EVALUATION OF FORAGE MILLET CULTIVARS ON THE PERFORMANCE OF DAIRY COWS

Tania Brunette

Department of Animal Science
McGill University, Montreal

December 2014

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Master of Science

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ADICP	acid detergent insoluble crude protein
ADF	acid detergent fiber
ADL	acid detergent lignin
A :P	acetate to propionate ratio
AOAC	association of Official Analytical Chemists
BW	body weight
CHO	Carbohydrate
CHU	corn heat units
CNCPS	Cornell net carbohydrate and protein system
CP	crude protein
CS	corn silage
DM	dry matter
DMD	dry matter digestibility
DMI	dry matter intake
ECM	energy corrected milk
ED	Energy digestibility
EM	early boot stage millet
eNDF	effective neutral detergent fiber
F :C	forage to concentrate ratio
GDP	gross domestic product
GE	gross energy

GS	grass silage
In situ DMD	*in situ* dry matter digestibility
In situ NDFD	*in situ* neutral detergent fiber digestibility
IVDMD	*in vitro* dry matter digestibility
IVNDFD	*in vitro* neutral detergent fiber digestibility
MM	mature millet
MUN	milk urea nitrogen
NDF	neutral detergent fiber
NDFD	neutral detergent fiber digestibility
NDFI	neutral detergent fiber intake
NDSC	neutral detergent soluble carbohydrate
NEL	net energy lactation
NPN	non-protein nitrogen
NSC	non structural carbohydrate
peNDF	physical effective neutral detergent fiber
RM	regular millet
SAS	statistical analysis method
SC	structural carbohydrate
SCM	solid corrected milk
SCP	soluble crude protein
SM	sweet millet
SNF	solid non fat

TMR	total mixed ration
TS	total solids
VFA	volatile fatty acids
WSC	water soluble carbohydrate

ABSTRACT

The present study evaluated the potential of forage pearl millet in the nutrition of lactating dairy cows. Experiment 1 investigated the effects of replacing corn silage (**CS**; control) with 2 millet cultivars [i.e., regular millet (**RM**) and sweet millet (**SM**)] in cow's diet, whereas Experiment 2 evaluated stage of maturity of sweet millet [i.e., early boot stage (**EM**) and dough to ripe seed stage (**MM**)] in comparison with grass silage (**GS**; control). The parameters evaluated in both studies were milk yields, apparent total-tract digestibility and ruminal fermentation characteristics. Both experiments used fifteen lactating dairy cows in a replicated 3 x 3 Latin square experiment and fed a high forage to concentrate ratio (**F:C**) (i.e., 68:32 and 60:40, respectively) in total mixed rations. Dietary treatments included CS (control), RM and SM diets for Experiment 1, and GS (control), EM and MM diets for Experiment 2. Ruminally fistulated cows (n=3) were used to evaluate the effects of dietary treatments on ruminal fermentation and total tract nutrient utilization.

In Experiment 1, cows fed CS consumed more dry matter (**DM**; 24.4 *vs.* 22.7 kg/d) and starch (5.7 *vs.* 3.7 kg/d), but less neutral detergent fiber (**NDF**; 7.9 *vs.* 8.7 kg/d) than cows fed RM or SM. But, intakes of DM, starch and NDF were not affected by millet silage type. Feeding RM compared to CS reduced milk yield (32.7 vs. 35.2 kg/d), energy-corrected milk (**ECM**; 35.8 vs. 38.0 kg/d) and solid-corrected milk (**SCM**; 32.7 *vs.* 35.3 kg/d). However, milk, ECM and SCM yields of cows fed SM were similar to cows fed CS or RM. Milk protein concentration was greatest for cows fed the CS, intermediate for cows fed SM and lowest for cows fed RM. Milk concentration of solids non fat **(SNF)** was lesser, whereas milk urea nitrogen **(MUN)** was greater for cows fed RM than those fed CS. However, milk SNF and MUN levels were similar among millet silage type. Milk fat, lactose and total solids **(TS)** concentrations were not affected by silage type. Ruminal pH and ruminal NH_3-N level were greater for cows fed RM and SM than for cows fed CS. Total tract digestibility of DM, NDF, crude protein **(CP)** and gross energy were not influenced by dietary treatments.

In Experiment 2, cows fed GS consumed more DM (22.9 *vs.* 21.7 kg/d) and CP (3.3 *vs.* 3.1 kg/d), but similar starch (4.9 kg/d) and NDF (1.3 kg/d) than MM-fed cows.

Advanced maturity of sweet millet (EM *vs.* MM) did not affect DM, NDF and CP intakes. But, compared to GS, MM reduced milk yield (29.1 *vs.* 26.1 kg/d), ECM (30.4 *vs.* 28.0 kg/d) and FCM (28.3 *vs.* 26.4 kg/d). However, milk, ECM and FCM yields were similar between cows fed EM and GS. Milk protein yield and milk protein concentration were higher for cows fed GS compared to EM or MM. Milk concentrations of SNF, TS, fat and lactose were not influenced by dietary treatments. However, MUN was lower among cows fed GS than MM but not different between cows fed EM and GS or MM. Ruminal pH and total tract-digestibility of DM, NDF, CP and gross energy were not affected by dietary treatments. But, ruminal NH_3-N was higher for cows fed EM than GS, but similar between cows fed MM and GS or EM.

It was concluded that cows performed better when fed CS than RM or SM because of higher starch and lower NDF intakes. However, cow's performance was equivalent between GS and EM, whereas MM significantly reduced milk yield probably because sweet millet was harvested at mature (seed setting) stage. Our findings indicate that sweet millet may represent a reliable silage source for dairy cows, especially in temperate regions.

ABRÉGÉ

Deux projets de recherche ont été menés afin de déterminer le potentiel du millet perlé fourragé pour la nutrition des vaches laitières. Pour l'étude 1 nous avons évalué les effets de remplacer l'ensilage de maïs (**CS**; contrôle) par 2 cultivars de millet [c.-à-d., le millet régulier (**RM**) et le millet sucré (**SM**)] dans l'alimentation des vaches; alors que pour l'étude 2 nous avons évalué 2 différents stades de maturité du millet sucré [c.-à-d., stade gonflement (EM) ainsi que stade pâteux au stade mature (MM)] en les comparant à l'ensilage de graminée (**GS**; contrôle). Les paramètres évalués dans les deux études étaient la production laitière, la digestibilité totale et les caractéristiques de fermentation ruminale. Les deux expériences ont utilisé 15 vaches en lactation dans un 3 x 3 carré Latin répliqué. Les vaches ont été nourris des rations totales mélangées avec un ratio fourrages : concentrés (**F:C**) élevé (c.-à-d., 68:32 et 60:40, respectivement). Trois vaches ruminalement fistulées ont été utilisées pour évaluer la fermentation ruminale et la digestibilité totale.

L'étude 1 a démontré que les vaches consommant CS ont ingéré plus de matière sèche (**MS**; 24,4 *vs*. 22,7 kg/j) et d'amidon (5,7 *vs*. 3,7 kg/j), mais moins de fibre a détergent neutre (**NDF**; 7,9 *vs*. 8,7 kg/j) que les vaches consommant RM ou SM. Par contre, l'ingestion de MS, d'amidon et de NDF n'ont pas été affectées par les types d'ensilage de millet. En alimentant les vaches avec CS par rapport à RM cela à augmenté le rendement en lait (35.2 *vs*. 32.7 kg/j), l'énergie du lait corrigée (**ELC**; 38.0 *vs*. 35.8 kg/j) et les solides du lait corrigé (**SLC**; 35.3 *vs*. 32.7 kg/j). Cependant, les rendements en lait, ELC et SLC des vaches nourries avec SM étaient semblables à celles nourries avec CS et RM. La concentration en protéines du lait était plus élevée pour les vaches consommant CS, intermédiaire pour les vaches consommant SM et plus basse pour les vaches consommant RM. La concentration en solides de lait sans gras (**SSG**) était moins élevé, alors que l'urée d'en le lait (**MUN**) était plus élevé pour les vaches nourries avec RM que celles nourries avec CS.. Le pH du rumen et le niveau NH3-N du rumen étaient plus élevé pour les vaches consommant RM et SM que pour les vaches consommant CS. La digestibilité totale de MS, NDF, protéine brute (**PB**) et d'énergie brute n'ont pas été influencés par les traitements alimentaires.

Dans l'étude 2, les vaches soignées avec GS comparativement à celles soignées avec MM ont consommé plus de MS (22,9 *vs*. 21,7 kg/j) et de PB (3,3 *vs*. 3,1 kg/j), mais une quantité similaire d'amidon (4,9 kg/j) et de NDF (1,3 kg/j). Par contre, la maturité avancée du millet sucré (EM *vs*. MM) n'a pas affecté l'ingestion de MS, NDF et PB. Mais, par rapport à GS, MM a réduit le rendement en lait (29,1 *vs*. 26,1 kg/j), ELC (30,4 *vs*. 28,0 kg/j) et SLC (28,3 *vs*. 26,4 kg/j). Cependant, les rendements en lait, ELC et en gras corrigé du lait étaient similaires entre les vaches nourries avec EM et GS. Le rendement en protéines du lait et la concentration en protéines du lait étaient plus élevés chez les vaches nourries GS par rapport à celles nourries MM ou EM. Cependant, MUN était plus bas chez les vaches nourries GS que MM mais semblable entre les vaches nourries EM et GS ou MM. Le pH ruminal et la digestibilité total en MS, NDF, PB et énergie brute n'ont pas été affectés par les traitements alimentaires. Par contre, le niveau de NH3-N dans le rumen était plus élevé pour les vaches nourris avec EM que GS, mais semblable entre les vaches consommant MM et GS ou EM.

Il a été conclu que les vaches on mieux performé lorsqu'elles ont été nourris avec CS que RM ou SM dû a l'apport supérieur en amidon et inférieur en NDF. Cependant, la performance des vaches était équivalente entre GS et EM, alors que MM a réduit significativement la production de lait sans doute parce qu'il a été récolté au stade mature.

ACKNOWLEDGEMENT

I would like to acknowledge everyone who has helped me bring this master thesis to completion. Foremost, I would like to express my deepest appreciation to Dr Arif Mustafa, my supervisor, and Dr Shyam Bushansingh Baurhoo, a member of my graduate committee, for their scientific advice, guidance, support and encouragement to complete this project. I also wish to thank Dr Philippe Seguin, member of my committee, for his encouragement and interest in my work.

I would like to sincerely thank Somaia Amer, Farial Karimian and Wucheng Liu for their precious help, hard work, encouragements and joyful company in the laboratory. I also wish to thank Hélène Lalande for technical support with ammonia analysis.

I extend my appreciation to my graduate friends from Animal Science Department for their encouragement and company. My thanks also go to the staffs and students working at McGill's farm for all their support and hard work.

I am very grateful to Belisle Solution Nutrition Inc. for all technical support, advice and to have given me the opportunity to pursue my Masters Degree.

I am very thankful to Bélisle Solution Nutrition Inc., Fonds Québecois de la Recherche sur la Nature et les Technologies (FQRNT), and Natural Science and Engineering Research Council of Canada (NSERC) for the financial aid via the Industrial Innovation Scholarship.

Finally, my special thanks go to the love of my life Sebastien, my family and friends who supported me throughout the entire process. Without their love, support and patience it would have been impossible to finish this project.

CONTRIBUTION OF AUTHORS

Authors of the presented manuscript

Tania Brunette, Bushansingh Baurhoo, and Arif Mustafa

Dr. Arif Mustafa and Dr. Bushansingh Baurhoo designed experiments, analyzed data, wrote the manuscript and supervised the primary author. Tania Brunette designed and conducted all the experiments, analyzed data and wrote the manuscript.

CHAPTER I. GENERAL INTRODUCTION

The agriculture and agri-food system plays an important role in the Canadian economy accounting for 8% of the country's total gross domestic product **(GDP)** and 12% of Canadian employment. Among all agricultural commodities, the dairy sector ranks third after red meats, and grains and oilseeds representing 12.6% of the total agricultural commodities market receipts and worth $46.2 billion (AAFC, 2013a). In 2012, the dairy industry contributed $5.9 billion to the Canadian economy with total milk production of 79.5 million hectoliters produced on 12,529 dairy farms (CDIC, 2013a).

In Canada, as much as 81.4% of the dairy farms are located in the provinces of Ontario and Quebec (Canadian Dairy Commission, 2012). But, Quebec ranks first in terms of milk production in Canada producing about 29.3 million hectoliters of milk annually (CDIC, 2013b). In 2012, Quebec had 6,189 dairy farms, which represented almost half of the total dairy farms in Canada, and 37% of the total Canadian dairy cow population (FPLQ, 2008). The dairy industry plays a major role in Quebec's economy, but also constitutes 37% of the Canadian dairy industry's net income (FPLQ, 2008).

Forages are a major source of nutrients for dairy cows and constitute 40 to 70% of the cow's ration depending on its stage of lactation (NRC, 2001). Forages are of important value to producers. In 2010, according to the OMAFRA, forages accounted for 41% of feed costs or 16% of the total cost for keeping a dairy cow per year (Yungblut, 2012). Concentrates which generally contain high amounts of ruminally-available carbohydrate (i.e., corn grains) and/or CP (i.e., soybean meal) constitute the remaining portion of the diets. In dairy cattle diets, forages help to improve rumen health, milk fat and yields, reduce risks of acidosis, and laminitis (Mertens, 1997; NRC, 2001). Forages are the principal source of fibre in dairy cow's diet. However, too much fiber or forages in the ration, at the expense of concentrates, may lower energy density, reduce intake and productivity of cows (Mertens, 1997). On the other hand, concentrates increase palatability, energy density, and tend to increase dry matter intake (**DMI**), and milk production and milk protein concentrations (Llamas-Lamas and Combs, 1991; Weiss and Shockey, 1991). However, high levels of concentrates in the ration, at the expense of forages, is associated with rumen acidosis which may lead to rumenitis and eventually to

ruminal parakeratosis and erosion (Garry, 2002), and also to laminitis, sole abscesses, and sole ulcer (Weiss and Shockey, 1991; Johnson and Combs, 1992; Nocek, 1997). Therefore, adequate forage to concentrate ratio is critical when formulating dairy cow diets.

Forages are defined as the vegetative portion of plants (stem and leaves) that are fed to livestock as pasture, hay, silage, haylage or pellets. Forage crops can be annual or perennial and the majority of forage species used in dairy cow's nutrition belongs to the grass (Graminae) and legume (Leguminosae) families. As annual crop, corn silage is most commonly and extensively used on dairy farms because it is high yielding and provides a good source of energy for the cows. Whether corn silage can be termed as 'forage' is debatable, considering that it usually contains approximately 30% grains. The most common perennial grass species grown in Canada are timothy, bromegrass, orchardgrass and fescues, whereas the main legumes are alfalfa, red clover and to some extent birdsfoot trefoil (Bélanger et al., 2005; AAFC, 2013b).

Over the past years, perennial grasses and legumes forages were harvested over 4 to 8 years. However, today, alfalfa fields on many farms only last for about 3 to 4 years as a consequence of high incidences of alfalfa winterkill and damage to perennial crops. Alfalfa winterkill is caused by lack or absence of snow cover, ice encasement or diseases due to changes in climatic conditions (Bélanger et al., 2005). The winterkill of perennial forages causes lower yields, forage shortages and economic losses on dairy farms. Unfortunately, climatic changes and extreme weather conditions, such as heat waves, storm events, drought, flood and water availability, will occur more frequently in the coming years (OMAFRA, 2011). Therefore, incidences of winterkill of perennial crops and forage shortages will become even more dramatic on dairy farms.

Annual forages are high yielding and may help to solve forage shortages or low yields that frequently prevail on dairy farms, especially during conditions of elevated winterkill of perennial forages. For instance, with regards to its high yielding capacity, there is interest to increase corn silage production. However, in many temperate regions of Canada, corn silage production is very limited to practically impossible given that corn requires high heat units (2100 corn heat units, **CHU**) for corn grain setting and maturity. Therefore, other annual forage types, including peas, cereals (oat and barley), sudangrass,

sorghum and triticale are frequently grown as alternative silages in these cold regions (Bélanger et al., 2005). Despite their contribution as additional forage sources, these annual forages possess few limitations with regards to dairy cow's nutrition. For example, sorghum contains high prussic acid levels, a cyanogenic and toxic compound for ruminants, whereas peas and cereals yields are much lower than corn. Therefore, it is clear that dairy farms located in the more temperate regions of Canada requires new annual forage types that can generate high yields of high quality silages and free from any toxic compound for feeding cows. To this end, we hypothesize that forage pearl millet may represent a reliable annual forage adaptable to the temperate regions of Canada.

Pearl millet is an African origin warm season annual grass and of special interest as a new forage for dairy cattle since it adapts well to poorly fertilized and acidic soils, and is heat and drought resistant. Millet can grow in conditions where corn and sorghum may not be able to. In addition, unlike sorghum, pearl millet does not contain toxic compounds such as prussic acid (Bélanger et al., 2005). The capacity of pearl millet to grow rapidly in brief periods of favourable conditions (Andrews and Kumar, 1992) and to produce interesting yields of high quality forages, makes it suitable for the temperate regions of Canada. Whereas most published studies have researched grain cultivars of pearl millet for feeding cows, we were particularly interested in forage pearl millet cultivars. Moreover, we investigated a variety containing high water soluble carbohydrates **(WSC)**, given that soluble sugars of forages are rapidly utilized as energy source by microorganisms to considerably improve forage fermentation and silage quality (Woolford, 1984). Finally, factors affecting the feeding value of millet silage such as types of cultivars, stage of maturity at harvest, and high forage to concentrate ratio in the nutrition of dairy cows have not been studied yet.

Therefore the objectives of this study were to:

1. Determine the chemical compositions, ensiling characteristics, and in situ degradability of two forage pearl millet varieties, namely a conventional or regular **(RM)** and high WSC or sweet **(SM) cultivar**, and to compare these with corn silage (Experiment 1).

2. Evaluate the effects of RM and SM on milk yield, milk composition, total-tract nutrient digestion, and ruminal fermentation of lactating cows in comparison with corn silage when fed high forage: concentrate diets (Experiment 1).

