. However, some starch in dried grains may be insoluble and, hence, slowly digested within the rumen. Pectins are important in legume forages, seed products, and beef pulp (Sniffen et al. 1992). All NSC fractions are fermented by ruminal bacteria that utilize either ammonia or peptides as a nitrogen source.

The CNCPS divides protein into five fractions. Fraction A is non-protein nitrogen (NPN), including ammonia, peptides and amino acids. These are rapidly converted to ammonia in the rumen. Most of the soluble protein in silages and forages is NPN. Fraction B is subdivided estimate rate of degradation in the rumen (Sniffen et al. 1992). Fraction B1 is rapidly degraded in the rumen and is a small part of the total soluble protein. The B2 Fraction of protein is the amount of buffer insoluble protein minus the protein insoluble in neutral detergent. This fraction is fermented in the rumen, but also may escape to the lower gut depending on rate of passage. Cereal grains contain B2 protein. Fraction B3 is soluble in neutral detergent but insoluble in acid detergent solution. Fraction B3, protein associated with the cell wall, is slowly degraded in the rumen. A small amount of B3 protein is found in forages, fermented grains and larger amounts of B3 are found in by-product feeds. Unavailable or bound protein is considered to be Fraction C protein. It is the protein which is insoluble in acid detergent. This commonly includes proteins associated with lignin, tannin-protein complexes, and Maillard products (Sniffen 1992). Fraction C is not degraded by ruminal bacteria and is unavailable for post-ruminal digestion and absorption. Feeds that have significant amounts of Fraction C protein include: hay-crop silages, dehydrated alfalfa, brewers dried grains and corn distillers grains.

2.5 Clostridium Perfringens

Clostridium perfringens is ubiquitous in the environment and may be considered commensal organism of mammalian intestinal tracts as well as an opportunistic pathogen. This unique status of *C.perfringens* challenges Koch's postulates as *C. perfringens* is isolated from healthy cattle. Moreover, Ewolt and Anderson (2005) did not induce clinical disease when they inoculated 12 non-lacating dairy cows with *C. perfringens* in the abomasums or jejunem. *C. perfringens* is provisionally identified as a non-motile Gram-positive bacillus spore former. The genus *Clostridium* is described as rod-shaped and ranging from 0.3-2.0 x 1.5-2.00 μm in size (Holt et al. 1994). Bacterial cells are commonly arranged in pairs or short chains. Most species are chemoorganotrophic, but some species may be chemoautotrophic or chemolithotrophic. *Clostridia* may be saccharolytic, proteolytic, neither or both (Holt et al. 1994). The genus is metabolically diverse with optimum growth temperatures ranging from 10-65^{o}C. Optimal temperature for colony growth is approximately 40 ^{o}C consistent with colonization of the intestinal tracts of most mammals. Quinn et al. (2002) recommends culturing *C. perfringens* anaerobically on blood agar enriched with neomycin at 37 ^{o}C for 48 hours. Colonies appear up to 5 mm in diameter, circular, flat, greyish and surrounded by a double ring of haemolysis.

There are five strains of *C. perfringens*, classified as types A, B, C, D, and E. Each strain produces four major extra cellular toxins known as α, β, ε, and ι, however an additional 12 toxins have been identified (Carter et al. 1995). Alpha-toxin is an enzyme also known as phospholipase-C which hydrolyzes lecithin to phosphoryl-choline and a diglyceride (Niilo 1980). Alpha toxin destroys lecithen, a key component

in cellular membranes. Targeted tissues exhibit hemolysis, necrosis or death. Commonly, in dairy cattle suffering from hermorrhagic bowel syndrome (HBS), intestinal and liver necrosis due to *C. perfringens* colonization and α-toxin production is observed. *C. perfringens* Type A is likely more widespread than any other pathogenic bacterium, being present in air, soil, dust, water and manure (Carter et al. 1995). It has been isolated from vegetables, milk, cheese, canned food, fresh meat, and shellfish . Types B, C, D, and E strains are also found in the gastrointestinal tracts of animals. Tschirdewahn et al. (1991) identified the presence of *C. perfringens* in the feces of horses, cattle, poultry and swine at an prevalence of 24%, 36%, 80% and 2% respectively. Fecal samples containing enteropathogenic strains amounted to 14%, 22%, 10% and 0% respectively. Van Metre (2005) reported that diets high in concentrates have been shown to increase the rate of isolation of *C.perfringens* from the rumen and cecum of healthy cattle.

2.5.1 *C. perfringens* associated enteritis

C. perfringens has been identified as an enteric pathogen in humans, domestic animals, and wildlife (Songer 1999). *C. perfringens* associated enteritis is a multifactorial disease, most prevalent as necrotic enteritis in domestic poultry. It commonly occurs in broiler chickens 2-4 weeks post hatching (Dahiya et al. 2006). Necrotic enteritis in chickens shows necrosis of the jejunum and ileum, which can extend the entire width of the mucosa (Songer 1999). Infection may cause decreased weight gain, depression, inappetence, anorexia, and diarrhoea. *C. perfringens* intestinal colonization in clinically normal broilers is low (about 10^4 cfu/g digesta), however when colonization levels increase to 10^7-10^9 cfu/g digesta, clinical disease develops

(Kondo, 1988). Outbreaks of necrotic enteritis are sporadic and may result in high mortality and severe economic losses (Dahiya et al. 2006). Necrotic enteritis is a multi-factorial disease and pre-disposing factors are numerous and poorly understood. Dahiya et al. (2006) reported five predisposing factors for necrotic enteritis: *Eimeria* infection, removal of coccidiostats or antibiotic growth promotants from feeds, environmental and managerial conditions, physiological stress and immunosuppression, and the nature and form of the diet.

A diet effect on level of *C. perfringens* colonization and the prevalence of necrotic enteritis in chickens has been noted. Riddell and Kong (1992) reported that mortality due to necrotic enteritis was higher among chickens fed rations based on wheat, rye, barley and oat groat than chickens fed corn-based rations. Branton et al. (1997) noted that chicks fed wheat diets enriched with dietary fibre had fewer lesions than chicks fed a wheat diet without dietary fibre. This implies that the amount of available carbohydrate in the intestine may affect the level of *C. perfringens* colonization in the intestinal tract. Riddell and Kong (1992) hypothesized that the carbohydrates in corn are less available to microbial digestion than those in other grains. Wagner and Thomas (1978) noted anaerobe counts in the ileum of chicks fed diets containing rye or pectin were two or three logarithmic cycles greater than chicks fed a corn-soybean diet. Branton et al. (1987) showed that chicks fed wheat diets are more susceptible to necrotic enteritis than chicks fed corn based diets. Kaldhusal and Hofshagen (1992) found that chicks fed barley had a significantly higher prevalence of subclinical necrotic enteritis than chicks fed a corn based diet. Moreover, a strong correlation between subclinical necrotic enteritis and growth depression was noted.

Hofshagen and Kaldhusal (1992) demonstrated that the addition of arvoparcin, a feed antibiotic, decreased the number of *C. perfringens* in the small intestine of chicks fed corn or barley at two to four weeks of age. No difference was noted between corn or barley diets. This may have been due to the amount of oat and barley in the corn diet, which may have affected *C. perfringens* counts.

C. perfringens is the most common pathogenic agent associated with enteric clostridiosis in horses (Tillotson 2002). *C. perfringens* types A and C are most often recovered from foals with bloody diarrhoea. Type A is also associated with enterocolitis in adult horses. Low amounts of grass hay and grain fed postpartum to mares may have increased the risk of disease (East et al. 2000).

Enterotoxemia in suckling Belgian Blue calves appears to be associated with *C. perfringens* (Manteca et al. 2001). In sick calves, *C. perfringens* was isolated more often and in higher quantities than the control group. 99% of the *C. perfringens* isolates belonged to the A type toxin. *C. perfringens* Type A is commonly associated with abomasal ulceration and tympany in unweaned calves (Jelinski et al. 1995; Songer 1999).

2.6 Hemorrhagic Bowel Syndrome

HBS is defined as a sporadic disorder of adult cattle characterized by acute necrohemorrhagic enteritis that primarily affects the small intestine (Dennison et al., 2001). HBS is also know to as jejunal hemorrhage syndrome and dead gut. Pathogenesis has been linked to *C. perfringens*, however, the disease model is multi-factorial and poorly understood. The prevalence of HBS has increased in recent years (Figure 2.7) as producers have become increasingly intensive in dairy management

strategies, adopting total mixed rations, lower forage to concentrate ratios and higher production goals (Seglar, 2001). De Ondarza hypothesizes that a large amount of starch reaching the intestine, altering the pH may trigger overgrowth of *C. perfringens.* Abutarbush and Radostits (2005) noted that seven of eight dairy cow affected with HBS were on a diet of barley silage, alfalfa or brome chopped hay as well as 9-12 kg of grain (dairy ration or barley). One heifer affected with HBS was on pasture without any grain. HBS is thought to be induced by an acute overgrowth of *Clostridium perfringens* Type A and subsequent α toxin production in the proximal small intestine (Songer 1999). Additionally other enteric pathogens such as Salmonella and bovine viral diarrhea virus are rarely present in HBS cases, further supporting the theory that *C. perfringens* Type A is associated with HBS (Scholtz, 2006). However, similar to necrotic enteritis in chicken, HBS is a multifactorial disease. Van Metre (2005) reported that diets high in concentrates have been shown to increase the rate of isolation of *C. perfringens* from the rumen and cecum of healthy cattle. Scholtz (2006) suggests that HBS is more prevalent in dairy herds over 500 cows with a minimum rolling herd averaged of 20,000 lb. Additionally, HBS is found more frequently in western operations and more frequently in the winter and fall. De Ondarza (2004) reports older cows in the first 100 days of lactation are most commonly affected by the disease. Abutarbush and Radostits (2005) reported 7 HBS cases being between 1 and 3.5 months post partum.

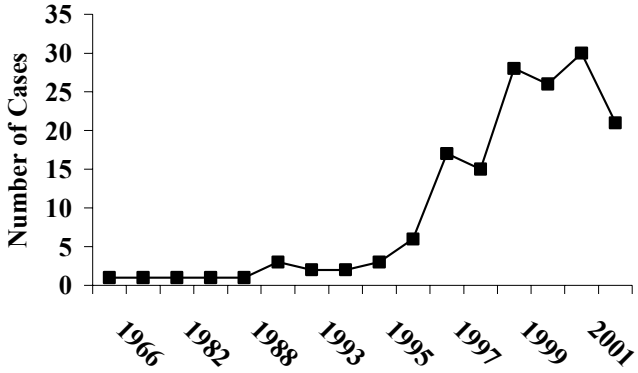

Figure 2.7 The prevalence of Hemorrhagic bowel syndrome in U.S.A. from 1966-2002. Adapted from: APHIS 2003.

Studies comparing the occurrence of *C. perfringens* type A in normal and diseased animals have consistently shown the presence of *C. perfringens* in both groups, however, increased levels of colonization are sometimes found in diseased animals (Songer 1999). Dennison et al. (2002) reported *C. perfringens* was isolated from 85% of the animals diagnosed with HBS.

HBS may be linked to feeding spoiled silages (Seglar 2001). Some suggest the HBS may be related to *Aspergillus fumigatus* contaminated silage in combination with *C. perfringens* colonization in the intestinal tract but there is little published research to support this theory (Puntenney et al. 2003). Furthermore, we typically see more mold challenges in warmer weather while HBS is more common in cooler weather (De Ondarza, 2004). It was also reported that De Ondarza (2004) utilized a direct fed microbial containing *Lactobacillus* bacteria in addition to mannan oligosaccharides and this appeared to reduce the prevalence of HBS in one high producing herd.

HBS appears to be sporadic in morbidity, affecting individual dairies, and affecting mature cows. It is estimated that HBS accounts for 1-2% of morbidity of the mature cow population (Seglar, 2001). The mortality rate of affected cows is high ranging from 85-100% due to the severe nature of this disease. Commonly, producers will see no symptoms and either find the cow dead or in systemic collapse (Kirkpatrick et al. 2001b).

The prevalence of HBS was evaluated in 2002 by the National Animal Health Monitoring System's Dairy 2002 study in the United States. Of the 1,009 dairy operations surveyed, twelve percent reported at least one case of HBS in 2002 (APHIS 2003). When the study was analyzed by herd size for the past five years, 31.2% of the

large operations (> 500 cows) reported at least one case of HBS compared to 6.4% of the small operations (< 100 cows) reporting at least one case of HBS. On average 9.1% of all farms have at least on case of HBS in the past five years. When the study was assessed by rolling herd average the prevalence of HBS increased with increasing production. The prevalence of HBS was shown to be 13.7% of the farms with herd producing greater than 9,000 kg of milk per year, 7.3% of herds producing 7,250 to 8,999 kg milk per year and 3.2% of herd producing less than 7,250 kg milk per year. A survey of the prevalence of HBS conducted in Minnesota revealed that 61% of HBS cases reported occurred in the first one hundred days of lactation, 22% in mid lactation (100-200 DIM) and 11% in late lactation (greater than 200 DIM). No cases were reported in the far off dry period, and one case was reported during the close up dry period (Godden et al. 2001).

HBS is less commonly reported in beef cattle. However, Abutarbush and Radostits (2005) reported three beef cows affected with jejunal hemorrhagic syndrome. Glock and DeGroot (1998) noted enterotoxemia in feedlot cattle commonly caused by *Clostridium chauvoeii* and *Clostridium novyii*. It was also reported that *C. perfringens* is commonly identified upon necropsy, however they questioned the role of *C. perfringens* in the disease complex as it a part of the normal bovine intestine.

Abutarbush et al. (2004) reported two beef cows necropsied to reveal the presence of a segmental area of ulceration, necrosis, and intraluminal blood clots in the lumen that were subsequently diagnosed with HBS. This was the first report of HBS in beef cattle. One 3 year old Simmental cow was on a ration consisting of free-choice legume hay and 1.4-1.8 kg of oat per head per day. The other cow was a 5 year old

Simmental presented from a herd where 2 other cows died previously exhibiting similar clinical signs. This herd was fed oat hay from round bales with no grain supplement. Both cows presented hadscant feces at the time of euthanasia. *Clostridium perfringens* was cultured from the intestines of both cows.

2.6.1 Clinical Signs

Clinical signs associated with HBS include clotted blood in the feces, abdominal distension, severe depression, acute decrease in milk production, dehydration, loss of appetite, extreme weakness and death (Dennison et al., 2002; Ceci et al. 2006). Appendix A contains a pathology report of a cow from the University of Saskatchewan dairy herd diagnosed with HBS. This animal was a second lactation cow approximately one hundred days in lactation. Upon admission to the Western College of Veterinary Medicine, she was exhibiting decreased milk production, melena, distended ventral abdomen and kicking at her belly. Cow 400 tested negative for bovine coronavirus and bovine virus diarrhoea virus. *C. perfringens* alpha and beta toxins were detected by polymerase chain reaction. Histopathology revealed massive haemorrhage in the submucosa in the jejunum. The haematoma was covered by remnants of necrotic mucosa infiltrated by degenerate inflammatory cells. The final diagnosis for the cow was segmental hemorrhagic jejunitis. Utlrasonography of HBS cows show dilated loops of the intestine with clotted blood in the lumen.

