Improving Phosphorus Utilization by Dairy Cows

By

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ABSTRACT

The primary objective of the thesis research was to identify strategies to improve the utilization of dietary phosphorus (P) on commercial dairy farms. In the first study, a survey was carried out on 19 commercial dairy farms in Manitoba to determine relationships between dietary, animal, and management factors and the P utilization. Multiple regression analysis of variables showed that high dietary P concentrations and free-stall instead of tie-stall housing were most associated with a high P concentration of feces. The second study determined the effects of SARA and *Saccharomyces cerevisiae* fermentation products (SCFP) on the rumen pH and apparent total tract digestibility coefficient (ADC) of dry matter (DM), neutral detergent fiber (NDF), starch and P in 32 lactating Holstein cows. Cows were assigned to four treatments; 1) control and three SCFP supplementations, including 2) 14 g/d Diamond V Original XPC™ (XPC, Diamond V, Cedar Rapids, IA), 3) 19 g/d NutriTek® (NTL, Diamond V), or 4) 38 g/d NutriTek® (NTH, Diamond V). Subacute ruminal acidosis (SARA) challenges were conducted during wk 5 and wk 8 by switching from a moderate-grain diet containing 18.6% of DM starch to a high-grain diet containing 27.9% DM starch. On average, the SARA challenges increased the duration of the rumen pH below pH 5.6 from 13.6 to only 179 min/d. In cows on the NTH treatment, this increase was shorter ($P<0.05$) at 79.3 min/d. Across SCFP treatments, the SARA challenges reduced the ADC of NDF and tended to increase that of starch. The combination of both SARA challenges reduced the ADC of DM and P in all treatments. Our results show that NTH can increase rumen pH during high grain feeding and increase NDF digestion, which is particularly important when cows are at risk of SARA, and that prolonged high grain feeding can reduce the P digestibility of dairy cows. The results of these studies show that the utilization of dietary P by dairy cows on

Manitoba dairy farms can be improved by more closely matching the demands and requirements of P by dairy cows and reducing the risk of SARA.

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FOREWORD

This dissertation is written in manuscript style and is comprised of an introduction, a literature review, two manuscripts on scientific studies, and a general discussion. The two manuscripts have been submitted for publication to the Canadian Journal of Animal Science.

Chapter 2: Senaratne, V. and J.C. Plaizier. 2019. Relationships between dietary and cow factors with the milk and feces phosphorus concentrations of dairy cows in Manitoba. Can J. Anim Sci.

Chapter 3: Senaratne, V., H. Khalouei, K. Fehr, J. Guo, I., Yoon, E. Khafipour and J.C. Plaizier. 2019. Saccharomyces cerevisiae fermentation products (SCFP) reduce the impact of Subacute Ruminal Acidosis (SARA) on rumen pH and increase fibre digestibility in lactating dairy cows. Can J. Anim Sci.

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ABBREVIATIONS

ADC	Apparent total tract digestibility coefficient
ADF	Acid detergent fiber
AIA	Acid insoluble ash
BHBA	Beta-hydroxybutyric acid
CF	Crude fiber
CP	Crude protein
DM	Dry matter
DIM	Days in milk
DMD	Dry matter digestibility
DMI	Dry matter intake
MY	Milk yield
NDF	Neutral detergent fiber
NEFA	Non- esterified fatty acid
NFC	Non- fiber carbohydrate
NTH	NutriTek® high dosage
NTL	NutriTek® low dosage
OS	Other solids
OM	Organic matter
SARA	Subacute ruminal acidosis
SCFP	*Saccharomyces cerevisiae* fermentation product
SSC	Somatic cell count
TMR	Total mixed ration
VFA	Volatile fatty acids
XPC	Diamond V Original XPC™

1. GENERAL INTRODUCTION

With the expansion and intensification of the livestock sectors, there is increased public concern regarding the resources utilized, and the waste material produced, such as wastewater, manure and bedding, have increased substantially (Schindler e al., 2012). Phosphorus (P) is of particular interest, as it contributes to eutrophication of water bodies, a major environmental problem attracting much attention locally and globally. Lake Winnipeg, for example, experienced a two-fold increase in nuisance blooms of heterocystous cyanobacteria between 1990 to 2000 (Kling et al., 2011; McCullough et al., 2012). Although the factors that affect eutrophication are complex, one of the main factors causing the surge of eutrophication is the runoff of P from agricultural lands. One of the culprits for P loss from agricultural lands is the application of animal manure and synthetic fertilizers (Schindler et al., 2012). Recognising this issue, regulatory bodies all over the world introduced/are introducing rules and regulations to manage P use in agriculture and are moving legislation of manure application from N-based to P-based manure application rates. Hence, strategies to manipulate and increase the utilization of P within the cows and within the farms and, thereby, to reduce the output of P to the environment through waste materials must be found.

According to a survey conducted by Plaizier et al. (2004), the average dietary P supply exceeds the estimated P requirement of dairy cows by 51.9% on Manitoba dairy farms. Furthermore, it was found that the P efficiency of farms, calculated as the farm P exports as a percentage of farm P imports, averaged around 48%, suggesting that 52% of P is not utilized (Plaizier et al., 2012a). The P outputs from farms can be reduced by increasing the P utilization by cows and, consequently, by reducing the P concentration in manure. Generally, cows excrete 50 to 70% of their intake P through manure (Dou et al., 2002). Several factors can influence the absorption efficiency of P,

including age, P intake, source of P, physiological state of the animal (lactating *vs.* dry cow), dietary concentration of minerals, intestinal pH and dietary Ca: P ratio (Irving, 1964; Peeler, 1972; Soares, 1995). Therefore, when developing best management practices for farms, it is important to consider these dietary and animal factors and understand how they affect P utilization. In order to achieve that purpose, we undertook a survey to investigate the impact of different factors that contribute to P utilization in dairy cows.

A large proportion of P in the dairy cattle diet is in the form of phytate P, which is not directly available to monogastrics. However, in ruminants, this form of P is digested via symbiosis with microbiota residing in the digestive tract. These microbiota break down phytate P, thereby making P available for ruminants to absorb. This symbiosis between ruminants and microbiota in the rumen can be disturbed by current feeding practices, which include using high-grain diets to satisfy the high energy needs of high-yielding cows, ultimately resulting in subacute ruminal acidosis (SARA). The prevalence of SARA is quite extensive and several surveys conducted in North America have observed that SARA occurs in 19 to 25% of early and mid-lactation herds (Garrett et al., 1997; Oetzel et al., 1999). There is evidence suggesting that SARA can reduce the functionality of the rumen, as well as the symbiosis with microbiota, resulting in a reduction of nutrients digestibilities (Plaizier et al., 2017; Plaizier et al., 2018). Dietary supplementation with *Saccharomyces cerevisiae* fermentation products (SCFP) has been suggested to alleviate these adverse effects of SARA and achieve a better symbiosis between the host cow and its gut microbiota (Desnoyers et al. 2009; Plaizier et al., 2018). Therefore, the second study was carried out to investigate the effects of SARA on digestibility of nutrients such as P, DM, NDF and starch and to investigate the effectiveness of dietary supplementation of SCFP in alleviating adverse effects of SARA.

2. LITERATURE REVIEW

2.1 Why look at P?

With advances in dairy technology and increased global demand for dairy foods, the dairy industry has realized substantial growth in terms of total milk production, farm size, and milk production per cow. Consequently, nutrients, including minerals are released by these farms into the environment (Plaizier et al, 2004; Plaizier et al. 2012a). One of the key examples of the effects of large amounts of P excretion from various sources into the environment is Lake Winnipeg. Since mid-1990, the frequency of blooms of Cyanobacteria in Lake Winnipeg has nearly doubled due to eutrophication due to increases in the rate of loading and concentration of P (Schindler et al., 2012). The main environmental problem that is associated with P is eutrophication (NRC, 2001). Of all the essential micro and macro mineral elements for animals, phosphorus (P) represents the greatest risk if it is released to the environment (Smith et al., 1999). The reason that P is considered the most significant risk is that P is, in most situations, the primary limiting factor for eutrophication in inland lakes and reservoirs (Powers et al., 1972). Thus, less P contamination results in less risk of eutrophication. Accumulation of P in ecosystems such as water bodies can result in accelerated growth of cyanobacteria, which are also known as algae blooms (Smith et al., 1999). These algae will block the sunlight for the plants underwater and will compete for oxygen and other nutrients with the other life forms in the aquatic community, such as fish and vascular plants (Powers et al., 1972; Smith, 2003). When these large masses of algae decompose, water oxygen concentration drops dramatically and can create anoxic conditions in water, resulting in deterioration of water quality and biodiversity, smelly odours, increased fish mortality and increased turbidity (Powers et al., 1972).

The utilization of P by ruminants is not efficient, as 50 to 70% of total ingested P is excreted through manure (Dou et al., 2002). This can result in lower amounts of P exported out of the farm by the ways of milk and meat, compared to that excreted in manure (Dou et al., 2002). Solid manure produced on dairy farms in Manitoba contains, on average, 0.59% P (DM basis Plaizier et al., 2014). Ebeling et al. (2002) compared the P runoff of land that received manure from dairy cows on a diet containing 0.31% DM P with that of cows receiving a 0.49% DM P diet and observed that the high P diet increased this runoff more than 10 times. Also, Dou et al. (2002) reported that increases in dietary P increase not only the total feces P output but also the proportion of the water-soluble P in the feces, which increases the risk of run-off. Understanding the significant risk of water contamination by manure, authorities such as federal as well as provincial governments, have urged the livestock industry to manage P nutrition in an efficient manner to maintain an environmentally sustainable industry.

Subacute ruminal acidosis (SARA) reduces the utilization of dietary nutrients by cows (Plaizier et al., 2008; Plaizier et al., 2012), and may reduce the utilization of P also. Hence, strategies to reduce SARA may enhance the utilization of dietary P and reduce P excretion by cows.

2.2 What is P?

Phosphorus is an essential nutrient for life. Because of its extreme reactivity, P does not occur in its elemental form in the soil, and it is mostly found in phosphate mineral salts and in its maximum oxidized form as phosphate ($PO_{4)}$ (Bieleski, 1973). Phosphorus is present in feedstuffs in different forms and compounds. These compounds can be divided into two different groups, i.e., organic and inorganic phosphorus. Orthophosphate monoesters such as phytic acid and orthophosphate diesters, phospholipids, DNA and phosphonates are examples of organic P, whereas

orthophosphate, pyrophosphate compounds are considered inorganic. Among these different forms, phytic acid and orthophosphates are the most common (Bieleski, 1973). Different forms of organic and inorganic P are added to the diet of ruminants to provide them with P. Plants mainly store P in the inorganic form of orthophosphate and the organic form of phytic acids. Generally, in grains and legumes, phytic acids dominate, while in grasses orthophosphate compounds dominate (Bieleski, 1973). Farmers also supplement dairy rations with P in inorganic salts, such as dicalcium phosphate, monosodium phosphate, monocalcium phosphate, to enrich the diet (Knowlton and Herbein, 2002; Knowlton et al., 2004).

2.2.1 Importance of P in biological systems

Phosphorus is one of the mineral elements that is essential for life. In general, it is assumed that animal carcasses contain approximately 0.7% of P (Payne and Pugh, 2011), and P is a major structural component in the bones of humans and animals. In dairy cows, approximately 80% of P in the body is contained in the skeletal tissue and teeth. In the bones, P is present as calcium phosphate such as apatite salts (NRC, 2001). It is also an important component in milk, with a concentration of approximately 1.14 g/L of P (Cerbulis and Farrell, 1976). Furthermore, P is vital for the transportation of fatty acids in the body, formation of cell membrane, and production of nucleic acids, phospholipids, and proteins (NRC, 2001). It also plays a vital role in energy metabolism, involving formation and breaking of high energy bonds that link oxides of phosphate to carbon or to carbon-nitrogen compounds, such as adenosine triphosphate and adenosine diphosphate (NRC, 2001). Furthermore, it is crucial in enzyme systems (Knowlton et al., 2004). In an average dairy cow, between 1 and 2 g of inorganic phosphate is circulating in blood plasma, and the blood plasma P concentration ranges from 1.3 to 2.6 mmol/L. Due to the high P

concentration in red blood cells, whole blood can contain 6 to 8 times more P than the blood plasma (Goff, 1998).

Phosphorus is also considered as one of the mineral elements that is essential for ruminal microorganisms, including those that digest fiber (Burroughs et al., 1951), as well as for microbial protein synthesis (Breves and Schroder, 1991). For proper functionality of rumen microbes in terms of fiber digestion, cows are required to maintain a minimum available P concentration of 5 g per kg of organic content digested in the rumen (Durand and Komisarczuk, 1988). Maximum cellulose digestion *in vitro* can be acquired when the rumen fluid contains P between 20 to 60 mg/L of rumen liquid digesta (Hall et al., 1961). Rumen fluid concentrations above this threshold are typically maintained through P recycling from saliva and the P from the diet. Cows fed a very deficient P diet (0.12% DM of P), can experience a rumen fluid P concentration of over 200 mg/L, which are substantially higher than the maximum threshold (60 mg/L) proposed by Hall et al. (1961).

2.2.2 Effect of different forms of dietary P

Grains and legumes have higher concentrations of total phosphorus (TP) than grass forages (Table 1). The average TP concentration in grain concentrates ranges from 0.11 to 1.20% among these feeds, while in legume-grass forages this ranges from 0.07 to 0.74%. Most P in grain concentrates is phytate bound. Typically, approximately 65-85% of TP in seeds is present in the form of phytic acid, which is a form of P that is not directly available to monogastrics (Reddy et al., 1982; Eeckhout and De Paepe, 1994). This is because monogastric animals do not produce enzymes that can break down phytic acid (Ravindran et al., 1999). However, ruminants have microorganisms with phytase enzyme activity in their digestive tract (Yanke et al., 1998). The phytase-producing

microflora in the rumen are mostly comprised of bacteria, such as *Selenomonas rumenantuim, Megasphaera elsdenii* and by several protozoa (Yanke et al., 1998). Although rumen microbes can also produce phytase, not all the phytate P may be available to dairy cattle as the efficiency of phytase activity can vary due to several factors. The optimal pH and temperature for phytase are in the range of 4.5-6.0 and 45-60 ^{o}C, respectively (Lei and Porres, 2003). In addition, temperature and several dietary factors affect the efficiency of phytase. A high Ca:P ratio is believed to result in low P absorption in the intestine and low efficacy of phytase (Sandberg et al., 1993; Lei et al., 1994). Moderate to high concentrations of inorganic P can negatively affect the production of phytase by rumen microbes (Lei and Porres, 2003). Supplementation of organic acids, such as citric acid and acetic acid, can increase the solubility of P and result in high phytase activity (Han et al., 1998; Maenz et al., 1999). The inclusion of exogenous phytase with hydrolytic enzymes in the diet can also increase phytate digestibility (Zyla et al., 1995).

The total phosphorus (TP) concentration in feedstuffs (Table 1) is paramount when formulating diets, because of the direct relationships between the TP concentration in the diet, the utilization of dietary P, and the concentration of P in feces. Hence, to satisfy the animal's P requirement and at the same time reduce P excretion, the P concentration of feeds is commonly measured. Grain by-products, such as brewers' grains (0.67 % TP, DM basis) and corn distillers' grain (0.90 % TP, DM basis) contain high concentrations of TP, making them a rich source of P (NRC, 2001), as indicated in Table 1.