3. Determine the effects of stage of maturity (vegetative *vs.* mature) of SM on chemical compositions, ensiling characteristics, and in situ degradability compared with grass silage (Experiment 2).

4. Evaluate the effects of stage of maturity of SM on milk yield, milk composition, total-tract nutrient digestion, and ruminal fermentation of lactating cows compared with a mixed grass silage (Experiment 2).

CHAPTER II. LITERATURE REVIEW

2.1. FEED CLASSIFICATION OF DAIRY COWS

2.1.1. Feed components

Feed is composed of water, ash, fat, carbohydrates (**CHO**), and protein (Cornell net carbohydrate and protein system (**CNCPS**, 2014). Protein and carbohydrates are further divided according to chemical composition, physical characteristics, solubility, and fermentation characteristics (Sniffen et al., 1992). In North America, typical composition of dairy rations consists of 60 to 80% carbohydrates, 15 to 18% crude protein, and 2 to 5% fat depending on cow's stage of lactation (Ishler et al., 1996; NRC, 2001).

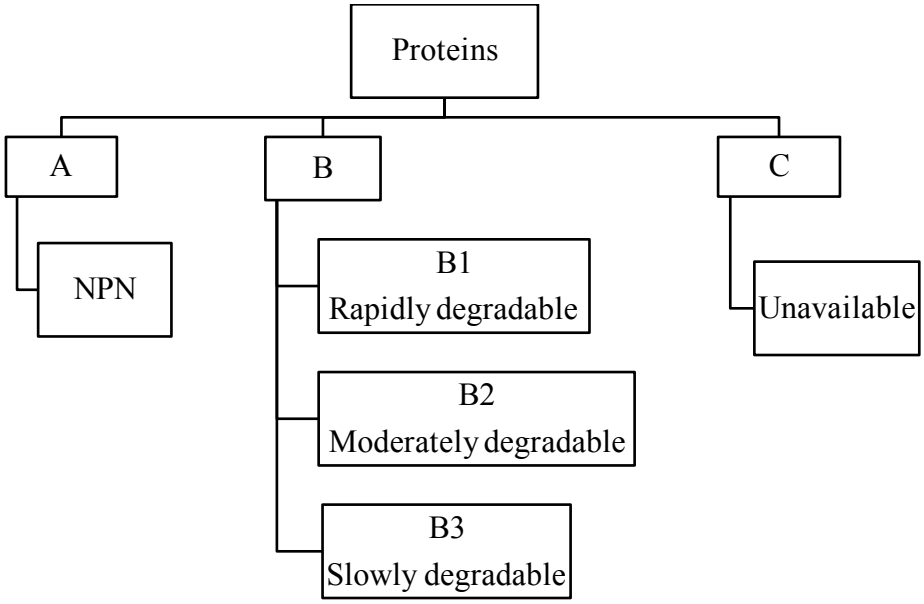
2.1.1.1. Proteins

2.1.1.1.1. Protein fractions

According to the CNCPS, proteins are divided into 3 main fractions: non protein nitrogen (**NPN**), true protein and unavailable nitrogen. True protein is further divided into 3 subcategories according to their rate of degradation in the rumen, namely into rapidly, intermediately and slowly degradable fractions (Sniffen et al., 1992). The CNCPS divides proteins into a similar scheme based on their solubility in protein precipitant agents, buffers and detergent solutions (Lanzas et al., 2007) (Figure 1). The protein fraction A includes NPN compounds such as peptides, amino acids, NH_3, amides, amines, nucleotides and nitrate (Licitra et al., 1996). Fraction A can be determined by precipitating NPN using sodium tungstate (Licitra et al., 1996). Sub fraction B1 represents the rapidly degradable protein (1.0/h) and is soluble, whereas sub fraction B2 is the intermediately degradable fraction. Degradation rate of protein fraction A varies between 0.03 and 0.6/h suggesting that this protein fraction could be partially degraded or escape the rumen degradation based on rumen flow rate (Van Soest et al., 1980; Lanzas et al., 2007). The slowly degradable B3 fraction (i.e., $\leq$ 0.02/h) represents the protein bound to neutral detergent fiber (**NDF**) with a high ruminal escape value. The fraction C

is the nitrogen bound to lignin, tannin-protein complex, and Maillard products which has a ruminal degradation of 0 (Sniffen et al., 1992; Lanzas et al., 2007).

Figure 2.1 : CNCPS protein fractions[1]



[1]Adapted from Sniffen et al. (1992)

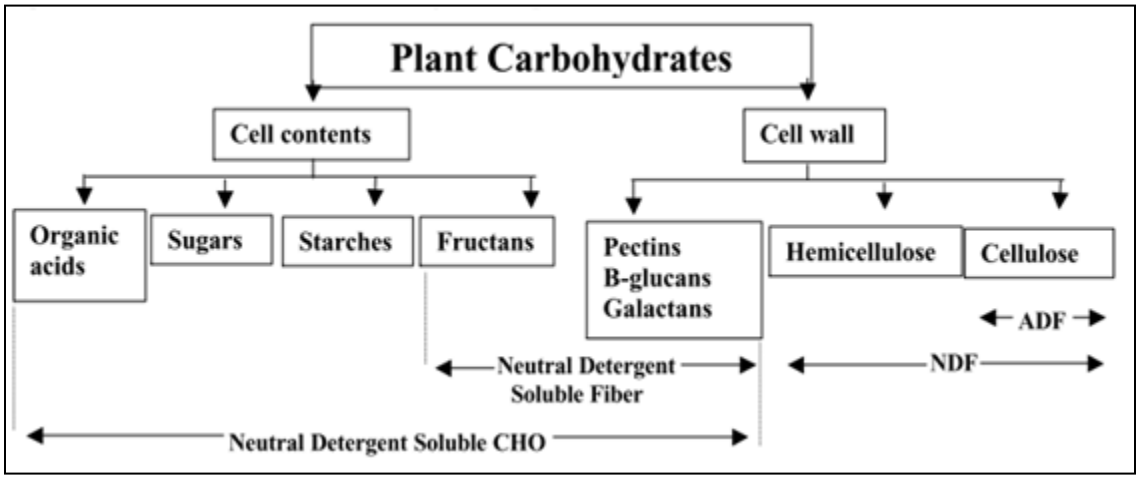
2.1.1.2. Carbohydrates

2.1.1.2.1. Structural and non-structural carbohydrates

Carbohydrates make up the largest portion of dairy rations and are an important source of energy for the rumen microbes. In general, carbohydrates are partionned into structural CHO (**SC**) and non-structural CHO (**NSC**) (Figure 2). Structural carbohydrates include cellulose, hemicellulose and lignin and are found in the plant cell wall. Cellulose, hemicellulose and lignin are chemically known as NDF. The NDF fraction is the insoluble part of plant carbohydrates and is also known to be negatively correlated with dietary intake of cows. Cellulose and lignin are the components that remain after treatment with an acid detergent solution and therefore termed as acid detergent fiber (**ADF**). Acid detergent fibre is negatively correlated with digestion (Mertens, 1997; Lanzas et al., 2007). Lignin, a non-carbohydrate compound that reduces the availability of cellulose and hemicelluloses to the rumen microbes, is determined with a strong acid

treatment which solubilises cellulose (Van Soest et al., 1991). On the other hand, pectic substances and beta-glucans, although not categorized as NDF or ADF, are defined as fiber because they are not digested by mammalian enzymes (Lanzas et al., 2007). Pectic substances and beta-glucans are neutral detergent soluble carbohydrate (**NDSC**) together with organic acids, monosaccharide, oligosaccharide, fructans, and starch which are all found within plant cells and known as NSC (NRC, 2001; Hall, 2003). In general, depending on cow's stage of lactation, the carbohydrate composition in a diet for high producing dairy cows should be 28 to 38 % NDF and 32 to 38 % NSC (Ishler et al., 1996).

Figure 2.2 : Structural and non-structural carbohydrates of plants[1]



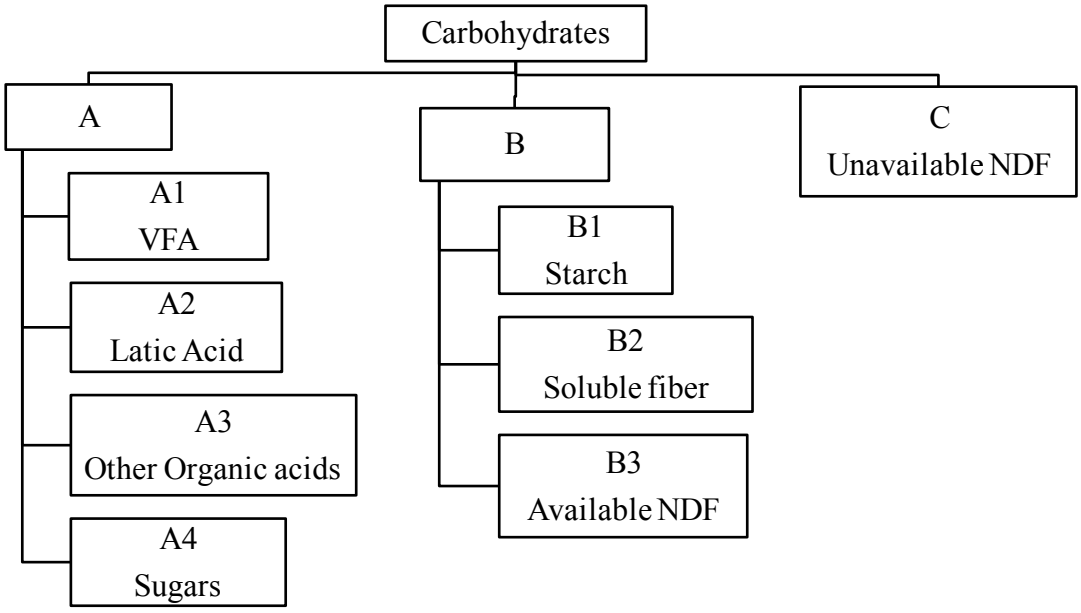
[1]Source: Ischler et al. (1996)

2.1.1.2.2. Carbohydrate fractions

The CNCPS further divides carbohydrates based on their ruminal fermentation characteristics into fractions A (organic acids and sugars), B (starch and cell wall), and C (unavailable CHO) (Figure 2). Fraction A was recently subdivided to represent more accurately the fermentation of carbohydrates in the rumen (Lanzas et al., 2007). Category A1 represents the volatile fatty acids which are rapidly available to the rumen microbes. Fraction A2 is lactic acid and has a ruminal degradation rate of 0.7/h (Benitez and Osman, 2002). Fraction A3 represents all the other organic acids with a ruminal degradation rate of 0.05/h (Benitez and Osman, 2002). Fraction A4 represents sugars

such as glucose, fructose and sucrose. The degradation rate of sugars ranges between 0.16/h and 0.4/h (Benitez and Osman, 2002). The B fraction is subdivided into: B1 (starch), B2 (soluble fiber), and B3 (cell wall). The B1 gradation ranges between 0.04 to 0.4/h depending on partical size, grain type, processing effect and method of preservation of the feed (Offner et al., 2003). The B2 fraction includes soluble fibers such as β-glucans and pectic substances with a fermentation rate ranging between 0.2 and 0.4/h (Hatfield and Weimer, 1995; Hall et al., 1998). Beta-glucans are found in barley and oat (Engstrom et al., 1992) whereas pectic substances are found in high concentrations in by products such as beet pulp, soybean hulls and cell wall of legume forages (Van Soest, 1994). The B3 fraction is the available NDF that are slowly degraded in the rumen, whereas the C fraction is fiber associated to lignin which is not available for digestion (Sniffen et al., 1992). Ruminants usually extract one third of the energy in structural carbohydrates while they digest almost completely the non structural carbohydrates (Buxton, 1996).

Figure 2.3: CNCPS carbohydrate fractions[1]



[1]Adapted from Sniffen et al. (1992) and Lanzas et al. (2007)

2.1.1.2.3. Effective fiber

The concepts of effective fiber and physical effective fiber have been developed to help assessing fiber needs in dairy cattle diets. Effective fiber (**eNDF**) is defined as the ability of a feed to effectively maintain milk fat percentage (Mertens, 1997). Therefore supplying a dairy cow with adequate amount of structural carbohydrate is important to maintain milk fat percentage. Physical effective fiber (**peNDF**) is defined as a feed's ability to promote chewing activity and maintain the rumen mat. Therefore, providing adequate peNDF requires feeding the dairy cow proper forage particle size and bulkiness (i.e., NDF content) (Ishler et al., 1996; Mertens, 1997). Therefore, fiber is required in the rations of dairy cattle for their chemical and physical characteristics. Chemical characteristics of fiber (i.e., NDF and NSC) are related to milk fat concentrations whereas physical characteristics of fiber (i.e. particle size and density) influence the rumen fiber mat, chewing activity and saliva production (Mertens, 1997; NRC, 2001). Hence, source of NDF as well as particle size of the fiber are important factors for ration formulation (NRC, 2001). For instance, concentrates such as beet pulp, soybean hulls and distillers grain that usually contain up to 35% NDF or more are excellent sources of highly digestible small particle fiber (Ishler et al., 1996) which may maintain milk fat percentage but are not effective to promote chewing activity. In order to supply proper eNDF as well as peNDF, 21 to 28 % of the NDF in the diets of dairy cattle should come from forages with adequate quality and particle length (Ishler et al., 1996).

2.2. EFFECTS OF FORAGE : CONCENTRATE RATIO ON PERFORMANCE OF DAIRY COWS

2.2.1. Chewing activity, saliva secretion, and ruminal pH

Dietary F:C proportion may significantly impact dairy cow's performance and health. Feeding cows high concentrate diet increases DMI and milk yield (Weiss and Shockey, 1991; Reis and Combs, 2000; Yang and Beauchemin, 2006). However, feeding cows high concentrate diet with low levels of effective fiber may reduce chewing activity saliva production and therefore ruminal pH (Beauchemin et al., 2003; Kononoff and Heinrichs, 2003). Ruminal pH $\leq$ 5.8 has been associated with metabolic disorder such as

milk fat depression, reduced fiber digestion, rumen malfunction, displaced abomasum, lameness, borderline and acute acidosis, and fat cow syndrome (Mertens, 1997; Maekawa et al., 2002; Ametaj et al., 2010).

It is well documented that fiber from forages with adequate particle size is necessary in dairy cattle diets (Mertens, 1997; Beauchemin et al., 2003; Beauchemin and Yang, 2005; Yang and Beauchemin, 2006, 2007). Unlike concentrates, forages stimulate chewing as these need to be reduced in sufficiently small particle size in order to move through the gastro-intestinal tract and chewing stimulates the release of saliva which acts as a buffer in the rumen (Beauchemin, 1991; Ishler et al., 1996; NRC, 2001; Yang and Beauchemin, 2006; Plaizier et al., 2008; Kargar et al., 2010). Saliva accounts for about 30 to 40% of the neutralization of fermentation acids in the rumen (Allen, 1997). Nonetheless, the impacts of F:C and peNDF on chewing and ruminal pH are inconsistent. Maekawa et al. (2002) reported that increasing the F:C ratio from 40:60 to 60:40 increased rumination time (min/d), total chewing time (min/d), salivary secretion while ruminating and resting (L/d), and ensalivation of the feed (L/kg DM) without affecting the rumen pH. Similarly, Yang et al. (2001) reported no effect of F:C or forage particle size on ruminal pH. However, Llamas-Lamas and Combs (1991) (F:C of 86:14 *vs.* 71:29 *vs.* 56:44), Yang and Beauchemin (2007) (F:C of 60:40 *vs.* 35:65), and Kendall et al. (2009) (F:C of 62:38 *vs.* 58:42) reported that high F:C increased ruminal pH. It was also reported that feeding lactating dairy cows chemically identical diets with similar F:C ratios but different proportion of peNDF lead to a decrease of 13.5% in total chewing time per day for the low peNDF diet compared to the high peNDF diet (Yang and Beauchemin, 2006). Furthermore, although rumen pH was not affected by forage particle size, the pH variation was more apparent in the medium and low peNDF diet compared to the high peNDF diet (Yang and Beauchemin, 2006). Similar findings were reported by Beauchemin and Yang (2005) and Kahyani et al. (2013). Hence, forages and adequate forage particle size seems to indirectly help to maintain rumen pH through the action of chewing, saliva secretion and ensalivation of the feed.

2.2.2. Ruminal fermentation

In the rumen, feed particles are fermented by the rumen microbes. According to the CNCPS, rumen microbes can be divided into two groups according to their fermentation substrate. The structural carbohydrate bacteria degrade cellulose and hemicellulose whereas non-structural carbohydrate bacteria degrade starch and pectin. Bacteria that ferment cellulose and hemicellulose grow slowly and utilize ammonia as a source of nitrogen while non-structural carbohydrate bacteria grow rapidly and utilize peptides and amino acids (Russell et al., 1992). End products of the fermentation of carbohydrates by bacteria include volatile fatty acids (**VFA**; acetate, propionate and butyrate) (NRC, 2001). Fermentation of structural carbohydrates yields acetate whereas digestion of non-structural carbohydrate yields propionate. Hence, increased forage to concentrate ratio or increased forage particle size (i.e., peNDF increased) caused the acetate:propionate ratio in cow's rumen to increase (Beauchemin, 1991; Llamas-Lamas and Combs, 1991; Yang et al., 2001; Krause et al., 2002; Beauchemin and Yang, 2005; Kendall et al., 2009; Neveu et al., 2013) or remain unchanged (Yang and Beauchemin, 2007). Feeding highly degradable concentrate or forage with low peNDF often leads to VFA accumulations in the rumen. Total ruminal VFA was increased with reducing dietary peNDF (Yang and Beauchemin, 2006), and decreased with increasing F:C ratio or increasing forage particle size (Krause et al., 2002; Yang and Beauchemin, 2007). However, ruminal ammonia increased with increasing F:C or peNDF (Beauchemin, 1991; Yang and Beauchemin, 2006).