The clinical findings from Cow 400 are similar to those reported in many cases (Ceci et al. 2006, Van Metre et al. 2005, Dennison et al. 2002). Brenner et al. (2002)

described cows affected HBS as showing no clinical signs other than a severe drop in milk production on the same or previous day to the appearance of profuse bloody diarrhea and death. The cows had a normal rectal temperature, but tenaciously resisted rectal palpation. Common clinical features reported by Autbarbush and Radostits (2005) include: decreased feed intake, depression, milk depression, tachycardia, dehydration, scant or reduced feces, melena, and abdominal distention.

HBS affected cows also demonstrate changes in blood biochemistry. These animals are hyperglycemic, hypermagnesic, hypokalemic, hyponatremic, and hypochloremic (St. Jean and Anderson 1999; APHIS 2003, Appendix A). In addition to theses findings, Dennison et al. (2002) found cows with HBS had high serum bicarbonate concentrations and a high anion gap indicating metabolic alkalosis. As a result of dehydration, packed cell volume and total protein levels in the blood are also increased (St. Jean and Anderson 1999). Several affected cows had elevated serum enzyme activities. Fifteen of 19 cows exhibited high serum creatine kinase activity indicating musculoskeletal damage due to systemic disease and myodegeneration associated with recumbency. Elevated levels of aspartate aminotransferase and g-glutamy-transferase were found in several cows indicating liver damage associated with intestinal obstruction and absorption of bacteria and toxins. (Carlson 2002, Lehninger et al. 1993).

Small intestinal biopsies of HBS affected animals show severe, segmental submucosal hemorrhage and edema of the small intestine (Appendix A, Dennison, 2002). Affected cows also commonly have mixed inflammatory or cellular infiltrate. Upon necropsy, the predominant finding in cows succumbing to HBS is severe

necrohemorrhagic enteritis or jejunitis with intraluminal hemorrhage or clots (Dennison et al. 2002). Animals may also have fibrinous peritonitis. Liver necrosis maybe noted due to septicemia and α toxin entering the bloodstream (Dennison 2002).

2.7 Summary of literature review

The source of concentrate utilized in a modern dairy ration is important in terms of animal production and health. Moreover, the level of inclusion of the cereal grain and method of processing is an important factor to consider when formulating dairy rations. Too high a level of concentrate in a ration may lead to acidosis and laminitis. Increasing the amount of starch bypassing the rumen may lead to changes in the microbial community in the intestinal tract. An increase in the prevalence of Hemorrhagic Bowel Syndrome has been noted in North America in recent years. It is hypothesized that the lower amount of forage and higher levels of concentrate may lead to an increased amount of nutrient bypassing the rumen. This may allow for opportunistic growth of pathogens such as C. perfringens and subsequent disease. Understanding nutrient utilization by the dairy cow in combination with using modeling tools to predict animal performance may allow for increased productivity of dairy cattle without compromising animal health.

3.0 EFFECT OF CEREAL GRAIN CHARACTERISTICS ON PRODUCTION PERFORMANCE OF LACTATING DAIRY CATTLE

3.1 Introduction

Ration formulation on a commercial dairy operation strives to find an optimal balance between economics and production performance. Often, various cereal grains are incorporated based on price and availability. Most of the grains entering the feed market are commodities that failed to enter the human food market. Many grain farmers tend to grow barley cultivars meant for malting as they yield a higher value commodity. When grain fails to meet malting quality, it is shunted into the feed industry. These grains have different feed characteristics compared to grain grown specifically for the animal feed industry. Harrington barley has been selected for rapid rates of brewhouse fermentation coupled with low protein content to allow efficient malting. Valier, a feed-type barley, has been selected for slow rate of ruminal fermentation and larger dry rolled particle size. Decreasing the rate of fermentation in the rumen is favourable for dairy production to avoid carbohydrate overload and subsequent ruminal acidosis. Oat grain is less common in the dairy industry due to its lower digestible energy., Oat is a good source of fat for dairy cattle, and the hull of the grain provides more fibre than other cereal grains. Derby is an oat cultivar developed with low fat content for food millers. AC Assiniboia is an oat with very low acid detergent lignin levels in the hull allowing for more extensive digestion of the hull.

The objective of this study was to evaluate the effect of feeding two different cultivars of barley (cv. Harrington and Valier) and oat (cv. Derby and AC Assiniboia) grain on feed intake, milk yield and milk constituents. It is hypothesized that diets

formulated to contain grains with varying nutritive composition will affect milk yield and composition.

3.2 Materials and Methods

3.2.1 Animals and Diets

Eight Holstein cows were randomly assigned to one of four treatments in a replicated 4 x 4 Latin square design with 28 day periods. The experiment utilized four multiparous cows and four primiparous cows averaging 86 DIM in order to investigate the effect of parity on production and nutrient digestibility. Cows were housed at the University of Saskatchewan Dairy Barn, Saskatoon, SK in individual tie stalls. Cows were cared for according to the guidelines of the Canadian Council for Animal Care (1993).

Treatments consisted of fifty percent of the concentrate portion of the ration as Valier Barley, Harrington barley, AC Assiniboia oat or Derby oat (Table 3.1). Rations were 47:53 forage: concentrate ratio on a dry matter basis. Cows were fed to appetite, allowing for five percent orts. Feed intake and orts were recorded daily. The concentrate consisted of 62.5% dry rolled grains and 37.5% supplement pellets. The composition of the supplement pellets is described in Table 3.2. Pellet ingredients were hammer milled and pellet diameter was 0.48 cm. The forage portion of the TMR consisted of 60% Rosser barley silage and 40% chopped alfalfa hay on a dry matter basis. The silage and hay fraction of the TMR were mixed daily in a Harvestore stationary mixer. Concentrate was top dressed onto the forage in individual cow mangers and hand mixed to form a 3). TMR. Diets were based on NRC Dairy 2001 ration model in order to predict production performance (Table 3.3)

Table 3.1 Composition of experimental diets formulated for cows producing 40 kg milk per day with 3.6% fat and 3.2% protein.[1] (kg DM*day^{-1}).

	Barley Cultivar		Oat Cultivar	
	Harrington	**Valier**	**AC Assiniboia**	**Derby**
Valier Barley	-	6.62	-	-
Harrington Barley	6.62	-	-	-
AC Assiniboia Oat	-	-	6.62	-
Derby Oat	-	-	-	6.62
Dolly Barley	2.90	3.34	2.45	2.45
Barley Silage	7.05	7.05	7.05	7.05
Alfalfa Hay	4.70	4.70	4.70	4.70
Wheat Distillers Grain	0.40	0.40	0.40	0.40
Soybean Meal	0.90	0.68	1.13	1.13
Corn Gluten Meal	0.73	0.73	0.73	0.73
Canola Meal	0.90	0.69	1.13	1.13
Canola Oil	0.07	0.07	0.07	0.07
Molasses	0.13	0.13	0.13	0.13
Sodium Bicarbonate	0.08	0.08	0.08	0.08
Cobalt-Iodized Salt	0.08	0.08	0.08	0.08
Dynamate[2]	0.04	0.04	0.04	0.04
Vitamin-Mineral Premix[3]	0.40	0.40	0.40	0.40

[1]Proximate composition
[2] Contains 22% S, 18% K, and 11% Mg (International Minerals and Chemical Corp., Mundelein, Ill.)
[3]Supplied kg^{-1} premix (DM basis): 330,000 IU of vitamin A, 60,000 IU of vitamin D, 1,000 IU of vitamin E, 16% Ca, 8.5% P, 6.3% Na, 4.5% Mg, 2,100 mg/kg Mn, 535 mg/kg Cu, 12 mg/kg Se and 45 mg/kg I.

Table 3.2 Pellet supplement composition for each treatment .

Ingredient	Inclusion Level (% of DM)			
	Barley Cultivar		Oat Cultivar	
	Valier	Harrington	AC Assiniboia	Derby
CDC Dolly Barley	35.2	26.4	17.3	17.3
Soybean Meal	13.6	18.1	22.7	22.7
Canola Meal	13.9	18.2	22.7	22.7
Corn Gluten Meal	14.7	14.7	14.7	14.7
Wheat Dried Distillers Grains	8.0	8.0	8.0	8.0
Cobalt Iodine Salt	1.6	1.6	1.6	1.6
Canola Oil	1.3	1.3	1.3	1.3
Molasses	1.3	1.3	1.3	1.3
Sodium Bicarbonate	1.6	1.6	1.6	1.6
Dynamate[1]	0.8	0.8	0.8	0.8
Vitamin-Mineral Premix[2]	8.0	8.0	8.0	8.0

[1]Contains 22% S, 18% K, and 11% Mg (International Minerals and Chemicals Corp., Mundelein, Ill.
[2]Supplied kg^{-1} premix (DM basis): 330,000 IU of vitamin A, 60,000 IU of vitamin D, 1,000 IU of vitamin E, 16% Ca, 8.5% P, 6.3% Na, 4.5% Mg, 2,100 mg/kg Mn, 535 mg/kg Cu, 12 mg/kg Se and 45 mg/kg I.

Table 3.3 Formulated rations using Dairy NRC 2001 assuming 40 month old cows with 40 kg milk production, 3.6% milk fat, 3.0% milk true protein, 90 DIM, and 650 kg BW.

	Diet			
	Barley Cultivar		Oat Cultivar	
Nutrient	**Harrrington**	**Valier**	**Derby**	**AC Assiniboia**
DMI (kg/day)	24.5	24.5	24.5	24.5
ME (Mcal/kg DM)	2.48	2.48	2.48	2.48
Nel (Mcal/day)	38.2	38.2	38.2	38.2
Nel (Mcal/ kg DM)	1.56	1.56	1.56	1.56
NEg (Mcal/day)	1.09	1.1	1.06	1.06
Undiscoutned TDN (% DM)	71	71	70	70
MP	2579	2459	2535	2541
Ether Extract	3.0	3.0	3.8	3.8
NDF	34.9	34.9	37.4	37.4
Forage NDF (% DM)	24.4	24.4	24.4	24.4
ADF	20.7	20.5	22.8	22.3
NFC	39.7	39.8	36.3	36.3
Ca (% DM)	0.6	0.6	0.6	0.6
Ca (g/day)	65	64	69	69
P (% DM)	0.5	0.5	0.5	0.5
P (g/day)	80	79	82	82
K (g/day)	351	346	354	354
DCAD (mEQ/kg)	179	177	177	177

3.2.2 Sample Collection

Feed intake and milk yields were recorded daily. Eighteen days were allowed for adaptation and ten days were used for data collection. Milk yield and feed intake data were colleted on days 18 to 27 of each period. Milk composition was sampled twice daily on days 25 to 27. After pooling morning and evening samples, milk was stored at 4^{o}C until analyzed. Cow bodyweights were measured on days 25 to 27. Blood sampling for urea analysis occurred 2 hours post feeding on days 27 and 28. Blood samples were collected from the tail vein. Forage samples for analysis were collected between days 18 and 27 for chemical analysis. The concentrates were sampled each period for chemical analysis.

3.2.3 Chemical Analysis

TMR and silage constituents of rations were dried in a forced air oven for 48 hr prior to grinding and chemical analysis. Forage and TMR samples were ground through a 1 mm screen of a Christy Norris hammer mill (Christy & Turner, Suffolk, United Kingdom). Concentrate samples were ground through a 1 mm screen using a Table Top Reutsch hammer mill (Reutsch ZM 100, Haan, Germany). All samples were analyzed for the parameters of the Cornell Net Carbohydrate and Protein System (Sniffen et al. 1992). Dry matter (AOAC official method 930.15), crude protein (AOAC official method 954.01), ash (AOAC official method 924.05), and ether extract (AOAC official method 973.18) were determined using methodologies described by the AOAC (1990). Neutral detergent fibre with amylose and sulphate, acid detergent fibre, lignin, neutral

detergent insoluble nitrogen and acid detergent insoluble nitrogen were determined utilizing methodology described by Van Soest et al. (1991).

Milk samples were analyzed in duplicate form totals solids (AOAC official method 925.23). Milk fat was measured using the Babcock procedure (AOAC official method 989.04). Milk protein was analyzed suing the Kjeldahl procedure (AOAC official method 984.13). Lactose was analyzed using infrared spectroscopy (O-scan 605, Foss Foods, Denmark) at the Provincial Dairy Laboratory (Regina, SK). Milk urea nitrogen (MUN) was measured using a Beckman analyzer (Beckman Instruments, CA) at the Provincial Dairy Laboratory (Regina, SK). Blood urea nitrogen was measured using enzymatic/kinetic UV assay absorbance in a Roche/Hitachi anaylazer (Roche Diagnostics, Laval, QU) at the Clinical Pathology Laboratory Western College of Veterinary Medicine (Saskatoon, SK).

3.2.4 Statistical Analysis

Data was analyzed as a 4 x 4 Latin square using the general linear model of SAS (SAS Institute Inc., 1999). Animal and period were the block factors for the trial. Animal and period were treated as random and fixed respectively. Significance was noted at $P<0.05$. Mean separation was based on the Student-Newman-Keuls Test.

3.3 Results

3.3.1 Chemical Analysis

Chemical analyses were conducted on diet constituents and averaged over all four periods (Table 3.4). The silage-hay mix was determined to be 44.9% dry matter, 14.4%

protein, 30.2% ADF and 44.5% NDF. The protein contents of the Valier and Harrington barley were similar and Valier had a slightly higher level protein. The oat grains had lower protein percentages compared to the barley grain. Differences in grain protein content were adjusted in the supplement pellets by the addition of canola meal and soybean in order to produce isonitrogenous diets. Dry matter content of the supplement pellets was similar for all diets. Rations were formulated to be isonitrogenous. To adjust for the difference in protein percentages in the grains, the supplement pellets ranged from 30.5-36.2 % protein.

3.3.2 Dairy NRC 2001 Modelling

Dairy NRC 2001 modeling was conducted in order to predict production performance for the four dietary treatments. A summary of the modelling results are shown in Table 3.5. Little differences were seen among the four treatments. The Valier and Harrington barley treatments were predicted to have slightly lower dry matter intakes compared to the oat treatments. Net energy for lactation was very similar among all the dietary treatments. Target milk production for the diets was 40 kg/d. Harrington barley was predicted to yield slightly more metabolizable protein for milk production than Valier barley and the oat treatments. Dietary crude protein and rumen degradable protein were similar for all treatments. Harrington barley was predicted to have higher rumen undegradable protein than all other treatments.

Table 3.4 Chemical composition of grains, supplement pellets, and forages used in the diets.