Table 1. Total P in common feedstuffs (NRC, 2001)

Feedstuffs	Total P, % of DM (± SD)
Distillers grains with solubles, dried	0.83 ± 0.14
Cotton seed hulls	0.12±0.06
Cotton seed meal, solvent, 41% CP	1.15±0.10
Feather meal	0.50
Grass hay	0.23±0.06
Grass silage	0.29±0.08
Legume hay	0.26±0.05
Legume silage	0.32±0.06
Linseed meal	0.83
Meat meal	4.20±1.14
Meat and bone meal	4.73±1.06
Sugar cane molasses	0.10±0.02
Beet sugar molasses	0.03±0.01
Oats grain	0.40±0.06
Rice bran	1.78±0.36
Sorghum	0.35±0.07
Soybean meal, 44% CP	0.71±0.04
Sunflower meal	1.00±0.25
Sunflower oil	0.51±0.18
Wheat bran	1.18±0.23

The absorption of P by dairy cows also depends on several factors, such as total protein intake, Ca to P ratio, pH in the intestine, disease and parasites, environment, age of the animal and dietary concentrations of calcium, iron, aluminium, manganese, potassium, and magnesium, which can result in formation of insoluble and unabsorbable complexes with P (Wise et al., 1963; Field et al., 1983; NRC, 2001; Ekelund, 2003).

The availability of feed P is also dependent upon the dietary protein content, starch degradability, and grain content of the diet (Braithwaite, 1976; Guyton et al., 2000). A high concentration of dietary protein may reduce the availability of P from phytate, as chemical bonds between protein and P in phytate must be broken down before phytase can remove inorganic P from phytate (Fontaine et al., 1946; Morse et al., 1992a). According Guyton et al. (2000), increase in digestible starch source resulted in a decrease of fecal P excretion as a percentage of intake. However, effects of SARA due to a high grain content on P utilization were not examined in these studies. These effects may exist, as SARA may reduce the functionality of rumen bacteria that breakdown phytate (Knowlton et al., 2007). Addition of grain by-products such as brewers' grain and corn distillers' grain, which both have high phytic P concentrations can help meet P requirements. However, feeding these products can result in a reduction in feed intake and therefore, P intake (Morse et al., 1992a). This reduction in feed intake may be due to the high water holding capacity of grain by-products, such as wheat bran (Ramanzin et al., 1994).

2.2.3 Homeostasis of P in dairy cows

Homeostasis of P includes digestion of dietary P, absorption of P in the small intestine, resorption of P from the bones, recycling blood P through saliva, and the excretion of P through feces, milk and urine (NRC, 2001). In ruminants, the primary route of P excretion is through feces. According to Chapuis-Lardy et al. (2004), TP excreted in feces accounted for 55, 67, and 76% of P intake from the feed when cows were fed diets containing 0.31, 0.39, and 0.47% P. These proportions are affected by the activity of the phytase produced from the rumen microflora, P secretion in saliva, dietary P concentration, and P absorption from digestive tract (Morse et al., 1992b; Tamminga, 1996). Primarily, the required P necessary for maintenance, pregnancy, and production is absorbed in the small intestine, even when the body contains excess P (Dou et al., 2003). Microbial P

availability is dependent on the pH of the intestine, which may become unavailable to ruminants when the pH in the small intestine is greater than 6 (Playne, 1976). The absorption of P occurs mainly in two sites of the small intestine, the duodenum and the jejunum (Scott et al., 1984; Reinhardt et al., 1988). The upper parts of the small intestine have a relatively low pH, which can maintain phosphates in solution and prevent their precipitation. In contrast, phosphates in the lower parts of the small intestine can precipitate when the digesta pH exceeds 6.5 (Poppi and Ternouth, 1979). The absorption of P in the small intestine occurs via two main mechanisms, an active and a passive mechanism. Active absorption is vitamin D dependent and mostly occurs when the animals are fed a low P diet, resulting in low P concentrations in the blood. Low P concentrations in the blood stimulate the production of 1,25-dihydroxyvitamin D, which will, in turn, increase active P absorption (Horst, 1986). Passive absorption predominates when animals are fed diets containing adequate and excessive concentrations of P, and the process has a direct relationship with the concentrations of P in the digesta in the small intestine and the blood P (Wasserman and Taylor, 1976). According to Braithwaite (1983), there is an inverse relationship between P intake and the P absorption rate. This author suggested that the P absorption rate is reduced by saturation or inhibition of the absorptive mechanism when blood serum levels are within the normal range (4-8 mg/dL). A study has also shown that the apparent digestibility of dietary P decreases with the increase of P in the diet, as increases in the dietary P content from 0.34 to 0.51, and to 0.67% DM reduced the apparent digestibility of P from 49.0, to 34.4 and to 32.8% respectively (Knowlton and Herbein, 2002). Further, when the P concentration of the diet exceeds NRC (2001) recommendations (Table 2), 50 to 70% of the dietary P is excreted with feces (Dou et al., 2002).

The resorption of P from the bones during early lactation occurs to replenish blood mineral concentration and is triggered by Ca mobilization. When triggered, osteoclasts break down the bone, which results in releases of Ca and other compounds, including P, in the form of phosphates from the bone to the blood. Bone resorption usually occurs in the early lactation period when the milk production is high, and feed intake and blood Ca concentration are relatively low. The recycling of P is mainly achieved through saliva in ruminants, as saliva contains large amounts of P, mainly in the inorganic form such as orthophosphate (Braithwaite, 1983). The P concentration in saliva can be 4 to 5 times higher than the blood plasma concentration and will secrete 30 to 90 g/d of salivary P (Reinhardt et al., 1988; Scott, 1988). Absorbed P from the small intestine and P from bone resorption will re-enter the digestive tract through saliva to the gastrointestinal tract (Ternouth, 1990). The P in the saliva is dominated by water-soluble P, unlike the diet which mainly contains water-insoluble P.

The excretion of P occurs via three main routes, namely through feces, urine and milk. Generally, the milk P concentration shows little variation and is approximately 1 g/kg of milk (Wu et al., 2001). Urinary P concentrations are very low compared to the P concentration in feces and milk. As a result, urinary P excretions are only approximately 1 g/d, even when high P diets are fed (Morse et al., 1992b; Wu et al., 2001). According to the NRC (2001), a cow weighing 600 kg secretes on average 1.2 g/d of urinary P. The major route for excretion of P is through feces, which can contain between 60 to 70% of TP intake (Morse et al., 1992b; Wu et al., 2001).

Spiekers et al. (1993) divided feces P into three components. The first is the unavailable dietary P, which is not available to the animal under any conditions. Dou et al. (2002) suggested that the unavailable P in the diet mainly include organic and water-insoluble plant cell wall residues that are not digestible. The second P component consists of microbial residue P and metabolic P, which

can be digested but escape digestion is referred to as inevitably lost P. Most of this P fraction is includes insoluble, organic P from microbial residues, sloughed gut tissue and the digestive secretions from the intestine. The third component is regulated P. The proportion if the P in feces that is regulated depends on the P concentration in the diet, thereby making this component important in regard to the P utilization efficiency of the cow. When a cow receives a diet containing less P than the recommended 0.34% P (DM basis), the animal will utilize P efficiently, which reduces the proportion of regulated P out of the TP (Morse et al., 1992a). Similarly, when the cow is fed diets containing P in excess of the NRC (2001) recommendations, P utilization will be inefficient, and excess P will be recycled to the digestive system through saliva and other digestive secretions, which in turn increases the size of the regulated P fraction (Wu et al., 2000). The regulated P fraction largely consists of water-soluble P because it represents the portion which is fed in excess of requirements and is recycled to the gut through saliva in the form of inorganic P after digestion and absorption in the rumen (Scott et al., 1985; Ternouth and Coates, 1997). The P entering the digestive tract through the saliva can be as high as 80% of the endogenous P entering the digestive tract (Care, 1994; Bravo et al., 2003).

When the dietary P concentration increases, the P intake by the cow also increases, resulting in an increase in the excretion of P through urine and feces (Morse et al., 1992b; Wu et al., 2000; Wu et al., 2001). This will not only increase the TP concentration of feces, but also its water-soluble P concentration. This increase results in higher P concentration in feces, and reduce the apparent digestibility of dietary P. In agreement, Satter and Wu (1999) observed that increasing the P concentration in the diet from 0.40% to 0.49% increased the P excretion through feces by 23%. Dairy cows excrete P through feces, which mainly consist of the regulated P fraction. When dairy cow diets contained 0.34, 0.51 or 0.67% DM basis, the water-soluble fraction from feces TP

accounted for 56, 77 and 83% respectively (Dou et al., 2002). These authors also reported that a when fed excess, almost all of P excretion occurred consisted of water-soluble fraction. Therefore, feces of animals fed high P diets contain more water-soluble P compared to feces from low P concentration diet fed animals (Dou et al., 2002). This will affect the fate of the P that is spread through the manure, by increasing the risk of P in runoff.

2.2.4 Effects of excessive P usage

According to Knowlton et al. (2004), dietary P concentrations in excess of NRC (2001) recommendations (Table 2) are used in dairy cow diets for various reasons. There is a misconception that high inclusions of P ensure high reproductive performance, even though there is little evidence to support this hypothesis (Lopez et al., 2004).

Table 2. Dietary P requirements of lactating dairy cows as recommended by NRC (2001)

	Holstein = 680 kg BWT, Mature BWT = 680 kg, BCS = 3.3, 58 mos. age Milk fat = 3.5%, Milk true protein = 3.0%, Milk lactose = 4.8% Default environmental conditions			Jersey = 454 kg BWT, Mature BWT = 454 kg BCS = 3.3, 58 mos. age Milk fat = 4.2%, milk true protein = 3.6%, Milk lactose = 4.8% Default environmental conditions		
Days in milk	11	11	11	11	11	11
Milk production (kg)	25	25	35	35	25	25
Dry matter intake (kg)	13.5	16.1	15.6	18.8	11.9	14.3
Absorbable phosphorus (g/ day)	37.3	40.0	49.0	52.0	35.0	37.7
Dietary P % DM	0.38	0.34	0.42	0.37	0.40	0.36

Further, a review that summarized 13 studies that had higher concentrations of P than the NRC (2001) recommendations did not show that these excesses affected the days from birth to first estrus, days open, services per conception, days to first artificial insemination, and pregnancy rate (Satter and Wu, 1999).

High variation of P concentration in feed ingredients is another reason that results in excessive P in animal diets (Kertz, 1998). Furthermore, the inconvenience of testing new feedstuffs and the high cost of testing makes it impractical to measure P concentration in all feed ingredients. In addition, the inconsistency between NRC recommendations and the advice that farmers receive from nutritionist/consultants is another major factor resulting high P concentrations in the diet (Knowlton et al., 2004). Furthermore, the inclusion of high P feed ingredients in cattle diets such as by-products from corn processing and ethanol production also results in higher P concentrations than the NRC (2001) recommendations (Koelsch and Lesoing, 1999). These by-products are becoming more popular because of their high protein and energy contents and comparative low cost. Most published data support that feeding high P diets do not improve animal performance (NRC, 2001; Dou et al., 2002). These authors also observed that different concentrations of dietary P did not exert any effect on milk yield, milk fat yield or other milk components.

2.2.5 Effects of P deficiency

Reduced feed intake, body weight and milk production were observed when ruminants were fed lower P concentration diets than that of recommended by NRC (2001) for prolonged periods (Goff, 1998; NRC, 2001). It has been suggested that negative effects due to P deficiency may be due to the impaired microbial activity in the rumen or/and changes in cellular metabolism (Ternouth, 1990). During a 21-month trial, 24 multiparous cows were fed diets containing 3.3, 2.8,

2.4 g/kg P, DM basis (Valk and Sebek, 1999). The authors observed that cows fed the lowest P diet had a significant reduction in feed intake in the first dry period and that milk yield and BW were reduced during the second lactation. The other treatment groups did not show any significant differences within the two lactations and two dry periods with regards to milk yield, feed intake and BW. In addition, no differences in the milk composition and reproductive performance were observed among the diets.

In a study conducted by Wu et al. (2001), cows were fed three diets, containing 0.31, 0.39, or 0.47 % P on a DM basis. It was found that the shear strength of bones did not differ among treatments. Concentrations of ash and P in the bone did not differ between the two treatments with higher dietary P but there was a tendency towards lower bone ash and P concentrations with the 0.31% P diet. However, this was not severe enough to be reflected in bone strength.

2.3 P status in dairy farms in Manitoba

In 2004, dairy farms in Manitoba exceeded the P requirement as per NRC (2001) by 52% according to a survey carried out on 40 farms (Plaizier et al., 2004). According to that survey, dairy farms in Manitoba fed predominantly a total mixed ration (TMR) with a mean dietary P concentration of 0.44 % of DM, 25^{th} percentile of 0.37 % DM and a 75^{th} percentile of 0.48 % DM. Of the forages used on the dairy farms, alfalfa silage contained the highest average P concentration of 0.32 % (DM basis), and straw had the lowest P concentration with a P concentration of 0.10% (DM basis). As seen in Table 3, feed is the major source of P imports to the farm, representing 65 % of TP imported, while milk was the major source of P export from the farm followed by manure (Plaizier et al., 2014). The average net retention of P on dairy farms in Manitoba was 7290 kg per year, and farms with a land base which can produce feed for the cows as well as spread their manure, had an average surplus of P per hectare of 14.3 kg/yr. The average P efficiency on farms was 47.9%,

and the average milk P efficiency was 26.2%. The P efficiency was calculated as total exports divided by the total imports, and milk P efficiency was calculated as the ratio between P in milk exports and total exports of P.

Table 3. Status of whole farm P imports and exports of dairy farms in Manitoba

Item	Import average (1000 kg per year/farm)	Export average (1000 kg per year/farm)
Feeds	6.78	0.29
Fertilizer	2.31	
Animals	0.36	3.23
Bedding	0.32	-
Manure	0.24	0.24
Milk	-	2.36
Total Imports	10.44	-
Total Exports	-	3.15

Source: Plaizier et al. (2014).

2.4 Phosphorus fertilization in Manitoba

High soil P concentrations in Manitoba are not yet a widespread concern, and the majority of the fields require supplemental P for optimum yields of crop production (Johnston and Roberts, 2001; Heard, 2019). In the past several years the soil P levels in Manitoba have gone down further calling for new action plan to mitigate the low soil P level (Heard, 2019). Reason for this is that in recent years the crop removal of P has surpassed the application rate of fertilizer P. This have resulted in more soil P test values declining into the low range (less than 10 ppm Olsen P test) in some areas of Manitoba while the P application regulations are not updated since 1992 (Heard, 2019).

Similar to animals, P plays a critical role in plant functions, including photosynthesis, respiration, energy metabolism, cell division and enlargement, seed formation, early root growth, and

expansion. Therefore, the productivity of plants declines when P supply declines. As such, the adequate P supply for optimum plant growth requires the sufficient combination of organic P from the soil and inorganic P fertilizer. This was demonstrated by a study conducted in Saskatchewan in which wheat production was increased by the combination of high soil P fertility and annual P fertilizer application, compared to high soil P fertility or annual P fertilization alone (Wagar et al., 1986).

Accumulation of P in water sources, such as Lake Winnipeg may originate from livestock manure and inorganic fertilizer (Schindler et al., 2012). To reduce the potential for eutrophication, the Government of Manitoba introduced P-based regulations for livestock manure application on livestock farms in 2006 (Table 4). Previously, only nitrogen- (N) based manure application regulations were applied, but with the addition of P-based manure application regulations, farmers have had to put more emphasis on managing dietary, manure, and soil P concentrations.