2.2.3. Dry matter and NDF intake

The effects of high dietary NDF on DMI are inconsistent. Diets containing high dietary NDF tend to limit DMI because of the rumen fill effect (Mertens, 1997). Several studies have investigated the effects of dietary NDF on DM and NDF intakes by feeding cows with different sources of forage (alfalfa silage, barley silage or corn silage) and by varying the F:C ratio of the diets. Feeding lactating dairy cows diets with increasing amount of dietary NDF decreased DMI and increased NDF intake (Llamas-Lamas and Combs, 1991) or had no effect on DMI (Yang et al., 2001; Maekawa et al., 2002; Neveu et al., 2013) whereas lower dietary NDF increased DMI and decreased NDF intake

(Weiss and Shockey, 1991). Moreover, peNDF level had no effect (Beauchemin and Yang, 2005; Yang and Beauchemin, 2006) or when reduced had an increasing (Kononoff and Heinrichs, 2003) effect on DM and NDF intake.

Other studies observed the influence of NDF content and digestibility on DM and NDF intake. Ivan et al. (2005) found that DMI was increased by 4.72% for the diet with the highest NDF content and highest NDF digestibility. Similar findings were reported by Oba and Allen (1999) who conducted a meta-analysis with 13 sets of forage comparisons as reported in the literature. Feed intake was increased by 2.92% when cows were fed diets with higher NDF digestibility than lower NDF digestibility. Thus, fiber digestibility, NDF content and peNDF are important factors that directly influence DMI.

2.2.4. Milk yield

In general, high forage diets contain higher NDF and lower starch relative to low forage diets. As a result, high forage diets have lower net energy lactation ($\mathbf{NE_L}$) contents (Yang et al., 2001; Maekawa et al., 2002; Andersen et al., 2003). Thus, energy intake which is limited by DMI and energy density of diet may affect the performance of the animal (Mertens, 1997). Studies have reported a decline in milk yield when lactating cows were fed a high forage diet (i.e., more than 55%) (Llamas-Lamas and Combs, 1991; Yang et al., 2001). In contrast, feeding cows low (i.e., 40% forage) or high (i.e., 60% forage) did not affect milk yield (Neveu et al., 2013). Moreover, feeding diets with different forage particle size (i.e., peNDF) had no effect on milk yield (Krause et al., 2002; Kononoff and Heinrichs, 2003). When cows were fed a high NDF (32%) diet compared to a low NDF diet (28%), milk yield was reduced by 6.65% (Kendall et al., 2009). Moreover, milk yield declined when dietary NDF content increased over 25% of DM (Beauchemin and Rode, 1997). In contrast, no effect of high fiber diets on milk yield were reported (Weiss and Shockey, 1991; Maekawa et al., 2002). On the other hand, when digestibility of NDF was increased, Dado and Allen (1996), Oba and Allen (1999) and Ivan et al. (2005) have reported higher milk yields for high forage diets.

2.2.5. Milk composition

Milk compositions may be affected by forage levels in the diet. When feeding cows high forage diets, milk fat concentration was increased (Yang et al., 2001; Maekawa et al., 2002), decreased (Kendall et al., 2009), or unchanged (Llamas-Lamas and Combs, 1991; Weiss and Shockey, 1991). Moreover, high forage diets had no influence on 4% FCM (Llamas-Lamas and Combs, 1991; Weiss and Shockey, 1991; Yang et al., 2001; Maekawa et al., 2002).

2.3. FORAGES CROPS

Canada's forage resources include native range and cultivated crops. In 2011, the reported area of land in crops (field crops, vegetables, fruits and nursery crops) was 35 million ha accounting for 54.6% of the total farm area (Statistics Canada, 2011). The land used for pasture which includes pasture under cultivation and seeded pasture as well as native range pasture represented 31.2% of the cropland (Statistics Canada, 2011). Western provinces possess 96% of Canadian rangelands, 82% of cultivated pastures, and 64% of forage crop area which are mostly used for beef production whereas Eastern provinces have the majority of remaining cultivated forage and forage crop area for dairy and beef production (McCartney, 2011).

According to a report by the Conseil Québécois des Plantes Fourragères over the last decade, Quebec's cultivated forage land has declined by 23% while the number of dairy cows has decreased by 53%. But, improved cow's performance has been able to keep milk production relatively stable. However, it is not the case for forages. In the last 30 years, forages yields have remained stable or declined when compared to other crops such as corn and canola. Moreover, forage breeding and developmental research has declined dramatically in the public sector (Yungblut, 2012) despite the fact that climate change continues to challenge forage cultivation and production.

2.3.1. Warm season vs. cool season forages

2.3.1.1. Agronomic characteristics

Based on their photosynthetic pathway, forages are categorized into warm and cool season plants. Warm season plants utilize the C_4 photosynthetic pathway whereas cool season plants utilize the C_3 photosynthetic pathway (Waller and Lewis, 1979). Plants using the C_4 pathway utilize available resources such as water, light, CO_2 and soil more efficiently (Bewley, 2006). In contrast to C_3 plants, warm season plants are well adapted to high light intensities, high temperature and dry conditions but have a lower tolerance to cold temperature (Raven, 1999). For instance, C_4 plant's photosynthetic rate may be 3 to 4 times greater than the one of C_3 plants under the same environmental conditions (Samson). Moreover, C_4 plants have greater dry matter production per unit of water transpired (Brown, 1978). The C_4 plants have optimum growth at temperatures ranging between 25 to 35 °C whereas C_3 plants have an optimum growth at temperatures of 15 to 25 °C (Nelson, 1996). Examples of warm season plants include corn, sudangrass, sorghum, pearl millet, bermudagrass and switchgrass. Wheat, rye, oats, rice, orchardgrass and fescues are examples of cool season plants (Oregon State University, 2008a).

2.3.1.2. Chemical composition of seasonal forages and its impact on intake and digestibility

Experiments regarding the comparison between cool and warm season grasses for chemical composition, intake and digestibility are limited. Assoumaya et al. (2007) conducted a meta-analysis regrouping 236 scientific publications selected for specific criteria (632 cool season and 721 warm season forages). For instance, the analysis reported that temperate forages contained 30% less NDF, 20% less ADF, 46% less lignin, and 27% more CP than tropical forages. Nutrient intakes as percentage of body weight followed the same trend as the chemical composition of the forages. Ruminants fed temperate forages had greater DM and CP intakes, and lower NDF and ADF intakes. However, DMI as a percent of body weight was similar for temperate and tropical forages when the forages contained similar CP content. Total tract digestibility and ruminal digestibility of NDF were not different between temperate and tropical forages at different and similar NDF and CP contents (Assoumaya et al., 2007).

2.3.2. Grasses *vs.* legumes forages

2.3.2.1. Agronomic characteristics of legume and grass forages

The majority of forage crops grown for livestock production belong to two plant families, namely legumes (Fabaceae previously called Leguminosae) and grasses (Poaceae formely called Gramineae) (Encyclopædia Britannica, 2013). About 75% forage crops are from the grass family while legumes are the second most typical forages. Both legumes and grasses may be annual or perennial and have roots, stems and leaves. The main characteristic differentiating legumes from grasses is that legumes have the capacity to fix nitrogen from air by means of a symbiotic relationship with the bacteria rhizobium which transforms atmospheric nitrogen into a form available to the plant. For this reason, legume forages require less nitrogen fertilizer. However, legumes tend to require more management than grass for survival and productivity (OMAFRA, 2009; Oregon State University, 2008b). Nevertheless, legumes and grasses are distributed worldwide and represent an important source food and feed. Legumes (soybean, garden pea and alfalfa), and grasses (i.e., wheat, barley, rye, oat and corn) are good examples (Encyclopædia Britannica, 2013). The most widely grown forage legume in Canada and United States is alfalfa whereas corn is the most common grass type. Other forage legumes include red and white clover, and birdsfoot trefoil. For grass, timothy, bromegrass, fescues and orchardgrass are most common in Canada whereas bromegrass, orchardgrass and switchgrass are frequently cultivated in Northern US (Bélanger et al., 2005; Olmstead and Brummer, 2008; AAFC, 2013c)

2.3.2.2. Chemical composition of forages and its impact on animal performance

Crude fiber and protein are the two main factors that differentiate grasses from legumes (Table 1). In general, grasses have higher NDF and lower CP contents than legumes. The higher NDF concentration of grasses than legumes may be attributed to the different fiber content in the leaves. Indeed, leaves of legumes such as alfalfa and red clover at mid flowering stage contained 25% fiber compared to 50% in leaf blades of grasses (i.e., tall fescue, smooth bromegrass and orchardgrass) (Buxton et al., 1995). Likewise, in the same study, stems of the same legumes and grass species contained 40 to

55% and 70% fiber, respectively (Buxton et al., 1995). The higher CP content of legume is likely due to its ability to fix nitrogen. In fact, alfalfa produces more protein per unit of area than any other forages (OMAFRA, 2009).

Several studies have investigated the effects of feeding grass, legumes or a mixture of both in the nutrition of lactating dairy cows (Hoffman et al., 1997; Hoffman et al., 1998; Broderick et al., 2002; Cherney et al., 2004; Halmemies-Beauchet-Filleau et al., 2014). Intake of DM was reduced (Hoffman et al., 1998; Broderick et al., 2002), similar (Cherney et al., 2004), or increased (Hoffman et al., 1997) when cows were fed grass silage than legume silage. Milk yield was reduced (Hoffman et al., 1998; Broderick et al., 2002) or similar (Hoffman et al., 1997; Cherney et al., 2004) when cows were fed grass than legume silage. However, a mixture of grass and legume silages increased both milk yield and DMI compared with feeding cows with grass or legume silage solely (Halmemies-Beauchet-Filleau et al., 2014). Milk fat and protein concentrations were similar (Hoffman et al., 1998; Cherney et al., 2004) or reduced with grass silage (Broderick et al., 2002).

Table 2.1: Chemical compositions of grass and legume silages[1]

	Grass silage	Legume silage
Chemical Composition, % DM		
DM	29.0-42.0	36.2-54.2
CP	12.7-24.7	20.0-24.3
NDF	45.0-66.0	33.9-50.0
ADF	29.2-41.1	34.1-40.9
ADL	3.0-7.4	5.9-8.4
NE_L, Mcal/kg	1.12-1.41	1.22-1.39

[1]Adapted from Weiss and Shockey (1991); Hoffman et al. (1998); NRC (2001); Broderick et al.,(2002); Cherney et al. (2004); Filleau et al. (2013)

2.3.3. Perennial forages

2.3.3.1. Agronomic characteristics

Perennial plants are plants that persist for several years. Depending on soil types, management practices, crop rotation system and climatic conditions, perennial forages may produce feed for livestock over 1 to 8 years (OMAFRA, 2009). In addition, perennial forages are well known for their ecological benefits such as adding organic matter, improving soil structure and water infiltration, reducing erosion and leaching of chemical compounds, as well as controlling weeds and pests (Olmstead and Brummer, 2008). The majority of forage plants cultivated for livestock are perennial legumes and grasses, including alfalfa, bird's-foot trefoil, alsike clover, red clover (single cut), red clover (double cut), sweet clover, white clover, timothy, meadow brome grass and smooth brome grass (Bélanger et al., 2005; AAFC, 2013c).

2.3.3.2. Impact of climate change on perennial forages

In Canada, our harsh climatic conditions do not always favour growth and survival of perennial forages. In order to survive winter, a plant must go through the process of hardening which is the cessation of the meristem (part of plant containing actively dividing cells that forms new tissue) activity and the establishment of a dormant state (Rohde and Bhalerao, 2007). Basically, the hardening process is triggered in the fall by cool temperatures and accelerates when temperatures below 5 °C are reached (Bélanger et al., 2002). However, hardening is severely affected when fall temperatures are above 5 °C or when soil moisture content is excessively high. But most importantly, in the winter, the dormant state of a plant may be negatively affected by the fluctuation of temperature above and below freezing point. Hence, the level of hardening reached in the fall is directly related to the plant's ability to withstand and survive winter's environmental stresses. Nonetheless, an adequate snow cover (>10 cm) helps to prevent plant injury and protects the plant from winter's freezing weather conditions thus increasing its chances of survival (Bélanger et al., 2005). However, due to global climate change, winter survival of perennial forages will severely be affected with time. For instance, warmer falls and winters as well as increasing temperature fluctuation will

affect the hardening process and dormancy of perennial plants thus reducing winter survival rates (Bélanger et al., 2002). In Canada, climate change already has negative repercussions on the survival of perennial forages. In 2012, alfalfa majorly suffered from frost damage in south-western Ontario as a result of mild and freezing temperatures in early- to mid-March (OMAFRA, 2012a). Moreover, in spring and summer 2011, excess moisture and flooding in the prairies (Alberta, Saskatchewan and Manitoba) caused significant damage to different crops, hay and pastures (AAFC, 2013c). In summer 2012, producers in Southern Ontario and Gatineau, Pontiac and Témiscamingue regions of Quebec were severely affected by drought conditions (less than 40% the normal precipitation) (OMAFRA, 2012b). In 1998, an ice storm in the corridor extending from Kingston Ontario to New Brunswick led to ice encasement of the vegetation and caused massive damage to crops and trees (Statistics Canada, 2007, 2008). Consequently, the survival and productivity of perennial forages largely depends on climatic conditions (i.e., temperature and precipitation). Unfortunately, climatic conditions are predicted to become more unstable and unpredictable in the coming years. However, climate change will bring longer growing season and increase forage productivity because of warmer temperatures, and hence the chance to diversify and grow new crops (Olivier, 2013).

2.3.4. Annual forages

Annual plants complete their life cycle in one growing season (Encyclopædia Britannica, 2013). Annual forages are versatile, and may be used as cover crop for the establishment of perennial forages, as green manure, for silage production, to extend the grazing season or to replace damaged perennial crops while providing similar ecological benefits as perennial forages (Carr, 2006). Therefore, annual forages provide flexibility and are an important forage source in dairy production. The major crops of the world are annual. Corn is the most widely grown annual forage, especially for silage production. However, corn requires at least 2100 CHU per growing season and is not drought tolerant (Bélanger et al., 2005). Because of insufficient CHU, corn cannot be grown to maturity in the US Northern Great Plains, and in the Canadian prairies and northern regions. Alternatively, in these regions, small-grains cereals such as barley, oat and triticale, and to lesser extent spelt and rye are mainly used for silage production and grazing

(Khorasani et al., 1997; Carr, 2006). But yields of small cereals alone or when seeded together with peas are extremely poor when compared to corn. When targeted for silage production, small cereals are usually harvested at the milk to soft dough stage at which quality, yield and conservation are best compromised (Khorasani et al., 1997; Bélanger et al., 2005; Séguin, 2010). Nevertheless, quality of small grains silage is generally not equivalent to that of alfalfa or corn silage. Peas, sudangrass and sorghum are other annual crops grown for forages in Canada. However, sudangrass and sorghum are confined to areas with 2500 CHU or greater. Therefore, high quality and high yielding annual forages may alleviate the problems of forage shortages on dairy farms that more frequently occurs in consequence of rapid climate change and winter kill of perennial forages.

2.3.5. Effect of stage of maturity on forage quality

2.3.5.1. Effect of maturity on chemical composition

Forage maturity directly influences its chemical compositions (Buxton, 1996; Buxton and Redfearn, 1997; Khorasani et al., 1997; Elizalde et al., 1999; Lewis et al., 2004; Bélanger et al., 2005; Kuoppala et al., 2009; Morales et al., 2011; Kammes et al., 2012). In the field, developmental stages of forages are routinely used as assessment of their nutritive values. The common stages of development for grasses are tillering, stem elongation, heading and flowering, and vegetative, early bud, early flower and late flower for legumes (Elizalde et al., 1999). Moreover, environmental conditions, forage type, and forage variety majorly influence the chemical compositions of forages. In general, the highest nutritive values of forage are obtained at the stem elongation early heading stage for grasses and at the early bud/early flower stage for legumes (Bélanger et al., 2005).

Advancement in forage maturity reduces CP but increases fiber contents. This is the result of higher SC in the stem and leaves, and lower leaf:stem ratio when plant matures. Because stems are higher in fiber than leaves, NDF concentration in the total plant increases with maturity (Buxton and Redfearn, 1997). On the other hand, as forage matures, CP content decreases in the leaves and stems, but leaf blades may contain twice as much CP than stems. Therefore, lower leaf:stem ratio greatly affect the CP contents of forages (Buxton, 1996). For legumes, CP contents decline by 0.25 units/d when the

budding stage is reached such that CP contents will have declined by 1.75 units after 1 week (Bélanger et al., 2005). When alfalfa matured from the late vegetative to late flowering stage, a 30% reduction in CP contents and a 24% increase in NDF contents were observed (Elizalde et al., 1999). Similarly, CP levels decreased when bromegrass (-60%) and tall fescue (-58%) matured from the tillering to flowering stage, whereas NDF contents of bromegrass (+23%) and tall fescue (+28%) were significantly increased (Elizalde et al., 1999). The reduction in CP contents with maturity was more pronounced in grasses than legumes. But, fiber increase was similar between grasses and legumes. In a study Yu et al. (2003), when alfalfa was compared at the early bud and early bloom stages, results indicate lower CP (-2.6 units) and NPN (-41.9 units) contents, higher soluble crude protein (**SCP**; +10.6 units), acid detergent insoluble crude protein (**ADICP**; +13.6%) and CHO (+3.85%) levels, but fibre contents were not affected. When timothy was compared at the stem elongation and flowering stage, CP level was reduced by 3.15 units whereas SCP, NPN, CHO, NDF, and ADF were increased by 8.75, 28.15, 3.55, 4.1 and 2.75 units, respectively.

2.3.5.2. Effect of maturity on digestibility

Fibre (especially lignin) content is the principal factor that affects the digestibility of forages. In contrast to legume leaves, the digestibility of grass leaves declines more rapidly with maturity (Buxton, 1996). Regardless of whether the forage is annual, perennial, tropical, temperate, a grass or legume, in vivo and/or in situ digestibility of NDF, CP and DM are significantly reduced with advanced maturity of the forage (Elizalde et al., 1999; Yu et al., 2003; Ammar et al., 2010).