		% of dry matter[1]				
	Dry matter (%)	**CP**	**ADF**	**NDF**	**Ca**	**P**
Forage (silage/hay)	44.9	14.4	30.2	44.5	0.84	0.27
Grains						
Dolly barley	90.5	13.7	ND[2]	ND	ND	ND
Harrington barley	86.9	13.5	ND	ND	ND	ND
Valier barley	89.6	15.3	ND	ND	ND	ND
AC Assiniboia oat	87.3	12.0	11.7	23.0	ND	ND
Derby oat	87.4	11.1	ND	ND	ND	ND
Supplement pellets for						
Valier	90.0	30.5	ND	ND	1.77	1.21
Harrington	90.5	32.9	ND	ND	2.15	1.33
AC Assiniboia	90.6	36.2	ND	ND	1.81	1.41
Derby	90.6	36.2	ND	ND	ND	ND

[1] CP = crude protein, ADF = acid detergent fiber, NDF = neutral detergent fiber, Ca = calcium, and P = phosphorus.
[2] ND = not determined.

3.3.3 Milk Production Parameters

Milk production results are summarized in Table 3.6. No differences were noted in milk, fat, protein, and lactose yields between dietary treatments. A trend ($P<0.10$) was noted for cows fed AC Assiniboia oat to have higher milk yields. Persistency of milk yield was not significantly affected by dietary treatment. However, a trend ($P<0.10$) was noted for increased persistency with oat diets. Numerically, Derby oat and Valier barley resulted in lower milk yields than Harrington barley and AC Assiniboia oat. Milk fat percentages were similar among Harrington, Valier and Derby treatments and significantly lower for the AC Assiniboia oat treatment. Feeding the Assiniboia oat ration resulted in numerically lower 3.5% fat corrected milk yields. Barley fed cows had significantly higher levels of total milk solids ($P<0.05$). No differences were noted for the dry matter intakes of forage or concentrates among diets (Table 3.7). Crude protein intakes were not significantly affected by treatment, but a trend ($P<0.10$) was noted for AC Assiniboia oat fed cows to have higher total crude protein intakes due to higher ($P<0.10$) concentrate crude protein intakes. Although not significantly, AC Assiniboia fed cows had higher total dry matter intakes per 100 kg 3.5 % fat corrected milk than all other treatments. Body weight changes were similar across all dietary treatments.

Table 3.5 NRC Dairy 2001 Predictions for experimental diets.

	Diet			
	Barley Cultivar		Oat Cultivar	
	Harrington	Valier	Derby	AC Assiniboia
Predicted DMI (kg/d)	24.5	24.5	25.1	25.1
NEl Milk (kg/d)	39.8	39.8	39.9	39.9
MP Milk (kg/d)	38.2	37.6	37.3	37.3
Target Milk Yield (kg/d)[1]	40.0	40.0	40.0	40.0
CP in Diet (% DM)	18.2	18.1	18.2	18.2
Rumen degradable CP (%DM)	12.4	12.5	12.6	12.6
Rumen undegradable CP	5.8	5.6	5.6	5.6

[1] Target milk yields predicted for milk containing 3.6% fat and 3.2% protein.

Table 3.6 Effect of feeding barley and oat cereal grain on milk production and composition.

	Barley cultivar		Oat cultivar			
	Harrington	**Valier**	**AC Assiniboia**	**Derby**	**SEM**	**P-value**
Yield, kg/d						
Actual milk	42.5	42.4	44.1	42.5	0.49	0.087
3.5 % FCM	39.9	39.5	38.7	39.9	2.41	0.348
Fat	1.32	1.3	1.21	1.32	0.04	0.096
Protein	1.38	1.34	1.4	1.46	0.06	0.269
Lactose	1.94	1.96	2.05	1.97	0.04	0.549
Persistency, %						
Actual milk	97.6	95.5	100.4	100	1.28	0.093
3.5% FCM	99.2	98.6	91.7	98.5	2.41	0.438
Milk composition, %						
Fat[1]	3.11a	3.08a	2.74b	3.10a	0.09	0.046
Protein[2]	3.26	3.18	3.19	3.45	0.14	0.119
Lactose[2]	4.58	4.63	4.66	4.66	0.05	0.147
Total solids[2]	12.3a	12.3a	11.9b	12.0b	0.08	0.026

[abc] Means with different superscripts within rows significantly different ($P < 0.05$).

3. 4 Discussion

Milk yields were not affected by dietary treatment. Milk yields for Valier barley fed cows were similar to yields reported for cows fed Steptoe barley, a feed-type cultivar (Grings et al. 1992). Valier fed cows produced 42.4 kg milk with 3.08% fat and 3.18 % protein (Table 3.6), while Steptoe fed cows produced 41.1 kg milk with 3.42 fat and 3.12% protein (Grings et al. 1992). Valier barley, a feed-type barley developed specifically for cattle (Bowman et al. 2001) was expected to support higher milk production versus Harrington barley. In a similar study Fuhr (2006) studied the difference in production performance of high producing dairy cows fed CDC Dolly barley, Derby oat or a low acid detergent lignin, high ether extract (LLH-HOG) oat. Fuhr (2006) noted similar milk production between cows fed rations containing oat and barley in the concentrate of the ration. In this study, cows fed a low LLH-HOG oat had numerically higher milk production than Derby Oat or CDC Dolly barley rations. Although not significant, LLH-HOG fed cows also had lower milk fat concentrations. Similar to Assiniboia oat, LLH-HOG has lower ADL levels in the hull and higher ether extract content. This different in composition of the grain may affect the rumen kinetics, shifting ruminal metabolism to encourage higher milk production and lower fat concentrations. Fuhr (2006) noted significantly different milk protein percentages and a trend to (P=0.10) different total solids in cows. Cows fed Derby Oat had significantly lower milk protein than CDC Dolly fed cows and cows fed LLH-HOG oat fed had significantly lower milk protein than both Derby oat and CDC Dolly barley fed cows.

Table 3.7 Dry matter intake data for cows fed various cereal grains.

	Diet					
	Barley Cultivar		Oat Cultivar			
	Harrington	Valier	AC Assiniboia	Derby	SEM	P-value
Dry matter intake, kg/d						
Forage	12.1	11.9	12.1	11.9	0.09	0.256
Concentrate	12.9	13.0	13.1	12.8	0.15	0.168
Total	25.0	24.9	25.1	24.7	0.21	0.479
Crude protein intake, kg/d						
Forage	1.75	1.71	1.74	1.71	0.01	0.159
Concentrate	3.01	3.00	3.12	2.99	0.04	0.095
Total	4.76	4.71	4.86	4.7	0.04	0.075
DM intake, kg/100 kg 3.5% FCM	63.6	63.3	65.6	62.9	1.2	0.327
Bodyweight change, kg/day	0.12	0.12	0.17	0.20	0.10	0.682
Blood urea[1], mmol/L	7.54	7.57	7.64	7.78	0.24	0.183
Milk urea[2], mmol/L	6.00	6.37	6.27	6.47	0.17	0.218

[1] Clinical Pathology Laboratory, Western College of Veterinary Medicine, Saskatoon, SK.
[2] Provincial Dairy Laboratory, Regina, SK.

Ekern et al. (2003) reported significantly higher milk production in cattle fed oat compared to barley grain. Oat fed cows also produced lower ($P<0.05$) concentrations of milk fat and milk protein. Although these findings are significant, the difference in performance may be a result of the variation in ration protein and fat composition.

Moran (1986) reported that cows fed oat produced more milk and milk fat compared to cows fed wheat and barley. However, it must be noted that cows in Moran's experiment had lower dry matter intakes (16.89-18.10 kg/d) than cows in this trial (24.7-25.1 k/d). Furthermore, milk yield was much lower (22.9-26.4 kg/d) compared to the current study (42.4-44.1 kg/d).

Yu et al. (2003) reported that Valier had lower levels of non-structural carbohydrate than Harrington barley. Also, Valier had lower rapidly degradable carbohydrate, higher intermediately degradable carbohydrate and higher levels of unavailable carbohydrate compared to Harrington barley.

Moreover, Valier had lower effective dry matter degradability than Harrington in in-situ studies (Yu et al. 2003). The AC Assiniboia and Derby oat treatments resulted in numerically higher milk production than Valier barley. The increased milk production by AC Assiniboia oat may be related to its high ruminal degradability. Thompson (2000) reported *in vitro* dry matter degradability of AC Assiniboia hulls at 68.2% to be significantly higher than that of other oat cultivars including Derby.

NRC predictions of milk production, dry matter intake and digestibility for the Harrington and Valier barley diets were similar to values reported by Meier (2002). The oat diets were predicted to result in higher dry matter intakes than the barley diets, but in the feeding trial no difference in dry matter intakes were noted. NRC Dairy 2001

DMI predicted intakes were within two percent of actual intakes demonstrating that the model is valid for predicting milk yield and intakes for high production cattle. For all dietary treatments actual milk yield exceeded the NRC predictions, indicating that the tabular values for oat and barley may be a good indicator of animal performance fed western Canadian dairy rations. Derby oat showed the highest milk production paired with significantly lower milk fat percentage than all other treatments. The fat content of the oat in combination with low forage intake may have depressed milk fat production. However, all milk fat percentages were similar to values reported by Beauchemin et al. (1997) but lower than other reported values for barley based diets (Moran 1986; Grings et al. 1992; Yang et al. 2000). The decrease in milk fat may have been affected by subclinical ruminal acidosis, as the cows were on a ration with a low forage to concentrate ratio. As ruminal pH decreases the proportion of volatile fatty acids (VFA) change shifting ruminal digestion from acetate production to propionate production (McAllen et al. 1994). Propionate is less efficiently converted into milk fat than acetate and hence, milk fat percentages drop. Furthermore, changes in rumen biohydrogenation to increase the concentration of trans 10 cis 12 conjugate linoleic acid results in a reduction in milk fat syntheseis (Bauman & Kiinari 2003).

Dry matter intakes were high for all dietary treatments, as the cows were producing large quantities of milk. Cows fed AC Assiniboia oat tended ($P<0.10$) to have higher concentrate and total crude protein intakes despite similar crude protein composition of supplement pellets between oat diets. This may be a result of numerically higher concentrate and total dry matter intakes. Furthermore, the increased

dry matter intake of AC Assiniboia fed cows resulted in higher milk yields and low milk fat percentages.

3.5 Conclusions

Barley may be successfully replaced with oat grain in western Canadian dairy rations. This trial shows that feeding oat grain may yield slightly higher milk production than barley, depending on the cultivar. Feeding Valier barley resulted in lower milk yield than other dietary treatments without affecting feed intake or milk constituents. Based on the current milk pricing structure, feeding oat that result in lower milk fat and total solids results in a lower SNF ratio which is not desirable for Western Canadian dairy economics. Feeding diets with a low (47:53) forage to concentrate ratio did not have adverse effects on animal production or feed intakes.

4.0 THE EFFECT OF CORN AND BARLEY SUPPLEMENTATION ON DAIRY PRODUCTION AND FECAL SHEDDING OF C. PERFRINGENS

4.1 Introduction

The use of corn grain in dairy diets is common in Western Canada depending on the availability and the relative cost of other grains such as barley, oat, and wheat. Due to inherent differences in the chemical and physical characteristics between corn and barley, one may expect differences in production performance in dairy diets when one replaces the other. However, very little research has been conducted investigating the effect of cereal type on gut microbiology.

A recent increase in Hemorrhagic Bowel Syndrome (HBS) has been noted in North American dairy herds. HBS is a sporadic disorder of adult cattle characterized by acute necrohemorrhagic enteritis that primarily affects the small intestine (Dennison et al. 2002). HBS is caused by a sudden overgrowth of *Clostridium perfringens* in the small intestine causing hemorrhagic jejunitis and sudden death. It is hypothesized that changes in nutrient composition of the diet by altering major cereal grain ingredients will affect the type and availability of nutrients in the rumen and/or small intestine and the level of colonization of *C. perfringens.*

The primary objective of this study was to investigate the effect of the different diets on fecal shedding of *C. perfringens.* A secondary objective was to investigate the effect of replacing barley with corn in the concentrate of the dairy ration on milk production and milk constituents.

4.2 Materials and Methods

4.2.1 Animals and Diets

Eight lactating dairy cows averaging 83 DIM were randomly assigned two treatments in a Lucas switchback design with 28 d periods. Cow were fed to appetite, allowing approximately 5% orts. Cows were housed at the University of Saskatchewan Dairy Barn, Saskatoon, SK in individual tie stalls. All animals were treated in compliance to the standards developed by the Canadian Council on Animal Care (1993). Cows were vaccinated with Covexine 8 against *Clostridium chauvoei, C. septicum, C. novyi* Type B, *C. haemolyticum*), *C. tetani* and *C. perfringens* Types C and D (Scherring-Plough Animal Health, Omaha, NE, USA).

Individual cow intakes and weigh back was recorded daily. The control diet was based upon the standard University of Saskatchewan dairy concentrate. The corn diet was formulated to contain corn as 10% of the TMR on a dry matter basis. A detailed composition of the diets is shown in Table 4.1. The concentrate portion of the ration contained 65% rolled grains and 35% supplement pellets. Rations were formulated using Dairy NRC 2001. Both rations were formulated to 48:52 forage: concentrate on a dry matter basis. The forage portion of the ration consisted of 55% barley silage and 45% chopped alfalfa hay on a dry matter basis. The forage and concentrates was fed twice daily as a TMR. Forage and concentrates were hand mixed in individual mangers. Diet transition was achieved over the first six days of each period.

Table 4.1 Ration composition (%) on an as fed basis of corn and control treatments[1].

	Treatment	
Ingredient	**Corn**	**Barley**
Alfalfa Hay	21.91	21.82
Barley Silage	25.98	25.87
Barley Grain	23.35	29.44
Corn	10.16	-
Wheat	-	2.55
Oat	-	2.62
Soy Bean Meal	6.76	6.40
Canola Meal	5.77	5.24
Wheat Dried Distiller's Grain	1.06	1.06
Corn Gluten Meal	1.06	1.06
Molasses	0.90	0.90
Mineral-Vitamin Premix[2]	1.75	1.75
Cobalt-Iodine Salt	0.37	0.36
Sodium Bicarbonate	0.37	0.36
Canola Oil	0.40	0.40
Dynamate[3]	0.15	0.15

[1]Proximate composition
[2] Supplied kg^{-1} premix (DM basis): 330,000 IU of vitamin A, 60,000 IU of vitamin D, 1,000 IU of vitamin E, 16% Ca, 8.5% P, 6.3% Na, 4.5% Mg, 2,100 mg/kg Mn, 535 mg/kg Cu, 12 mg/kg Se and 45 mg/kg I.
[3]Contains 22% S, 18% K, and 11% Mg (International Minerals and Chemicals Corp., Mundelein, Ill.)

4.2.2 Sample Collection

Feed intake and milk yields were recorded daily throughout out the experiment. Eighteen days were allowed for adaptation and ten days were used for data collection. Milk yield and feed intake data was collected on days 18 to 27 of each period. Milk composition was sampled twice daily on days 25 to 27. After pooling morning and evening samples, milk was stored at 4^{o}C until analyzed. Cow bodyweights were measured on days 25 to 27. Blood sampling for urea analysis occurred 2 hours post feeding on days 27 and 28. Blood samples were collected from the tail vein. Forage samples for feed composition were collected between days 18 and 27 for analysis. Forage samples were composited for each period for further chemical analysis. The concentrates were sampled each period for chemical analysis.