2.4.1 Guidelines regarding manure application in Manitoba

The N:P nutrient ratio (5-7:1) in cow manure is smaller than the N: to P ratio removed by crops (Eghball and Power, 1999). Therefore, applying manure based on crop N requirements results in excessive application of P. Eventually, this will result in build up of soil P. One of the common methods to analyse plant available P in soil is the Olsen P test (Olsen et al., 1954). The Olsen P test measures soil P fertility. Though the test does not measure TP in soil, it provides an estimate of the plant available P in neutral to alkaline soils. Thus, this test is popular among farmers in Manitoba and is well recognized in the livestock industry.

Table 4. Soil phosphorus regulatory thresholds for livestock manure application on cropland in Manitoba, as stated in the Livestock Manure and Mortalities Management Regulation (2006)

Soil Test P Threshold (Olsen P - ppm in the 0 to 6 inch depth)	Intent of Threshold	Manure P Application
Less than 60 ppm P	No restriction of P application	Apply on the basis of crop nitrate nitrogen (N) requirements. Soil N concentrations are subject to section 12 of the Livestock Manure and Mortalities Management Regulation
Between 60 and 119 ppm P	Control soil P accumulation rate	Apply P up to 2 times the crop removal rate of P_2O_5
Between 120 and 179 ppm P	Prevent further increase in soil P concentration	Apply P up to 1 time the crop removal rate of P_2O_5
180 ppm or greater P	Depletion at a rate controlled by crop removal	No manure application without written consent of the Director

2.5 Dietary and digestive tract health-related factors that affect nutrient utilization

Several dietary and digestive tract-related factors may affect the utilization of dietary P in ruminants. These include the dietary content of carbohydrates and subacute ruminal acidosis (SARA).

2.5.1. Carbohydrates

Carbohydrates are the largest part of the dairy cow diets, consisting up to 60 to 70 % DM, and are divided into structural and non-structural carbohydrates. They are the main source of energy for the animal, as well for its rumen microflora. The carbohydrate fraction of the diet is a complex mixture of different types of monomers and polymers, broadly classified as non-structural

carbohydrate and structural carbohydrates. Non-structural carbohydrates are relatively easy to digest and found in the interior of plant cells, whereas structural carbohydrates are mostly found in the walls of plant cells. Other than acting as the energy source, structural carbohydrates are essential for the development of the rumen and for the proper function of the gut (Cerrilla and Martínez, 2003).

One of the major sources of energy in ruminant concentrates comes from grain in the form of starch. The starch DM percentage of wheat, corn and sorghum is approximately 72% and that of barley and oats is approximately 57-58% (Huntington, 1997). Processing these grains by dry rolling or steam processing, can increase the ruminal digestibility of the starch from 52% to 78% for sorghum and from 75% to 85% for corn (Huntington, 1997).

2.6 What is subacute ruminal acidosis?

Subacute ruminal acidosis (SARA) is a condition that arises when the rumen environment is exposed to high grain diets that contain excessive amounts of easily digestible carbohydrates without sufficient coarse fiber. Feeding high grain diets can result in a release of volatile fatty acids (VFA) and lactic acid at rates that are higher than the absorption rates of these acids and the buffering capacity of the rumen (Nordlund et al., 1995). This will lead to a rumen pH depression that disrupts the normal function and activity of the rumen microbiota and, therefore, the proper function of the rumen. The ruminal papillae play a key role in absorption of VFA and lactic acid. These VFA, especially butyrate, stimulate the growth of rumen papillae. However, rumen papillae are not yet well developed in cows that are transitioning from a dry cow diet to a high grain lactation diet, limiting their absorptive capacity. Therefore, during this transition period, cows are more susceptible to SARA (Kleen et al., 2003).

Even though an exact definition for SARA has not been established, a drop in the rumen between pH 5.2 to 5.6 for periods of 3 hours or more per day is generally considered to be indicative of SARA (Gozho et al., 2005). A rumen pH depression below 5.2 is considered indicative of acute ruminal acidosis, but this disorder is uncommon in dairy cows (Plaizier et al., 2008). The pH criteria for SARA can differ according to the methods used for monitoring of the rumen pH, which can be measured by collecting rumen fluid using a stomach tube, by rumenocentesis, and by placing a pH probe inside the rumen of rumen-cannulated cows. These different methods measure the pH from different areas of the rumen which can result in different values for the same cow, at the same time (Duffield et al., 2004; Plaizier et al., 2008).

Rumen fluid samples collected using rumenocentesis can have pH values that are 0.35 and 0.33 pH units lower, compared to samples collected using a stomach tube and samples collected from the ventral sac of rumen-cannulated cows, respectively (Table 5). Therefore, it is suggested to use pH values less than 5.5, 5.8 and 5.9 to define and diagnose SARA when collecting rumen fluid using rumenocentesis, through rumen cannula from ventral sac and stomach tube, respectively.

Table 5. Methods and pH criteria used to define SARA in previous studies

Study	pH	Sample collection method
Garrett et al. (1999)	5.5	Rumenocentesis
Plaizier (2004)	6.0	Stomach tube - 4 h post feeding
Gozho et al. (2005)	5.2-5.6 for at least 3h/day,	Indwelling pH probes
Cooper et al. (1999)	5.2 – 5.6	Indwelling pH probes
Beauchemin et al. (2003)	< 5.8	Indwelling pH probes

2.6.1 Prevalence of SARA

The risks of SARA during the early and mid lactation periods are considerably higher compared to late lactation. A study in Wisconsin reported 19% and 26% prevalence of SARA in the early and mid lactation stage, respectively (Garrett et al., 1997). Further, a 20.1% prevalence of SARA in early- and mid-lactation herds was observed in a study conducted on 14 dairy farms in Wisconsin (Oetzel et al., 1999). Cows in early lactation are susceptible to SARA for several reasons. They suffer from a considerable amount of stress due to calving, changes in housing, onset of milk production, and low feed intake. These changes can result in a severe negative energy balance, and expose the cow to ketosis, depress immunity, and increase the susceptibility to other diseases (Kleen et al., 2003). In addition, the transition of the diet from low-energy, dry cow diet to high-energy, lactating diet can increase the susceptibility to SARA, as well (Brand and Warner, 1996; Nocek, 1997; Plaizier et al., 2008). A reason for this is that the rumen papillae of dry cows are not yet fully developed during early lactation, after the transition from the dry period (Kleen et al., 2003). At this time, the rumen papillae are relatively short and have less surface area to absorb the increasing amounts of VFA that are produced in the rumen compared to later in lactation (Nordlund et al., 1995). In addition, high energy diets generally contain insufficient coarse fiber to stimulate rumination, saliva secretion and rumen buffering (Mertens, 1997). When the diet lacks coarse fiber, the buffering activity through saliva secretion will be insufficient. Ultimately, these changes lead to accumulation of fatty acids in the rumen, and result in depressed rumen pH, thereby affecting acid-sensitive microbiota of the rumen (Nordlund et al., 1995).

2.6.2 Implications and signs of SARA

Many clinical symptoms have been identified, and studies on additional physiological parameters to diagnose SARA are still in progress. None of these symptoms can individually be used to diagnose SARA and, therefore, a combination of signs and symptoms needs to be considered when diagnosing SARA in the field. Several signs and symptoms are discussed below.

2.6.2.1 Reduced feed intake

Feed intake depression is often considered as a consistent clinical sign associated with SARA. This depression in DMI can be more than 25%, during a SARA-induced period compared to normal feeding (Krajcarski-Hunt et al., 2002). Similarly, Khafipour et al. (2009b) observed that grain-induced SARA reduced DMI of lactating dairy cows from 19 to 17 kg/day. Olsson et al. (1998) also reported that cows fed a high-grain diet had lower DMI compared to cows fed a low grain diet.

Several reasons have been suggested for this depression in feed intake. It has been suggested that reduced fiber digestion, high osmolality of rumen digesta, and the reduction of rumen motility in the rumen due to high-grain feeding reduce feed intake (Slyter, 1976; Allen, 2000). Reduced *in situ* fiber digestion, resulting in higher rumen fill, was observed when SARA was induced by feeding a high-grain diet (Plaizier et al., 2001; Krajcarski-Hunt et al., 2002). However, it has also been reported that DMI of dairy cows was reduced when SARA was induced by feeding pellets of ground alfalfa without increasing the dietary grain content. This suggests that factors other than fiber digestion, rumen VFA and high osmolarity might be affecting the feed intake depression during SARA (Khafipour et al., 2009a). Another mechanism suggested for the reduction in feed intake due to SARA is inflammation, as inflammation commonly reduced feed intake (Weingarten,

1996). It is suggested that the pH depression in the rumen due feeding a high-grain diet triggers the release of vasoactive substances, such as histamines and lipopolysaccharide (LPS) of bacterial origin. These substances can induce a systemic immune response. According to Khafipour et al. (2009a), grain-induced SARA increases concentrations of inflammation markers, such as the acute phase proteins, serum amyloid A, and haptoglobin in peripheral blood plasma (Gozho et al., 2007). Further, prolonged pH depression can increase the concentration of free rumen LPS in digesta in the rumen and large intestine, and cause ruminitis which can result in reduced barrier function of rumen mucosa and translocation of free LPS from rumen into blood stream leading to inflammation in other organs (Dirksen et al., 1985; Nocek, 1997; Kleen et al., 2003). It has also been reported that SARA is associated with an inflammatory response of the liver (Rossow, 1984; Oetzel, 2000), as well as with inflammation and abscesses on kidneys (Rossow, 1984; Oetzel, 2000), lungs (Nordlund et al., 1995), and the heart (Oetzel, 2000).. This suggests that inflammation during grain-induced SARA may be playing a role regarding the feed intake reduction.

2.6.2.2 Milk fat depression

Depression of milk fat during SARA have been often documented (Oetzel, 2000; Kleen et al., 2003; Oetzel, 2003; Stone, 2004). Many studies have shown that depression of milk fat is associated with changes in the diet including increased grain feeding. Such increases may be due to errors in the feeding strategy, such as inclusion of large amounts of grain, processing of roughages, and supplementation of fatty acids (Gürtler and Schweigert, 2000). Changes in the milk fat are difficult to observe, as they occur in individual cows and will go undetected during bulk milk testing (Garrett, 1996). However, milk fat depression due to SARA is still inconclusive, as some studies have not observed any changes in milk fat level due to induction of SARA (Keunen

et al., 2002; Gozho et al., 2007). A field study conducted in New York State to demonstrate the impacts of SARA reported a reduction of milk production and milk fat percentage by 2.7 kg/day and 0.3%, respectively, in response to SARA (Stone, 1999). Further, this was accompanied with increase in milk protein by 0.12% (Stone, 1999). Khafipour et al. (2009a) also observed that grain-induced SARA reduced milk production, milk fat percentage, and increased milk protein percentage by 3.3 kg/day, 0.37% and 0.13%, respectively.

Milk fat depression is attributed to rumen environment changes, namely a reduction of acetate production, increase in propionate production, and increases in long chain trans fat polyunsaturated fatty acids, such as trans 12-cis 12 C18:2, increased blood insulin, and reduced *de novo* milk fat synthesis (Murphy et al., 2000; Khorasani and Kennelly, 2001; Bauman and Griinari, 2003).

2.6.2.3 Diarrhea

Diarrhea is commonly reported in relation to SARA (Oetzel, 2000; Garry, 2002). The rate of rumination, rumen microflora and ruminal passage all affect the feces structure and its consistency (Garry, 2002). Feces of cows with SARA produce bright and yellowish feces that has a sweet-sour smell (Kleen et al., 2003). The feces may also appear foamy and may have relatively high quantities of undigested fiber or grain (Kleen et al, 2003; Plaizier et al., 2008). This is a result of the absence of a sufficient fiber mat in the rumen. As a result, feed particles are not retained long enough in the rumen, resulting in a fast digesta passage, and feces that contain 1–2 cm sized fiber particles, compared to a normal size of 0.5 cm (Hall, 2002). These changes generally suggest occurrence of hindgut fermentation (Nordlund et al., 2004). When there is high concentration of starch in diet, the outflow of undigested carbohydrates from the rumen that may undergo fermentation in the hindgut is increased. This will increase the production of VFA and carbon

dioxide in the hindgut. In this process, the hindgut absorbs the VFA, while carbon dioxide will be trapped in feces giving a foamy appearance (Nordlund et al., 2004; Plaizier et al., 2008). The VFA production during hindgut fermentation may also result in depression of pH in the hindgut (Plaizier et al., 2012b), causing damage to its epithelial cells. To reduce this damage, the animal will secrete more mucus and fibrin, resulting in diarrhea (Argenzio et al., 1988; Hall, 2002).

2.6.2.4 Reduction in fiber digestion

A reduction in fiber digestion is commonly observed during SARA. A study conducted by Krajcarski-Hunt et al. (2002) reported an average 20.5% and 24.8% reduction in *in-situ* NDF digestibility after feed sample incubations of 24 and 48 h, respectively, during grain-induced SARA. Another study also reported that grain-induced SARA reduced *in-situ* NDF digestibility of mixed hay by 19.6% and 21.8% after 24 and 48 h, respectively (Plaizier et al., 2001). The decreased fiber digestion during SARA is suggested to be due to acid sensitivity of cellulolytic rumen microbes. The naturally residing cellulolytic bacteria in the rumen cannot tolerate the low pH in the rumen that occurs during SARA, resulting in a reduction in microbial population and functionality (Shi and Weimer, 1992).

2.6.2.5 Laminitis and inflammation

Laminitis is a clinical symptom that has been associated with SARA (Garrett, 1996; Oetzel, 2000). This sign is scientifically known as "pododermatitis aseptic diffusa". Laminitis is defined as inflammation of the dermal layer of the hoof (Nocek, 1997), and causes considerable economic losses, animal welfare problems and pre-disposes cattle to other diseases (Nelson and Cattell, 2001). Laminitis, which contributes to feet and leg problems, is the 4th major reason for the culling

of dairy cows in North America (Canwest, 2004). Feeding high-grain diets to cattle increases the risk of laminitis (Kelly and Leaver, 1990). The amount and frequency of grain consumption influence the development of laminitis (Bergsten, 1994; Valk and Sebek, 1999). The exact mechanism that is causing laminitis is still not well understood, but is believed to be multifactorial (Nocek, 1997; Ruegg, 2000). One such proposed mechanism is that the low pH during SARA periods triggers the release of vasoactive substances, such as histamine and lipopolysaccharide endotoxins (LPS) in the rumen and in the large intestine (Nocek et al., 1997; Plaizier et al., 2012). The barrier function of the epithelia of the rumen and large intestine may also be compromised by SARA, which results in the translocation of these substances from the digestive tract to the blood circulation. These substances may damage capillaries of the lamellae in the hoof resulting in hemorrhages, inflammation, anatomical changes and lameness (Nocek, 1997). In agreement, Khafipour et al. (2009a), observed that grain-induced SARA in lactating dairy cows increased the concentration of LPS in peripheral blood plasma. In contrast, Gozho et al., (2007) did not observe any increase in LPS in peripheral blood during SARA, but that contrast may be explained by relatively poor sensitivity of the LPS assay of Gozho et al. (2007) and detoxification of LPS in the liver. Grain-induced SARA is commonly associated with increases in the concentrations of acute phase proteins in peripheral blood (Plaizier et al., 2012; Plaizier et al., 2018). These increases may also be caused by the translocation of LPS and other immunogenic substances out of the digestive tract (Plaizier et al., 2012; Plaizier et al., 2018).