2.4. WATER SOLUBLE CARBOHYDRATES CONTENTS OF FORAGES

Fructose, glucose, sucrose and fructosans are the major WSC in forage crops (Woolford, 1984). These sugars are important source of energy for microbes responsible for silage fermentation. Therefore, forage WSC is vital for proper ensiling and fermentation of forages. High moisture in forages increases competition between organisms for nutrients and allows undesirable microbes to withstand higher concentration of acids (Woolford, 1984). When WSC concentration is low, growth of

lactic acid bacteria is affected. Under such conditions, lactic fermentation of forages is impeded and pH is relatively high such that growth of undesirable microorganisms and spoilage of the forage is likely to occur. On the other hand, an excess of WSC may be used by undesirable microorganisms and increase clostridial fermentation of the plant material (Cherney and Cherney, 2003). Therefore, in order to optimize ensiling, fresh plant materials need to contain a minimum concentration of 30 g/kg WSC with a minimum DM content of 20 g/kg (Haigh and Parker, 1985).

The WSC content in plants is influenced by species, cultivar, stage of maturity, weather conditions and harvest time (McDonald et al., 1991). For example, a high sugar rye grass cultivar had 40% more WSC than a regular rye grass cultivar at same stage of maturity (Moorby et al., 2006). For pearl millet, 90% of the total WSC is stored in its stem. The WSC concentration in pearl millet leaves was 35.7 to 48.1 g/kg DM compared to 146.8 to 162.4 g/kg DM in stems (Bouchard et al., 2011). Therefore, as the stem:leaf ratio increases with maturity, pearl millet shall contain higher WSC levels. Indeed, WSC contents of pearl millet harvested at 65 d *vs.* 106 d of growth were 86.5 g/kg DM compared to 136 g/kg DM, respectively (Leblanc et al., 2012). However, for grain forage types, WSC content will decline at the grain maturation stage because starch contents will increase at the expense of WSC concentration (Johnson et al., 2003; Slewinski, 2012). Rainfall also influences WSC content in forages. For alfalfa, when no rain was compared with 11.7, 23.3, 35.0, and; 46.7 mm of simulated rain, increasing rainfall linearly decreased WSC content of the cut alfalfa (Coblentz and Muck, 2012). In general, plants accumulate sugars during the day upon photosynthesis and utilize sugars at night through respiration. Tall fescue and alfalfa had higher WSC contents when harvested in the afternoon than in the morning (Fisher et al., 1999; Ficher et al., 2002; Brito et al., 2008; Oba, 2011).

2.4.1. Effect of water soluble carbohydrate content on silage fermentation

During silage fermentation, WSC serves as substrate for lactic acid bacteria that produces organic acids causing a rapid decline in pH which is essential in the early process of fermentation (McDonald et al., 1991).

In a study, Yang et al., (2006) evaluated the impact of adding glucose to wheat straw in order to determine the optimum initial level of WSC required for natural fermentation of the material. Glucose was added to wheat straw at the following concentrations: 1.4, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0; and 10 % DM. Results indicated that pH of the material was more rapidly reduced when glucose was added at 5.0 to 10.0% compared with 1.4 to 4.0%. Moreover, after 30 d of fermentation, materials with an initial WSC contents ranging between 7.0 to 10.0 % had a pH close to 4.0 whereas materials with initial WSC content ranging between 4.0 to 6.0 % had a pH close to 5.1. The association between higher initial WSC content and lower pH of ensiled materials have previously been reported (Hassanat et al., 2006; Merry et al., 2006; Downing et al., 2008). In addition, increasing WSC levels significantly increased lactic and acetic acid concentrations of silages whereas butyric acid level was decreased (Yang et al., 2006). Similar observations were reported by Amer et al. (2012). In contrast, Merry et al. (2006) found that a high WSC ryegrass (87.5 WSC % DM) had 49% greater lactic acid content but contained 33% less acetic acid compared to a conventional ryegrass cultivar (30.1 WSC % DM).

2.4.2. Effect of feeding high water soluble carbohydrate forage on animal performance

The fermentation rate of WSC occurs very rapidly in the rumen. According to the CNCPS, the rate of rumen fermentation for WSC is approximately 300%/h. But, the effects of sugars on dairy cow's performance are inconsistent (Oba, 2011). For example, in contrast to a regular WSC variety, feeding cows a high WSC variety of perennial ryegrass that was freshly cut did not influence intake, digestibility, milk yield or milk components except that milk urea N was lower (-14%) for the high WSC diet (Taweel et al., 2005). However, Moorby et al. (2006) reported increased total DMI, forage intake, milk NPN concentration, and milk yields of true protein, casein and whey when a fresh high WSC perennial ryegrass was fed to cows compared with a conventional variety. In addition, nitrogen utilisation efficiency by cows was increased by feeding the high WSC diet. In another study, PM cut alfalfa 19% higher WSC contents than AM cut alfalfa (Brito et al., 2008). When fed to late lactating cows, the freshly cut PM cut alfalfa

significantly increased DMI, milk yield, FCM, EMC, and milk fat, protein and lactose yields whereas MUN was reduced by 9%. The PM cut alfalfa also tended to increase ruminal pH, isobutyrate and valerate molar proportions, and the P/(A+B) ratio whereas total VFA, acetate and the acetate to propionate ratio **(A:P)** were decreased.

2.5. PEARL MILLET

2.5.1. Introduction

Pearl millet was domesticated approximately 4000 to 5000 years ago from the semi-arid tropics of West Africa and then spread to eastern and southern Africa before reaching the India subcontinent about 2000 to 3000 years ago (Maiti and Ebeling, 1997). Millet was introduced in Europe and USA in 1566 and 1850, respectively. Pearl millet may be grown as grain, forage or as a dual purpose crop. It is one of the most important source of food and forage for many African and Asian countries, but is of less importance as a forage crop in the United States, Australia, South Africa and South America (Andrews and Kumar, 1992; Andrews et al., 1993; Maiti and Ebeling, 1997; Myers, 2002; Bewley, 2006). In 2009, India was the largest producer of pearl millet with 9 million ha accounting for 50% of the cultivated area and a yield of 8.3 million tons (ICRISAT; Bewley, 2006).

2.5.2. Agronomic and economics characteristics of pearl millet

Pearl millet [*Pennisetum glaucum* (L.) R.Br.] is an annual warm season bunch type grass classified in the Poaceae family and Panicoideae subfamily (Maiti and Wesche-Ebeling, 1997). Millet is a C4 plant that grows optimally at temperatures ranging between 25 and 30 °C (Maiti and Wesche-Ebeling, 1997; Newman, 2010). Millet prefers light sand to loamy soils, tolerates soil pH as low as 5.5, is drought resistant and possess a well-developed root system that may grow more than 2 m laterally or horizontally to reach water and soil nutrients (Maiti and Wesche-Ebeling, 1997). Pearl millet grows well in arid and dry (<350 mm of rainfall) areas (Newman, 2010). But, for maximum biomass, millet requires about 600 to 800 mm of rainfall (Maiti and Wesche-Ebeling, 1997).

2.5.3. Dry matter yield of pearl millet forages

Forage DM yield of pearl millet ranges between 3.0 to 19.2 t/ha depending on planting date, harvest management, soil fertility, cultivar, temperature and available moisture (Jaster et al., 1985; Hill et al., 1999; AERC, 2009; Bouchard et al., 2011; Leblanc et al., 2012). Higher yields are obtained when pearl millet is harvested at the heading (9.0 t/ha) than vegetative (3.7 t/ha) stage (Hassanat et al., 2006). Yields are even lower for brown midrib (**BMR**) cultivar (3.89 *vs*. 5.57 t/ha) than conventional pearl millet cultivar (Mustafa et al., 2004; Hassanat et al., 2006). Moreover, increasing the seeding rate of sweet pearl millet from 5 to 20 kg/ha reduced (-18%) DM yield, whereas seeding millet at row spacing of 18 cm than 36 cm significantly increased (+11%) DM yield (Bouchard et al., 2011).

2.5.4. Chemical composition of pearl millet forage and silage

2.5.4.1. Crude protein contents

Crude protein content of pearl millet ranges between 6.5 to 22.5% DM for forages and 7.1 to 17.1% DM for silages (Table 2) with little differences between cultivars (Hassanat et al., 2007a). In general, millet's CP concentration is higher than corn forage, lower than alfalfa and similar to cool season grasses (Table 2). Several factors affect the CP content of millet forages. For example, low soil N fertilization (Ahmadi et al., 2009; Leblanc et al., 2012) increased plant density (Bouchard et al., 2011) and advanced maturity (Hassanat et al., 2006) may reduce millet's CP contents. A 16% loss in CP content was recorded when pearl millet was harvested at the dough grain stage than milk grain stage (Morales et al., 2011).

2.5.4.2. Fiber contents

Forage pearl millet contains higher NDF levels compared with corn or cool season grasses. The NDF content of forage pearl millet ranges between 52.7 and 68.2 % of DM (Table 2). Cultivars, maturity stage at harvest and conservation methods are important factors that influence NDF contents of millet. When harvested at same maturity stage, BMR cultivars of pearl millet contained lower NDF than regular millet cultivars

(Cherney et al., 1988; Mustafa et al., 2004; Hassanat et al., 2007b). In general, advancement of maturity of forages typically increases the NDF contents as well as NDF compositions, namely acid detergent fiber (**ADF**) and acid detergent lignin (**ADL**) (Albrecht et al., 1987). However, lignin content is negatively correlated with the digestibility of forages (Van Soest, 1983). Both the NDF (+9.5%) and ADL (+20%) contents were increased when pearl millet was harvested at the boot stage compared with dough grain stage (Morales et al., 2011). However, when pearl millet was harvested at the vegetative than heading stage, NDF contents were either not affected (Hassanat et al., 2006) or increased by 12% (Hassanat et al., 2007b). Moreover, the method of conservation greatly affects the fibre contents of millet. For example, in contrast to hay, millet silage contained lower NDF, ADF and ADL concentrations when millet was harvested at any stage of development (Morales et al., 2011). In general, fibre contents of pearl millet silage are as follows: NDF (52.0 to 73.1%), ADF (29.9 to 48.3%), and ADL (0.6 to 6.6%) (Table 2).

Table 2.2: Chemical composition of pearl millet, corn, alfalfa and cool season grass mix forages and silages[1]

Chemical Composition, % DM	Pearl millet*		Corn	Alfalfa**		Cool season grasses**+	
	Forage	Silage	Silage	Forage	Silage	Forage	Silage
DM	11.9 – 32.4	18.2 – 38.5	19.5 – 44.2	16.9 – 47.7	27.2 – 55.8	14.9 – 25.0	36.2 – 44.3
Ash	6.1 – 12.2	6.6 – 14.9	2.9 – 7.2	8.3 – 12.2	7.0 – 14.8	9.4 – 13.0	8.0 – 10.6
CP	6.5 – 22.5	7.1 – 17.1	6.8 – 10.2	14.8 – 22.6	14.4 – 23.2	8.2 – 25.8	11.5 – 22.5
NDF	52.7 – 68.2	52.0 – 73.1	35.0 – 54.1	28.2 – 50.1	34.4 – 51.0	46.5 – 68.9	41.5 – 68.4
ADF	30.2 – 44.6	29.9 – 48.3	20.8 – 34.1	23.9 – 40.0	28.7 – 41.2	28.1 – 46.6	19.4 – 41.1
ADL	0.8 – 6.3	0.6 – 6.6	2.1 – 3.6	4.4 – 6.7	5.8 – 8.7	1.5 – 5.2	3.2 – 9.7
SCP, % of CP	22.4 – 68.2	52.8 – 79.2	48.8 – 67.9	-	51.4 – 71.3	-	-
NPN, % of CP	20.2 – 61.2	51.0 – 74.6	48.8 – 58.0	-	43.4 – 68.4	-	47.9 – 60.5
NDICP, % of CP	18.9 – 41.5	15.9 – 21.6	12.9 – 15.3	4.9 – 9.1	7.1 – 18.8	14.3 – 25.3	17.2 – 25.2
ADICP, % of CP	1.9 – 14.2	2.4 – 12.7	6.4 – 10.6	3.3 – 5.1	5.2 – 10.3	1.1 – 4.6	6.5 – 12.4
Starch	0.9 – 7.6	1.1 – 10.7	22.1 – 35.4	2.8 – 3.1	0.6 – 3.2	-	-
WSC	7.3 – 17.8	1.1 – 4.1	0.9 – 3.0	3.5 – 6.4	3.6	12.3 – 13.9	0.9 – 13.9
EE	1.4 – 2.3	1.6 – 3.4	2.5 – 3.2	-	2.1 – 4.8	-	2.4 – 3.0
NE_L, Mcal/kg	1.40 – 1.62	1.36 – 1.64	1.35 – 1.72	-	1.08 – 1.52	-	1.05 – 1.34

[1]Adapted from Jaster et al. (1985); Fisher and Burns (1987); Cherney et al. (1988); Weiss and Shockey (1991); Messman et al. (1992); Hoffman et al. (1997); McAllister et al. (1998); Elizalde et al. (1999); Hill et al. (1999); Mustafa et al. (2000); NRC (2001); Ward et al. (2001); Kochapakdee et al. (2002); Kung Jr et al. (2003); Mustafa et al. (2004); Bélanger et al. (2005); Ipharraguerre and Clark (2005); Hassanat et al. (2006); Filya et al. (2007); Hassanat et al. (2007a,b); Guo et al. (2008); Kendall et al. (2009); Amer and Mustafa (2010); Brassard and Desmeules (2010); Gencoglu et al. (2010); Amer et al. (2012); Coblentz and Muck (2012); Simili da Silva et al. (2014).

*chemical composition of forage and grain (harvested for forage) cultivars from USA and Canada harvested at different maturity stages **chemical composition of forages harvested at different maturity stages + Orachardgrass, bromegrass, timothy, tall fescue

2.5.5. Ensiling characteristics of pearl millet silage

2.5.5.1. Dry matter content

The DM content of pearl millet forage ranges between 11.9 and 32.4%. Forage DM is an important factor for proper ensiling. A minimal moisture level is required for lactic bacteria to thrive and ferment soluble sugars into by-products such as lactic acid which in turn reduces pH of the plant material and inhibit undesirable organisms such as mold, yeast and spoilage bacteria (Woolford, 1984). Pearl millet is ensiled at lower DM content than other conventional forages such as corn (30 to 35 % DM) and alfalfa (30 to 50 % DM) (Allen et al., 2003; Albrecht and Beauchemin, 2003). Nonetheless, well-preserved pearl millet silage can be achieved with a low DM content (23.4 to 26.83 %) (Messman et al., 1992; Ward et al., 2001; Hassanat et al., 2006).

2.5.5.2. Water soluble carbohydrates

The WSC contents of forage pearl millet ranges between 7.3 to 17.8% (Table 2), whereas most perennial grass forages contain approximately 10 to 20% WSC (Pitt and Leibensperger, 1987). High WSC in forages is desirable because WSC helps in proper fermentation and in the making of good quality silage. Several factors affect the WSC contents of forage pearl millet. For instance, higher WSC are obtained when pearl millet is seeded at higher rate (5kg/ha *vs.* 10 kg/ha) (Bouchard et al., 2011), delaying harvest date by 15 d at the vegetative stage (Leblanc et al., 2012), and harvested at heading (+30%) than vegetative stage (Hassanat et al., 2006). Millet cultivar also affects WSC contents. Whereas conventional pearl millet contained 22% more WSC than the BMR cultivar (Hassanat et al., 2007a). Amer et al. (2012) reported that a high WSC millet cultivar contained 26% more WSC than the conventional cultivar.

2.5.5.3. pH

In general, well preserved silages have a pH around 4.0 (McDonald et al., 1991), whereas putrid odors and abnormal organic acid profile are reported when silages stabilized at pH between 4.6 and 6.04 (Jaster et al., 1985; Hill et al., 1999). But, well preserved pearl millet silages were reported at pH ranging between 3.86 and 4.5 (Messman et al., 1992; Ward et al., 2001; Amer and Mustafa, 2010; Amer et al., 2012).

2.5.5.4. Lactic acid

Lactic acid is generally the main fermentation acid in most well-preserved silages. For well-preserved pearl millet silage, lactic acid concentration ranges between 3.33 and 8.5% (Messman et al., 1992; Ward et al., 2001; Amer and Mustafa, 2010; Amer et al., 2012). Higher lactic acid concentrations are reported in tropical or temperate corn and sorghum silages than pearl millet silage (Ward et al., 2001; Kochapakdee et al., 2002). However, Amer and Mustafa (2010) found that a high WSC pearl millet silage contained 7% higher lactic acid concentration than corn silage.

2.5.5.5. Aerobic stability

Aerobic stability is defined as the time required for the silage temperature to rise by 2˚C above ambient temperature, and therefore the duration (time) that the silage may remain stable (Seppälä et al., 2013). A wide range of aerobic stability (18 to 156 h) were reported in the literature for pearl millet silage (Hassanat et al., 2006, 2007a; Amer and Mustafa, 2010). Harvesting pearl millet at the vegetative than heading stage reduced aerobic stability by 95%. Similarly, when treated with an inoculant, millet silage had lower (-12%) aerobic stability than the untreated pearl millet forage (Hassanat et al., 2007a). Moreover, the aerobic stability of corn silage was 71% greater than pearl millet silage (Amer and Mustafa, 2010).

2.5.6. *In vitro*, *in situ* and total tract degradability of pearl millet forage and silage

2.5.6.1. *In vitro* dry matter and neutral detergent fiber degradability

In vitro dry matter degradability **(IVDMD)** ranges between 57.1 to 77.2% for fresh pearl millet forage (Cherney et al., 1988; Han et al., 2006; Hassanat et al., 2006, 2007a), and between 49.3 to 79.0% for pearl millet silage (Hassanat et al., 2006, 2007a; Amer and Mustafa, 2010; Amer et al., 2012). Cultivars and stage of maturity at harvest are the principal factors affecting IVDMD of forage millet. For example, harvesting millet at the vegetative (56d) than heading (84d) stage increased IVDMD by 33% (Hassanat et al., 2006). When compared to a conventional pearl millet cultivar, a BMR cultivar had higher IVDMD (Cherney et al., 1988; Hassanat et al., 2006, 2007a) due to lower lignin concentrations in the stems and leaves of the BMR forage millet (Cherney et al., 1991; Mustafa et al., 2004; Hassanat et al., 2007a). Moreover, IVDMD was higher in a high WSC pearl millet silage than in conventional pearl millet silage (Amer et al., 2012). However, pearl millet silage had lower IVDMD than corn (Amer and Mustafa, 2010) and sorghum (Amer et al., 2012) silages.