Microbial sampling for *C. perfringens* was conducted daily at approximately 09:00 during days 25 to 28 of each period. Three samples, each representing approximately 50 g of fecal matter was collected directly from the rectum into 50 ml conical tubes for analysis.

4.2.3 Chemical Analysis

TMR and silage constituents of rations were dried in a forced air oven for 48 hr prior to grinding and chemical analysis. Forage and TMR samples were ground through a 1 mm screen of a Christy Norris hammer mill (Christy & Turner, Suffolk, United Kingdom). Concentrate samples were ground through a 1 mm Table Top Reutsch hammer mill (Reutsch ZM 100, Haan, Germany). All samples were analyzed for the

parameters of the Cornell Net Carbohydrate and Protein System (Sniffen et al. 1992). Dry matter analysis (ACOAC official method 930.15), crude protein (ACOAC official method 954.01), ash (ACOAC official method 924.05), and ether extract (ACOAC official method 973.18) were determined using methodologies described by the AOAC (1990). Neutral detergent fibre with amylose and sulphate, acid detergent fibre, lignin, neutral detergent insoluble nitrogen and acid detergent insoluble nitrogen were determined utilizing methodology described by Van Soest et al. 1991).

Milk samples were analyzed in duplicate for totals solids (ACOAC official method 925.23). Milk fat was measured using the Babcock procedure (ACOAC official method 989.04). Milk protein was analyzed using the Kjeldahl procedure (ACOAC official method 984.13). Lactose was analyzed using infrared spectroscopy (O-scan 605, Foss Foods, Denmark) at the Provincial Dairy Laboratory (Regina, SK). Milk urea nitrogen (MUN) was measured using a Beckman analyzer (Beckman Instruments, CA) at the Provincial Dairy Laboratory (Regina, SK). Somatic Cell Count was analyzed at the the Provincial Dairy Laboratory (Regina, SK). Blood urea nitrogen was measured using enzymatic/kinetic UV assay absorbance in a Roche/Hitachi analyzer (Roche Diagnostics, Laval, PQ) at the Clinical Pathology Laboratory Western College of Veterinary Medicine (Saskatoon, SK).

4.2.4 Microbial Enumeration

From each 50 g fecal sample, subsamples of approximately 1 g were weighed and mixed and diluted to 10^{-1} and 10^{-3} (w/v) in sterile water containing 0.1% peptone and 0.05% cysteine HCl. Diluted samples were spread plated on blood agar (Blood

Agar Base, Bectin, Dickinson and Company, Sparks MD) containing 5% sheep blood and 0.01% neomycin (Neomix, The Upjohncompany, Orangeville, Ont.). Plates were incubated under anaerobic conditions (Gas-Pak system, Becton Dickinson and Company, Oakville, ON) at 40^{o}C for 24 hr. Samples were plated in triplicate on each day. Colonies surrounded by a double zone of haemolysis and identified as gram-positive rods after Gram staining were presumed *C. perfringens* and enumerated. Subsequently colonies were verified as *C. perfringens* using PCR typing.

4.2.5 Statistical Analysis

Dietary treatments of corn or barley were applied to animals in a Lucas-Switchback Design. Statistical analysis of production parameters was conducted as a Lucas-Switchback Design using the General Linear Model of SAS (1990). Animal and period were treated as random and fixed respectively. Significance was noted at $P<0.05$. Mean separation was based on the Student-Newman-Keuls Test (SAS 1990).

Microbial data was unavailable for the first period of the switchback design and therefore analyzed using the Mixed Procedure of SAS, utilizing day as a repeated measurement. *C .perfringens* counts were considered the dependant variable. Treatment and cow were subject effects. Sources of variation were period and day of sampling within period. An analysis of covariate structure was conducted and it was determined that compound symmetry was most effective. Degrees of freedom were determined by the Kenward-Roger method.

4.3 Results

4.3.1 Chemical Analysis

Chemical analysis of the forages and concentrates used in the study was conducted (Table 4.2). Analysis of the forage portion of the TMR was determined as 37.6 % dry matter, 15.4% protein, 34.1% ADF and 54.1% NDF. The corn and barley grain had similar dry matter percentages. The barley grain had higher percentage of protein than the corn grain. Supplement pellets were formulated to be isonitrogenous by adjusting levels of canola meal and soybean meal.

4.3.2 Dairy NRC 2001 Modelling

The corn and barley diets were evaluated using the NRC Dairy 2001. A summary of the model results are presented in Table 4.3. Production predictions as summarized in Table 4.4. The barley diet was predicted to have slightly higher predicted milk yield in terms of NEl and MP. Diets were similar for predicted dry matter intake and protein. Protein fractions of rumen degradable protein (RDP) and rumen undegradable protein (RUP) were also similar for corn and barley diets. No significant differences were noted for duodenal amino acid supply between diets.

Table 4.2 Chemical composition of grains, supplement pellets, and forages used in diets.

		% of Dry Matter[1]		
	Dry Matter (%)	**CP**	**ADF**	**NDF**
Forage (silage/hay)	37.6	15.4	34.1	54.1
Grains				
Corn	89.7	9.16	ND[2]	ND
Barley	90.5	13.7	ND	ND
Supplement pellets for				
Corn	92.07	36.8	8.5	13.6
Barley	91.61	38.7	15.0	8.7

[1] CP= crude protein, ADF= acid detergent fibre, NDF= neutral detergent fibre
[2] ND= not determined

4.3.3 Microbial Enumeration

Enumeration of *C. perfringens* was conducted in periods two and three of this study (Table 4.5). Mean (±SEM) level of colonization in the feces of barley- and corn-fed cows was 1.30±0.27 and 2.47±0.27 log cfu/g feces, respectively and was significantly different ($P<0.05$). All cows switching from corn to barley showed a decrease in fecal shedding whereas 2 of 4 cows switching from barley to corn showed a decrease, and the remaining two showed a increase. Cows were found to consistently shed either high (>1.0 log cfu/g feces) or low (<1.0 log cfu/g feces) levels of *C. perfringens* over the three day sampling period (data not shown). One cow shed low levels of *C. perfringens* throughout the experiment.

4.3.4 Production Parameters

Replacing a portion of a barley based diet with corn had no effect on milk yield and persistency of milk yield (Table 4.6). Chemical analysis of supplement pellets and TMR confirmed that the diets were iso-nitrogenous. No differences were noted between treatments for dry matter intakes or body weight change. Cows fed corn or barley also had similar somatic cell counts. There was a minimal effect on milk composition, with a significant difference in milk protein percentage, but no difference in milk fat or lactose. Cows fed corn produced a higher milk protein percentage than barley fed cows. It was also noted that corn fed cows had significantly lower milk urea and blood urea levels compared to barley fed cows (Table 4.7). Dry matter and crude protein intakes were similar for corn and barley fed cows. Corn fed cows had numerically higher dry matter intake per 100 kg 3.5 % fat corrected milk.

Table 4.3 Formulated rations using Dairy NRC 2001 assuming 40 month old cows with 40 kg milk production, 3.6% milk fat, 3.0% milk true protein, 90 DIM, and 650 kg BW

	Diet	
Nutrient	**Barley**	**Corn**
DMI (kg/day)	26.1	26.1
ME (Mcal/kg DM)	2.47	2.47
Nel (Mcal/day)	40.9	40.5
Nel (Mccal/ kg DM)	1.55	1.55
NEg (Mcal/day)	1.11	1.12
Undiscounted TDN (% DM)	71	71
MP (g/day)	2775	2757
Ether Extract (% DM)	3.1	3.2
NDF (% DM)	33.5	32.8
Forage NDF (% DM)	22.8	24.0
ADF (%DM)	20.5	20.3
NFC (%DM)	39.2	40.5
Ca (% DM)	0.6	0.6
Ca (g/day)	73	71
P (% DM)	0.5	0.5
P (g/day)	91	86
K (g/day)	393	393
DCAD (mEQ/kg)	193	202

Table 4.4 Summary of the NRC Dairy 2001 performance predictions for corn and barley diets.

	Diet	
	Barley	**Corn**
Predicted DMI (kg/d)	26.4	26.1
NEl Milk (kg/d)	43.7	43.1
MP Milk (kg/d)	41.3	41.1
Target Milk Yield (kg/d)	40.0	40.0
CP in Diet (% DM)	19.8	19.0
Rumen degradable protein (%DM)	13.9	13.1
Rumen undegradable protein (%DM)	5.9	5.9

Table 4.5 Summary of fecal shedding *C. perfringens* shedding for cows on corn or barley based diets.

Treatment	Period 2		Period 3	
	Cow	Log cfu/g	Cow	Log cfu/g
Corn	414	2.567	363	2.611
	387	3.577	421	2.059
	464	3.539	460	0.046
	462	3.602	463	1.744
Barley	363	0.823	414	0.347
	421	1.388	387	2.666
	460	0.523	464	1.859
	463	3.602	462	1.000

4.4 Discussion

4.4.1 Production Parameters

This study shows that corn and barley based rations result in similar production levels in high producing dairy cows. No significant differences were noted for milk yield, milk fat percentage or lactose percentage. The milk fat percentages for both treatments were similar to the Saskatchewan average milk fat percentage of 3.6 %. Corn fed cows had significantly higher milk protein percentage than barley fed cows. It must be noted that diet effects are confounded by the inclusion of wheat and oat in the barley ration. Higher milk protein in combination with high butterfat percentages may be of economic importance to producers paid using the multiple components pricing system and solids:non-fat ratio targets in Canada. Khalili et al. (2001) reported that corn fed cows had higher milk yield, milk protein and milk lactose than barley fed cows. Similarly, higher milk yield and dry matter intakes have been reported when feeding corn than barley (Casper et al. 1999; McCarthey et al. 1989). However, many have reported no difference in production parameters between cows fed corn or barley-based diets (Yang et al. 1997; Gringset al. 1992; Eisenbeisz et al. 1990; DePeters and Taylor 1985). Compared to the NRC model predictions, both corn and barley fed cows had higher dry matter intakes than predicted and production was slightly lower than predicted. Actual dry matter intakes were within 4% of predicted values and milk production was within 3% of predicted values. This indicates that the application of NRC Dairy 2001 for formulation of Western Canadian rations may be accurate for high producing dairy cows.

Table 4.6 Effects of replacing cereal grains in a dairy concentrate with corn on milk yield and composition.

	Diet			
	Barley	**Corn**	**SEM**	**P-Value**
Milk Yield, kg/day	38.9	39.2	0.483	0.368
Persistency of yield, %	92.3	95.5	1.523	0.083
Milk fat[1], %	3.76	3.67	0.108	0.202
Milk fat yield, kg/day	1.44	1.42	0.0194	0.197
3.5% FCM yield, kg/day	40.2	39.9	0.3515	0.430
Persistency of FCM, %	96.3	95.1	1.660	0.443
Milk protein[2], %	3.39[a]	3.42[b]	0.009	0.0213
Milk protein yield, kg/day	1.3	1.32	0.015	0.1446
Milk total solids, %	12.9	12.9	0.136	0.9395
Lactose, %	4.5	4.51	0.0139	0.688
Somatic Cell Count, (1000)	39.5	39.3	3.765	0.956

[abc] Means with different superscripts within columns significantly different ($P < 0.05$).

Table 4.7 Effects of replacing cereal grains in a dairy concentrate with corn on feed intake and utilization.

	Diet			
	Control	**Corn**	**SEM**	**P-Value**
Silage/Hay DM intake, kg/day	12.9	12.9	0.207	0.657
Concentrate DM intake, kg/day	14.2	14.4	0.299	0.611
Total DM intake, kg/day	27	27.2	0.502	0.626
DM intake, kg/100 kg 3.5% FCM	67.4	68.5	0.898	0.201
Forage CP intake, kg/day	1.95	1.96	0.024	0.631
Concentrate CP intake, kg/day	3.07	3.05	0.051	0.791
CP Intake, kg/day	5.02	5.01	0.071	0.462
Body weight change, kg/day	0.51	0.57	0.244	0.786
Blood urea, mmol/L	7.89[a]	7.16[b]	0.133	<0.001
Milk urea, mmol/L	6.57[a]	6.32[b]	0.099	0.026

[abc] Means with different superscripts within columns significantly different (P <0.05).

Corn fed cows had lower levels of blood urea and milk urea than barley fed cows, suggesting a difference in protein digestibility and conversion efficiency between corn and barley grain. This may be attributed to the zein protein in corn compared with more rapidly digestible protein in barley. Khalili et al (2001) noted a similar diet effect on milk urea in corn fed cows compared to barley fed cows.

Dry matter intakes were similar between dietary treatments, although intakes were higher than reported values (Yang et al. 1997, Khorasani et al. 2001). Corn fed cows showed numerically higher dry matter intakes per 100 kg 3.5 % fat corrected milk. This implies the corn based diet was not as efficiently utilized for milk production. Also, corn fed cows had a slightly higher body weight change throughout the study, which may indicate a difference in feed conversion efficiency between corn and barley fed cows.

NRC Dairy 2001 predictions for the corn and barley diets were compared to actual performance levels. Actual milk yields for barley and corn fed cows were similar to the target milk production of 40 kg per day. However actual milk production was lower than predicted values (43.7 kg and 43.1 kg, respectively) based on NE_L values, but milk production predictions based on metabolizable protein for lactation were only slightly higher than actual milk production. In contrast, NRC under predicted milk yields in previous chapters. Actual dry matter intakes were slightly higher for barley and corn fed cows compared to predicted dry matter intakes for the NRC Dairy modeling.

4.4.2 Microbial Analysis

Corn fed cows had significantly higher levels of *C. perfringens* in feces than cows fed the barley diet. Little research has been conducted on the effect of diet upon *C. perfringens* colonization in cattle; however, a diet effect upon necrotic enteritis has been well documented in poultry (Thomas 1978; Branton et al. 1987; Kaldhusal and Hofshagen 1992). Riddell and Kong (1992) noted that birds fed barley, wheat, rye and oat groats were more susceptible to necrotic enteritis than corn-fed birds. Similarly, Kalhudsal and Hofshagen (1992) reported a higher occurrence of sub-clinical necrotic enteritis in birds fed diets with large amounts of barley. However, due to significant differences in gut physiology, feeding regimes and rates of passage between monogastrics and ruminants it is difficult to predict diet effects on *C. perfringens* colonization in cattle. It also may be possible that there are potential genetic effects on *C. perfringens* colonization in cattle; however, there has been no research conduct on genetic effects on microbial colonization of cattle.