2.7 Feed supplements to reduce the effects of SARA

SARA disturbs natural residing microflora and its natural balance particularly to beneficial fibrolytic bacteria, including those that breakdown phytate, in the rumen which can result in

reduced nutrient digestibilities and incomplete breakdown of phytate (Plaizier et al, 2001; Plaizier et al., 2008; Knowlton et al., 2007). This can result in a poor release and digestion of P from the fiber in the diet, and lead to a reduction in P utilization. Direct fed microbials (DFM) may overcome some of the adverse effects of SARA (Plaizier et al., 2018). However, no studies have been conducted to investigate the effects and effectiveness of these probiotics and DFM on the utilization of dietary P by cows on high-grain diets.

2.7.1 Probiotics

There are several definitions for the term "probiotic". According to Vanbelle et al. (1990) probiotics are "natural intestinal bacteria that, after oral administration in effective doses, are able to colonize the animal digestive tract, thus keeping or increasing the natural flora, preventing colonization of pathogenic organisms and securing optimal utility of the feed". Fuller (1991) also defined them as "live microbial feed supplements that beneficially affect the host animal by improving its intestinal microbial balance."

Considering these definitions, there are several criteria that a group of microorganisms must fulfill to be considered as an effective probiotic. According to Dunne et al. (1999) probiotic microorganisms must demonstrate one or more of the following qualities:

1. Must not exert any pathogenic or toxic activity to the live host
2. Must be durable to withstand the technological process related to diet preparation
3. Should be immune to the gastric juice
4. Demonstrate adhesive capabilities to the GI tract of the host
5. Able to resist and remain in the gastrointestinal tract in large numbers
6. Able to secrete substances that either supress or promote other microbiota

7. Induce beneficial immune reactions to the host
8. Capability to stimulate/induce beneficial metabolic activities

Probiotics are considered to be “alive” (Dunne et al., 1999). Several “dead” microbial-based feed supplements are also available, which are considered direct fed microbials (DFM) (Martin and Nisbet, 1992; Nocek and Kautz, 2006).

2.7.2 Direct-fed microbials in ruminants

Manipulating the microbial ecosystem of the rumen to improve production efficiency in domestic ruminants has been studied in recent years. Growth stimulants and antibiotics have been utilized in the feed industry for maintaining optimal animal performance and microbial profile. Much research has been aimed at reducing feed energy losses associated with methanogenesis in the rumen, thereby increasing energy utilization. To this purpose, DFM are considered. This involves feeding of beneficial microbes and microbial products to farm animals when they are under stress conditions such as disease, ration changes, environmental or production changes. For ruminants, microbial cultures have been used to potentially replace or reduce the use of antibiotics in neonatal and stressed calves, to enhance milk production in dairy cows, and to improve feed efficiency and daily gain in beef cattle (Krehbiel et al., 2003).

The term DFM and probiotics are sometime used interchangeably, which creates confusion. The U.S. Food and Drug Administration (FDA) recommends manufacturers to use the term DFM rather than the probiotics and defined DFM as ‘products that are purported to contain microorganisms such as bacteria, fungi, and yeast’.

Few studies have been done to evaluate the effects of DFM on ruminal microbial fermentation and ruminant performance. The ruminant gastrointestinal tract is constantly challenged by a large

number of bacteria, virus, protozoa found in feed, bedding and the environment (Adams et al., 2008). The composition and functionality of microbiota in the intestines are affected by stress caused by weaning, transportation, changes in diet or weather, and treatments with antimicrobials that change the composition and functionality of microbiota in the intestines creating unfavorable conditions in the rumen for the animals. As a result, intestinal defensive mechanisms are impaired, making the animal more susceptible to disease. Cows undergoing nutritional stress have responded quite favorably to large doses of DFM (Krehbiel et al., 2003). This can be due to inhibition of the growth of potential pathogens in the intestine by producing organic acids which create a low pH environment during the fermentation (Leeuw et al., 2009; Kenney et al., 2015; Vyas et al., 2015). Furthermore, some DFM produce antimicrobial (bacteriocins) compounds while controlling pathogens and strengthen the immune system which can help minimise the nutritional stress and stabilise the gut health (Williams and Newbold, 1990).

Most commercial DFM products used specifically for calves contain one or more types of bacteria combinations such as *Lactobacillus acidophilus, L. lactis, L, plantarum, L. casei, Bacillus subtilis, B. lichenformis, Bifidobacterium bifidum, B. longum,* and *B. thermophilum* (Seo et al., 2010). Commensal bacteria ferment carbohydrates and produce short fatty acids which can reduce intestinal pH and inhibit the growth of some pathogens while improving digestion and absorption. DFM provide a barrier effect against pathogens by competitive exclusion and improve the immune system of young animals. According to Kung Jr (2001) *Megasphaera elsdenii* prevents severe rumen acidosis when diets change to those with highly fermentable carbohydrates and *Lactobacillus acidophilis* increases the milk yield when feed intake is depressed. Furthermore, *Propionibacterium freudenrechii* and *L. acidophilis* improve the feed efficiency in cattle and *Propionibacteria* and *L. acidophilis* improves feed efficiency during adaptation to higher starch

diets. *Propionibacterium acidipropionic* increases propionic acid in the rumen, whereas *Propionibacterium freudenrechii* improves weight gain in calves (Kung Jr, 2001; Seo et al., 2010). Non-bacterial DFM, such as fungi and yeast, generally consist of *Aspergillus oryzae* extracts or *Saccharomyces cerevisiae* cultures, or both. These products contain viable cells plus the growth medium (Williams and Newbold, 1990). Van Horn et al. (1984) reported that cows receiving *Aspergillus oryzae* supplementation exhibited significant improvements in the digestibility of DM and ADF, but no differences in milk production or feed intake were observed. In addition, Gomez-Alarcon et al. (1990) found that *Aspergillus oryzae* treatment increased milk production in early lactation cows. Furthermore, Martin and Nisbet (1992) reported that total tract digestibility of CP and hemicellulose was increased by *Saccharomyces cerevisiae* supplementation in non-lactating Holstein cows, but digestibility of DM and ADF remained unchanged.

According to Martin and Nisbet (1992), DFM can increase weight gain, milk production, and total tract digestibility of feed components. Furthermore, Dawson et al. (1990) observed that DFM increases cellulolytic bacterial numbers in the rumen and stimulated the fermentation end products while providing growth factors for the ruminal microbia. According to Savage (1977), the primary objective of supplementing DFM for dairy calves is for the rapid adaptation to solid feed by accelerating the establishment of ruminal and intestinal microorganisms and avoiding the establishment of enteropathogens, which often results in diarrhea. In the neonate and in stressed calves, the microbial population is in transition and extremely sensitive and abrupt changes in diet or the environment can cause alterations in microbial populations in the gastrointestinal tract (Warner, 1962). By summarizing all the findings, most results suggested that DFM improves body weight gain. However, the efficiency of DFM may be limited in young milk-fed calves. Under

stress conditions, DFM may reduce the severity of scours caused by a disturbance of the normal intestinal flora of the calf.

When supplemented to calves, DFM is mixed with milk or milk replacer. When water is used instead of milk or milk replacer, chlorination, temperature, minerals, digesta flow rates, ionophores, and antibiotics must be considered to avoid killing or reducing the effectiveness of DFM products (Krehbiel, 2014).

Adding DFM to pelleted feeds may be difficult when the pellets are produced at high temperatures and pressure, as this can kill and denature microorganisms. Although some products contain purified strains of individual microorganisms, most products are a combination of species of bacteria, yeast and fungi and typical feeding rates of DFM for ruminants range between 3 and 110 g/d per animal (Williams and Newbold, 1990).

2.7.1.1 Saccharomyces cerevisiae fermentation products

Products from *Saccharomyces cerevisiae* yeast are used commonly in the dairy industry. *Saccharomyces cerevisiae* fermentation products (SCFP) are dried end products of *Saccharomyces cerevisiae* fermentation grown on culture media under standardized conditions and are therefore are classified as DFM. This dried yeast culture contains many beneficial metabolites that facilitate the growth and activity of fiber-digesting bacteria and fungi in rumen (Hristov et al., 2010). It is believed that these beneficial metabolites include soluble growth factors, such as amino acids, organic acids such as malic acid, B vitamins, as well as oxygen scavenging compounds (Newbold et al., 1996; Callaway and Martin, 1997). Many strains of yeast have been used with variable success to enhance the ruminal environment and cow performance, especially when the rumen environment is stressed during disorders such as SARA (Desnoyers et al., 2009; Robinson and

Erasmus, 2009; Hristov et al., 2010). Therefore, it is believed that supplementing with these SCFP can attenuate several symptoms of SARA, including the disturbed functionality of rumen microbiota.

It has been suggested that SCFP stabilize the ruminal pH through decreased lactate production (Marden et al., 2008). Also, Longuski et al. (2009) reported that *Saccharomyces cerevisiae* fermentation products improved milk production when the rumen was challenged by feeding highly fermentable carbohydrates. Another study reported that yeast culture products increased dry matter (DM) digestion, propionic acid production, and protein digestion, suggesting that these yeast products can alter the microbial metabolism in the rumen (Miller-Webster et al., 2002). Li et al. (2012) also revealed that SCFP reduced ruminal LPS concentration under SARA conditions, suggesting that SCFP can stabilize the rumen environment under these conditions.

In order to develop and implement effective strategies to enhance gut health in cattle, the modes of action of SCFP must be understood. However, the mechanisms regarding how these SCFP increase bacterial counts in the rumen are not yet clearly understood. Nisbet and Martin (1991) and Martin and Nisbet (1992) showed that aqueous extracts prepared from *S. cerevisiae* stimulated the growth and activity of the lactic acid-producing rumen bacteria *Selenomonas ruminantium* in pure culture. This stimulation is believed to be due to be high concentration of malic acid in the yeast product (Kung Jr. et al., 1982; Nisbet and Martin, 1991; Martin and Nisbet, 1992). Newbold et al. (1996) demonstrated that the stimulation of rumen bacteria by *S. cerevisiae* is, at least partly dependent on its respiratory activity and is not solely mediated by malic acid. However, they observed that that malic acid in yeast stimulates growth and metabolic activities of pure cultures of common rumen micro-organisms in vitro, but not in vivo. Yeasts also provide vitamins for the growth of rumen fungi (Chaucheyras et al., 1995).

Hristov et al. (2010) suggested that the overall utilization of ruminal ammonia for microbial protein synthesis is enhanced by yeast product supplementation. Ruminal bacteria have a strong preference for ammonia as their N source. Hence, yeast supplementation can result in more efficient conversion of ruminal ammonia into microbial protein, reduce urinary N losses, and improve the overall utilization of dietary N in the ruminant animal. The study of Hristov et al. (2010) demonstrated that SCFP reduce ruminal ammonia concentration and increased microbial protein synthesis in the rumen of dairy cows provided with the yeast supplemented.

2.8 Summary

Phosphorus utilization has a significant impact on the environmental sustainability of cattle operations. Reasons for this include the common practice of exceeding dietary P requirements, the increasing use of high P feeds, and the increased risk of eutrophication of water sources when excess P is excreted into the environment. Cattle, including dairy cows, are commonly fed in large groups, which makes meeting the individual nutrient requirements of cattle challenging. In formulating diets for such large groups, meeting the P requirements of the highest milk producing cows appears to get more attention than the prevention of excess P feeding to lower producing cows. Also, the increased use of high P feeds, such as distillers grain, wheat bran, and brewers grain creates a risk of overfeeding P, especially when meeting the energy and protein requirements are given priority in diet formulation. The use of inorganic P supplements in dairy cow diets appears to be decreasing due to the increased availability of other less expensive high P feeds, such as grain by-products. Hence, from a feed cost point of view, feeding excess P to dairy cows may not be a significant issue. However, increases in the P concentration in manure due to excessive P feeding may lead to excessive manure P application rates in Manitoba. This may not have large

financial and logistical impacts for Manitoba dairy farmers in the short term. However, by looking at the intensive dairy industries in many other industrialized countries, it can be predicted that these impacts in Manitoba will increase, as dairy farmers may require more land for the spreading of their manure. As a result, the efficiency of the utilization of dietary P on dairy farms in Manitoba, and elsewhere in Canada must be improved. Current best management practices for P nutrition on Manitoba dairy farms include matching the supply and requirements of P of individual cows, but these practices may not always be implemented. We believe that increasing the total tract digestibility of dietary P will enhance P utilization, but more research is needed to develop feeding strategies to achieve this.

The utilization of dietary phytate P by monogastric animals are low, as they do not have the enzymes to break down this compound. However, ruminants differ from monogastrics in that, through symbiosis with microbiota in the digestive tract, they can breakdown phytate P and therefore, can utilize phytate P. Evidence exists that current feeding practices, such as high-grain feeding that can result in subacute ruminal acidosis (SARA), may reduce this breakdown by reducing the functionality of beneficial phytase-producing gut microbiota, thereby reducing the utilization of dietary P. It is assumed that supplementation with yeast products, such as *Saccharomyces cerevisiae* fermentation products (SCFP) attenuates the impact of high-gain diets and SARA on the functionality of these beneficial gut microbiota, including their phytase production. Testing these assumptions are among the main goals of this thesis.

3. HYPOTHESES

Study 1.

I. Dietary, animal, and farm factors, including the dietary fiber, P, and starch concentrations, affect P utilization and digestibility in lactating dairy cows on commercial dairy farms.

Study 2.

I. Grain-induced SARA reduces the total tract digestibility of dietary P in lactating dairy cows
II. A reduction in the total tract digestibility of nutrients due to grain-induced SARA can be attenuated by supplementation with *Saccharomyces cerevisiae* fermentation products (SCFP)

4. OBJECTIVES

Study 1.

I. Investigate the relationships between dietary and animal factors and P utilization on commercial dairy farms in Manitoba.

Study 2:

I. Investigate the effects of grain-induced SARA on the total tract nutrients digestibilities of DM, P, starch, and NDF in lactating dairy cows.
II. Determine whether supplementation with *Saccharomyces cerevisiae* fermentation products affect the total tract digestibility of DM, P, starch, and NDF and if these effects differ between control and grain-induced SARA in lactating dairy cows.
III. Investigate the effects of different types and dosages of supplementing SCFP on nutrient digestibility.

5. MANUSCRIPT 1 (SHORT COMMUNICATION) – RELATIONSHIP BETWEEN DIETARY, PRODUCTION AND ANIMAL FACTORS REGARDING PHOSPHORUS UTILIZATION IN DAIRY FARMS IN MANITOBA

5.1 Abstract

A survey was carried out on 19 dairy farms in Manitoba that varied in size, diet composition, housing, and feeding strategy to identify factors that affect the phosphorus (P) concentration of feces and the excretion of P in milk. On each farm 10 early, 10 mid and 10 late lactation cows were included. Multiple regression analysis showed that high dietary P concentrations and free-stall instead of tie-stall housing were associated with higher P concentrations of feces. This suggest that a closer matching of the requirements and supply of P of dairy cows, especially in free stall-housing, is needed to enhance the utilization of dietary P on these farms.