Ensiling is another factor that increases IVDMD of pearl millet (Hassanat et al., 2007a). Data regarding the *in vitro* NDF degradability **(IVNDFD)** of fresh pearl millet forage is limited. The IVNDFD ranges between 56.6 and 68.2% for pearl millet forage (Han et al., 2006) and between 39.0 and 66.1% for millet silage (Hassanat et al., 2006, Amer and Mustafa, 2010). BMR pearl millet had greater IVNDFD than regular pearl millet (Hassanat et al., 2006). Likewise, harvesting conventional (+7.8%) and BMR (+9.2%) millet at the vegetative than heading stage significantly increased IVNDFD. Both, the ensiling of pearl millet and increased maturity stage reduced IVNDFD of pearl millet silage (Hassanat et al., 2006). But, IVNDFD of pearl millet silage was 65% greater than corn silage (Amer and Mustafa, 2010).

2.5.6.2. *In situ* dry matter and neutral detergent fiber degradability

In situ DM degradability **(*in Situ* DMD)** of fresh pearl millet ranges between 45.2 and 57.5% (Mustafa et al., 2004; Hassanat et al., 2007b). But, *in situ* DMD of fresh alfalfa, bromegrass and tall fescue were 66.3, 52.3 and 56.0, respectively (Elizalde et al., 1999). Fresh BMR pearl millet had higher *in situ* DMD compared to fresh regular pearl millet (Mustafa et al., 2004; Hassanat et al., 2007b). However, for both fresh BMR and fresh conventional pearl millet, leaves and stems had greater i*n situ* DMD when harvested at the vegetative (seven fully developed leaves) than at the heading (50% of plants at heading stage) stage (Hassanat et al., 2007b). There are limited data for *in situ* DMD of pearl millet silage. Amer et al. (2010) reported *in situ* DMD (48h) of 62.3% whereas Kochapakdee et al. (2002) found that *in situ* DMD (72h) of millet was 80.9%. Pearl millet had similar *in situ* DMD (72h) than temperate corn, tropical corn and white lupin (Kochapakdee et al., 2002). However, *in situ* DMD (48h) was 11.7% lower for pearl millet compared to corn silage (Amer and Mustafa, 2010).

In situ NDF degradability **(*in situ* NDFD)** of pearl millet forage ranges between 27.0 and 76.5%, and is mainly affected by stage of maturity, cultivar and leaf:stem ratio (Mustafa et al., 2004, Hassanat et al., 2007b). In contrast to corn silage, *in situ* NDFD (48h) was 54% greater (Amer and Mustafa, 2010) and *in Situ* NDFD (72h) was 20% greater for pearl millet silage (Kochapakdee et al., 2002).

2.5.6.3. Total tract nutrient degradability

Total tract degradability of DM (**DMD**) of forage millet ranges between 51.6 to 67.1% whereas total tract degradability of NDF (**NDFD**) ranges between 44.5 to 72.1% (Cherney et al., 1991; Henry et al., 2013). The main factors affecting DMD and NDFD

include forage cultivar, stage of maturity and differences in animal species. When fed to wethers, DMD and NDFD were higher for fresh BMR pearl millet than a conventional cultivar (Cherney et al., 1991). In a study with Angus and Angus crossbred heifers, it was found that fresh pearl millet had 16.5% lower DMD than fresh sorghum whereas NDFD was 21% lower than sorghum (Henry et al., 2013). When fed to Holstein heifers, pearl millet silages had greater DMD and NDFD than oat silage, barley silage and pea silage (Jaster et al., 1985). Moreover, results indicate that both DMD (+6.5%) and NDFD (+11.4%) were higher with pearl millet silage than sorghum silage. In another study Holstein heifers, Ward et al. (2001) reported higher DMD for sorghum and tropical corn silages than pearl millet silage, but NDFD was not different between the 3 silages. When lactating dairy cows were fed a mixture of corn and alfalfa silages (control diet) compared to a mixture of pearl millet and alfalfa diets, DMD was similar between both diets but NDFD was higher for the pearl millet silage diet (Messman et al., 1992). When feeding lactating cows pearl millet silage or temperate corn silage as sole forage, DMD (72.1% *vs.* 62.1%) and NDFD (68.7% *vs.* 51.1%) were found to be higher with pearl millet silage (Kochapakdee et al., 2002).

2.5.7. Performance of ruminants fed pearl millet silage

2.5.7.1. Dry matter intakes

In high forage diets, dry matter intake (**DMI**) is greatly affected by physical characteristics such as rumen fill (Allen, 2000). Although DMI may be limited by excessive metabolic fuel it is more often limited by NDF content (Mertens, 1997; Allen, 2000). However, increased NDF digestibility of forages or silages allows greater NDF intake (**NDFI**) and therefore increased DMI (Oba and Allen, 1999). In a study with sheep, DMI was similar between the first cut of conventional and BMR pearl millet (Cherney et al., 1991). However, DMI was reduced by 4.8% for the conventional cultivar and by 2.9% for the BMR cultivar when the second was fed. Beef cattle consumed more corn silage than pearl millet silage (Hill et al., 1999). In contrast, Ward et al. (2001) reported that DMI was higher for pearl millet silage than sorghum or tropical corn silages by 19.7% and 21.1%, respectively. Moreover, DMI was greater for beef cattle fed pearl millet compared to cool season forage silages (Jaster et al., 1985), but similar between beef cattle fed millet and sorghum silages (Henry et al., 2013). Feeding diets with pearl millet silage relative to diets with corn silage to lactating dairy cows reduced DMI by 32% (Kochapakdee et al., 2002) and by 22% (Messman

et al., 1992). But, Amer and Mustafa (2010) found no difference in DMI between cows fed pearl millet and corn silage diets.

Pearl millet had higher NDFI than cool season forages, tropical corn and sorghum (Jaster et al., 1985; Ward et al., 2001). However, Henry et al. (2013) found no difference in NDFI between pearl millet and sorghum silage.

2.5.7.2. Milk yield and composition

Milk yield (average = 23.9 kg/d) was similar for cows fed pearl millet and alfalfa silages diets when compared with corn and alfalfa silage diets (Messman et al., 1992). In contrast, cows fed millet silage produced 17% less milk than those fed corn silage as the sole forage (Kochapakdee et al., 2002). Fat corrected milk was greater (Amer and Mustafa, 2010), similar (Messman et al., 1992) and lower (Kochapakdee et al., 2002) when corn silage was substituted with pearl millet silage in cow's diet. Milk fat concentration was similar between cows fed millet and corn silage diets (Messman et al., 1992) and millet and corn silages (Kochapakdee et al., 2002). However, Amer and Mustafa (2010) reported higher milk fat concentration for cows fed millet silage diet relative to cows fed corn silage diet. Milk protein concentration was reduced as a result of feeding millet silage diets (Messman et al., 1992) and millet silage (Kochapakdee et al., 2002). But, milk urea nitrogen (MUN) was not affected when cows were fed pearl millet silage than corn silage (Kochapakdee et al., 2002) or pearl millet silage diet than corn silage diet (Amer and Mustafa, 2010).

2.5.7.3. Ruminal fermentation

Data regarding the ruminal fermentation parameters of ruminants fed pearl millet are limited. Ruminal pH was similar between cows fed millet silage than corn silage (Kochapakdee et al., 2002). Higher ruminal molar proportion of acetate but lower ruminal molar proportion of propionate has been reported when cows were fed pearl millet silage compared to cows fed corn silage (Messman et al., 1992; Kochapakdee et al., 2002). Similar findings were observed between heifers fed pearl millet than sorghum forages (Jaster et al., 1985).

2.5.8. Canadian forage pearl millet cultivars

2.5.8.1. Agronomic characteristics

In Canada and northern USA, pearl millet is grown between late May to September. Millet is seeded from late May to mid July because hybrids require soil temperature above 12 °C with no risk of frost to germinate and survive (Newman, 2010). Two new hybrids of forage pearl millet are available: the Canadian Forage pearl millet (regular millet) and the Canadian sweet pearl millet (sweet millet). The former was developed for grazing, as green chop and silage, whereas sweet millet was developed for ethanol and silage production. When used in the rotation of potato, tobacco and vegetable crops, forage millet decreased root lesion nematode and increased soil organic matter (Ball-Coelho et al., 2003; Bélair et al., 2005). Regular millet is used in a multi cut system, however the second harvest yields less (-54.5%) biomass compared with the first cut (Mustafa et al., 2004).

Similar to other pearl millet cultivars, the Canadian pearl millet is known for its capacity to grow in semi arid regions and produce high yields in brief periods of cultivation. Based on these characteristics, pearl millet may be suitable production in areas of Quebec where corn silage is not successful. In Canada, average yields for pearl millet ranges between 1.95 and 9.6 t DM/ha for regular millet and between 9.5 and 12.0 t DM/ha for sweet millet (Table 3). In the Alma region of Quebec (CHU = 1940 units), yields of forage pearl millet was 2.4 t DM/ha only because of cool weather conditions (Brassard and Desmeules, 2010).

Table 2.3: Average yields (t DM/ha) of Canadian forage pearl millet grown at different locations in Canada[1]

	Average yields (t DM/ha)	
	Regular millet	Sweet millet
Quebec	9.6	12.0
Southern Ontario	9.0	9.5
New Brunswick	6.4	-
Saskatchewan	1.95	-

[1]Adapted from Hassanat et al. (2006); AERC (2007, 2009); Bouchard et al. (2011); Amer et al. (2012); Leblanc et al. (2012)

CHAPTER III. REPLACING CORN SILAGE WITH DIFFERENT MILLET SILAGE CULTIVARS: EFFECTS ON MILK YIELD, NUTRIENT DIGESTION, AND RUMINAL FERMENTATION OF LACTATING DAIRY COWS[1]

T. Brunette,* B. Baurhoo,† and A. F. Mustafa*

*Department of Animal Science, McGill University, Sainte-Anne-de-Bellevue, Quebec, H9X 3V9, Canada

†Bélisle Solution Nutrition Inc., St-Mathias-sur-Richelieu, Quebec, J3L 6A7, Canada

[1] Published in *Journal of Dairy Science*: 2014. 97:6440-6449.

3.1. ABSTRACT

This study investigated the effects of dietary replacement of corn silage with 2 cultivars of forage millet silages [i.e., regular millet (**RM**) and sweet millet (**SM**)] on milk production, apparent total-tract digestibility, and ruminal fermentation characteristics of dairy cows. Fifteen lactating Holstein cows were used in a replicated 3 x 3 Latin square experiment and fed (ad libitum) a high forage TMR (68:32 forage:concentrate ratio). Dietary treatments included CS (control), RM, and SM diets. Experimental silages constituted 37% of each diet DM. Three ruminally fistulated cows were used to determine the effect of dietary treatments on ruminal fermentation and total-tract nutrient utilization. Relative to CS, RM and SM silages contained 36% more crude protein, 66% more neutral detergent fiber (**NDF**), and 88% more acid detergent fiber. Cows fed CS consumed more dry matter (**DM**; 24.4 *vs.* 22.7 kg/d) and starch (5.7 *vs.* 3.7 kg/d), but less NDF (7.9 *vs.* 8.7 kg/d) than cows fed RM or SM. However, DM, starch and NDF intakes were not different between forage millet silage types. Feeding RM relative to CS reduced milk yield (32.7 *vs.* 35.2 kg/d), energy-corrected milk (**ECM**; 35.8 *vs.* 38.0 kg/d) and SCM (32.7 *vs.* 35.3 kg/d). However, cows fed SM had similar milk, energy-corrected milk, and solids-corrected milk yields than cows fed CS or RM. Milk efficiency was not affected by dietary treatments. Milk protein concentration was greatest for cows fed CS, intermediate for cows fed SM, and lowest for cows fed RM. Milk concentration of solids-not-fat was lesser, whereas milk urea nitrogen was greater for cows fed RM than for those fed CS. However, millet silage type had no effect on milk solids-not-fat and milk urea nitrogen levels. Concentrations of milk fat, lactose and total solids were not affected by silage type. Ruminal pH and ruminal NH_3-N were greater for cows fed RM and SM than for cows fed CS. Total tract digestibility of DM (average = 67.9%), NDF (average = 53.9%), crude protein (average = 63.3%) and gross energy (average = 67.9%) were not influenced by dietary treatments. It was concluded that cows fed CS performed better than those fed RM or SM likely due to the higher starch and lower NDF intakes. However, no major differences were noted between the 2 forage millet silage cultivars.

Key words: corn silage, forage millet silage, dairy cow, milk yield

3.2. INTRODUCTION

Corn silage (**CS**) is a preferential and abundantly used forage in dairy cow nutrition, principally due to its high DM yield, single-cut harvest at optimum DM contents, high NE_L concentration, capacity to sustain high milk yields, and good ensiling characteristics. However, in many temperate regions of Canada, the production of CS is risky and low-yielding, despite the use of short-season cultivars. Indeed, the growing season with warm temperatures (above 15°C) is only between 70 and 90 d. Moreover, the high N fertilizer application rate that CS necessitates makes it uneconomical for cold regions. Alfalfa and perennial grasses are the most commonly used forages on such dairy farms. But, dairy producers are often challenged with on-farm forage shortages, especially during conditions of alfalfa winter kill. Therefore, we hypothesize that forage pearl millet may be used as an emergency forage or routinely as a new forage option by dairy producers located in temperate regions.

Pearl millet [*Pennisetum glaucum* (L.) R.] is an annual semi-arid tropical grass with high biomass yield and low N fertilizer requirement, and is drought resistant and adaptable to low soil pH (Maiti and Wesche-Ebeling, 1997). Because of its adaptability to harsh conditions, millet can be grown in areas unfavourable to other cereals, such as corn (Hanna, 1995). Data regarding the feeding value of pearl millet silage to lactating cows are limited. Moreover, from the few published studies that have investigated the nutritive values of pearl millet, findings are highly inconsistent. For example, pearl millet (harvested after 66 d of growth; 23% DM) silage fed to lactating cows in place of alfalfa silage plus CS had no effect on milk yield or milk fat concentration, but reduced DMI and milk protein levels (Messman et al., 1992). Kochapakdee et al. (2002) have shown reduced milk production and milk protein levels when cows were fed diets containing pearl millet silage (30% DM) compared with temperate CS. In contrast, feeding pearl millet (harvested at 80 d of growth; 27% DM) silage relative to CS had similar effects on feed intake, milk yield, and milk efficiency (Amer and Mustafa, 2010).

Pearl millet is mostly grown to grain in many African and Asian countries. However, unlike grain millet cultivars, forage pearl millet offer flexible harvest dates. Indeed, forage pearl millet may be harvested from vegetative (i.e., 24% DM) to more mature (32% DM) stages, thus making it extremely suitable for the cold regions. In this study, we were also interested in testing pearl millet cultivars containing high levels of water-soluble carbohydrates (**WSC**). High WSC is reported to improve ensilability of forages by accelerating lactic acid production (Adesogan et al., 2004). Therefore, the objectives of this

study were to determine the effects of replacing CS with 2 different forage millet silage cultivars [i.e., regular millet (**RM**) and sweet millet (**SM**)] on milk yield, milk composition, apparent total-tract nutrient digestibility, and ruminal fermentation characteristics of lactating dairy cows.

3.3. MATERIALS AND METHODS

This study was conducted at the MacDonald Campus Farm of McGill University (Ste Anne de Bellevue, QC, Canada; 45°N, 73°W). All animal procedures were conducted under approval of the Animal Care Committee of the Faculty of Agriculture and Environmental Sciences of McGill University.

3.3.1. Silage preparation

Two forage pearl millet hybrids, namely RM and SM, were seeded on June 1, 2012 and harvested on July 24, 2012 at the vegetative stage and approximately 1.65 m high. Millet seeds were provided by Belisle Solution Nutrition Inc. (Saint-Mathias sur Richelieu, QC, Canada). Prior to millet seeding, 100 kg of urea N/ha (46% N) was evenly applied to each field. Millet (70% moisture) was chopped into at least 12-mm particle size using a New Holland forage harvester (model 900; New Holland, PA) and ensiled under high pressure into 45-m-long horizontal Ag-Bag silos (2.1-m diameter and approximately 50 t each; AgBag, Miller-St. Nazianz Inc., St. Nazianz, WI) for approximately 2 mo. The initial WSC content of fresh RM and SM were 74 ± 7.46 and 80 ± 0.77, g/kg, respectively. The compositions of experimental silages are given in Table .

3.3.2. Animals, experimental design, and diets

Fifteen multiparous Holstein cows in early to mid lactation [milk yield: 39.9 ± 5.60 kg; DIM: 75.2 ± 54.51 d; BW: 660.2 ± 77.41 kg (average ± SD)] were used in a replicated 3 x 3 Latin square experiment with 21-d periods (14 d of diet adaptation and 7 d of data collection). Cows were housed in individual tie-stalls and had free access to water. Five cows were allotted to each treatment and blocked into 5 groups of 3 by parity, milk yield and DIM. Three high forage iso-nitrogenous diets (68:32 forage:concentrate ratio) were formulated to meet nutrient requirements of lactating dairy cows in early lactation (NRC, 2001; Table 6). Experimental treatments were the replacement of CS with RM or SM silages. In all diets, the silage portion consisted of 70% CS, RM, or SM, and the remaining 30% consisted of alfalfa silage. For the objectives of this study, the proportions of experimental silages were kept

constant in all dietary treatments (Table 6). Diets were offered as a TMR once daily in the morning (0800 h) for ad libitum intake. Orts were measured daily to determine daily feed intake per cow. Total mixed rations and silages were sampled daily during the data-collection periods (d 15-21 of each period) and composited by period.