It is hypothesized that the differences in a cow's fecal shedding of *C. perfringens* is related to carbohydrate structure. McAllister et al. (1990) hypothesized that corn may be less available to rumen microbial digestion than the carbohydrates in wheat. Moreover, the zein protein in the horny endosperm and high oil content of corn may limit microbial digestion (McAllister et al. 1990). Barley, similar to wheat, is rapidly degradable in the rumen. Differences in degradation patterns between corn and barley may affect lower gut *C. perfringens* colonization. De Ondarza (2004) hypothesizes increased amount of starch reaching the intestine may trigger an intestinal pH change to trigger *C. perfringens* growth. Since barley grain is much more ruminal

degradable seeing lower levels of *C. perfringens* shedding compared to corn fed cows may be reasonable as less starch reaches the jejenum. In this trial dry matter intakes were high (27.0 and 27.2 kg for barley and corn fed cows). It is likely that rates of ruminal passage were increased, allowing for more starch to reach the intestine in the corn based diet. Interestingly, differences in availability of carbohydrate fractions or in the composition of carbohydrates in each cereal grain which may have altered *C. perfringens* colonization in the digestive tract did not significantly alter nutrient availability through rumen fermentation and intestinal digestion absorption as measured by production performance.

Van Metre (2005) reported that diets high in concentrates have been shown to increase the rate of isolation of *C. perfringens* from the rumen and cecum of healthy cattle. Furthermore, Van Metre (2005) suggested that rations fed in early stages of lactation tend to be rich in energy and protein and low in fibre relative to rations later in lactation, increased the risk of HBS in early lactation. It was also reported that *C. perfringens* Type A and A + B_2 can be isolated from multiple sites of the intestinal tract of cows affected with HBS at a significantly higher level than unaffected herdmates. This indicates that fecal levels of *C. perfringens* may be a good indicator of colonization in the intestine.

4.5 Conclusions

Corn could be successfully integrated into a western Canadian dairy ration while supporting high levels of dry matter intake and milk production. Corn fed cows produced higher milk protein percentages than barley fed cows. High milk protein percentage in combination with above average milk fat percentage production may

result in lower revenues for producers when targeting solids to non-fat (SNF) ratios. The difference in milk protein percentage may be linked to protein characteristics of the grains, as the diets were formulated to be isonitrogenous.

An effect of diet was noted upon *C. perfringens* colonization in dairy cows. Corn fed cows had higher *C. perfringens* counts than barley fed cows. Higher levels of *C. perfringens* in corn fed cows may be related to the amount of starch reaching the jejunum. However, there are many factors that may predispose cattle to HBS. Further research must be conducted to investigate the effect of diet composition on *C. perfringens* colonization in cattle and predisposition to hemorrhagic bowel disease.

5.0 A SURVEY OF THE PREVALENCE OF FECAL SHEDDING OF CLOSTRIDIUM PERFRINGENS IN CATTLE

5.1 Introduction

Hemorrhagic bowel syndrome (HBS) is an emerging disease in dairy cattle. HBS is a segmental hemorrhagic jejunitis causing sudden death commonly noted in high producing dairy cattle. HBS is associated with a sudden overgrowth of *C. perfringens* Type A in the intestine. Subsequent α toxin production induces acute jejunal haemorrhaging and intestinal obstruction (Godden 2001). It is hypothesized that 1-2 percent of the morbidity in the mature cow population in North America is associated with HBS (Seglar 2001). Godden (2001) reported that 61 % of the HBS cases occurred in the first 100 days of lactation, 22% occurred in mid-lactation, 11% in late lactation, no cases were reported in far-off dry cows and one cow was reported to have HBS in the close up dry cow period. Dennison et al. (2002) reported cows with HBS ranging from 9 to 319 DIM (mean, 107.5 DIM; mode, 100 DIM). Furthermore, it was determined that *C. perfringens* was isolated from 85% of cows with HBS, with 14 of 17 cultures yielding moderate to heavy growth (Deinnison et al. 2002) *C. perfringens* is normally present in the intestinal tract of cattle; however, the level of colonization is not well documented in bovine species. Abutarbush and Radostits (2005) investigated cows 30-105 days in lactation exhibiting HBS. There was no lactation relationship determined in the cows with HBS. Also one pregnant heifer and three beef cows were diagnosed with HBS in the same study. Van Metre (2005) reported that diets high in concentrates have been shown to increase the rate of isolation of *C .perfringens* from the rumen and cecum of healthy ruminants.

Little research has been conducted on *C. perfringens* colonization and associated enteritis in feedlot cattle. Abutarbush et al. (2004) reported two beef cows with jejunal hemorrhagic syndrome. One of the cows was from a herd in which two other cows that had died previously of similar clinical signs. This was the first record of HBS in beef cattle. Abutarbush and Radostits (2005) reported three beef cows (two female, one male) with HBS. The cows were diagnosed with enteritis and an obstructive blood clot in the jejunum, consistent with HBS. Glock and DeGroot (1998) discussed Clostridial enterotoxaemia as a key cause of sudden death in the feedlot. Nevertheless, some research that has been conducted on dairy cattle may apply to feedlot animals. Seglar (2001) hypothesized that ruminal acidosis may be a predisposing factor to the onset of HBS. Since ruminal acidosis is common in the finishing steers on high grain rations, one may hypothesize that feedlot animals would also be susceptible to HBS.

The objective of this study was to determine the level of shedding of *C. perfringens* in feces of dairy cattle at different ages and stages of lactation and in feedlot cattle through the backgrounding and finishing phase. Information on the prevalence of C. perfringens in cattle will be useful to determine a link between *C. perfringens* and Hemorrhagic Bowel Syndrome.

5.2 Materials and Methods

5.2.1 Animals and Survey Design

5.2.1.1 Dairy Cows

Twenty four cows were surveyed to determine the prevalence and level of fecal shedding of *C. perfringens* in the University of Saskatchewan dairy herd over a six month period. In February 2003 six cows in early (0-100 DIM), mid (101-200 DIM),

and late (201-305 DIM) lactation as well as six dry cows were rectally sampled for *C. perfringens* at monthly intervals. Cows in early and mid lactation were fed a TMR with a 50:50 forage to concentrate ratio. Cows in late lactation were fed a TMR with a 55:45 forage to concentrate ratio. Dry cows were fed a TMR with a 90:10 forage to concentrate ratio. Cows were housed at the University of Saskatchewan Dairy Barn, Saskatoon, SK. All animals were treated in compliance to the standards developed by the Canadian Council on Animal Care (1993). Cows were vaccinated with Covexine 8 against *Clostridium chauvoei, C. septicum, C. novyi* Type B, *C. haemolyticum*), *C. tetani* and *C. perfringens* Types C and D (Scherring-Plough Animal Health, Omaha, NE, USA).

5.2.1.2 Dairy Heifers

Eighteen heifers were surveyed monthly determine the prevalence and level of fecal shedding of *C. perfringens* in the University of Saskatchewan dairy herd over a six month period. Two heifers of each age: 4, 6, 8, 10, 12, 14, 16, 18 and 20 months of age were sampled. As heifers calved and began lactating sampling continued. Heifers 6 to 22 months in age were fed a ration consisting of approximately ninety percent forage. Forage was offered *ad libitum* and approximately two kilograms of barley-based concentrate was fed to the animals daily. Cows were housed at the University of Saskatchewan Dairy Barn, Saskatoon, SK in individual tie stalls. All animals were treated in compliance with the standards developed by the Canadian Council on Animal Care (1993). Heifers were vaccinated with Covexine 8 against *Clostridium chauvoei, C.*

septicum, C. novyi Type B, *C. haemolyticum*), *C. tetani* and *C. perfringens* Types C and D (Scherring-Plough Animal Health, Omaha, NE, USA).

5.2.1.3 Feedlot Steers

Twenty feedlot steers were surveyed through backgrounding and finishing periods. Fecal samples were collected every six weeks on November 25 2002, January 17 2003, February 28 2003 and March 28 2003 when the steers were sent for slaughter. During the first phase of the backgrounding period animals were fed a diet with a 57:43 forage to concentrate ratio. The second phase of backgrounding consisted of a diet with a 61:39 forage to concentrate ration. Both backgrounding diets contained barley silage, barley grain, barley greenfeed and a concentrate supplement. After the backgrounding phase steers were fed one of four diets containing zero, five, ten, or fifteen percent sunflower seeds. Five steers were allocated per diet. Sunflower seeds were used to replace barley silage in the ration. All finishing diets in the first phase consisted of a 46:54 forage to concentrate ratio. Finishing diets in phase two contained a 25:75 forage to ratio. Steers were housed at the University of Saskatchewan Feedlot, Saskatoon. All animals were treated in compliance to the standards developed by the Canadian Council on Animal Care (1993). Steers were vaccinated with Covexine 8 against *Clostridium chauvoei, C. septicum, C. novyi* Type B, *C. haemolyticum*), *C. tetani* and *C. perfringens* Types C and D (Scherring-Plough Animal Health, Omaha, NE, USA).

5.2.2 Fecal Sampling

Fecal samples were taken via the rectum to retrieve approximately 1 kg of fecal material. This sample was homogenized and 50 g of feces were collected in duplicate. Samples were stored on ice until analyzed for microbial content with 2-3 hours.

5.2.3 Microbial Enumeration

Duplicate, 50 g samples of fecal matter were further mixed and a (approximately 1 g) subsample diluted to 10^{-1} and 10^{-3} (w/v) in sterile water containing 0.1% peptone and 0.05% cysteine HCl. Dilutions were spread plated in duplicate on blood agar (Blood Agar Base, Bectin, Dickinson and Company, Sparks MD) containing 5% sheep blood and 0.01% neomycin (Neomix, The Upjohncompany, Orangeville, Ont.). Plates were incubated under anaerobic conditions (Gas-Pak system, Bectin Dickinson and Company, Oakvill, ON) at 40^{o}C for 24 hr. Colonies surrounded by a double zone of haemolysis and identified as gram-positive rods on staining were presumed *C. perfringens* and enumerated. Subsequently colonies were verified as *C. perfringens* using PCR typing.

Fecal cultures showing no growth were recorded as 0 log cfu/g feces.

5.2.4 Statistical Analysis

5.2.4.1 Dairy Cows

Statistical analysis for prevalence and level of fecal *C. perfringens* was conducted used the Mixed Procedures of SAS. All data including cows with no detectable levels of shedding (enter as 0 log cfu/g feces) was used. The model included the variables ‘stage of lactation at start of trial (SOLS), period and the interaction.

Period was the repeated variable and the SIMPLE covariance structure produced the lowest AIC and BIC scores. Means separation was achieved using the least significant difference at a significant level of $P<0.01$.

5.2.4.2 Dairy Heifers

Dairy heifer data was analyzed as a completely random design using time as a repeated measure in the mixed procedure of SAS. The main effect was age at start of trial. Mean separation used the least significant difference with significance deemed at $P<0.01$. Heifer data was also analyzed using the Regression Procedure of SAS Institute Inc. (1989) to investigate the relationship between heifer age and level of *C. perfringens* in the feces.

5.2.4.3 Feedlot Steers

Fecal shedding in feedlots animals was analyzed using a compound symmetry covariance structure in the mixed procedure of SAS. Means separation was achieved using the least significant difference at a significant level of $P<0.01$.

5. 3 Results

5.3.1 Dairy Cows

A summary of the level of shedding of *C. perfringens* in the feces of lactating dairy cattle over a six month period is noted in Figure 5.1. There was no period or period by SOLS interaction. This suggests that there is no SOLS effect on fecal levels of *C. perfringens*. It appears that the group of cows selected as mid lactation cows at the trial start had higher shedding over the study than others. Level of shedding was variable among cow groups and over the six month period. Late lactation cows tended

to exhibit lower shedding early in the survey compared to the other cows. Although not significant, late lactation cows exhibited the highest level of shedding in the July sampling period when the cows were in mid lactation. Cows in mid-lactation exhibited the highest level of shedding in April at 3.06 log cfu/g feces. Late lactation cows exhibited the lowest level of shedding at 0.25 log cfu/g. Most cows (83.3%) exhibited intermittent prevalence of shedding *C. perfringens* in the feces. Cow ranged from shedding *C. perfringens* at 0 cfu/g feces to 4.3 log cfu/g feces A percentage of sampled cows (16.7%) consistently shed high levels of *C. perfringens* in the feces through all stages of lactation. The mean level of shedding for these cows was 3.02 log cfu/g of feces, with a range of 2.00 to 5.50 log cfu/g of feces. The prevalence of fecal shedding of *C. perfringens* in the sample cows ranged from 33-35% during the study (Figure 5.2). It must be noted that all cows remained clinically normal during this survey.

5.3.2 Dairy Heifers

The results of the dairy survey for heifers are shown in Figure 5.3. Levels of *C. perfringens* in the feces ranged from 0.32 to 1.31 log cfu/g feces. All heifers remained clinically normal during this survey. The main effect was age at start of period. In June and July, levels of shedding increased as the animals aged. The prevalence of *C. perfringens* shedding is shown in Figure 5.4. Also, the prevalence of *C. perfringens* shedding increased in June and July. No relationship was found between age and fecal level of C. *perfringens*.

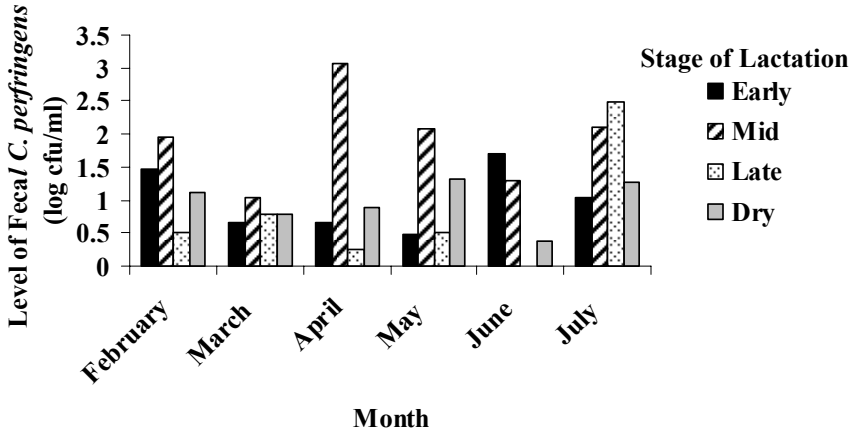

Figure 5.1 Number of *C. perfringens* (log cfu/g feces) in the feces dairy cattle over a six month period.

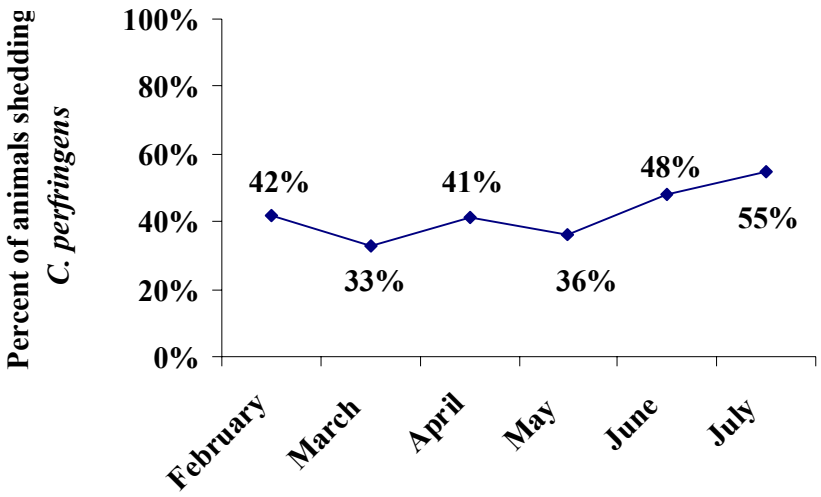

Figure 5.2 The prevalence of fecal shedding of *C. perfringens* in dairy cows (n=24) over a six month period.