Key words: phosphorus, dairy, cow

5.2 Introduction

Due to increases in the sizes of dairy farms and the milk production of dairy cows, these operations generate large quantities of manure. Among the components of manure, phosphorus (P) represents a great concern due to its contribution to eutrophication of water bodies (NRC, 2001). Dairy farmers must, therefore, adopt practices that reduce the P excretion in the manure of their cows. Increasing the dietary P concentrations above requirements reduces the total tract digestibility of P and increases P excretion in feces without increasing milk yields (Wu et al., 2000; Knowlton and Herbein, 2002). In an attempt to reduce P excretions, the dairy industry has reduced the P concentrations of dairy cow diets in recent years. Surveys on Manitoba dairy farms have suggested that the average P concentrations in dairy cow diets decreased from 0.48 % DM in 2002 (Plaizier

et al., 2004) to 0.45 % DM (Plaizier et al., 2014). The dietary P concentration from Plaizier et al. (2014) are still considerably higher than the recommended range of between 0.32 - 0.42 % of DM (NRC, 2001). Most studies on the factors affecting the utilization of dietary P by dairy cows have been conducted in controlled experiments at research institutions. The relationships between animal, management and dietary factors and the utilization of dairy P on commercial dairy farms with large variations in these factors have been less well studied. However, a better understanding of these relationships is needed to development strategies that enhance the utilization of dietary P.

5.3 Materials and methods

A survey was carried out on 19 dairy farms in Manitoba that varied in herd size, feeding, housing and management systems. Each farm was visited once to collect milk, feed, blood, and feces samples as well as production records from 10 early-lactation, 10 mid-lactation and late-lactation cows, defined as 1 – 100, 100 – 200 and 200 – 300 days in milk (DIM), respectively. Feed, milk, blood, and feces samples were collected, processed and analyzed as described by Plaizier et al (2014). Milk production data were obtained from Canwest Dairy Herd Improvement (DHI, Guelph, ON). Summary statistics were calculated with the MEANS procedure of SAS 9.4 software (SAS Institute, Cary, NC). Multiple regression models were developed using the MIXED procedure of the SAS 9.4 software (SAS Institute, Cary, NC) to test the relationships between the feces phosphorus concentration with parity, DIM, milk yield, milk fat concentration, milk protein concentration, body condition score, and diet composition, as described by Plaizier et al. (2004). Diet composition variables included dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), starch, crude fat (CF), ash, Ca and P. Farm variables included in the model were herd size, feeding practice (total mixed ration (TMR)- or component-fed), number of production groups, and

housing (tie stall or free stall). Independent variables with a probability greater than 0.25 were stepwise removed from the model. The R-square, CV, and root MSE of the final model of feces P concentration were 0.36, 19.94, and 0.15, respectively.

5.4 Results and discussion

Summary statistics are given in Table 6. These results show that, on average, the milk yields and milk protein concentrations of the cows included in the survey were very close to industry averages, whereas the milk fat concentration of these cows was, on average, higher than this average (Canwest DHI. 2017). The average dietary crude protein and crude fat concentration of the diets were 16.8 and 3.5 % of DM, respectively, with only small variations among farms. The dietary medians for NDF and NFC were 33.5 and 38 % of DM, respectively. NRC (2001) recommends that for barley grain-based dairy diets, such as these that are common in the Canadian prairies, the minimum NDF concentration should be 34% of DM. Hence, approximately half of the farms did not meet this NDF guideline, which would increase the risk of SARA. Diets contained less NDF than the maximum inclusion rate recommended by NRC (2001) for both corn grain and barley grain-based diets. The median, 25th percentile and 75th percentile of the starch concentrations of the diets were 22.6, 19.3, and 25.2% of DM, respectively. Recommendations for dietary starch concentrations are not provided by NRC (2001). However, 25% DM starch concentration has been suggested as close to optimal (Staples, 2007). Dietary starch concentrations of 27.9, 31.8, and 33.7 % DM have been used to induce SARA successfully. Hence, we can conclude the cows in 75th percentile for high starch diets are at risk of SARA.

Table 6. Summary statistics of dietary and cow variables

Variables	Min	25 pctl	Median	75 pctl	Max
Milk					
DIM, d	4	72	170	259	464
MY, kg/d	9.5	30.5	36.8	42.2	65.1
Fat, %	1.04	3.74	4.24	4.76	6.99
Protein, %	2.46	3.09	3.34	3.57	4.66
P, %	0.05	0.09	0.09	0.10	0.12
Feces					
P, % DM	0.30	0.64	0.77	0.88	1.79
starch, % DM	0.26	1.43	3.57	5.08	12.7
Blood					
P, mmol/L,	1.34	1.83	2.03	2.21	3.04
Diet					
CP, % DM	14.62	15.93	16.78	17.57	18.50
Diet NDF, % DM	21.26	30.52	33.62	35.79	43.50
Diet ADF, %DM	13.57	20.39	23.30	26.04	31.47
Diet starch, % DM	12.55	19.27	22.39	25.15	32.10
Diet NFC, % DM	21.75	35.46	37.48	40.45	43.60
Diet fat, % DM	2.23	3.06	3.78	4.22	5.93
Diet ash, % DM	6.72	7.59	8.60	9.12	14.92
Diet Ca, % DM	0.50	0.89	1.24	1.26	2.04
Diet P, % DM	0.34	0.38	0.42	0.45	0.53

The milk P concentration did not vary substantially among cows and averaged 0.9 +/- 0.04% (mean +/- SD), which is similar to the values reported by NRC (2001) and Plaizier et al. (2014). The median dietary P concentration was 0.42 % DM, whereas the 25th, and 75th percentile values for this variable were 0.38 and 0.45 % DM, respectively. This shows that more than half of the diets in our survey contained more P than recommended by NRC (2001). Nevertheless, Plaizier et al. (2004) reported that the median, 25th and 75th percentiles of the dietary P concentration were 0.48, 0.42, and 0.54% of DM. In a later survey, Plaizier et al. (2014) reported that the median, 25th and

75th percentiles of the dietary P concentration were 0.45, 0.37, and 0.48 %, respectively. This suggests that since this report, the dietary P concentration of dairy cow diets in Manitoba may have decreased.

We intended to determine total tract digestibilities P using acid insoluble ash as a marker to assess the utilization of dietary P. However, the data obtained with this technique were not reliable, as too many digestibility values were found to be outside of the expected physiological range. This technique has been found reliable in a controlled experiment setting, where several feces samples per animal were collected per experimental period (McGeough et al., 2010). However, in our survey, only one feces sample was collected per animal, and many sources of error and variations existed that may make this technique unreliable. As a result, the utilization of dietary P was assessed only by utilising feed and feces P concentrations.

The results of the multiple regression analyses of the feces P are given in Table 7. The number of production groups, dietary NDF concentrations, and feces starch concentrations were associated with lower feces P concentrations, whereas increases in DIM and the dietary concentrations of P and Ca were associated with higher feces P concentrations. Cows in tie stalls and component-fed cows had lower feces P concentrations than free stall-housed and TMR-fed cows. Of these factors, the dietary P concentration had the largest effect, followed by housing. The positive correlation between the dietary P and feces P concentrations was expected, as increases in the dietary P concentration above the P requirements decrease the total tract digestibility and P absorption efficiency (Wu et al., 2000; Knowlton and Herbein, 2002). The housing effect suggests that in a tie stall, matching of the supply and demand for nutrients of individual cows is easier than in a free stall as in a tie stall feed is delivered to individual cows, and competition for feed is absent. The same may be concluded for component feeding, and the number of production groups/diets.

Furthermore, DIM showed positive relationship with feces P, suggesting that the stage of lactation might be playing a role in P utilization. This may be due to the decrease in milk production and P requirements in late lactation, without a reduction in the dietary P concentration. The reductions of the feces P concentration due to increases in the dietary NDF concentration and the feces starch content in our study seems contradictory, as increases in the dietary grain content reduce the dietary forage content (NRC, 2001). Grain contains more phytate P compared to forages. Hence, replacing

Table 7. Parameter estimates of the equation between feces P concentration (% DM, dependent variable), and cow, herd, feeding, and diet variables (independent variables) determined by multiple regression. Only intercept, estimates of significant ($P<0.05$) independent variables and independent variables tending towards significance ($0.05<P<0.10$) are given

Independent variables / Intercept	Value	Estimate	SE	P-value
Intercept	-	1.162	0.132	<0.0001
Feeding	Component	-0.095	0.026	0.0002
	TMR	0	-	-
Housing	Free stall	0.2524	0.035	<0.0001
	Tie stall	0	-	
Herd size		-0.0007	0.0001	<0.0001
Number of groups/farm		-0.101	0.009	<0.0001
DIM		0.0003	0.00007	<0.001
Diet CP		0.0155	0.009	0.07
Diet Ca		0.019	0.006	>0.001
Diet P		0.936	0.201	0.05
Diet NDF		-0.009	0.002	<0.0001
Feces starch		-0.020	0.003	<0.0001

forages with grain increases the proportion of total P that consists of phytate P. Rumen microbes can degrade phytate P, but that degradation may not be complete (NRC, 2001; Knowlton et al., 2007). Assuming that to be true, then the increase in phytate P due to the increased grain feeding, could indeed reduce the total tract P utilization. Another explanation may be that increases in the

dietary grain concentration may increase the digestibility of the diet, resulting in less feces and less dilution of the excreted P in the feces. In that situation, a positive relationship between feces starch and feces P would be expected. That this did not occur, may be due to differences in the type and digestibility of diets starch among diets and farms. Confounding factors may also have been responsible, as the dietary starch, NDF, and ADF concentrations were correlated with the dietary P concentration.

Our results suggest that a closer matching of the demand and supply of dietary P of individual cows will contribute the most to enhancing the utilization of dietary P. Until recently this was challenging, as many Manitoba dairy farms used only one TMR for the entire herd. Even in farms which use more than one TMR designed according to high and low yielding groups, these TMRs did not vary greatly in their dietary P concentration (Plaizier et al., 2014). Further, many common feeds used on these farms, such as distillers' grains and wheat bran are high in P (NRC, 2001, Plaizier et al., 2004; Plaizier et al., 2014). Hence, reducing the inclusion rate of P supplements, such as dicalcium phosphate, is not always sufficient to reduce the dietary P concentration to the desired concentration. However, recently the use of a partial TMR (pTMR), with supplementation of concentrate in voluntary milking systems or programmable feeders have increased greatly (Canwest DHI, 2017 DHI). In addition to providing the required amounts of energy and protein of group-housed cows, this technology could also be used to prevent direst excesses and deficiencies of P.

6. MANUSCRIPT 2 - EFFECTS OF *SACCHAROMYCES CEREVISIAE* FERMENTATION PRODUCTS AND SUBACUTE RUMINAL ACIDOSIS (SARA) ON NUTRIENT UTILIZATION IN DAIRY COWS

6.1 Abstract

The effects of *Saccharomyces cerevisiae* fermentation products (SCFP) on digesta pH and on total tract nutrient digestibilities were determined in 32 lactating Holstein cows. Cows were assigned to four treatments: 1) a control and three SCFP supplementations that included 2) 14 g/d Diamond V Original XPCTM (XPC, Diamond V, Cedar Rapids, IA), 3) 19 g/d NutriTek® (NTL, Diamond V), or 4) 38 g/d NutriTek® (NTH, Diamond V) starting 4 wks before calving. Cows were monitored from wk 4 to wk 9 of lactation. SARA challenges were conducted during wk 5 and wk 8 by switching from a moderate grain (18.6% DM starch) to a high grain diet (27.9% DM starch). Across SCFP treatments, the two SARA challenges increased the duration of the rumen pH below 5.6 from 13.6 to 179 min/d ($P<0.05$). The NTH treatment attenuated this increase to 79.3 min/d, but decreased feces pH from 6.74 to 6.55 ($P = 0.01$). Across SCFP treatments, SARA challenges reduced the ADC of DM, NDF and P during the challenges, but tended to increase ADC of starch. Across diets, the NTH treatment increased the total tract NDF digestibility from 52.7 to 61.8% ($P<0.05$). Results show that the NTH treatment can attenuate some of the adverse impacts of SARA, but not a reduction in the total tract digestibility of dietary P.

Key words: Dairy cows, SARA, *Saccharomyces cerevisiae* fermentation products, digestibility

6.2 Introduction

Subacute ruminal acidosis (SARA) is a metabolic disorder characterized by reversible and temporary rumen pH depression and remains a common problem in high yielding dairy cows

(Kleen et al., 2003; Plaizier et al., 2008; Plaizier et al., 2018). Symptoms attributed to SARA include reduced and erratic feed intake, depressions of milk yield and milk fat, inflammation, and reduced functionalities of gastrointestinal microbiota (Kleen et al, 2003; Plaizier et al, 2012; Plaizier et al., 2018). Rumen pH depression can reduce microbial digestion, and especially that of fibre, in the reticulo-rumen and large intestine, as many rumen microorganisms and the enzymes that they produce are sensitive to a low rumen pH (Russell and Dombrowski, 1980; Shi and Weimer, 1992; Russell and Wilson, 1996). In agreement, Plaizier et al. (2001) and Krajcarski-Hunt et al. (2002) observed that experimental induction of SARA by high grain feeding reduced the in situ 24-h NDF degradability of forages in the rumen by between 19.6 and 20.5%. However, this does not imply that total tract NDF digestibility was also reduced, as increased hindgut fermentation may compensate the reduced fiber digestibility in the rumen (Demeyer, 1991). Nevertheless, as grain-induced SARA can increase the acidity and starch concentrations of hindgut digesta (Gressley et al., 2011; Li et al., 2012), this type of SARA may also reduce fibre digestion in the hindgut and its total tract digestibility. Several rumen bacteria, including *Selenomonas ruminantium*, *Megasphaera elsdenii*, and *Prevotella ruminicola*, have phytase activity (Yanke et al., 1998). These microbes are assumed to hydrolyze nearly all phytate, which makes most phytate P available to the cow (Morse et al., 1992a). Research regarding the effects of SARA on phytate P availability in cows is not yet available. However, as SARA affects the abundances and functionalities of many rumen microbes, an effect of SARA on phytate P availability may exist. In agreement, Godoy and Meschy (2001) did not observe that increasing the dietary grain concentration reduced the bacterial phytase activity in an in-vitro Rusitec system. However, the simulated rumen conditions in this study differed from experimentally induced SARA in live cattle. Most rumen bacteria identified by Yanke et al. (1998) as having phytase activity do not

appear to be very sensitive to SARA conditions (Russell and Dombrowski, 1980; Russell and Wilson, 1996). However, that does not mean that acid-sensitive microorganisms in the rumen with phytase activity do not exist. Hence, the effect of SARA on total tract P digestibility in dairy cows must still be determined experimentally in live animals.

In order to overcome adverse effects of SARA in dairy cows, supplementation of *Saccharomyces cerevisiae* fermentation products (SCFP) has been suggested (Allen and Ying, 2012; Li et al., 2016). These products are becoming popular as a dietary additive for ruminants to help stabilize and enhance the gut health, including improving the functionality of rumen microbiota (Li et al., 2016; Plaizier et al., 2016; Tun et al., 2019). It is assumed that these beneficial effects involve the provision of fermentation products and soluble growth factors such as amino acids, organic acids, minerals, and B vitamins by SCFP (Harrison et al., 1988; Callaway and Martin, 1997). It still needs to be determined if SCFP also attenuate the reduction in rumen and total tract nutrient digestibilities resulting from SARA.

The main hypotheses of our study are that grain-induced SARA reduces the rumen pH and apparent total tract digestibilities of DM, NDF, starch and P, and that supplementation with SCFP attenuates these impacts.