3.3.3. *In situ* ruminal nutrient degradabilities of experimental silages

One representative sample of CS, RM, and SM silages was obtained by mixing 200 g of the dried (65˚C for 48 h) silages from each of the 3 periods. A 5-g subsample of each mixture was then placed into nylon bags (20 × 10 cm, 50-μL pore size; Ankom Technology Corp., Macedon, NY) and incubated in the rumen of 3 lactating cows (1 bag per treatment per time period per cow) fed a single type of ration and fitted with rumen cannulas for 0, 3, 6, 12, 24, 48, 72, and 96 h. At the end of each incubation time, bags were removed from the rumens and manually washed under cold tap water until the water was clear. The 0-h incubation was determined by washing the bags containing the samples. The washed bags were then dried in a forced-air oven at 65˚C for 48h. In situ residues were analyzed for DM and NDF (Van Soest et al., 1991). Data of ruminal DM and NDF disappearances were used to determine nutrient kinetic parameters by using the equation of Dhanoa (1988):

$$p = a + b\ (1 - e^{-c\ (t-Lt)})$$

where p represents the nutrient disappearance at time t, a is the soluble fraction (%), b is the potentially degradable fraction (%), c is the rate of degradation of the b fraction (%/h), and Lt is the lag phase (h). The parameters were estimated by PROC NLIN of SAS (SAS Institute, 2008) using iterative least squares regression (Gauss-Newton method). Effective degradabilities (**ED**) of DM and NDF were calculated according to the equation of Ørskov and McDonald (1979):

$$ED = a + bc/(c + k)$$

where k represents the ruminal outflow rate (6.25%/h), and a, b, and c are as described previously.

3.3.4. Milk production and analysis

Cows were milked twice daily at 0500 and 1700 h. Milk yields were recorded at each milking by cow. Milk samples were collected twice daily on 2 consecutive days of each data-collection period, composited by cow according to volume, and analyzed for fat, protein, lactose, and MUN using an infrared analyzer (Valacta, Sainte-Anne-de-Bellevue, Canada) according to Association of Official Analytical Chemists (**AOAC**, 1990). Milk TS content was determined according to AOAC (1990).

3.3.5. Ruminal fermentation and apparent total-tract nutrient digestibility

Three multiparous lactating Holstein cows [milk yield: 37.8 ± 7.5 kg; DIM: 52.0 ± 25.51 d; BW: 671.5 ± 37.75 kg (average ± SD)] fitted with rumen cannulas were used in a 3x3 Latin square experiment to determine the effects of dietary treatments on ruminal fermentation and total-tract nutrient digestibility. Cows were kept in tie-stalls with free access to water. The cows were fed the same experimental diets and followed the same experimental protocol as in the production study.

Rumen fluid samples were collected from different areas in the rumen with a syringe screwed to a stainless steel tube ending by a fine metal mesh (RT Rumen Fluid Collection Tube, Bar Diamond Inc., Parma, ID). The collection began before the morning feeding (0h) and 2, 4, 6, 8, 10, and 12 h postfeeding on d 16 and 17 of each period. Ruminal pH was determined immediately using an Accumet pH meter (Fisher Scientific, Montreal, Canada). Thereafter, two 50-mL samples were immediately preserved by adding 5-mL of 25% metaphosphoric acid and 5-mL of 0.1 N HCL for measurements of VFA and NH_3-N, respectively. Samples were kept at -20˚C for later analysis.

Chromic oxide was used as an inert external marker to determine total fecal output. Gelatin capsules containing 10 g of Cr_2O_3 were inserted into the rumen of each cow twice daily in equal intervals starting on d 10 of the adaptation period. Grabbed fecal samples were collected 4 times daily on d 15, 17 and d 21 of each period. Samples were then dried at 60°C in a forced-air oven for 72 h and pooled by cow within each period.

3.3.6. Chemical analysis

Thawed samples of fresh and ensiled forages were homogenized with 500 mL of distilled water and the pH of the extract was immediately determined using an Accumet pH meter (Fisher Scientific). Extracts were centrifuged at 12,000 × g for 15 min at 4˚C and analyzed for organic acids (lactic, acetic, propionic, and butyric acid) by using HPLC

(Andersson and Hedlund, 1983). The conditions for the HPLC analysis were 0.013 M H_2SO_4 as mobile phase and a flow rate of 0.6 mL/min. Silage concentrations of WSC were determined colorimetrically within aliquots of filtered extracts using the phenolic-sulphuric acid reaction (Dubois et al., 1956).

Subsamples of silages and TMR were dried in a forced-air oven at 65˚C for 72h, subsequently ground through a 1-mm screen using a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA), and then analyzed for DM, ash, and ether extract following standard procedures (AOAC, 1990). Crude protein (N x 6.25) was analyzed using a Leco Nitrogen Analyzer (Truspec Nitrogen Determinator System; Leco Corp., St. Joseph, MI). Nonprotein N and soluble CP were determined for silages samples according to Licitra et al. (1996). Neutral detergent fibre (Van Soest et al., 1991) and ADF (AOAC, 1990) were determined using an Ankom Fiber Analyzer (Ankom Technology Corp., Macedon, NY). Analysis of NDF was performed using heat stable α-amylase and without the use of sodium sulfite. Acid detergent lignin of TMR and silage samples was determined according to AOAC (1990). Neutral and acid detergent-insoluble protein concentrations were estimated by analyzing NDF and ADF residues, respectively, for total N (Licitra et al., 1996). Starch was analyzed colorimetrically according to McCleary et al. (1997). Gross energy (**GE**) of feed samples was determined using an oxygen bomb calorimeter (model 6200; Parr Instrument Co., Moline, IL).

Samples of experimental silages for the three periods were agitated to ensure air exposure and individually packaged loosely into 500-mL plastic containers. Thermal insulator was wrapped around the sides of each container to prevent heat dissipation and 4 holes were made on top and bottom of each container to permit air exchange. Thermocouple probes were inserted in the core of each plastic container to detect temperature difference from the environment. Aerobic stability was defined as the time required to increase the temperature by 2˚C (Kung et al., 2000). Temperature, measured using a Hotmux data logger (DDC Corp., Pennsauken, NJ), was recorded every 5 min.

Dried fecal samples were analyzed for DM, NDF, and GE as previously described. Chromic oxide content was determined according to the procedure Fenton and Fenton (1979). Ruminal fluid samples were centrifuged at 12,000 × g for 15 min at 4˚C and analyzed for acetic, propionic, and butyric acids using HPLC, as described previously. Ruminal NH_3-N was determined colorimetrically with a multichannel Lachat Autoanalyzer (Lachat Instruments, Milwaukee, WI).

3.3.7. Statistical analysis

Data of the performance study and total tract nutrient utilization were analyzed as a replicated 3 x 3 Latin square design using PROC MIXED of SAS (SAS Institute, 2008) and the following model:

$$Y_{ijkh} = \mu + trt_i + block_j + animal_{jk} + per_h + e_{ijkh}$$

where Y_{ijkh} represents the observation, μ is the population mean, trt_i is the fixed effect of the i^{th} treatment (i = 1,2 or 3), $block_j$ is the fixed effect of the j^{th} block (j = 1, 2, 3, 4 or 5), $animal_{jk}$ is the random effect of the k^{th} animal (k = 1, 2 or 3) in the j^{th} block, per_h is the fixed effect of the h^{th} period (h = 1,2 or 3), and e_{ijkh} is the random error. In situ ruminal degradability data for experimental silages were analyzed using an ANOVA and a completely randomized design with treatment as main effect and cows as replicates.

Ruminal fermentation parameters data were analysed as repeated measures in time using PROC MIXED (SAS Institute, 2008) and the following model:

$$Y_{ijkh} = \mu + trt_i + animal_{ij} + per_k + time_h + trt_i * time_h + e_{ijkh}$$

where Y_{ijkh} represents the observation, μ is the population mean, trt_i is the fixed effect of the i^{th} treatment (i = 1, 2 or 3), $animal_{ij}$ is the random effect of the j^{th} cow (j = 1, 2 or 3) on the i^{th} treatment, per_k is the fixed effect of the k^{th} period (k = 1, 2 or 3), $time_h$ is the fixed effect of the h^{th} time (h = 1, 2, 3, 4, 5, 6 or 7), $trt_i \times time_h$ is the interaction effect between treatment and time, and e_{ijkh} is the residual error [$e_{ijkh} \sim N(0, \sigma^2_{cow})$]. Significant differences were declared at $P < 0.05$.

3.4. RESULTS

3.4.1. Chemical composition of millet silages and experimental diets

Relative to CS, forage millet cultivars contained 66% more NDF, 88% more ADF, and 36% more CP (Table 3.1). Starch was higher (approximately 85 times) in CS than RM and SM silages likely due to the grain content (30%) of CS. Corn silage also contained higher (+0.42 Mcal/kg) NE_L than millet silages. However, ether extract was similar among all silage types. None of these abovementioned analyzed parameters were different between the 2 forage millet silages. However, SM contained 1.8 times higher WSC and was aerobically

more stable than RM. In addition, both WSC concentrations and aerobic stability were comparable between CS and SM. Lactic and acetic acids were the main fermentation acids in all silage types, whereas butyric acid was generally undetectable (data not shown). However, a tendency existed for acetic acid concentration to be the highest in SM, whereas CS tended to contain high lactic acid levels. Both millet silages stabilized at a pH higher than CS (Table 3.1). However, no heat damage or putrid odors were noticed from millet silages.

3.4.2. In situ ruminal nutrient degradability of experimental silages

In contrast to forage millet silages, CS had greater ($P < 0.05$) in situ effective DM degradability but lower ($P < 0.05$) effective NDF degradability (Table 6). In situ ruminal degradability of soluble DM fraction was greater ($P < 0.05$), whereas the in situ slowly degradable DM fraction was lower ($P < 0.05$) for CS than for forage millet silages. However, CS, RM and SM had similar ruminal degradable rates for DM, soluble NDF fraction, and slowly degradable NDF fraction. Moreover, effective degradability as well as degradability of both soluble and slowly degradable fractions for DM and NDF were not influenced by forage millet cultivars.

3.4.3. Animal performance

Dry matter, NDF, starch and NE_L intakes were not affected by the 2 millet diets (Table 3.4). However, cows fed RM or SM consumed more ($P < 0.05$) NDF, but significantly less ($P < 0.05$) DM, starch and NE_L than CS-fed cows. However, CP intakes were similar across dietary treatments.

Milk, lactose, TS, SNF, ECM, and SCM yields were similar between cows fed CS and SM, but lower ($P < 0.05$) among cows fed RM than CS (Table 3.6). Milk protein yields were highest ($P < 0.05$) for cows fed CS, intermediate ($P < 0.05$) for cows fed SM, and lowest ($P < 0.05$) for cows fed RM. However, milk efficiency, and yields of fat and 4% FCM, were not affected by dietary treatments. With the exception of milk protein yields, none of the milk yields were different between RM and SM.

Protein concentration was highest in the milk of cows fed CS, intermediate for cows fed SM and lowest for cows fed RM ($P < 0.05$, Table 3.6). However, milk fat, lactose and TS levels were similar across experimental diets. Cows fed CS produced milk with greater ($P < 0.05$) SNF than RM; however, similar milk SNF levels were recorded between CS- and SM-fed cows. Cows fed CS produced milk with lower ($P < 0.05$) MUN levels than for those fed RM and SM.

3.4.4. Ruminal fermentation and nutrient digestibility

No treatments × time interactions were significant for any of the ruminal fermentation parameters (Table 3.5). Therefore, only main treatment effects were reported. Ruminal pH and NH_3-N concentrations were greater ($P < 0.05$) for cows fed RM or SM relative to cows fed CS. Total VFA levels were lower ($P < 0.05$) for SM-fed cows than for CS-fed cows. However, total VFA was not different between cows fed CS and RM or SM and RM. Feeding forage millet diets relative to CS increased ($P < 0.05$) molar proportions of acetate, whereas molar proportion of propionate was greater ($P < 0.05$) for cows fed CS than for the cows fed RM. Consequently, the acetate:propionate ratio was increased ($P < 0.05$) as a result of feeding forage millet diets relative to CS. Ruminal butyrate levels were not affected by dietary treatments. Apparent total-tract digestibility of DM, CP, NDF, GE and starch were not influenced by silage type, and averaged 67.93, 63.30, and 53.87, 67.92 and 90.37%, respectively (Table 3.5).

3.5. DISSCUSSION

This study investigated the effects of replacing CS with a regular or high-WSC forage millet silage in the diets of lactating dairy cows on milk production, rumen fermentation characteristics and total-tract nutrient digestibility. Corn silage contained approximately 30% grains (Chase, 2012). In contrast, unlike grain millet cultivars, the 2 millet cultivars were harvested at the vegetative stage and before seed setting. If experimental diets were to be balanced for starch, the RM and SM diets would have necessitated additional grains (i.e., high moisture corn). However, to better reflect dairy production in the more temperate regions whereby CS and corn grains are extremely limited, we deliberately formulated diets with replacement of equal proportions of CS with RM or SM. The CS diet contained 85 times more starch than the RM or SM diet (Table 3.2). Calcium salts of palm oil (Megalac; Church & Dwight Co. Inc., Princeton, NJ) were added to the RM and SM diets to balance for NE_L across dietary treatments.

In this study, forage millet silages contained 66% more NDF, 88% more ADF, and 36% more CP compared with CS (Table 3.1). However, the nutritive values of both forage millet cultivars were equivalent. The greater residual WSC concentration of SM relative to RM is likely due to the lower utilization of WSC by lactic acid bacteria. The greater concentration of acetic acid may explain the higher aerobic stability of CS and SM relative to RM. Increases in acetic acid concentration in silages treated with Lactobacillus buchneri

improved aerobic stability in barley silages (Kung et al., 2000). Values of NDF and CP of millet silages were in agreement with Messman et al. (1992) and Ward et al. (2001). In contrast to our previous study with high WSC millet (Amer and Mustafa, 2010), SM had comparable CP and ADF levels but lower (11%) NDF level. The lower NDF contents of SM may be attributed to differences in maturity stages (vegetative vs heading) at which millet was harvested and ensiled. Advancement in maturity of grass or gramineae forages is usually associated with reduced CP, and increased NDF and ADF contents (Rinne et al., 1997; Cone et al., 1999; Holtshausen et al., 2012). But, this was not evidenced when comparing findings of our study with those of Amer and Mustafa (2010).

Forage millet had a higher effective in situ degradability of NDF than CS (Table 3.3). Our findings were somewhat expected, given the fact that advanced maturity of forages is negatively correlated with fiber (NDF and ADF) digestibility (Rinne et al., 2002; Holtshausen et al., 2012). Whereas CS is normally harvested at mature stages, forage millet was harvested earlier at the vegetative stage. The higher CP contents and greater quantities of more effectively degradable fiber of forage millet make it an interesting silage in dairy cow diets. In contrast, the higher DM degradability of CS than RM and SM as observed in this study were mainly due to its higher WSC contents, and in particular starch. Starch of corn grains is a rapidly fermentable carbohydrate in the cow rumen. In agreements with our findings, Amer and Mustafa (2010) reported higher in vitro DM but lower in vitro NDF disappearance for CS than forage millet silage. However, RM and SM had similar nutritive values and in situ degradability of DM and NDF, given that these were harvested at same maturity (vegetative) stage.

Total tract digestibility of DM, CP and NDF were not affected by dietary treatments (Table 3.5). However, in the production study (Table 3.4), dairy cows fed forage millet diets consumed greater NDF than those fed CS likely because RM and SM were ruminally more degradable than CS or due to the higher NDF contents of millet diets. Similar findings have previously been reported. For instance, Amer and Mustafa (2010) reported that dairy cows fed millet silage consumed more NDF (1.35% vs 1.18% NDF of BW) than when fed a CS diet. Ward et al. (2001) observed that heifers fed pearl millet silage consumed more NDF than those fed sorghum or tropical CS. On the other hand, cows fed CS consumed higher DM as a result of its higher starch intake (Table 3.4). However, DMI was not influenced forage millet silages. Previous studies indicated that cows fed pearl millet consumed greater (Ward et al., 2001), similar (Amer and Mustafa, 2010), and less (Messman et al., 1992; Kochapakdee et al., 2002) DM than cows fed CS. Inconsistent findings between studies may

be related to factors such as the forage:concentrate ratio, maturity stage at which millet was harvested, and diet compositions.

Our findings indicate that milk yield was greater among cows fed CS than RM, but not compromised when cows were fed the SM diet (Table 3.6). The lower milk production recorded among RM-fed cows relative to CS-fed cows is most likely due to their lower DM (starch) and NE_L intakes. Kochapakdee et al. (2002) also reported 12% reduction in milk yield as a result of feeding cows with pearl millet silage compared with CS. However, despite the lower DM and NE_L intakes among cows fed SM compared with CS, their similarity in milk yield is difficult to explain at this time. In agreement with our findings, unaffected milk yields between cows fed high-WSC pearl millet silage and CS have previously been reported (Amer and Mustafa, 2010).

Higher milk yield and milk protein levels, but lower milk fat concentrations are typically observed among dairy cows fed high-starch diets (Table 3.6). The lower carbohydrate intake (i.e. lower supply of gluconeogenic precursors such as propionate), and lower intakes of MP (soybean meal) may explain the reduction in milk protein concentration due to feeding with forage millet silages (Broderick, 2003; Jenkins and McGuire, 2006). Our explanation about lower MP intake or amino acid supply is consistent with the increases in ruminal NH3-N concentrations (Table 3.5). Reductions in milk protein levels among cows fed pearl millet silage compared with CS were also observed by Messman et al. (1992) and Kochapakdee et al. (2002).