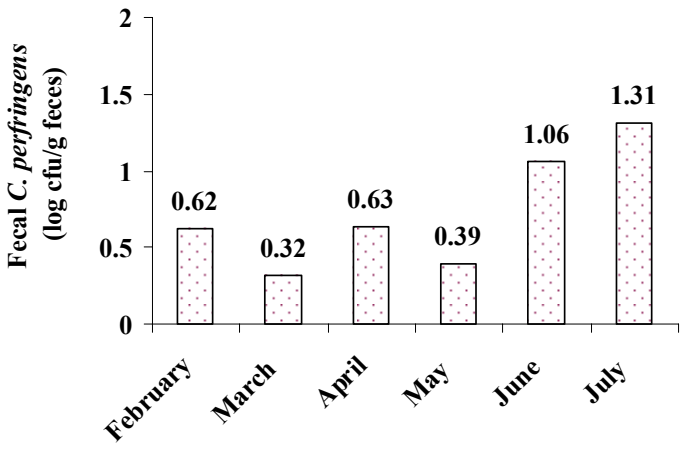

Figure 5.3 Level of fecal *C. perfringens* in dairy heifers (n=18) over a six month period.

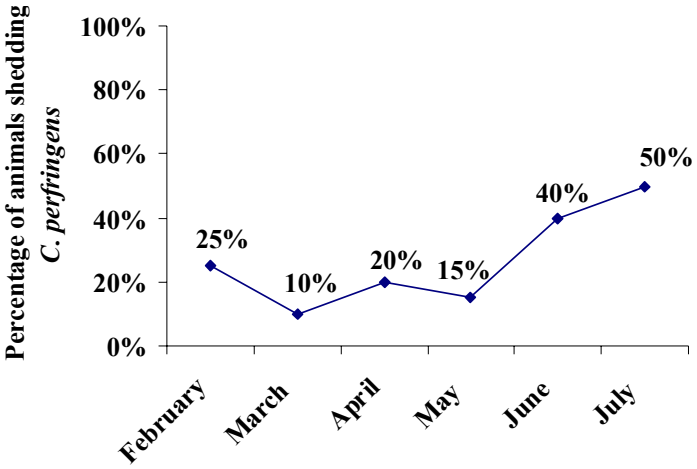

Figure 5.4 The prevalence of fecal shedding of *C. perfringens* in dairy heifers (n=18) over a six month period.

5.3.3 Feedlot Cattle

A survey of the prevalence and level of *C. perfringens* in feedlot steers was conducted to steers fed through two backgrounding and two finishing periods. All steers remained clinically normal during the survey. No significant treatment effect on *C. perfringens* shedding was noted for the amount of sunflower in the diets and there was no treatment by period interaction. The mean levels of *C. perfringens* in the feces are shown in Figure 5.5. Levels of *C. perfringens* were observed at 0.217, 0.908, 1.24, and 3.43 log cfu/g feces for the two backgrounding and two finishing phases,. No differences were noted between the two backgrounding phases. Steers on the final finishing diet had significantly higher fecal levels of *C. perfringens*..

The second finishing phase had the highest significantly higher levels of *C. perfringens* shedding than all other sampling periods ($P<0.05$). As steers were moved up through the backgrounding and finishing diets that the prevalence of fecal *C. perfringens* shedding increase (Figure 5.6). An exponential increase in the prevalence of shedding *C. perfringens* was observed as the animals were moved from one feeding phase to the next (Figure 5.7).

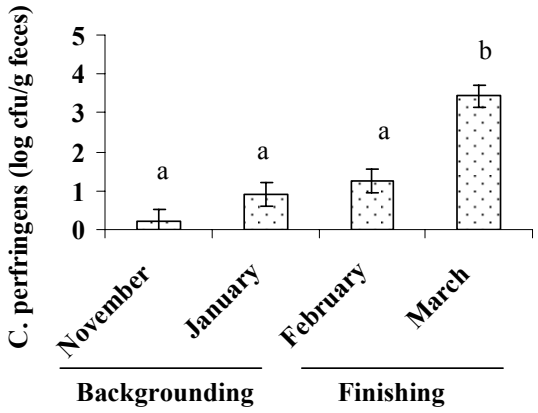

Figure 5.5 Mean level of *C. perfringens* in the feces of feedlot steers (n=20) sampled November through March.

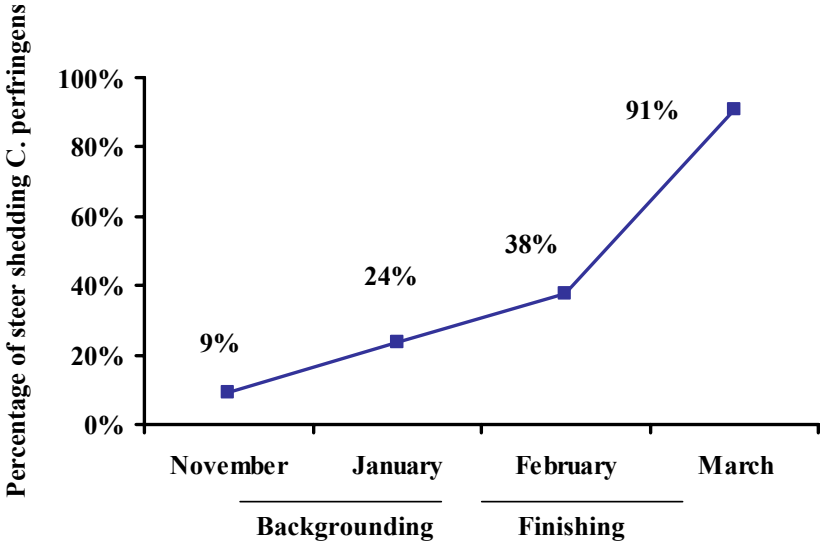

Figure 5.6 Prevalence of fecal shedding of *C. perfringens* by feedlots steers (n=20) over time.

5.4 Discussion

5.4.1 Dairy Cows

In the survey of fecal levels of *C. perfringens* in dairy cows, no clear effect of stage of lactation was observed. This is in contrast to common reports of an increased prevalence of HBS in fall and winter (Abutarbush and Radostits, 2005; De Ondarza, 2004). Over the six month sampling period the average prevalence of shedding in dairy cows was 43%. Tschirdewahn et al. (1991) reported a 36% prevalence of faecal *C. perfringens* shedding in cows, however, it was not stated if these were dairy or beef animals. It was also reported that counts of *C. perfringens* reached values up to 10^4 cfu/g feces, however, most (64%) values were at levels less than 10 cfu/g feces. Low levels of *C. perfringens* shedding were observed in 83% of the sample group observed.

In this study, the level and prevalence of fecal levels of *C. perfringens* were variable. Little research has been conducted on *C. perfringens*, however, *Escherichia coli* 0157:H7 shedding has been extensively studied in cattle. Many have noted a marked month to month variation in the prevalence of *E. coli* 0157 in cattle with rates being highest in spring and late summer (Champan et al. 1997; Van Donkersgoed et al. 1999; Elder et al. 2000; Miller et al. 2003). Chapman et al. (1997) also reported that *E. coli* 0157 was found in a higher percentage of dairy cattle (16.1%) than beef cattle (13.4%). It is of interest to note that there are a small percentage of cows that consistently shed *C. perfringens* in the feces through all stages of lactation similar to repeated isolation of *E. coli* 0157:H7 from healthy beef and dairy cattle. (Wells et al. 1991; Hancock et al. 1994; Zhao et al. 1995).

Many have reported an increased prevalence of HBS in early lactation (Dennison et al. 2002; Kirkpatrick et al. 2001; Seglar 2001); however, the survey results reported here do not indicate higher levels of *C. perfringens* in early lactation cows. Unfortunately, no statistical analysis has been performed in most papers examining the relationship between days in lactation and mean level of *C. perfringens*. Additionally, other possible predisposing factors such as nutrition, immune status and genetics have yet to be fully investigated. In Chapter Four of this thesis, it was noted that there was a significant diet effect on fecal level of *C. perfringens* in high producing dairy cows. The high degree of variation in prevalence and level of fecal *C. perfringens* shedding this study gives cause for further research in the field utilizing a larger number of animals and a longer survey period in order to address the possibility of a seasonal effect on *C. perfringens*.

5.4.2 Dairy Heifers

The prevalence and level of shedding *C. perfringens* in dairy heifers remained low until the warmer summer months of June and July however, no significant differences were noted among sampling dates. Moreover, no significant age and period effect or interaction was determined. Although not significant in this trial, it may be of merit to further investigate a seasonal effect on *C. perfringens* shedding in young cattle. Another possibility is that as the heifers aged, the level of shedding increased. A similar trend was noted in feedlots steers in this study. In general, the level of shedding heifers was low and may be associated with the high level forage in the diet. High forage rations have lower amounts of available carbohydrate for microbial digestion

reaching the small intestine, limiting the possibility for microbial growth and colonization.

5.4.3 Feedlot Cattle

The level of colonization and prevalence of shedding of *C. perfringens* were surveyed in feedlot steers. As the steers aged and were fed diets with an increasing amount of grain, the level of shedding increased. During the last finishing phase, steers had significantly higher *C. perfringens* counts than all other feeding phases. Also, as animals progressed through finishing, the percentages of animals shedding *C. perfringens* in their feces increased exponentially.

Although little research has been conducted on *C. perfringens*, many have investigated fecal *E. coli* shedding in feedlot cattle. When investigating genotype and diet influences of *E. coli* 0157:H7 in feedlot cattle, Berry et al. (2006) found no effect of diet or genotype on *E. coli* 0157:H7 shedding in feces. Generic *E. coli* fecal populations were greater ($P<0.001$) in the finishing period than the growing period of this trial. Berry et al (2006) hypothesized that the increase in generic *E. coli* is in response to higher starch loads in the colon. However, many confounding factors were noted including seasonal variation of shedding, chronic shedding of pathogens, and horizontal transmission of pathogens among animals. . Many have noted a marked month to month variation in the prevalence of *E. coli* 0157 in cattle with rates being highest in spring and late summer (Champan et al. 1997, Van Donkersgoed et al. 1999, Elder et al. 200, Miller et al. 2003).

Research conducted on intestinal *C. perfringens* colonization and associated enteritis in feedlot cattle is very limited. Nevertheless, some research has been

conducted on dairy cattle and the occurrence of HBS. Seglar (2001) hypothesized that ruminal acidosis may be a predisposing fact to the onset of HBS. Since ruminal acidosis is common in the finishing steers on high grain rations, one may hypothesize that feedlot animals would also be susceptible to HBS. Abutarbush et al. (2004) reported 2 beef cows with HBS, indicating that it may be a disease complex of interest to the feedlot industry. Glock and DeGroot (1998) noted enterotoxemia in feedlot cattle commonly caused by *Clostridium chauvoeii* and *Clostridium novyii*. The survey indicates that as steers move onto diets with increased energy density and starch levels, there may be an increased risk for cattle to develop HBS. In this survey, no seasonal effect was noted, however in dairy cattle, De Ordanza (2004) noted that HBS is found more frequently in western operations and more frequently in the winter and fall.

5.5 Conclusions

C. perfringens is a normal constituent of the intestinal microflora in cattle. A trend is noted that as cattle age, the level and prevalence of colonization increases. It was revealed the levels of *C. perfringens* shedding in dairy cattle are variable. Late lactation cows tended to have lower counts than other cows and mid lactation cows tended to have the highest level of shedding. Throughout the survey period all animals remained clinically normal. It has been proposed that rations rich in energy and protein and fibre depleted relative to diets fed later in lactation place cows at a greater risk for HBS (Van Metre et al. 2005). Dairy heifers tended to have low levels of *C. perfringens* colonization. This may be the result of age or the high forage ration these animals were fed.

Little research has been conducted in terms of characterizing *C. perfringens* in healthy cattle. Ewold (2005) attempted to induces HBS by infusing *C. perfringens* in the abomasums or jejunum or dairy cattle. None of the animals developed any clinical signs of HBS in the cows, indicating that HBS is a multi-factorial disease complex. Most published research on HBS are retrospective case studies with no controls and a possible necropsy bias. Further research needs to be conducted on cattle in respect to diet, stress, environmental and genetic effects upon the incidence and development of HBS in cattle.

This survey shows that *C. perfringens* is abundant in feedlot animals that are on high grain rations. These results are similar to studies investigating the level of *E. coli* in cattle on high grain rations. Moreover, as animals are fed increasing levels of concentrate, *C. perfringens* counts and shedding tends to increase. Further research is needed to validate these findings with more animals and possibly a longer sampling period in order to address possible seasonal differences in *C. perfringens* colonization and shedding.

6.0 GENERAL DISCUSSION AND CONCLUSIONS

The link between nutrition and disease has long been established in cattle. Exploring the link between specific feeding programs, production levels, and disease; merits further investigation. The Western Canadian commercial dairyman strives for higher production and health standards through aggressive feeding and management programs. The balance of maximum production and health is further complicated by variable feed costs, quality and availability. HBS is an emerging disease in cattle. Dennison et al. (2002) survey of 22 cases of HBS between 1997 and 2000 noting 77% of cows died or were euthanized. Determining predisposing factors to this disease is key as most cows die suddenly as a result.

In the first experiment it was determined that barley may be successfully replaced with oat grain in western Canadian dairy rations. The trial demonstrated that feeding oat grain may yield slightly higher milk production than barley, depending on the cultivar. Feeding Valier barley resulted in lower milk yield than other dietary treatments without affecting feed intake or milk constituents. Feeding diets with a low (47:53) forage to concentrate ratio did not have adverse effects on animal production or feed intake. NRC Dairy 2001 predictions for barley and oat diets fed in western Canada closely predicted dry matter intakes and milk production. These findings offer producers a means of flexibility in designing rations for high producing cows depending on feed availability and cost.

Experiment two determined that corn could be successfully integrated into a western Canadian dairy ration while supporting high levels of dry matter intake and

milk production. Corn fed cows produced higher milk protein percentages than barley fed cows. High milk protein percentage in combination with above average milk fat percentage production may result in lower revenues for producers when targeting solids to non-fat (SNF) ratios. The difference in protein efficiency may be linked to protein characteristics of the grains, as the diets were formulated to be isonitrogenous.