6.3 Materials and Methods

6.3.1 Animals and experimental design

The study was conducted at the Glenlea Research Station, University of Manitoba. It was approved by the University of Manitoba Animal Care Committee and followed the guidelines of the Canadian Council for Animal Care (CCAC, 1993). Thirty-two rumen-cannulated second lactation and older lactating Holstein dairy cows were assigned to a randomised complete block design

which contained 8 blocks. Cows were blocked based on parity, expected calving date and milk production during the previous lactation. Cows were housed and fed in individual stalls. Within each block, cows were randomly assigned to 4 treatments that started 4 wk before calving: 1) control, 2) 14 g/d Diamond V Original XPC™ (XPC), 3) 19 g/d NutriTek® (NTL), or 4) 38 g/d NutriTek® (NTH) mixed with 126, 121, and 102 g/d ground corn, respectively, while the cows under the control treatment received 140 g/d ground corn only. Differences between NutriTek® and XPC™ include enhanced bioactive compounds, new fermentation metabolite compounds, and additional propriety antioxidants and polyphenols present in NutriTek® (Diamond V Mills Inc. Cedar Rapids, IA). Supplements were top dressed on the TMR once daily immediately after the delivery of TMR during the entire experiment. Cows had unlimited access to fresh water.

6.3.2 Feeding and feed intake determination

Cows were fed a close-up total mixed ration (TMR) from 4 weeks pre-calving up to the calving date (Table 8, Table 9). After calving, cows were switched to a lactation TMR except for 5th and 8th weeks post calving, during which SARA challenges were conducted (Figure 1, Table 8, Table 9). These challenges involved replacing 20% of the DM of the lactation TMR with pellets containing 50% ground wheat and 50% ground barley. During the period between the SARA challenges, the lactation TMR was fed. Stages relative to SARA challenges during which cows were monitored included preSARA1, SARA1, postSARA1, SARA2, and postSARA2, corresponding with wk 4, wk 5, wk 6-7, wk 8, and wk 9 of lactation, respectively,

Diets were provided ad libitum, allowing for 5-10% orts, twice daily, at 0900 and 1500. Weights of the feed and orts were recorded each morning before the first feed delivery. Feed intake was

calculated daily by subtracting amount of feed left from the amount of feed delivered per day on a DM basis. Once weekly, feed samples were collected immediately after mixing.

6.3.3 Rumen pH and feces pH

Rumen pH was monitored continuously using indwelling pH data loggers (T7-1 LRCpH, DASCOR, Escondido, CA), placed in the ventral sac of the rumen of all cows as described by Li et al. (2012). Rumen pH was measured at 1-min intervals, and data were sent to a data logger for storage and used for subsequent analysis. The pH data were summarized as average daily pH and time below pH 5.6 for each 24-h period.

Feces grab samples of approximately 200 g were collected per rectum after cleaning of the perineal area as described by Li et al. (2012). The feces pH was determined immediately after thoroughly mixing 10 g of feces with 10 mL of distilled water using an Accumet Basic 15 pH meter (Fisher Scientific, Fairlawn, NJ), equipped with a Sensorex 450C Flat Surface Combination pH/Reference Electrode (Sensorex, Stanton, CA). Subsequently, feces samples were stored at -20 ^{0}C until further analysis.

6.3.4 Feed and feces analysis

Feed, orts, and feces samples were analyzed for DM by drying at 60°C for 48 h in a forced air oven. Dried samples were ground with a Wiley mill using a 1-mm screen (Thomas-Wiley, Philadelphia, PA) and kept in sealed bags for future analyses. Ground feed and feces samples were pooled by stage relative to SARA induction for each cow. Analytical DM for pooled samples for each stage was determined (method 934.01; AOAC, 1990). All feed and feces samples were analyzed for NDF according to Van Soest et al., (1991) using α-amylase (Sigma No. A3306, Sigma

Chemical Co., St. Louis, MO), and sodium sulfite, and corrected for ash concentration using an Ankom 200 Fiber Analyzer (Ankom Technology, Fairport, NY), P (AOAC methods 968.08 and 935.13A; AOAC, 2005), acid insoluble ash (AIA, AOAC method. 920.08 (AOAC, 2005), and starch using the UV method (method 996.11; AOAC, 2005). Feed samples were also analyzed for CP using the $CuSO_4$/ TiO_2 mixed catalyst Kjeldahl procedure (method 988.05; AOAC, 1990). Analyses of ADF, ether extract, and ash in feed samples were conducted using AOAC method 973.18 (AOAC, 1990), AOAC method 920.39 (AOAC, 1990), and AOAC method 923.03 (AOAC, 2005), respectively. Calcium, P, K, Mg, and Na in feed samples were measured by inductively coupled plasma emission spectroscopy (method 968.08; AOAC, 1990) using an Atom Scan 25 Plasma Spectrometer (Thermo Jarrell Ash Corp., Grand Junction, CO) after acid digestion. Feed, orts, and feces samples were analyzed for DM by drying at 60°C for 48 h in a forced air oven. Dried samples were ground with a Wiley mill using a 1-mm screen (Thomas-Wiley, Philadelphia, PA) and kept in sealed bags for future analyses. Ground feed and feces samples were pooled by stage relative to SARA induction for each cow. Analytical DM for pooled samples for each stage was determined (method 934.01; AOAC, 1990).

6.3.6 Statistical analysis

The apparent total tract dry matter digestibility (DMD) was determined by period relative to the SARA challenge and by cow as DMD = 100 x (1-Mfeed/Mfeces), where Mfeed = AIA concentration in the feed, and Mfeces = AIA concentration in the feces. The apparent total tract digestibility coefficient of nutrient (ADC, %) was determined relative to SARA challenge and by cow as ADC = 100 – 100 x (Mfeed/Mfeces) (Nfeces/Nfeed), where Mfeed = AIA concentration in the feed (% DM), Mfeces = AIA concentration in the feces (% DM), Nfeed = concentration of the nutrient in the feed (% DM), and Nfeces = concentration of the nutrient in the feces (% DM).

Data were analyzed with the MIXED procedure of SAS (Ver. 9.3, SAS Inst., Inc., Cary, NC). The model included the fixed effects of SCFP treatment (Control, XPC, NTL, and NTH), period relative to SARA challenge (preSARA, SARA1, postSARA1, SARA2, and postSARA2, corresponding with wk 4, wk 5, wk 6-7, wk 8, and wk 9 of lactation, respectively), and the interaction of SCFP treatment and period. The effect of block was considered random. The distributions of error terms were tested for normality using the Shapiro-Wilk's statistic of the UNIVARIATE procedure of SAS (Ver. 9.3, SAS Inst., Inc., Cary, NC). Rumen pH and DMI data were log transformed prior to statistical analysis. The PDIFF option and the Tukey test were applied as appropriate to evaluate pairwise comparisons between treatments and periods. The differences between means were generally regarded as significant if P values were less than 0.05.

6.4 Results

6.4.1 Rumen and feces pH

The effects of SCFP treatment, the stage of the SARA challenge, and their interaction on the average daily rumen pH and the daily duration of the rumen pH below 5.6 were significant (Table 10). The effect of SCFP treatment on the time below pH 5.6 was significant during the first and second SARA challenge, but not during other stages (Table 11). During the SARA challenges, only the NTH treatment attenuated the rumen pH depression (Table 11). Across these challenges, the duration of the rumen pH below 5.6 was greater than the threshold of 180 min/d proposed by Gozho et al., (2007) for the control, XPC, and NTL treatment, but not for the NTH treatment (79.3 min/d; Table 11). The effects of SCFP treatment on the average daily rumen pH was significant during the SARA1, SARA2, and postSARA1 stages, but not during the preSARA and postSARA2

stages. During the SARA1 and SARA2 stages, the average daily rumen pH of cows on the NTH treatment were higher than of those on the control treatment.

The effects of treatment and stage, but not that of their interaction, on the feces pH, were also significant (Table 10). Across treatments, the feces pH during the first and second SARA challenge was lower than those during the other stages (6.62 vs. 6.75, $P<0.05$). Across SARA stages, the feces pH of the NTH treatment was lower than those of the other treatments (6.55 vs. 6.74, $P = 0.02$).

6.4.2 Feed intake

The stage of SARA induction, SCFP treatment, and their interaction affected the DMI. This interaction was caused by an absence of an effect of SCFP on the DMI in the SARA2 and postSARA2 stages, whereas they reduced DMI in the earlier stages (Figure 2). The SARA challenges increased DMI, but these increases were greater in cows on the NTL and NTH treatment, compared to those on the Control and XPC treatments.

6.4.3 Total tract digestibilities

The SARA inductions reduced the ADC of NDF, DM, and P, and tended ($P = 0.064$) to increase that of starch (Table 14). The effects of SCFP treatment only affected the ADC of NDF. The interaction between this stage and treatment on the apparent total tract digestibility was not significant for any feed component. The ADC of NDF was lower during both SARA challenges, than during the preSARA1 stage, and the stages after both challenges. The ADC of DM was lower during the second SARA challenge, compared to all other stages. The ADC of P decreased between the preSARA1 and postSARA2 stages. Such effects were not observed for the other components.

The ADC of P was lower during the postSARA2 stage compared to the preSARA1 stage, whereas the ADC of starch was higher during both SARA challenges compared to the preSARA1, postSARA1, and the postSARA2 stages.

6.5 Discussion

The main objectives of the study were to determine if SCFP could reduce the impact of grain-induced SARA on the rumen pH and the total tract digestibilities of DM, NDF, starch, and P. Feces pH and DMI were also monitored to assess the severity of the induced SARA. The effects of yeasts and yeast products on the production, nutrient utilization, and rumen conditions of cattle have been studied extensively (Newbold et al., 1996; Desnoyers et al., 2009). These products can be divided into live yeast, dead yeast, and yeast culture fermentation products. In our study, yeast culture fermentation products obtained from fermentation of *Saccharomyces cerevisiae* were used. These fermentation products consist of dead yeast, fermentation medium, and compounds produced during the fermentation process. As a result, the mechanism of action of the supplementation with these products is different from that of supplementation with live or dead yeast alone (Newbold et al., 1996). Hence our results will only be compared with other studies on yeast culture fermentation products.

A meta-analysis by Desnoyers et al. (2009) revealed that supplementation of live yeast and yeast culture increased rumen pH, while increasing the lactic acid concentration in the rumen. However, the majority of the studies included in their analysis used live yeasts. Hence, it is not clear how representative their results are for our study. Yoon and Stern (1996) and Hristov et al. (2010) found that supplementation with Diamond V XP yeast culture (Diamond V Mills, Inc., Cedar Rapids, IA) does not affect the rumen pH of dairy cows. However, as both Yoon and Stern (1996) and

Hristov et al. (2010) did not use continuous pH monitoring via in-dwelling rumen pH probes, differences in the pH monitoring technology may have contributed to the differences in the results among these studies. Despite this, Li et al. (2016) concluded that Diamond V XPC yeast culture (Diamond V Mills, Inc., Cedar Rapids, IA) stabilized rumen pH in dairy cows during moderate and high grain feeding.

The pH threshold for SARA is not clearly defined, as different studies have used variable threshold values depending on the technique used to measure pH in the rumen. In previous studies, Cooper et al. (1999) used a threshold of rumen pH between pH 5.2 and 5.6, while Beauchemin et al. (2003) used rumen pH depression <5.8 as a threshold. In the present study rumen pH depression below 5.6 for more than 180 min/d was used as the threshold for SARA, as only equal or greater rumen depressions are able to reduce feed intake and create an inflammatory response (Gozho et al., 2007). This threshold was reached during the SARA challenges in cows on the control, XPC, and NTL treatments, but not in cows on the NTH treatment. In agreement, the average daily rumen pH of cows on the NTH treatment were also higher than those of cows on the control treatment. In contrast, the feces pH values during the SARA challenges were lower in cows on NTH treatment, than in cows on the control, XPC, and NTL treatments. This suggests that the NTH treatment shifted fermentation from the rumen to the large intestine. A reason for this shift may have been that this treatment reduced the rate of ruminal starch digestion, which has been reported previously for XPC (Allen and Ying, 2012). We were not able to confirm this, as this rate of digestion and the amylolytic activity of the rumen microbiota were not determined in our study. Such a shift in the site of starch digestion may be beneficial when the decrease in the amount of starch digested in the rumen is partly compensated for by an increase in the amount of starch digested in the small and large intestine (Remond et al., 2004). The absence of an SCFP effect on the total tract

digestibility in our study suggests that the increase in rumen bypass starch under the NTH treatment did not result in a decrease in total tract starch digestibility. The relatively low feces pH of cows on the NTH treatment, however, demonstrates that not all of this rumen bypass starch was digested in the small intestine, and that, as a result, fermentation of starch in the large intestine was increased. The SARA challenges increased DMI, especially for the NTL and NTH treatments. Results of earlier research on the effects of SARA on feed intake vary (Krause and Oetzel, 2006; Plaizier et al. 2008; Plaizier et al., 2012). Whereas severe SARA reduces feed intake, moderate SARA can increase DMI, possibly due to a reduction in physical rumen fill of the diet (Allen, 2000; Plaizier et al. 2008; Plaizier et al., 2012). In agreement with our study, the meta-analysis of Poppy et al. (2012) showed that SCFP decrease DMI by 0.78 kg/d beyond early lactation. These authors also showed that, due to an increased feed efficiency, SCFP increase milk yields, despite reduced feed intakes.

Apparent total tract digestibility coefficients (ADC) were determined using an internal marker, AIA (Van Keulen and Young, 1977). Chapuis-Lardy et al., (2004) concluded that this method produces satisfactory results, and it has the advantage over the total feces collection technique in that only aliquots of the feces have to be collected, the animal does not have to be restrained in special metabolism stalls for extended periods. Another advantage is that bladder catheters are not needed to collect the urine, and that the use of these catheters can lead to inflammation and the use of antibiotics (Plaizier, Personal Communication, 2019). McGeough et al. (2010) compared this method with the total feces collection method and reported an acceptable agreement between these methods. Hence, we believe that the digestibility coefficients that were determined in the current study are representative.

The total tract NDF digestibility was lower during the SARA challenges than during the preSARA and the postSARA1 and postSARA2 stages and did not differ between the two SARA stages and among the preSARA, postSARA1 and postSARA2 stages. This confirms our hypotheses that SARA reduces total tract fibre digestibility, and that this digestibility recovers quickly from a SARA challenge. These reductions in total tract NDF digestibility were similar to the reductions in rumen NDF digestibility due to grain-based SARA challenges conducted by Plaizier et al. (2001) and Krajcarski-Hunt et al. (2002). The reduction of fiber digestion resulting from SARA may be attributed to the sensitivity of the fibrolytic bacteria to low pH. The optimum environmental pH for fibrolytic bacteria ranges from 6.5 to 7, and environmental pH values below 6 are less tolerable (Shi and Weimer, 1992). In agreement, Calsamiglia et al. (2002) used a dual-flow continuous culture system and observed that SARA induction reduces simulated NDF degradability in the rumen from 53.8% at a constant pH of 6.4 to 34.3% at a constant pH of 5.7. However, by alternating cycles of low (5.7) and high pH (6.7), the reduction of the NDF digestibility was intermediate, suggesting that the populations of fibrolytic microorganisms in the rumen recovered quickly from the pH depression. Low pH values may cause reductions in the population of fibrolytic bacteria due to difficulty in attaching to feed particles (Cheng et al., 1980) and reduction in rate of their replication (Russell and Dombrowski, 1980). The reduction in rate of replication is attributed to an increase in the cost of maintenance (Russell and Dombrowski, 1980). In agreement, several studies have shown that SARA challenges reduce the abundance of many fibrotic bacteria in the rumen and hindgut (Khafipour et al., 2009a; Li et al., 2012; Mao et al., 2013). This may explain why these challenges reduce total tract NDF digestibility. However, as many genes are shared by various microbial taxa (Weimer, 2005), reduction of the abundance of a few fibrolytic bacteria in the rumen and hindgut does not have to result in a reduction of the

fibrolytic activity of the rumen and hindgut microbiomes. The absence of differences in the NDF digestions among the preSARA1, postSARA1, and postSARA2 periods suggests that the fibrolytic functionality of the rumen and hindgut microbiomes recovers rapidly from the SARA challenges. This recovery could be due to increases in the abundances of fibrolytic microorganisms, but the return to a better pH for fibrolytic enzymes may also contribute (Russell and Dombrowski, 1980). In our study, two successive SARA challenges were conducted, to determine if repeated SARA challenges have a more severe impact than single SARA challenges. This was done because earlier studies showed such a carryover and demonstrated that the duration of SARA challenges impacts their severity (Dohme et al., 2008; Plaizier et al., 2018). However, the absence of differences in total tract NDF digestibility among the preSARA1 stage and postSARA stages shows that there was no carryover of the first SARA challenge on the DM and NDF total tract digestibility during the second SARA challenge and the postSARA2 stage.