In the present study, we observed a reduction in ruminal NH_3-N for cows fed CS relative to those fed millet silages. Our findings are in agreement with Brito and Broderick (2006) and Hassanat et al. (2013), who also reported a decrease in ruminal NH_3-N concentration with increasing proportions of CS in the diets, and associated this effect with reduced urinary losses. In fact, when NH_3-N level in the rumen exceeds microbial uptake, excess NH_3-N is absorbed through the rumen, transferred to the liver, metabolized into urea, and excreted in urine (Van Soest, 1994). However, NH_3-N utilization in the rumen is mainly affected by carbohydrate availability (Russell et al., 1992). According to Hristov et al. (2005), higher intakes of fermentable carbohydrates may reduce NH_3-N synthesis in the rumen (by reducing the deamination of AA or enhancing microbial capture of released AA) or increase NH_3-N utilization by the rumen microbes.

Feeding cows forage millet silages compared with CS caused substantial changes in the ruminal environment (Table 3.5). The higher starch contents (23.5 vs 16.3 % of DM) of the CS diet led to an acidic ruminal environment (average pH of 5.77 vs 6.08) and shifted the

VFA pattern towards proportionally more propionate at the expense of acetate (Bradford et al., 2006). In the current study, cows fed the CS diet consumed 45% more starch than RM- or SM-fed cows. Ruminal fermentation of starch produces more propionate than fermentation of other carbohydrates such as glucose, fructose and sucrose (Heldt et al., 1999). In agreement with our findings, Messman et al. (1992) reported greater molar proportions of acetate and acetate:propionate ratio, but lower propionate proportions when cows were fed a combination of pearl millet and alfalfa silages than for cows fed a combination of corn and alfalfa silages. Reports indicate that low ruminal pH favors lower acetate:propionate ratio (Lana et al., 1998). In general, a higher acetate:propionate ratio is an indicator of lipogenic versus glycogenic VFA production (Messman et al., 1992). The significantly lower acetate proportion in the rumen of cows fed the CS diet may be associated with lower in situ ruminal NDF degradation. Fibrolytic activity in the rumen is very often impaired when feeding cows diets rich in fermentable carbohydrates. At low rumen pH (6.0 to 5.8), growth or activity of cellulolytic bacteria is compromised and hence fiber digestibility (Russell et al., 1992).

3.6. CONCLUSIONS

Under the condition of a high forage:concentrate diet, feeding cows forage millet silages in replacement of CS reduced DMI, milk yield (RM only) and milk protein concentration, likely because of higher NDF and lower starch contents. Nevertheless, cows fed pearl millet diets consumed more NDF because pearl millet silages were ruminally more degradable than CS. Forage millet diets necessitated less (<55%) soybean meal, given that pearl millet silages contained 36% higher CP than CS. The effects of forage millet cultivars had minimal influence on the performance of dairy cows due to similarity in chemical compositions, in situ degradability, nutrient digestibility and rumen fermentation. Finally, based on findings of this study, forage millet silages may be an alternative to CS, especially in the more temperate regions.

Table 3.1: Fermentation characteristics and chemical composition (mean ± SD) of millet and corn silages (DM basis)

	Experimental Silages[1]		
	CS	RM	SM
Chemical composition, %			
DM	34.7 ± 1.37	26.1 ± 1.71	25.3 ± 0.75
Ash	3.6 ± 0.25	12.1 ± 0.65	11.7 ± 0.26
NDF	35.6 ± 2.29	58.4 ± 1.62	60.1 ± 1.74
ADF	20.0 ± 1.57	37.6 ± 0.39	37.8 ± 0.90
ADL	1.8 ± 0.25	2.5 ± 0.15	2.6 ± 0.17
CP	9.6 ± 0.30	12.8 ± 0.34	13.4 ± 0.87
Soluble protein, % of CP	44.1 ± 4.66	61.5 ± 2.08	58.4 ± 2.72
Non-protein nitrogen, % of CP	41.5 ± 4.30	58.6 ± 2.03	56.8 ± 3.15
Neutral detergent insoluble CP, % of CP	16.8 ± 0.39	23.1 ± 1.76	22.8 ± 1.76
Acid detergent insoluble CP, % of CP	10.9 ± 0.10	9.0 ± 0.56	8.5 ± 0.72
Starch	29.7 ± 1.09	0.5 ± 0.52	0.2 ± 0.07
Ether extract	2.2 ± 0.25	2.0 ± 0.05	2.0 ± 0.10
NE_L[2], Mcal/kg	2.06 ± 0.025	1.65 ± 0.006	1.64 ± 0.021
Fermentation characteristics			
pH	3.95 ± 0.06	4.47 ± 0.08	4.56 ± 0.11
Water soluble carbohydrates, %	0.4 ± 0.01	0.1 ± 0.05	0.3 ± 0.10
Lactic acid, %	6.9 ± 0.17	5.8 ± 0.17	4.6 ± 0.20
Acetic acid, %	1.3 ± 0.16	0.8 ± 0.20	1.7 ± 0.03
Aerobic stability, hrs	151 ± 15.2	32 ± 8.1	125 ± 30.4

[1]Experimental silages: corn silage (CS), regular millet silage (RM) or high water-soluble carbohydrates millet silage (SM).

[2] Estimated according to Weiss et al. (1992).

Table 3.2: Ingredients and chemical composition (mean ± SD) of experimental diets with millet or corn silage

	Dietary treatments[1]		
	CS	RM	SM
Ingredients, %			
Pearl millet silage		36.98	
Pearl millet silage			36.58
Corn silage	38.58		
Alfalfa silage	30.39	30.11	30.01
High moisture corn	19.67	23.01	23.53
Soybean meal	9.8	6.35	6.33
Mineral premix[2]	1.56	1.54	1.54
Megalac[3]		2.01	2.01
DM, %	47.2 ± 0.83	40.3 ± 1.18	41.8 ± 2.35
Chemical Composition, % of DM			
Ash	5.9 ± 0.45	9.2 ± 0.26	8.4 ± 0.55
Ether extract	2.5 ± 0.30	3.0 ± 0.16	3.1 ± 0.38
NDF	32.4 ± 1.70	38.5 ± 2.99	37.9 ± 0.41
ADF	19.4 ± 1.97	24.1 ± 1.73	22.8 ± 0.59
ADL	2.9 ± 0.53	2.7 ± 0.55	3.0 ± 0.35
CP	15.2 ± 0.41	15.5 ± 0.36	15.9 ± 0.33
Neutral detergent insoluble CP, % of CP	14.9 ± 1.66	13.6 ± 1.83	16.1 ± 0.86
Acid detergent insoluble CP, % of CP	8.1 ± 0.08	7.9 ± 0.72	8.1 ± 0.45
Starch	23.5 ± 2.27	16.4 ± 2.41	16.1 ± 0.95
NDF:Starch	1.4 ± 0.20	2.4 ± 0.52	2.3 ± 0.11
NE_L[4], Mcal/kg	1.87 ± 0.055	1.75 ± 0.049	1.78 ± 0.023
RUP	22.8 ± 2.37	24.8 ± 2.19	27.7 ± 0.78

[1]Experimental diet (68:32 forage:concentrate ratio; DM basis) contained corn silage (CS), regular millet silage (RM) or high water-soluble carbohydrates millet silage (SM).

[2]Contained 38.84% sodium bicarbonate, 25.07% di-calcium phosphate, 15.10% sodium chloride, 5.35% Mg, 4.57% K, 1.56% Ca, 2.04% Na, 0.63% Zn, 0.54% Mn, 0.22% Cu, 0.02% Co, 0.01% I, 0.01% sodium selenite, 1.38% mineral oil, 3.63% canola meal, 2, 200 KIU of vitamin E/kg, 2, 900 KIU of vitamin A/kg, and 1, 450 KIU of vitamin D/kg.

[3] Manufactured by Church & Dwight Co., Inc. Princeton, NJ, USA.

[4] Estimated according to Weiss et al. (1992).

Table 3.3: *In Situ* Ruminal Degradability of Millet and Corn Silages

	Experimental Silages[1]				
	CS	RM	SM	SEM[2]	*P*-value[3]
DM					
Soluble fraction, %	57.0[a]	33.3[b]	34.7[b]	0.8531	<.0001
Slowly degradable fraction, %	23.4[b]	49.4[a]	45.6[a]	2.0873	0.0002
Degradable rate, % h-1	3.5	4.4	4.5	0.3825	0.2140
Lag time, h	1.1	0.8	1.9	0.3796	0.1885
Effective degradability	65.3[a]	53.7[b]	53.8[b]	1.2237	0.0008
NDF					
Soluble fraction, %	2.6	3.1	5.8	1.1656	0.2049
Slowly degradable fraction, %	69.5	70.7	66.4	4.2217	0.7419
Degradable rate, % h-1	1.9[b]	4.5[a]	4.0[a]	0.3761	0.0135
Lag time, h	1.2	0.4	1.6	0.5026	0.2622
Effective degradability	18.9[b]	32.5[a]	31.7[a]	2.1630	0.0153

[1]Experimental silages: corn silage (CS), regular millet silage (RM) or high water-soluble carbohydrates millet silage (SM).
[2] Pooled standard error of the mean.
[3]*P*-value for treatment effects ($P < 0.05$).

Table 3.4: Intake of lactating dairy cows fed millet or corn silages in the diet

Item	Dietary Treatments[1]			SEM[2]	*P*-value[3]
	CS	RM	SM		
Intake					
DM, kg/d	24.4[a]	22.7[b]	22.8[b]	0.63	0.0047
DM, % of BW	3.75[a]	3.49[b]	3.50[b]	0.130	0.0052
NDF, kg/d	7.9[b]	8.8[a]	8.6[a]	0.24	0.0012
NDF, % of BW	1.21[b]	1.35[a]	1.33[a]	0.050	0.0016
CP, kg/d	3.7	3.5	3.6	0.09	0.0613
CP, % of BW	0.57	0.54	0.56	0.020	0.0886
Starch, kg/d	5.7[a]	3.7[b]	3.7[b]	0.22	<.0001
NE_L, Mcal/d	45.5[a]	39.7[b]	40.6[b]	1.35	<.0001

[1]Experimental diet (68:32 forage:concentrate ratio; DM basis) contained corn silage (CS), regular millet silage (RM) or high water-soluble carbohydrates millet silage (SM).
[2] Pooled standard error of the mean.
[3]*P*-value for treatment effects ($P < 0.05$)

Table 3.5: Total-tract nutrient digestibility and ruminal fermentation parameters of lactating dairy cows fed millet or corn silages in the diet

	Dietary Treatments[1]				
	CS	RM	SM	SEM[2]	*P*-value[3]
Digestibility, %					
DM	70.43	66.02	67.34	1.822	0.5036
OM	71.72	68.78	69.86	1.748	0.6462
CP	65.68	61.48	62.75	2.128	0.5824
NDF	52.69	53.06	55.85	3.687	0.8175
GE	68.40	66.68	68.67	2.456	0.8270
Starch	91.27	90.39	89.44	1.924	0.8294
Fermentation					
pH	5.77[b]	6.04[a]	6.12[a]	0.063	<.0001
NH3-N, mg/dL	9.9[b]	15.0[a]	14.6[a]	1.09	0.0007
VFA, mM	134.9[a]	133.1[ab]	128.7[b]	4.88	0.0107
Molar proportion, %					
Acetate	56.4[b]	65.6[a]	63.9[a]	1.49	0.0002
Propionate	30.1[a]	22.9[b]	24.9[ab]	1.21	0.0308
Butyrate	13.5	11.5	11.2	1.09	0.2124
Acetate:propionate	1.98[b]	3.01[a]	2.74[a]	0.160	0.0002

[1]Experimental diet (68:32 forage:concentrate ratio; DM basis) contained corn silage (CS), regular millet silage (RM) or high water-soluble carbohydrates millet silage (SM).
[2] Pooled standard error of the mean.
[3]*P*-value for treatment effects ($P < 0.05$).

Table 3.6: Milk production and milk composition of lactating dairy cows fed millet or corn silages in the diet

Item	Dietary Treatments[1]			SEM[2]	*P*-value[3]
	CS	RM	SM		
Yield, kg/d					
Milk	35.2[a]	32.7[b]	34.0[ab]	1.57	0.0162
Fat	1.44	1.37	1.43	0.071	0.3459
Protein	1.15[a]	0.99[c]	1.06[b]	0.038	<.0001
Lactose	1.59[a]	1.48[b]	1.54[ab]	0.075	0.0370
TS	4.53[a]	4.15[b]	4.38[ab]	0.170	0.0046
SNF	3.10[a]	2.78[b]	2.94[ab]	0.116	0.0017
ECM	38.0[a]	35.2[b]	37.0[ab]	1.52	0.0184
SCM	35.3[a]	32.7[b]	34.4[ab]	1.38	0.0143
4% FCM	35.6	33.7	35.1	1.54	0.1036
Milk Efficiency[4]	1.46	1.46	1.51	0.070	0.3424
Composition, %					
Fat	4.09	4.25	4.27	0.185	0.3700
Protein	3.30[a]	3.04[c]	3.14[b]	0.092	<.0001
Lactose	4.50	4.53	4.52	0.043	0.6571
TS	12.93	12.76	12.95	0.273	0.5003
SNF	8.84[a]	8.51[b]	8.69[ab]	0.142	0.0352
MUN, mg/dL	8.6[b]	10.1[a]	10.8[a]	0.88	0.0001

[1]Experimental diet (68:32 forage:concentrate ratio; DM basis) contained corn silage (CS), regular millet silage (RM) or high water-soluble carbohydrates millet silage (SM).
[2] Pooled standard error of the mean.
[3]*P*-value for treatment effects ($P < 0.05$).
[4]Milk yield/DMI.

CHAPTER IV. CONNECTING STATEMENT

In Experiment 1, we compared the effects of corn silage with two different forage pearl millet cultivars [i.e., regular millet (RM) and sweet millet (SM)] on the performance of lactating dairy cows. Feeding cows millet silages relative to CS reduced DMI, milk yield (RM only) and milk protein concentration. However, when compared with CS, cows fed SM consumed greater NDF and had similar milk yield and milk efficiency. Cows consumed more NDF likely because SM was more ruminally degradable than CS. Therefore, based on findings of this first study, we further investigated the sweet millet cultivar as a potential alternative forage for the nutrition of lactating dairy cows.

CHAPTER V. EFFECTS OF REPLACING GRASS SILAGE WITH FORAGE PEARL MILLET SILAGE ON MILK YIELD, NUTRIENT DIGESTION, AND RUMIMAL FERMENTATION OF LACTATING DAIRY COWS[1]

T. Brunette,* B. Baurhoo,† and A. F. Mustafa*

*Department of Animal Science, McGill University, Sainte-Anne-de-Bellevue, Quebec, H9X 3V9, Canada

†Bélisle Solution Nutrition Inc., St-Mathias-sur-Richelieu, Quebec, J3L 6A7, Canada

[1] Ready to be submitted for publication in *Journal of Dairy Science*.

5.1. ABSTRACT

This study investigated the effects of dietary replacement of grass silage **(GS)** with forage sweet millet silage that was harvested at 2 different stages of maturity [i.e., vegetative stage **(EM)** and dough to ripe seed stage **(MM)**] on milk production, apparent total-tract digestibility, and ruminal fermentation characteristics of dairy cows. Fifteen lactating Holstein cows were used in a replicated 3 x 3 Latin square experiment and fed (ad libitum) a high-forage total mixed ration (60:40 forage:concentrate ratio). Dietary treatments included GS (control), EM and MM diets. Experimental silages comprised 24% of each diet DM. Three ruminally fistulated cows were used to determine the effect of dietary treatments on ruminal fermentation and total-tract nutrient utilization. Relative to GS, EM and MM contained 34% more NDF and 24% more ADF. However, MM contained less CP (6.2 *vs.* 10.4%), higher starch (2.7 *vs.* 0.5%) and higher ADL (4.3 *vs.* 2.9%) than EM. Cows fed the GS diet consumed more DM (22.9 *vs.* 21.7 kg/d), CP (3.3 *vs.* 3.1 kg/d), and similar starch (4.9 kg/d) and NDF (1.3 kg/d) than MM-fed cows. However, when comparing the two millets, intakes of DM, NDF and CP were not different. Feeding MM relative to GS reduced milk yield (29.1 *vs.* 26.1 kg/d), ECM (30.4 *vs.* 28.0 kg/d), and FCM (28.3 *vs.* 26.4 kg/d). But, cows fed EM had similar milk yield, ECM, FCM than cows fed GS, and similar SNF, SCM to cows fed GS or MM. Milk efficiency was greatest for cows fed GS than MM, but similar between cows fed EM and GS or MM. Milk protein yield and milk protein concentration was higher when cows were fed GS than EM or MM. Milk concentrations of SNF, TS, fat and lactose were not influenced by dietary treatments. But, MUN was lower for cows fed GS than MM but not different between cows fed EM and GS or MM. Ruminal pH was not affected by silage type. Ruminal NH_3-N was greater for cows fed EM than GS, but similar for cows fed MM and GS or EM. Total tract-digestibility of DM (average = 66.1%), NDF (average = 55.1%), CP (average = 63.6%), and gross energy (average = 64.5%) were not influenced by dietary treatments. It was concluded that cows fed GS and EM had comparable performance, whereas milk yield was significantly reduced with MM likely due to reduced DM and NE_L intakes.

Key words: forage millet silage, grass silage, dairy cow, milk yield

5.2. INTRODUCTION

A constant supply of high quality forages is essential for optimizing milk production on dairy farms. However, in Canada's temperate regions, forage production is limited due to the extremely cold winters. Indeed, the growing season with warm temperatures (above 15°C) is between 70 to 90 days only. In these cold regions, the production of corn silage is risky and low-yielding, despite the use of short-season cultivars. Perennial forages such as alfalfa and grass mixes are the most commonly utilized forages on such dairy farms. Often, legumes and grasses are cultivated as mix stands to optimize yield and nutritive values of harvested forages. But, the recurrence of alfalfa winter kill frequently causes low yield and on-farm forage shortage, whereas the quality of grasses is often reduced due to delayed harvesting at advanced maturity stages during intermittent periods of drought and/or rainy periods. In our previous study, we showed that, in contrast with corn silage, cows fed a high WSC forage pearl millet silage had higher NDF intake, similar milk and milk fat yields, and similar milk efficiency, but lower DMI and milk protein yield (Brunette et al., 2014). Therefore, we hypothesize that the sweet millet cultivar could be a good forage alternative for dairy cows, especially in temperate regions.