An effect of diet was noted upon *C. perfringens* shedding in dairy cows. Barley fed cows had lower *C. perfringens* counts than corn fed cows. The increased level of *C. perfringens* in corn fed cows may be a result of increased amounts of starch reaching the jejunum. Also, barley starch is more easily digested by ruminal microbes compared to corn starch, resulting in less starch reaching the jejunum. It was noted that some cows were consistently high or low shedders throughout this trial. Other factors influencing *C. perfringens* shedding may include feed intake behavior, immune status and genetics. Animals that have larger meals may have higher passage rates through the gut, allowing for more starch to reach the intestine. Immunosuppression may allow for opportunistic growth of *C. perfringens* in the intestine and genetics may predispose individual cows to higher *C. perfringens* fecal shedding and increased risk to contract HBS. Further research must be conducted to investigate the effect of diet composition on *C. perfringens* shedding in cattle and predisposition to haemorrhagic bowel disease.

C. perfringens is a normal constituent of the intestinal microflora in cattle. A trend is noted that as cattle age, the level and prevalence of colonization increases. It was revealed the levels of *C. perfringens* shedding dairy cattle are variable. Late lactation cows tended to have lower counts than other cows and mid lactation cows tended to have the highest level of shedding. Throughout the survey period all animals

remained clinically normal. It has been proposed that rations rich in energy and protein and fibre depleted relative to diets fed later in lactation place cows at a greater risk for HBS (Van Metre et al. 2005). Dairy heifers tended to have low levels of *C. perfringens* colonization. This may be result of age or the high forage ration these animals were fed. Little research has been conducted in terms of characterizing *C. perfringens* in healthy cattle. It may be of merit to survey a larger number of animals over a longer period of time to further quantify the prevalence and level of fecal *C. perfringens* shedding in dairy cattle.

It was determined that *C. perfringens* is abundant in feedlot animals that are on high grain rations. As animals are fed increasing levels of concentrate, *C. perfringens* counts and shedding tends to increase. These results are similar to findings of *E. coli* shedding in feedlot cattle. More research is needed to validate these findings with more animals and a longer sampling period in order to address possible seasonal differences in *C. perfringens* colonization and shedding.

This research is valuable to gain further insight to feeding effect on cow health and production. Investigating various feeding programs and their effect on product and fecal levels of *C. perfringens* allows one to develop rations to minimize the risk of HBS while maintaining high levels of milk production in dairy herd. Since HBS is usually a fatal disease, it is imperative to determine strategies to decrease the risk of contracting HBS. Although the link between HBS and high starch diets is not definitive, investigating feeding protocols to minimize HBS in cattle will allow the industry to move toward healthier, more productive cattle.

7.0 REFERENCES

Abutarbush, S.M. and Radostits, O.M. 2005. Jejunal Hemorrahge syndrome in dairy and beef cattle: 11 cases (2001 to 2003). Can. Vet. J. **46**:711-715.

Abutarbush, S.M., Carmalt, J.L., Wilson, D.G., O'Connor, B.P., Clark, E.G. and Naylor, J.M. 2004. Jejunal hemorrhagic syndrome in 2 Canadian beef cows. Can Vet. J. **45**:48-50.

Alberta Seed Industry. 2002. Spring Oat: Derby. Retrieved July 7, 2002 from http://www.seed.ab.ca/varieties/o-derby.shtml.

AMBAINC. 2001. Malting barley breeding guidelines: Ideal malt criteria. Retrieved on January 19, 2002 form www.ambainc.org Web address http://www.ambainc.org/ni/2001%20GUIDELINES.pdf

AOAC. 1990. Official Methods of Analysis (15th Ed.) Association of Official Analytical Chemists. Arlington, Va. USA.

APHIS. 2003. Hemorrhagic Bowel Syndrome. APHIS Info Sheet. United States Department of Agriculture, Animal and Plant Health Inspection Services. May 2003.

Bach, S. J., McAllister, T. A., Veira, D. M., Gannon, V. P. J., and Holley, R. A. 2002. Transmission and control of Escherichia coli O157:H7 - a review. Can. J. Anim. Sci. **84:**475-490.

Bauman, D.E. and Griinari, J.M. 2003. Nutritional regulation of milk fat synthesis. Ann. Rev. Nutr. **23**: 203-227.

Beauchemin, K.A. and Rode, L.M. 1997. Minimum versus optimum concentrations of fiber in dairy cow diets based on barley silage and concentrates of barley or corn. J. Dairy Sci. **80:**1629-1639.

Beauchemin, K.A., Rode, L.M., and Yang, W.Z. 1997. Effects on non-structural carbohydrates and source of cereal grain in high concentrate diets of dairy cows. J. Dairy Sci. **80**:1640-1650.

Benson, G.O. and Pearce, R.B. 1987. Corn perspective and culture. Pages 1-29. *in* Corn: Chemistry and Technology. S.A. Watson and P.E. Ramstad, eds. American Association of Cereal Chemists Inc. St. Paul, MN, U.S.A.

Berry, E.D., Well, J.E., Archibeque, S.L., Ferrel, C.L., Freetly, H.C., and Miller, D.N. 2006. Influence of genotype and diet of steer performance, manure ordor, and carriage of pathogenic and other fecal bacteria. II. Pathogenic and other fecal bacteria. **84**:2523-2532.

Boss, D.L., and Bowman, J.G.P. 1996. Barley varieties for finishing steers II. Ruminal characteristics and rate, site, and extent of digestion. J. Anim. Sci. **73**:1973-1981.

Boss, D.L., Bowman, J.G.P., Surber, L.M.M., Anderson, D.C., and Blake, T.K. 1999. Feeding value of two Lewis x Baronesse recombinant inbred barley lines, LB13 and LB30, for finishing steers. Proc. West Sect. Amer. Soc. An.Sci., Vol. **50**. Retrieved on July 27, 2001 from http://hordeum.oscs.montana.edu.cowdocs.cow299.htm

Boyer, C.D., and Shannon, J.C. 1987. Carbohydrates of the kernel. Pages 253-272. *in* Corn: Chemistry and Technology. S.A. Watson and P.E. Ramstad, eds. American Association of Cereal Chemists Inc. St. Paul, MN, U.S.A.

Bowman, J.G.P, Blake, T.K., Surber, L.M.M., Habernicht, D.K., Daniels, T.K., and Daniels, J.T. 2001. Genetic factors controlling digestibility of barley for ruminants. Proceedings, Western Section, American Society of Animal Science. Retrieved on June 29, 2001 from http://hordeum.oscs.monatan.edu/cowdocs/cow596.htm.

Branton, S.L., Lott, B.D., Deaton, J.W., Maslin, W.R., Austin, F.W., Pote, L.M., Keirs, R.W., Latour, M.A., and Day, E.J. 1997. The effect of added complex carbohydrates or added dietary fiber on necrotic enteritis lesions in broiler chickens. Poultry Sci. **76**:24-28.

Brenner, J., Orgad, U., Tiomkin, D., Vaksmann, I., and Perl, S. 2002. Adult bovine jejunal hemorrhagic syndrome- An emerging dairy disease? Israel Vet. Med. Assoc. **57**:1. Retrieved from http://www.isrvma.org/article/57_1_1.htm, on Jan 21, 2003.

Brown, P.D., Duguid, S.D., Haber, S., Chong, J., Harder, D.E., Menzies, J., Noll, J.S., and McKenzie, R.I.H. 2001. AC Assiniboia Oat. Can. J. Plant. Sci.**81**:77-79.

Buchko, S.J., Holley, R.A., Olson, W.O., Gannon, V.P.J., and Veira, D.M. 2000. The effect of different grain in diets on fecal shedding *Escherichia coli* 0157:H7 by steers. J. Food. Protect. **63:**1467-1474.

Can-Oat Milling. 1998. What are oat? Retrieved on Aug. 12, 2003 from http://www.can-oat.com/what/what.html

Canadian Council on Animal Care. 1993. Guide to care and use of experimental animals. Vol. 1. CCAC, Ottawa, Ont.

Carter, G.R., Chengappa, M.M, and Roberts, A.W. 1995. Clostridium. Pages134-141 i*n*: Essentials of veterinary microbiology 5th ed. G.R. Carter, M.M. Chengappa, and A.W. Roberts, eds. Williams and Wilkins. Philadelphia, USA.

Casper, D.P., Maiga, H.A., Brouk, M.J., and Schingoethe, D.J. 1999. Synchronization of carbohydrate and protein sources on fermentation and passage rates in dairy cows. J. Dairy Sci. **82**:1999.

Ceci, L, Paradies, P, Sasanelli, M, De Carpariis, D., Guarda, F., Capucchio, M.T., and Carelli, G. 2006. Haemorrhagic Bowel Syndrome in Dairy Cattle : Possible Role of *Clostridium perfringens* Type A in the Disease Complex. J. Vet. Med. **53:**518-523.

CGC. 2001. Grains from western Canada: 2000-01 crop year. Canadian Grains Commission. Winnipeg, MB, Canada.

Chapman, P.A., Siddons, C.A., Malo, A.T.C, and Harkin, M.A. 1997. A 1-year study of *Escherichia coli* 0157 in cattle, sheep, pigs and poulty. Epidem. And Infect. **119:**245-250.

Christensen, D.A., Mustafa, A., and McKinnon, J.J. 1995. Application of the Cornell Net Carbohydrate and Protein System to use of western Canadian feeds for beef and dairy ration formulation. Proc. Western Nut. Conf. **16**:223-244.

Dahiya, J.P., Wilkie, D.C., Van Kessel, A.G., and Drew, M.D. 2006. Potential strategies for controlling necrotic enteritis in broiler chickens in post-antibiotic are. Anim. Feed Sci. Tech. **129**:60-88.

Dennison, A.C., VanMetre, D.C., Callan, R.J., Dinsmore, P, and Mason, G.L. 2002. Hemorrhagic bowel syndrome in dairy cattle: 22 cases (1997-2000). J. Amer. Vet. Med. Assoc. **221**:686-689.

DePeters, E.J. and Taylor, S.J. 1985. Effects of feeding corn or barley on composition of milk and diet digestibility. J. Dairy Sci. **68:**2027-2032.

De Ondarza, M.B. 2004. Dead gut disease still perplexing. Hoards Dairyman **149**: 602-603

East, L.M., Dargatz, D.A.., Traub-Dargatz, J.L and Savage, C.J. 2000. Foaling management practices associated with the occurrence of entercolitis attributed to *Clostridium perfringens* in horses with intestinal disorders. Prev. Vet. Med. **46:**61-74.

Eisenbeisz, W.A., Schingoethe, D.J., Casper, D.P., Shaver, R.D., and Cleale, R.M. 1990. Lactational evaluation of recombinant bovine somatotropin with corn and barley diets. J. Dairy Sci. **73**:1269-1279.

Elder, R.O., Keen, J.E., Siragus, G.R., Barkocy-Gallagher, G.A., Koohmarie, M., and Laegreid, W.W. 2000. Correlation of enterohemorrhagic *Escherichia coli* 0157 prevalence in feces, hides and carcasses of beef cattle during processing. Proc. Natl. Acad. Sci. **97:**2999-3003.

Enevolden, B.S. 1984. Aspects of the fine structure of starch. Pages 55- 70. *in* Progress in Biotechnology: Volume 1 New approaches to research on cereal carbohydrates. R.D. Hill and L. Munck, eds. Elsevier. New York, NY, U.S.A.

Ewold, J.M., and Anderson, D.E. 2005. Determination of the effect of single abomasal or jejunal inoculation of *Clostridium perfringens* Type A in dairy cows. Can. Vet. J. **46**:821-824.

Fox, D.G., Sniffen, C.J., O'Connor, J.D., Russell, J.B., and Van Soest, P.J. 1992. A net carbohydrate and protein system for evaluating cattle diets: III. Cattle requirements and diet adequacy. J. Anim. Sci. 1992 **70**:3578-3596.

Godden, S., Frank, R., and Ames, T. 2001. Survey of Minnesota dairy veterinarians on the occurrence of and potential risk factors for jejunal haemorrhage syndrome in adult dairy cattle. Bov. Pract. **35**:97-103

Glock, R.D. and DeGroot, B.D. 1998. Sudden Death of Feedlot Cattle. J. Anim. Sci **76**:315-319.

Grings, E.E., Roffler, R.E., and Deitelhoff, D.P. 1992. Evaluation of corn and barley as energy sources for cows in early lactation fed alfalfa-based diets. J. Dairy Sic. **75:**193-200.

Hancock, D.D, Besser, T.E., Kinsel, M.L., Tarr, P.I., Rices, D.H. 1994. The prevalence of *Escherichia coli* 0157:H7 in dairy and beef cattle in Washington State. Epidemiol. Infect. **113:** 199-207.

Hartman, M. 1998. Oat production in Alberta- Adaptation. Alberta Agriculture and Rural Development. Retrieved on Aug. 12, 2003 from http://www.agric.gov.ab.ca/crops/oat/adapt01.html

Herrera-Sldana, R.E., Huber, J.T., and Poore, M.H., 1990. Dry matter, crude protein, and starch degradability of five cereal grains. J. Dairy Sci. **73:**2386-2393.

Hofshagen, M., and Kaldusdal, M. 1992. Barley inclusion and avoparcin supplementation in broiler diets 1. Effect of small intestinal bacterial flora and performance. Poultry Sci. **71**:959-969.

Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T and Williams, S.T. 1994. Bergey's Manual of Determinative Bacteriology. 9th Ed. Williams and Wilkins Baltimore, MD. USA.

Kahusdal, M. and Hofshagen, M. 1992. Barley inclusion and avoparcin supplementation in broiler diets. 2. Clinical, pathological, and bacteriological findings in a mild form of necrotic enteritis. Poultry Sci. **71**:1145-1153.

Kasha, K.J., Falk, D.E., and Ziauddin, A. 1993. Potential improvement of barley quality through genetic engineering page 421 *in* Barley chemistry and technology. A.W. MacGregor and R.S. Bhatty, eds. American Association of Cereal Chemists Inc. St. Paul. MN.

Khalili, H., **Sairanen, A., Hissa, K, and Huhtanen, P. 2001.** Effects of type and treatment of grain and protein source on dairy cow performance. Anim. Sci. **72**:573-584.

Kirkpatrick, M.A., Timms, L., Kesting, K.W., and Kinyon, J. 2001a. Jejunal hemorrage syndrome of dairy cattle. Amer. Assoc. Bov. Pract. Proc. **34**:135-136.

Kirkpatrick, M.A., Timms, L.L., Kersting, K.W., and Kinyon, J.M. 2001b. Case greport- Jejunal hemorrhage syndrome of dairy cattle. Bov. Pract. **35**:104-116.

Khorasani, G.R., Okine, E.K., and Kenelly, J.J. 2001. Effects of substituting barley grain with corn on ruminal fermentation characteristics, milk yield, and milk composition of Holstein cows. J. Dairy Sci. **84**:2760-2769.

Kondo, F. 1988. In vitro lecithinase activity and sensitivity to 22 antimicrobial agents of *Clostridium perfringens* isolated from necrotic enteritis of broiler chickens. Res. Vet. Sci. **45:**337-340.