The lower total tract P digestibility in the postSARA2 period compared to the preSARA1 period implies that, in contrast to NDF total tract digestibility, only prolonged SARA challenges and prolonged feeding of excessively high grain diets reduces the P total tract digestibility. During the SARA challenges, forages were replaced with grain. This dietary challenge increased the dietary P concentration slightly from 0.45 to 0.47 % of DM. However, as grains contain more phytate P than forages (NRC, 2001), the grain challenge would have increased the proportion of dietary P consisting of phytate P. This could have reduced the total tract digestibility of total P, but if that were so, this reduction would have occurred soon after the first SARA challenge, and the gradual decline in this digestibility that was observed in our study would not have occurred. Hence, we believe that an effect of the rumen pH depression associated with the SARA challenge on the phytase activity is a more likely explanation of this gradual decline. Godoy and Meschy (2001)

showed that ruminal phytase may not hydrolyze all phytate P. In support of this, Jarrett et al. (2014) observed that supplementation with phytase reduced the excretion of total P and phytate P in the feces of dairy cows, suggesting that the microbial breakdown of phytate P is incomplete, and that addition of phytase may increase the breakdown of phytate P. Konietzny and Greiner (2004) concluded that phytases are produced by several anaerobic rumen bacteria, including *Selenomonas ruminantium*, *Megasphaera elsdenii*, and *Prevotella sp*. Short grain-based SARA challenges do not reduce the abundances of these bacteria (Khafipour et al., 2009a; Petri et al., 2013; Plaizier et al., 2018). Mao et al., (2013) observed that such a challenge reduced the relative abundance of *Prevotella sp.* Hence, these studies suggest that phytase-producing bacteria do not respond as rapidly to increased grain feeding and a reduction in rumen pH, as the acid sensitive fibrolytic bacteria. Therefore, the response of phytate production in the rumen to grain-based SARA challenges could be slower than the response of fibre digestion to these challenges. However, in order to prove this, the direct monitoring of phytate activity in the rumen following these challenges will be needed. Nevertheless, it is assumed that the positive effects of yeast products are not through direct action on pH, but rather through a modulatory effect on the fermentation process and ruminal microbiome, such as by stimulation of lactate utilizers and an increase in certain cellulolytic bacteria and fungi (Calsamiglia et al., 2012). The soluble growth factors in SCFP have been shown to stimulate growth of pure cultures of ruminal bacteria that digest cellulose and utilize lactate in vitro (Callaway and Martin, 1997). Also, Calsamiglia et al. (2012) proposed that positive effects of yeast products have modulatory effects on the fermentation process and ruminal microbiome. One such effect proposed is the stimulation of lactate utilizing fibrolytic microorganisms in the rumen. This may explain why Mao et al. (2013) observed that XP (Diamond V, Cedar Rapids, IA) SCFP increased the populations of fibrolytic microorganisms

including protozoa, fungi, *F. succinogenes*, *R. albus*, and *R. flavefaciens* during in vitro incubations. In agreement, Harrison et al. (1988) found that supplementation with 114 g/d of a SCFP tended to increase the concentration of anaerobic bacteria and increased the concentration of cellulolytic bacteria in the rumen of dairy cows. Desnoyers et al. (2009) concluded that, on average, these supplementations increased total tract organic matter digestibility. However, as mentioned previously, the majority of the studies included in their analysis used live yeasts; hence, it is not clear how representative their results are for our study. Miller-Webster et al. (2002) observed that Diamond-V XP SCFP (Diamond V Mills, Inc., Cedar Rapids, IA) increased the total tract digestibility of DM, but not that of OM, NDF, ADF, and NSC. Yoon and Stern (1996) reported that supplementation with Diamond V XP SCFP (Diamond V Mills, Inc., Cedar Rapids, IA) increased the ruminal OM and CP digestibility, without affecting the ruminal NDF digestion. Hristov et al. (2010) observed that supplementation with 56 g/head per day of XP (Diamond V Mills Inc., Cedar Rapids, IA) did not affect the total tract digestibility of DM, OM, N, NDF and starch in lactating dairy cows. Similarly, Allen and Ying (2012) also reported that 56 g/head per day of XP (Diamond V Mills Inc., Cedar Rapids, IA) did not affect the total tract digestibility of NDF, and that this SCFP increased the total tract starch digestibility of lactating cows with a DMI of less than 26 kg/d prior to the study, whereas it decreased starch digestibility of cows that had higher feed intakes immediately before the study. This suggests that, despite promising effects on fibrotic microorganisms in the rumen, there is little evidence that at the low inclusion rates, SCFP increase total tract digestion of DM, OM, and NDF. However, the improvement of the NDF total tract digestibility by the NTH treatments suggest that at higher doses, SCFP may increase this digestibility, and that dosage is, therefore, an important consideration for the inclusion of SCFP in the diet.

A main finding from our study is that the NTH treatment is able to attenuate the impact of SARA on the rumen pH depression, and increase the total tract fibre digestibility, whereas the effects of other SCFP treatments on these impacts were not significant. NutriTek is different from XPC in that it functions differently by providing enhanced bioactive compounds which include those found in XPC, new fermentation metabolite compounds, and additional propriety antioxidants and polyphenols (Diamond V Mills Inc. Cedar Rapids, IA). De Nardi et al. (2014) found that supplementation with polyphenols attenuated the reticular pH depression of heifers during-experimentally induced SARA. High grain feeding and grain-induced SARA have been associated with oxidative stress and resulting inflammation (Gabel et al., 2002; O et al. 2011). Hence, the differences between the contents of antioxidants and polyphenols between XPC and NutriTek may explain why NutriTek had the largest effect of alleviating the symptoms of grain induced SARA.

6.6 Conclusions

Depressed pH, feed intake, and total tract fiber digestibilities showed that the SARA challenges induced a moderate form of SARA in cows under the control, XPC, and NTL treatment, but that the NTH treatment prevented SARA which may have been due to a shift of the digestion of starch from the rumen to the intestines. Cows recovered quickly from these challenges, and the first and second SARA challenges did not differ in their severity. The SCFP treatments did not affect the total tract digestibility of DM, CP, and P, but the NTH treatment increased the total tract digestibility of NDF across the stages of the SARA induction. The combination of both SARA challenges reduced the total tract digestibility of P. Our data suggest that the NTH treatment can limit the depressions of the rumen pH and fiber digestion that are commonly associated with high. grain feeding.

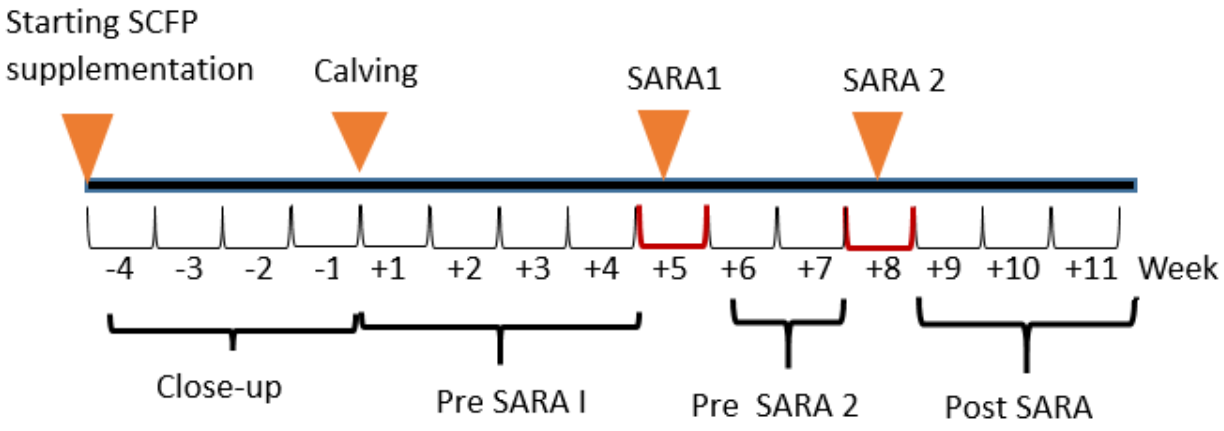

Figure 1. Timeline of the study with SARA induction stages by weeks relative to calving

Figure 2. Dry matter intake (DMI) for cows fed different Saccharomyces cerevisiae fermentation products (SCFP) by stage of SARA induction. Values marked with same letter within stages are not significantly different ($P<0.05$)

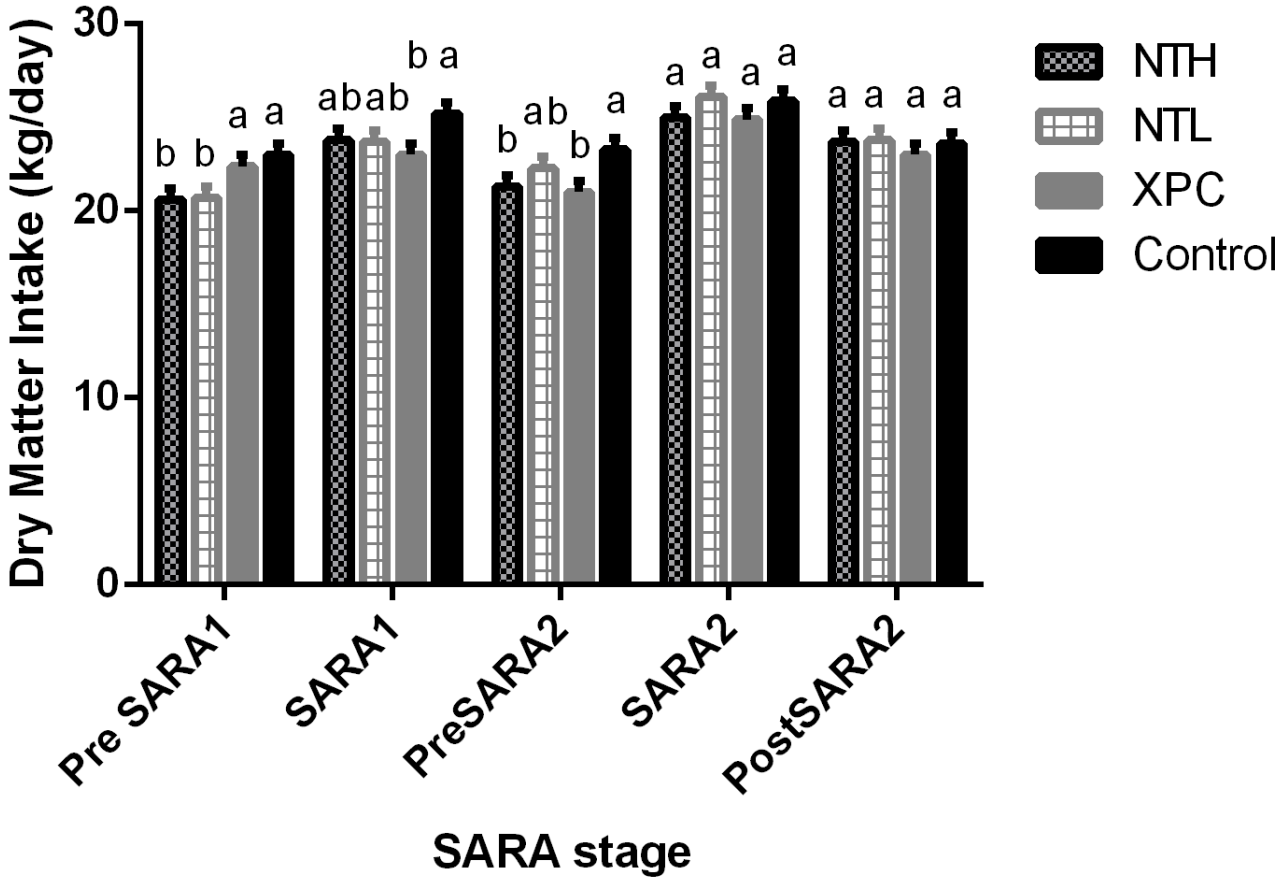

Table 8. Ingredient composition of experimental diets

	Lactating TMR	SARA TMR	Close-up dry cow TMR
Ingredient compositions (% of DM)			
Round bale mixed alfalfa /grass silage	35	28	20
Alfalfa 1st cut haylage	-	-	8
Corn silage	-	-	37
Barley silage	20	16	-
Straw	-		13
Ground corn	20	16	-
Dairy Aide[1]	25	20	
Close-up dry cow supplement[2]	-		22
Wheat-Barley pellets	-	20	-

[1]Dairy Aide contained: corn – flaked (65.2%), corn distillers grain (7.1%), APF fat plus (3.6%), feather meal (3.6%), porcine meat meal (2.9%), soybean meal (2.9%), canola meal (2.9%), AV fat Rothsay Feeders Choice (2.2%), sodium sesquicarbonate (SQ 810, 1.9%), dicalcium phosphate (1.5%), potassium chloride (DYNA K red, 1.5%), ground limestone (1.5%), salt – potash (1.5%), Dairy LMK Ultra micro (0.6 %), magnesium oxide (0.3%), and methionine analogue – Novus (0.3%).

[2]Dry cow supplement contained: Landmark close-up dry cow pellets[3] (50.0%), flaked corn (20.0%), beet pulp pellets (15.0 %), rolled barley (12.5%), liquid molasses (2%), soy oil (0.5%), and liquid caramel (0.01%).

[3]Landmark close-up dry cow pellets contained: barley (4.0%), limestone (7.0%), corn distillers' grain (34.9%), dicalcium phosphate (1.8%), canola meal (23.0%), soybean meal (8.5%), wheat (15.0%), niacin (0.3%), Biopowder SXC (0,05%), magnesium oxide (1.6%), Transition VB 25K (2.9%), Dry Cow MicroPX premix (1%).

Table 9. Chemical composition of experimental diets

	Lactating TMR	SARA TMR	Close-up dry cow TMR
DM, %	51	60	48
CP, % DM	17.9	17.2	15.5
Fat, % DM	4.3	3.6	2.8
NDF, % DM	34.9	28.2	38.7
ADF, % DM	26.0	19.9	26.7
Starch, % DM	18.6	27.9	17.6
Ca, % DM	1.32	1.06	1.26
P, % DM	0.45	0.47	0.37
Mg, % DM	0.37	0.34	0.41
Na, % DM	0.33	0.27	0.06
K, % DM	2.57	2.02	2.37

Table 10. Effects of SCFP treatment (control, XPC, NTL, and NTH) and stage of SARA induction (PreSARA, SARA1, PostSARA1, SARA2, and PostSARA2) on rumen and feces pH variables

	SCFP Treatment				Stage						ANOVA *P*-Values		
	Con	XPC	NTL	NTH	Pre SARA	SARA1	Post SARA1	SARA2	Post SARA2	SEM	SCFP Treatment	Stage	Treat*Stage
Avg. Rumen pH	6.28	6.25	6.26	6.36	6.35	6.04	6.46	6.09	6.5	0.02	<0.001	<0.001	0.004
Time< pH 5.6, min/d	82.3	99.1	106.1	33.2	13.6	179.3	19.0	178.0	10.8	22.1	<0.001	<0.001	<0.001
Feces pH	6.74a	6.75a	6.74a	6.55b	6.71a	6.61b	6.7a	6.63b	6.84a	0.05	0.01	0.02	0.87

Means followed by the same letter within treatments and stages are not significantly different ($P<0.05$)

Table 11. Effect of SCFP treatments (control, XPC, NTL, and NTH) by stage of SARA induction (PreSARA, SARA1, PostSARA1, SARA2, and PostSARA2) on average rumen pH

	SCFP Treatment					
Stage	Control	XPC	NTL	NTH	SEM	ANOVA *P*-Values
PreSARA	6.30	6.34	6.31	6.26	0.04	0.29
SARA1	5.54a	5.54a	5.58ab	5.66b	0.05	0.023
PostSARA1	6.35ac	6.30ab	6.26b	6.38c	0.03	0.001
SARA2	5.85a	5.77a	5.78a	6.00b	0.05	<0.001
PostSARA2	5.25	5.30	5.32	5.34	0.04	0.20

Means followed by the same letter within stages are not significantly different ($P<0.05$)

Table 112. Effect of SCFP treatments (control, XPC, NTL, and NTH) by stage of SARA induction (PreSARA, SARA1, PostSARA1, SARA2, and PostSARA2) on rumen time < pH 5.6 (min/d)

	SCFP Treatment					
Stage	Control	XPC	NTL	NTH	SEM	ANOVA *P*-Values
PreSARA	7.1	3.2	2.9	6.3	3.6	0.60
SARA1	228.4a	183.10ab	241.0a	104.6b	64.1	0.01
PostSARA1	26.58	6.28	24.08	4.26	10.1	0.10
SARA2	121.6b	284.4a	252.8a	53.9b	45.4	<0.001
PostSARA2	14.6	27.6	3.1	21.3	9.7	0.32

Means followed by the same letter within stages are not significantly different ($P<0.05$)

Table 12. Effects of SCFP treatment (control, XPC, NTL, and NTH) and stage of SARA induction (PreSARA, SARA1, PostSARA1, SARA2, and PostSARA2) on milk production variables

	SCFP Treatment				Stage					ANOVA *P*-Values		
	Con	XPC	NTL	NTH	Pre SARA	SARA1	Post SARA1	SARA2	Post SARA2	SEM[1]	SCFP Treatment	Stage	Treat× Stage
MY, kg/d	43.4a	44.7a	40.7b	41.3b	39.4b	43.6a	42.1a	43.1a	42.5a	1.25	0.01	<0.01	0.92
Fat yield, kg/d	1.59	1.46	1.47	1.62	1.66a	1.34b	1.55a	1.33b	1.54a	0.08	0.16	<0.001	0.97
Protein yield, kg/d	1.23	1.20	1.19	1.20	1.14b	1.24a	1.18ab	1.23a	1.23a	0.04	0.48	0.03	0.87

Means followed by the same letter within treatments and stages are not significantly different ($P<0.05$)

Table 13. Effects of SCFP treatments (control, XPC, NTL, and NTH) and stages of SARA induction (PreSARA, SARA1, PostSARA1, SARA2, and PostSARA2) on apparent digestibility coefficient (ADC) of dry matter (DM,) neutral detergent fibre (NDF), phosphorus (P) and starch

	SCFP treatments				Stages					SEM	ANOVA *P*-Values		
ADC	Con	XPC	NTL	NTH	PreSARA	SARA1	PreSARA2	SARA2	PostSARA2		SCFP Treatment	Stages	Treat×Stage
DM	67.2	70.0	71.65	66.6	71.8	72.9	71.1	59.6	69.0	3.40	0.6313	0.0464	0.2886
NDF	52.7a	52.2a	55.8ab	61.8b	61.1a	49.0b	59.9a	48.9b	59.1a	3.29	0.0138	<0.001	0.8161
P	50.8	52.6	52.91	53.9	57.7a	54.5ab	51.1ab	51.4ab	48.0b	3.48	0.7948	0.02	0.3983
Starch	91.5	89.8	91.2	90.8	89.6	92.9	89.5	92.4	89.7	1.34	0.8136	0.0644	0.3441

Means followed by the same letter within treatments and stages are not significantly different ($P<0.05$)

7. GENERAL DISCUSSION AND CONCLUSIONS

The utilization of dietary P in the dairy industry is a key factor for the environmental and economic sustainability of this industry. Excess P excretions harm the environment, increase feed costs, may increase the acreage needed to spread the manure thereby limiting opportunities for expansion, and may harm the fertility and health of dairy cows. As a result, strategies to enhance the utilization of dietary P must be developed. This development requires the understanding and quantification of the factors that affect this utilization. These factors have been studied in controlled experiments on research stations, but not in surveys on commercial dairy farms. Therefore, it is necessary to confirm if the effects of these factors are similar in controlled studies and field surveys.

Our first study was conducted to quantify the relationships between dietary, farm, and animal factors with the utilization of dietary P. Dietary factors included the concentrations of CP, ether extract, NDF, ADF, NFC, starch, ash, and Ca. Production parameters included milk yield, milk composition, SCC, BCS, DIM, and parity. Farm factors included herd size, type of housing, and feeding strategy (component feeding or TMR). These factors were chosen as it was assumed that they could affect the utilization of dietary P. The measures for the utilization of dietary P were the concentration of P in feces. However, the use of milk P as a biomarker of P status was not be feasible, as we observed that the milk P concentration was not correlated with the P concentration of the diet and of the feces.

A key observation in the first study was that the average P concentration in the diet of lactating dairy cows in Manitoba appears to have decreased over time. According to Plaizier et al. (2004), in 2002 the median dietary P concentration, 25^{th} and 75^{th} percentiles were 0.48, 0.42, 0.54% (DM

basis), respectively. A later study (Plaizier et al., 2012) revealed that in 2010/11, the median dietary P concentration, 25th and 75th percentiles were 0.45, 0.37, and 0.48 % (DM basis) respectively. In the current study, the median dietary P concentration, 25th and 75th percentiles were 0.42, 0.38, 0.45 % (DM basis), respectively. Even though it shows a decreasing trend, the dietary P concentration still exceeds the NRC (2001) recommendations on at least 50% of these farms. Our survey also showed that the average, 25th and 75th percentiles of the dietary starch concentrations were 22.4, 19.3, and 25.2 % of DM respectively. In previous studies, starch concentrations of 27.8%, 31.8, 33.7 % DM have been used to induce SARA. Therefore, cows on approximately 25% on the farms include in survey received diets that may have induced SARA (Plaizier et al., 2008; Plaizier et al., 2012). As SARA reduces the utilization of many nutrients by cows, we decided to investigate the effects of SARA on the utilization of dietary P in a follow-up study.

Multiple regression analysis was conducted to determine which cow, diets and farm factors affected the P concentration of the feces. This analysis showed that high dietary P concentrations, low dietary fiber concentrations, high DIM, and free-stall instead of tie-stall housing were associated with the high P concentrations of feces. The positive relationship between the dietary and feces P concentrations agrees with earlier studies, including Knowlton and Herbein (2002), who observed that when diets contained 0.34, 0.51, and 0.67% DM of P, the apparent digestibilities of P in dairy cows were 49.0, 34.4 and 32.8%, respectively. Similar observations were reported in other studies (Morse et al., 1992b; Wu et al., 2000; Wu et al., 2001; Dou et al., 2002). Therefore, it is crucial to formulate the diet of dairy cows with dietary P concentrations as recommended by NRC (2001), as excess dietary P is excreted.

The negative correlation between the feces P concentration and the dietary NDF and ADF concentrations indicates the importance of fiber in dairy cow diets. Replacing dietary fiber with

starch can result in increased P excretion. Reduction of dietary fiber concentration while increasing the dietary starch concentration can result in a more acidic rumen environment, which may reduce the breakdown of fiber and thus reduce the release and breakdown of phytate P from the diet (Plaizier et al., 2008; Plaizier et al., 2012b). Additionally, grain contains more P compared to forage and thereby feeding more grain increases the feed P resulting in lower utilization of P (NRC, 2001).

Another key observation on the first study was the positive correlation between feces P and DIM, suggesting that P utilization is reduced further during lactation. This can be the result of the reduction in milk production in the later stages of lactation or due to pregnancy. Lowering milk production reduces the P requirements, and thereby reduced the utilization of P by cows if the dietary P concentration is not adjusted. Therefore, farmers should formulate diets to meet requirements of cows in different stages of lactation, such as early/mid and late lactation.

The second study was conducted to determine the effects of *Saccharomyces cerevisiae* fermentation products (SCFP) and high grain feeding on the utilization of dietary P and other nutrients, such as NDF, DM, and starch, by lactating dairy cows. Earlier studies have shown that SCFP can increase milk production efficiency and attenuate the adverse effects of SARA (Poppy et al., 2012; Li et al., 2016). Also, a new SCFP, NutriTek (Diamond V Diamond V Mills Inc., Cedar Rapids, IA), had become available, and this new product was compared to an existing SCFP, XPC (Diamond V Diamond V Mills Inc., Cedar Rapids, IA) and the most beneficial dose of NutriTek was determined. An additional objective of the second study was to assess if a second SARA challenge would have a more severe impact than the first SARA challenge, as earlier studies had suggested.

As expected, induction of SARA decreased the NDF digestibility on average from 61 to 49%, compared to the lactation diet. However, no difference was observed between the two SARA challenges on the NDF digestibility. After switching the diets from the SARA challenge diet back to the normal lactation diet, the NDF digestibility returned to normal within a few days, suggesting that the two SARA challenges were not sufficient to reduce fiber utilization over the long term. This result agrees with the findings of Calsamiglia et al. (2002), who also reported that the NDF digestibility returned to normal after the rumen pH recovered after an *in vitro* SARA challenge. This also suggests that the disturbance caused by low pH is temporary and the fibrolytic microbes can survive short periods of low pH without compromising the overall rumen fiber fermentation process.

Interestingly, the total tract digestibility of P decreased from the PreSARA period until the postSARA2 period. This suggests that the first SARA challenge was not sufficient to reduce P digestibility and that the combination of the two challenges was needed for this reduction. The reason for this combination of challenges may be that fibrolytic bacteria are more sensitive to a low pH than phytase-producing bacteria, as phytase activity does not appear to be very sensitive to SARA conditions (Russell and Dombrowski, 1980; Russell and Wilson, 1996; Yanke et al., 1998).

The SCFP treatments did not affect the total tract digestibilities of DM, P, and starch. However, the NTH treatment increased the NDF digestibility by 9.1% across control feeding and the SARA challenges. However, no similar effects were observed for the other SCFP treatments. According to Zhu et al. (2017), increases in dosage of SCFP, such as NutriTek, may result in increases in rumen volatile fatty acids and the abundance of cellulolytic bacteria, while resulting in a decrease in the abundance of lactic acid bacteria. Even though the two treatments, NTL and NTH included

the same strains of SCFP, the dosage was different. Only NTH at a dosage of 38 g/day showed positive results on the attenuation of the rumen pH and NDF digestibility during SARA. This emphasizes the importance of the dose of SCFP that is administered to the diet in order to obtain an effective response from the cow. No difference between SCFP treatments or between SARA challenges was observed for DM and starch digestibility. The reason may be because unlike fibrolytic bacteria, amylolytic bacteria can tolerate a more acidic environment (Russell and Dombrowski, 1980). Therefore, the SARA challenges do not cause an adverse effect on amylolytic bacteria. Additionally, the rumen escape starch may also be subjected to hindgut digestion and fermentation resulting in efficient utilization of starch by ruminants, even when the degradability of starch in the rumen is reduced.

Considering these observations of study 2, we can accept our 1st hypothesis, that the grain-induced SARA will reduce the total tract digestibility of dietary P in lactating dairy cows, as well as the 2nd hypothesis that the reduction in the total tract digestibility of NDF due to grain-induced SARA can be attenuated by supplementation with SCFP, but not for DM and starch. However, for P we accept only the 1st hypothesis that grain-induced SARA reduces the total tract digestibility of dietary P in lactating dairy cows. We reject the 2nd hypothesis, that the reduction in the total tract digestibility of P due to grain-induced SARA can be attenuated by supplementation with *Saccharomyces cerevisiae* fermentation products). The basis for accepting the 1st hypothesis for P is that the lower total tract P digestibility in the postSARA2 period compared to the preSARA period implies that prolonged SARA challenges and prolonged feeding of high grain diets can reduce the P digestibility.

The most important findings of our research related to P utilization are that many dairy farmers still provide more P to their dairy cows than needed, leading to increased P concentration of feces,

and probably the P output in feces, without benefitting the milk production of these cows. Results also showed that approximately 25% of farms feed high dietary starch diets which can lead to risk of SARA, and that prolonged SARA reduces the utilization of dietary P. We also observed that NutriTek at a high dose (38 g/day) prevents the rumen pH depression in cows on high grain diets, and may, therefore, improve P utilization of these cows.

Considering our findings, we conclude that there are still opportunities to reduce the P concentration of dairy cow diets without adverse effects. A challenge to this is that most cows are managed in large groups and that the P concentration of the diets is formulated to meet the requirements of the highest producers. Another challenge is that Manitoba dairy farmers use many by-products feeds, such as distillers' grain, brewers grain, and wheat bran, that have high dietary P concentrations. As a result, dietary inclusion rates of inorganic P supplements, such as dicalcium phosphate, have been reduced to such an extent and that removing these inorganic P supplements from the diets may still not reduce to the desired P concentration. Therefore, the P concentration of the by-products that are included in the dairy cow diets must be considered carefully.

Free-stalls are becoming more common, and tie stalls are phased out in most parts of Canada (Canwest DHI, 2018). This implies that dairy cows will be managed in large groups. Until recently, cows in these groups received the same TMR. The P concentration of those TMRs was closer to that required for the high production cows than for the low producing cows in the group, resulting in excess P intake for most cows. The increase of voluntary milking systems (VMS) and programmable feeders (Lishin et al, 1995; Halachmi et al., 2006) offers the opportunity of feeding a partial TMR (pTMR) and individual supplementation in the VMS, or via programmable feeders. Currently, these supplementations mainly consider providing additional energy, fiber, or protein to cows, but this precision farming technology can be adapted for P, as well (Cerosaletti et al.,

2004; Berckmans, 2006). Therefore, we believe that the adaption of precision farming technology for the supply of P to dairy cows will contribute greatly to the reduction of P excretion by these cows.

Our studies also suggest that prolonged high grain feeding and the resulting SARA, can reduce the utilization and total tract digestibility of dietary P. Several recommendations have been made to prevent grain-based SARA, including balancing diets for rumen-available starch, feeding high quality forages to reduce the need for grain feeding, the use of inorganic buffers, frequent feed delivery, the prevention of sorting, the provision of sufficient coarse (physical effective) fiber, and the supplementation with yeast and yeast culture fermentation products (Plaizier et al., 2008; Plaizier et al., 2012; Plaizier et al., 2018). Our studies confirm that the use of NutriTek at a dose of 38 g/d reduces the rumen pH depression associated with high grain feeding. However, the present study also shows that NutriTek at a lower dose and XPC do not have a similar effect on the rumen pH. Hence, not all yeast and yeast culture products are equally effective in attenuating the impact of SARA.

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