Lehninger, A.L., Nelson, D.L.,and Cox, M.M. 1993. Principles of Biochemistry 2nd Ed. Worth Publishers. New York, NY.

MacGregor, A.W. and Fincher, G.B. 1993. Carbohydrates of the barley grain pages 73- 130 *in* Barley chemistry and technology. A.W. MacGregor and R.S. Bhatty, eds. American Assoication of Cereal Chemists Inc. St. Paul. MN.

Manitoba Agriculture and Food. 2003. Oat production and management. Retrieved on Aug. 12, 2003 from http://www.gov.mb.ca/agriculture/crops/cereals/bfc01s01.html.

Manteca, C., Daube, G., Jauniaux, T., Linden, Al, Pirson, V., Detilleux, J., Ginter, A., Coppe, P., Kaeckenbeeck, A., and Mainil, J.G. 2002. A role for *Clostridium pergringens* β2 toxin in bovine enterotxemia? Vet. Microbiol. **86**:191-2002.

Manteca, C., Daube, G., Pirson, V., Limbourg, B., Kaeckenbeeck, A., and Mainil, J.G., 2001. Bacterial intestinal flora associated with enterotoxaemia in Belgian Blue calves. Vet. Microbiol. **81**:21-32.

McAllen, A.B., Sutton, J.D., Beever, D.E., and Napper, D.J. 1994. Rumen fermentation characteristics and duodenal nutrient flow in lactating cows receiving two types of grass silage with two levels of concentrate. Anim. Feed Sci. Tech. **46**:277-291.

McAllister, T.A., Rode, L.M., Major, D.J., Cheng, K.-J., and Buchanan-Smith, J.G. 1990. Effect of ruminal microbial colonization of cereal grain digestion. Can. J. Anim. Sci. **70:**571-579.

McCarthy, R.D. Jr., Klusmeyer, T.H., Vicine, J.L, Clark, J.H., and D.R. Nelson. 1989. Effects of source of protein and carbohydrate on ruminal fermentation and passage of nutrients to the small intestine of lactating cows. J. Dairy Sci. **72**:2002-2016.

Meier, J.-A. 2002. Barley type and processing: Effect on rumen degradation characteristics. B.S.A. Thesis, University of Saskatchewan. Canada.

Meier, J.A., Yu, P., Christensen, D.A. and McKinnon, J.J. 2003. Effect of cereal grain characteristics on production performance of lactating dairy cattle. Paper Presentation. J. Dairy Sci. Vol 86 Suppl. 1

Miller, J.J., Beasley, B.W., Yanke, L.J., Larney, F.J., McAllister, T.A., Olson, B.M., Selinger, L.B., Chanasyk D.S., and Hasselbak, P. 2003. Bedding and seasonal effects on chemical and bacterial properties of feedlot cattle manure. J. Environ. Qual. **32**:1887-1894.

Moran, J.B. 1986. Cereal grain in complete diets for dairy cows: A comparison of rolled barley, wheat and oats and of three methods of processing oats. Anim. Prod. **43**:27-36.

Mustafa, A.F., Christensen, D.A., and McKinnon, J.J. 1998. Chemical characterization and ruminal nutrient degradability of hulled and hull-less oat. J. Sci. Food Agric. 77:449-455.

Niilo, L. 1980. *Clostridium perfringens* in animal disease: A review of current knowledge. Can. Vet. J. **9**:141-148.

NRC. 2001. Nutrient requirements of dairy cattle. 7th Revised Ed. National Research Council. National Academy Press. Washington, D.C., U.S.A.

Ørskov, E.R. 1986. Starch digestion and utilization in ruminants. J. Anim. Sci. **63:**1624-1633.

Puntenney, S., **Wang, W.-Q., and Forsberg, N. 2003.** Detection of *Aspergillus fumigatus* in hemorrhagic bowel syndrome in dairy cattle. J. Dairy Sci. **81** Suppl. 1:85.

Quinn, P.J., Markey, B.K., Carter, M.E., Donnelly, W.J., and Leonard, F.C. 2002. Verterinary microbiology and microbial disease. Blackwell Science Ltd. Malden, MA. USA.

Riddell, C., and Kong, X.-M. 1992. The influence of diet on necrotic enteritis in broiler chickens. Avian Dis. **36**:499-503.

Russell, J.B., O'Conner, Fox, D.G., Van Soest, P.J., and Sniffen, C.J. 1992. A net carbohydrate and protein system for evaluating cattle diets: I. Ruminal fermentation. J. Anim. Sci. **70**:3551-3561.

Russel, J.B., Diez-Gonzalex, F., and Jarvis, G.N. 2002. Invited review: Effects of diet shifts on *Escherichia coli* in cattle. J. Dairy Sci. **83**:863-873.

Seglar, B. 2001. Host, pathogen, and environmental interactions resulting in hemorrhagic jejunal syndrome in dairy cattle. Proc. 36th Annual Pacific Northwest Anim. Nut. Conf. pages 9-28.

Sholz, D. 2006. HBS: potential causes, prevention discussed. Feedstuffs, **78**:16.

Sniffen, C.J., O'Connor, J.D., Van Soest, P.J., Fox, D.G., and Russel, J.B. 1992. A net carbohydrate and protein system for evaluating cattle diets: I. Carbohydrate and protein availability. J. Anim. Sci. **70**:3562-3577.

Songer, J.G. 1999. *Clostridium perfringens* Type A infection in cattle. Amer. Assoc. Bov. Pract. Proc. **32**:40:43.

St. Jean, G., and Anderson, D.E. 1999. Intraluminal-intramural hemorrhage of the small intestine in cattle. page 539 *in*: J.E. Howard and R.A. Smith, eds. Current veterinary practice, 4th ed. Philidelphia. W.B. Saunders Co..

Tillotson, K. Traub-Dargatz, J.L., Dickenson, C.E., Ellis, R.P., Morley, P.S., Hyatt, D.R>, Magnuson, R.J, Riddle, W.T., Bolte, D., and Salman, M.D. 2002. Population-baseds tudy of fecal shedding of *Clostridium perfringens* in broodmares and foals. J. Amer. Vet. Med. Assoc. **220**:342-348.

Tschirdewahn, B., Notermans, S., Wernars, K., and Untermann, F. 1991. The presence of enterotoxigenic *Clostridium perfringens* strains in faeces of various animals. Internat. J. Food Microbiol. **14**:175-178.

Van Donkersgoed, J., Graham, T., and Gannon, V. 1999. The prevalence of verotoxins, *Esherichia coli* 0157:H7, and *Salmonella* n the feces, and rumen of cattle at processing. Can. Vet. J. **40:**332-338.

Van Metre, D. C. 2006. Clostridial Infections of the Ruminant GI Tract. L. An. Proc. Of N. Amer. Vet. Conf. **2006:**52-55.

Van Metre, D. C., Callan, R.J., Dennison, A.C., and Ellis, R.P. 2005. Hemorrhagic Bowel Syndrome: What We Do and Don't Know. Proc. N. Amer. Vet. Conf. Large Animal. **19**:47-48

Van Soest, P.J., Robertson, J.B., and Lewis, B.A. 1991. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. J.Dairy Sci. **74**:3583-3597.

Wagner, D.D., and Thomas, O.P. 1978. Influence of diets containing rye or pectin on the in testinal flora of chicks. Poultry Sci. **57**:971-975.

Watson, S.A. 1997. Industrial utilization of corn. Pages 721-763. *in* Corn and corn improvement. G.F. Sprague, eds. Am. Soc. Agron. Madison WI. As seen in Corn: Chemsitry and Technology. Watson and Ramstad, 1987.

Watson, S.A. 1987. Structure and Composition. Page 53-82 *in* S.A Watson and P.E. Ramstad, eds. Corn: Chemistry and Technology. American Association of Cereal Chemists Inc. St. Paul, MN, U.S.A.

Weiss, W. P., Conrad, H.R., and St.Pierre, N.R. 1992. A theoretically based model for predicting total digestible nutrient values of forages and concentrates. Anim. Feed Sci. Tech. **39**:95-100.

Wilson, C.M. 1987. Proteins of the kernel. Pages 273-275. *in* S.A Watson and P.E. Ramstad, eds. Corn: Chemistry and Technology. American Association of Cereal Chemists Inc. St. Paul, MN, U.S.A.

Yang, W.Z., Beauchemin, K.A., Koenig, K.M., and Rode, L.M. 1997. Comparison of hull-less barley, barley or corn for lacatation cows: Effects on extent of digestion and milk production. J. Dairy Sci. **80**:2475-2486.

Yang, W.Z., Beauchemin, K.A. and Rode, L.M. 2000. Effects of barley grain processing on extent of digestion and milk production of lactating cows. J. Dairy Sci. **83**:554-568.

Yu, P., Meier, J.A., Christensen, D.A., Rossnagel, B.G., and McKinnon, J.J. 2003. Using the NRC-2001 model and the DVE/OEB system to evaluate nutritive values of Harrington (malting-type) and Valier (feed-type) barley for ruminants. Anim. Feed Sci. and Tech. **107**:45-60.

Zhao, T., **Doyle, M.P, Shere, J., and Garber, L. 1995**. Prevalence of enterohemorrhagic *Escherichia coli* 0157:H7 in a survey of dairy herds. Appl. Enviro. Microbiol. **61**:1290-1293.

8.0 APPENDIX A POSTMORTEM OF COW 400

NECROPSY/BIOPSY

Submitted 13-Feb-02

Owner: Animal Sciences Dairy Barn
Animal: 400
Species: Bovine-Holstein-Freisian
Sex: Female
Age: 4 years
Pathologist: Brendan O'Connor

HISTORY
Disease suspected: Pylonic Obstruction? Jejunitis?
Euthanized with captive bolt.
Presented to WCVM Feb 11/02 for kicking at belly, distended ventral abdomen. Decreased milk production, melena, and straining to defecate. She calved 2.5, and is on her second lactation. Flocculent material was obtained on abdomincentesis. Her CBC revealed a moderate elevation in bands, mild lymphopenia, and mild monocytosis. She had an exploratory surgery performed, and found an adhesion on the cranial floor of the abdomen. The small intestines serosal surface appeared inflamed. Over her stay here, she was over thirsty, but anorexic. She was given approximately 60 L of saline with KCL addition. IV over the last ~30 hours. She stood a few times, but mainly remained in sternal recumbency. She milked out ~18 lb last night. Her blood gas revealed high PCO2, high HCO2-, low K+ and low Cl-.

FINAL DIAGNOSIS:
1. Segmental Hemorrhagic Jejunitis

ETIOLOGY:
Toxigenic *Clostridium perfringens* suspected

COMMENTS:
The postmortem findings are consistent with the syndrome know as Segmental Hemorrhagic Jejunitis. A Clostridial infection is suspected as the cause. Toxigenic strains of *Clostridium perfringens* were isolated from the intestine and confirmed by PCR in this case. This supports the hypothesis that this infection is responsible for the intestinal damage. However, the pathogenesis of this syndrome is unclear. There was no convincing evidence that *Clostridia* were the cause of the lesion on examination of the intestine with Special strains. No *Campylorbacter*-like organisms were seen in the mucosa.

I sent tissues for Immunohistochemistry test for BVDV and Bovine Caronavirus and the results were negative. This helps to rule out these viral agents as playing a role in the

condition. The initiating cause of the focal ulceration, extensive hemorrhage and associated ileus remains to be confirmed in this syndrome.

GROSS NECROPSY

The cow was in good body condition. The abdomen was noticeable distended. When the abdomen was opened a segment of swollen dark red-black intestine was visible in the lower right abdominal area. There was fibrin adherent to a portion of serosa that was very hyperemic over a15 cm long segment of this area of bowel. When the alimentary tract was removed from the abdomen, it was found that the dilated portion of intestine involved the duodenum and proximal jujunem and extended for approximately 6.5 m distal from the pylorus. In the upper half this distended segment the content was green fluid and fine roughage. In the distal half of the segment, the content was composed of free and clotted blood. At the site where there was fibrin on the serosal surface, there was a large irregular blood clot firmly attached to the mucosal surface for over a 8 cm length. It appeared that this blood clot was at least partially located beneath the mucosa. The intestine distal to this point was empty except for a small amount of thin watery black fluid. The fore-stomachs were unremarkable except for the fluid nature of the content. The heart and lungs were unremarkable. The liver was normal except for two discrete 1 x 1.5 cm sugerficial areas of pallor.

HISTOPATHOLOGY:

JEJUNUM: There was massive hemorrhage in the submucosa. The large haematoma was covered by remnants of necrotic mucosa infiltrated by degenerate inflammatory cells. Within the hematoma, there was an extensive neutrophil infiltration in some areas. In most cases, these were associated with traces of plant material. There was moderate interstitial hemorrhage, edema and neutrophil infiltration in the remaining submucosa overlying the muscularis layer.

The mucosa on the adjacent portion of the intestine was intact. There was a moderate lymphocyte infiltration in the lamina propria and the villi appeared hyperplastic. There was sever hemorrhage in the lamina propria of many villi. In other sections there was brown pigment, most likely hemosiderin, in the lamina propria near the tips of villi. There were also scattered lymphoid nodules in the lamina propria in the jejunum. There was necrosis in a single crypt and it was filled with degenerating inflammatory and epithelial cll.

LIVER: Mild locally extensive subcapsular area of fatty infiltration; Single milk periacinar mixed inflammatory cell infiltration.

LUNG: Mild congestion

KIDNEY: Single moderate, focal, subcapsular hemorrhage with a few nutrophils in the adjacent tubules.

SPLEEN: Moderate congestion; widespread pigment, most likely hemosiderin, in macrophages in the sinuses.

ADRENAL GLAND: Mild congestion

Special Strains: The results were not diagnostic. There was a moderate mixed population of gram positive and gram negative cocci and bacilli on the surface of the damaged intestine. Small numbers of large gram positive bacilli were seen within the

wall of the intestine. Now *Campylobacter*-like organisms were seen on the Warthin-Faulkner staining. No fungi were detected in the tissues using Grocott's strain.

IMMUNOLOGY

RESULTS & INTERPRETATION

Specimen	Examination	Method	Diag/Titre
Formalin tis. Sections	Bovine Caronavirus	Avidin biotin complex + Peroxidase	Negative
	Bovine Virus Diarrhea Virus	Avidin biotin complex + Peroxidase	Negative

BVDV and BCV were not detected

BACTERIOLOGY

CULTURE RESULTS

Jejunum 3+ alpha *Streptococcus spp*.
3+ *Escherichia coli*
No *Samonella spp.* Isolated
2+ *Clostridium perfringens*

Clostridium perfringens is potentially significant. Please see PCR for Typing

PCR

RESULTS

Specimen	Exanimation	Target	Results/Interpretation
Plate	Poymerase Chain Reactoin	*Clostridium Perfringens*	alpha and beta-2 toxins

Clostridium perfringens alpha and beta-2 toxins were detected by PCR