Pearl millet [*Pennisetum glaucum* (L.) R.] is a tropical annual grass possessing the C4 photosynthetic pathway with high biomass yield, low N fertilizer requirement, drought resistant, and adaptable to low soil pH. Moreover, it has the capacity to grow rapidly under ideal climatic conditions (Maiti and Wesche-Ebeling, 1997). Pearl millet offers flexible harvest dates, an important factor influencing yield and nutritive values of most forages. In general, the nutritive values and digestibility of forages decline rapidly with advancement in maturity. Indeed, a 26% increase in NDF content was recorded when bromegrass and tall fescue were harvested at the flowering than tillering stage (Elizalde et al., 1999). However, millet harvested from 56 to 106 d had lower or similar NDF contents (Hassanat et al., 2006; Leblanc et al., 2012), whereas in vitro digestibility of NDF was only 18% lower after 106 d of growth compared to 63 d (Leblanc et al., 2012), and 8% lower after 84 d compared to 56 d (Hassanat et al., 2006).

The majority of published studies have compared millet silages with corn silages. To the best of our knowledge, this is the first study that has compared millet silages with grass silages. Nevertheless, data regarding the performance of lactating dairy cows fed pearl millet silage is limited and inconsistent. Messman et al. (1992) reported no effect on milk yield or milk fat concentration but lower DMI and milk protein levels when lactating cows were fed pearl millet silage (harvested after 66 d of growth; 23% DM) in replacement of alfalfa silage

plus corn silage. Moreover, feed intake, milk yield, and milk efficiency were similar when lactating cows were fed pearl millet (harvested at 80 d of growth; 27% DM) silage in replacement of corn silage (Amer and Mustafa, 2010). But, milk production and milk protein levels were lower when cows were fed diets containing pearl millet silage (30% DM) compared with temperate corn silage (Kochapakdee et al., 2002).

Unlike other gramineae forages, the WSC content of sweet millet increases with advancement in maturity (Leblanc et al., 2012). High WSC is highly desirable because it improves the ensilability of forages by accelerating lactic acid production (Adesogan et al., 2004), and increases the efficiency of rumen microbes (Merry et al., 2006) and N utilization (Miller et al., 2001). Therefore, the objectives of this study were to evaluate a grass mix silage with high WSC forage millet silage when harvested at two stages of maturity (early *vs.* late) on milk yield, milk composition, apparent total-tract nutrient digestibility and ruminal fermentation characteristics of lactating dairy cows.

5.3. MATERIALS AND METHODS

5.3.1. Forage material and ensiling

Forage pearl millet was seeded on May 31st, 2013 in a sandy loam soil at the MacDonald Campus Farm of McGill University (Ste Anne de Bellevue, Quebec, Canada; 45^{o}N, 73^{o}W). Millet seeds were provided by Bélisle Solution Nutrition Inc. (Saint-Mathias-sur-Richelieu, QC, Canada). A pre-sowing supply of 100 kg of urea N/ha (46% N) was evenly applied to the fields. Millet fields were harvested at two different intervals, on August 5th, 2013 (65 d) at early boot stage (early millet; **EM**) approximately 1.83 m high and, on September 17th, 2013 (108 d) at dough to ripe seed stage (mature millet; **MM**) approximately 3.66 m high. Early and mature millets were chopped to a theoretical length of 12-mm particle size using respectively, a New Holland forage harvester (model 900; New Holland, PA) and a John Deere self-propelled forage harvester with camper head (harvester model series 7380, corn header model series SPFH 770, John Deere, DE). Harvested millets were ensiled under high pressure into horizontal Ag-Bag silos (2.1 m diameter; AgBag, Miller-St. Nazianz Inc., St. Nazianz, WI) for approximately 6 months for EM and 8 months for MM.

In this study, the first cut (at early boot stage) of a 4 year-old alfalfa-grass field containing >90% of a mixture of grasses (tall fescue, orchard grass and brome grass) was used as control silage (**GS**). The initial WSC content of fresh GS, EM, and MM were 60 ±

6.67, 149.7 ± 8.02 and 138.5 ± 13.23, g/kg, respectively. Chemical composition and fermentation characteristics of experimental silages are shown in Table 1.

5.3.2. Experimental design and cows

All animal procedures were approved by the Animal Care Committee of the Faculty of Agriculture and Environmental Sciences of McGill University. Fifteen lactating Holstein cows (BW: 620.6 ± 79.90 kg) of mixed parities in early to mid-lactation (DIM: 133.7 ± 64.11 d) producing 30.7 ± 6.00 kg/d (average ± SD) of milk prior to the trial were used in a replicated 3 x 3 Latin square experiment with 21-d periods (14 d of diet adaptation and 7 d of data collection). Five cows were assigned to each treatment and blocked into 5 groups of 3 by parity, milk yield and DIM. Cows were housed in tie-stalls with free access to water.

5.3.3. Milk production and analysis

Cows were milked twice daily at 0500 and 1700 h. Milk yields were recorded at each milking by cow. Milk samples were collected on d 16 and 17 of each data collection period at both milkings and combined by cow according to volume. Milk samples was analyzed for fat, protein, lactose, and MUN using an infrared analyzer (Valacta, Sainte-Anne-de-Bellevue, Canada) according to AOAC (1990, method no. 972.16). Milk total solids were determined according to AOAC (1990).

5.3.4. Dietary treatments and sample collection

Three high forage iso-nitrogenous diets (60:40 forage:concentrate ratio) were formulated to meet nutrient requirements of lactating dairy cows in early lactation (NRC, 2001; Table 5.2). Dietary treatments were the replacement of GS with EM or MM silage. The forage portion of all diets consisted of 41% of experimental silages (GS, EM or MM), 34% corn silage and 21% dry hay. Diets were offered as a TMR once daily in the morning (1000 h) for periods 1 and 2 and twice daily for period 3 (0900 h and 0200 h) to prevent spoilage of the diets. Cows were fed for >5% residual.

During data collection (d 15-21 of each period), feed offered and orts were measured daily to determine daily feed intake per cow, and TMR and silages were sampled daily and pooled by period.

5.3.5. *In vitro* and *in situ* DM and NDF disappearances of experimental silages

One representative sample of experimental silages from each period was dried separately in a forced-air oven at 65˚C for 48h, and subsequently ground through a 1-mm screen using a Wiley mill (A.H. Thomas Philadelphia, PA). A 0.25g of each sample was then placed into acetone pre-rinsed F57 filter bags (Ankom Technology Corporation Macedon, NY) and incubated with rumen fluid from a lactating cow for 48h at 39˚C in a DAISYII fermenter (Ankom Technology, Fairport, NY) to determine in vitro DM disappearances (**IVDMD**). In vitro NDF digestibility (**IVNDFD**) was determined by analyzing for NDF residue in samples before and after the 48 h incubation with rumen fluid.

Equal portions (200g) of dried and ground (2-mm) replicates of GS, EM and MM silages were composited to obtain one sample for each experimental silage. A 5 g sub-sample of each mixture was then placed into nylon bags (20 x 10 cm, 50 µL pore size, Ankom Technology Corporation Macedon, NY) and incubated in the rumen of 3 lactating cows (one bag per treatment per cow) fitted with rumen fistula for 0, 3, 6, 12, 24, 48, 72, and 96 h. Following each incubation time, bags were removed from the rumen and manually washed under cold tap water until the rinsing water was clear. The 0 h incubation was determined by washing the bags containing the samples. The washed bags were then dried in a forced-air oven at 65˚C for 48h. Data of ruminal DM and NDF disappearances were used to determine nutrient kinetic parameters by using the equation of Dhanoa (1988):

$$p = a + b\,(1 - e^{-c\,(t-Lt)})$$

where *p* represents the nutrient disappearance at time *t*; *a*: soluble fraction (%); *b*: potentially degradable fraction (%); *c*: rate of degradation of the *b* fraction (%/h); and *Lt*: lag phase (h). The parameters were estimated by PROC NLIN of SAS (SAS, 2008) using iterative least squares regression (Gauss-Newton method). Effective degradabilities (**ED**) of DM and NDF were calculated according to the equation of Ørskov and McDonald (1979):

$$ED = a + bc/(c + k)$$

where k represents the ruminal outflow rate (6.25%/h), and a, b, and c are as described previously.

5.3.6. Ruminal measurements and apparent total-tract nutrient digestibility

Three lactating Holstein cows [milk yield 38.2 ± 9.19 kg/d; DIM 64.0 ± 96.44 d; BW 701.8 ± 51.31 kg (average ± SD)] of mixed parities fitted with rumen fistulas were used in a 3x3 Latin square experiment to determine the effects of dietary treatments on ruminal fermentation and total-tract nutrient digestibility. Cows were kept in tie-stalls and had free access to water. The cows were fed the same experimental diets and followed the same experimental protocol as in the production study.

Rumen fluid samples were collected from different parts of the rumen on d 17 and 18 of each period using a syringe screwed to a stainless steel tube ending with a fine metal mesh (RT Rumen Fluid Collection Tube, Bar Diamond Inc., Parma, ID). Rumen fluid collection started before the morning feeding (0h) and 2, 4, 6, 8, 10, and 12 h post-feeding. Ruminal pH was determined immediately using an Accumet pH meter (Fisher Scientific, Montreal, Canada). After pH determination, two 50 mL samples were immediately preserved by adding 5 mL of 25% metaphosphoric acid for VFA analysis, and 5 mL of 0.1 N HCL for NH_3-N analysis. Samples were stored at -20˚C for later analysis.

Chromic oxide was used as an inert external marker to determine total fecal output. Gelatin capsules containing 10 g of Cr_2O_3 were placed into the rumen of each cow twice daily in equal interval starting on d 12 of the adaptation period. Grabbed fecal samples were collected 4 times daily on d 17, 18 and d 19 of each period. Samples were then dried at 60°C in a forced-air oven for 72 h and pooled by cow within each period.

5.3.7. Samples preparation and chemical analysis

A 50g thawed sample of fresh and ensiled forages were homogenized with 1000 mL of distilled water and thoroughly ground in a blender (Oster blender, model 6811-33, Oster, FL) for 1 min, thereafter the pH of the extract was immediately determined using an Accumet pH meter (Fisher Scientific, Montreal, Canada). Extracts were centrifuged at 12,000 x *g* for 15 min at 4˚C and analyzed for organic acids (lactic, acetic, propionic, and butyric acid) by using high performance liquid chromatography (HPLC) (Andersson and Hedlund, 1983). The conditions for the HPLC-analysis were mobile phase (0.013 M-H_2SO_4) and flow rate (0.6 mL/min). Silage extracts were analyzed for NH_3-N colorimetrically with a multichannel Lachat Autoanalyzer (Lachat Instruments, Milwaukee, WI). Extract of feed samples were obtained and centrifuged as described above. The WSC concentrations of forages and silages

were determined colorimetrically within aliquots of filtered extracts using the phenolic-sulphuric acid reaction (Dubois et al., 1956).

Composited TMR and silages sample were dried in a forced-air oven at 65˚C for 72h, then ground through a 1-mm screen using a Wiley mill (A.H. Thomas Philadelphia, PA), and analyzed for DM and ash according to AOAC (1990) procedure. Crude protein (N x 6.25) was analyzed using a Leco Nitrogen Analyzer (Truspec Nitrogen Determinator System, Leco Corp., St. Joseph, MI). Soluble CP was determined according to Licitra et al. (1996). Neutral (Van Soest et al., 1991) and acid (AOAC, 1990) detergent fiber were determined using an Ankom Fiber Analyzer (Ankom Technology Corp., Macedon, NY). The NDF analysis was performed using heat stable α-amylase and without the inclusion of sodium sulfite. Acid detergent lignin and ether extract of TMR and silage samples were analyzed following standard procedures (AOAC, 1990). Neutral and acid detergent insoluble protein were estimated by analyzing NDF and ADF residues, respectively, for total N (Licitra et al., 1996). Starch was analyzed colorimetrically as per McCleary et al. (1997). Gross energy (GE) of feed samples was determined using an oxygen bomb calorimeter (model 6200; Parr Instrument Co., Moline, IL).

Representative samples of experimental silages for each period were thoroughly agitated to ensure air exposure and packed loosely into 500-mL plastic containers. Thereafter, each container was wrapped around the sides with thermal insulator to prevent heat dissipation, and four holes were made on top and bottom of each container to permit air exchange. Thermocouple probes were then inserted in the core of each plastic container to detect temperature difference from the environment. Aerobic stability was assessed as the time required to raise the temperature of test silages by 2˚C (Kung et al., 2000). Temperature was measured using a Hotmux data logger (DDC Corp., Pennsauken, NJ) and recorded every 5 min.

Dried fecal samples were analyzed for DM, ash, CP, NDF, and GE as previously described. Chromic oxide content was analyzed following Fenton and Fenton (1979) procedure. Frozen rumen fluid samples were thawed, centrifuged at 12,000 x g for 15 min at 4˚C and then analyzed for acetic, propionic, and butyric acids using the HPLC, as described previously. Ruminal NH_3-N was determined as detailed previously.

5.3.8. Statistical analysis

Data of the performance study and total tract nutrient utilization were analyzed as a replicated 3 x 3 Latin square design using PROC MIXED of SAS (SAS Institute, 2008) and the following model:

$$Y_{ijkh} = \mu + trt_i + block_j + animal_{jk} + per_h + e_{ijkh}$$

where Y_{ijkh} represents the observation, μ is the population mean, trt_i is the fixed effect of the i^{th} treatment (i = 1,2 or 3), $block_j$ is fixed effect of the j^{th} block (j = 1, 2, 3, 4 or 5), $animal_{jk}$ is the random effect of the k^{th} animal (k = 1, 2 or 3) in the j^{th} block, per_h is the fixed effect of the h^{th} period (h = 1,2 or 3), and e_{ijkh} is the random error. In situ ruminal degradability data for experimental silages were analyzed using an ANOVA and a completely randomized design with treatment as main effect and cows as replicates.

Ruminal fermentation parameters data were analyzed as repeated measures in time using PROC MIXED of SAS (SAS Institute, 2008) and the following model:

$$Y_{ijkh} = \mu + trt_i + animal_{ij} + per_k + time_h + trt_i \times time_h + e_{ijkh}$$

where Y_{ijkh} represents the observation, μ is the population mean, trt_i is the fixed effect of the i^{th} treatment (i = 1, 2 or 3), $animal_{ij}$ is the random effect of the j^{th} cow (j = 1, 2 or 3) on the i^{th} treatment, per_k is the fixed effect of the k^{th} period (k = 1, 2 or 3), $time_h$ is the fixed effect of the h^{th} time (h = 1, 2, 3, 4, 5, 6 or 7); $trt_i \times time_h$ is the interaction effect between treatment and time, and e_{ijkh} is the residual error ($e_{ijkh} \sim N(0, \sigma_{cow}^2)$). Significant differences were declared at $P < 0.05$.

5.4. RESULTS

5.4.1. Compositions of silages and experimental diets

On DM basis, MM contained higher starch (2.7 *vs.* 0.4%) and ADL (4.3 *vs.* 2.6%), but lower ash (5.2 *vs.* 8.6%) than GS and EM silages (Table 5.1). However, CP contents of both EM (10.4%) and MM (6.2%) were lower than GS (17.7%). Ether extracts were also higher in GS (4.7%) than EM (2.7%) and MM (1.7%). Relative to GS, forage millet silages contained 34% more NDF and 24% more ADF but lower NE_L. Despite advancement in

maturity, NDF and ADF contents did not differ between EM and MM. In vitro DM and NDF disappearances were highest ($P < 0.05$) for GS, intermediate ($P < 0.05$) for EM, and lowest ($P < 0.05$) for MM. The WSC concentrations of MM were 2.4 times higher than EM, but quite comparable to GS (3.9 *vs.* 3.0%). Lactic and acetic acids were the main fermentation acids in all silage types. However, there was a tendency for acetic acid concentration to be highest in EM, while GS and MM tended to contain higher lactic acid levels. Both GS and EM stabilized at a higher pH than MM. On the other hand, all experimental diets had similar CP contents (Table 5.2). However, the GS diet contained higher NE_L (1.69 *vs.* 1.64, Mcal/kg) and DE (3.0 *vs.* 2.7 Mcal/kg), but lower NDF (35.2% *vs.* 38.6%) and ADL (2.2 *vs.* 2.6%) than EM and MM diets.

5.4.2. *In situ* DM and NDF degradability of experimental silages

In situ degradability of soluble DM was greater ($P < 0.05$) for GS than for forage millet silages (Table 5.3). However, slowly degradable fraction and rate of degradation were similar across silage types. Relative to forage millet silages, GS had greater ($P < 0.05$) in situ effective DM degradability. But, effective DM degradability was similar between EM and MM. There was no effect of experimental silages on in situ soluble NDF, in situ slowly degradable NDF fraction or rate of NDF degradation. However, in situ effective NDF degradability was greater ($P < 0.05$) for GS and EM than for MM (Table. 5.3).

5.4.3. Feed intake

Dry matter intake was similar for cows fed GS and EM diets (Table 5.4). But, cows fed the GS diet consumed more ($P < 0.05$) DM than cows fed MM diet. However, NDF intake was not influenced by dietary treatment. Crude protein and NE_L intakes were greater ($P < 0.05$) for cows fed GS than cows fed millet silages. But, advanced stage of maturity for millet silages did not affect cow's DM, CP and NE_L intakes.

5.4.4. Milk production and composition

Milk, lactose, TS, SNF, ECM, SCM and 4% FCM yields were greater ($P < 0.05$) for cows fed GS than MM, but not different than EM-fed cows (Table 5.4). When comparing EM- and MM-fed cows, milk, protein, lactose, ECM and 4% FCM yields were lower ($P < 0.05$) when cows were fed the MM diet. Milk protein yield was highest ($P < 0.05$) for cows fed GS, intermediate ($P < 0.05$) for cows fed EM and lowest ($P < 0.05$) for cows fed MM.

However, milk fat yield was not influenced by dietary treatment. Milk efficiency was similar between GS and EM, but lower with MM.

Milk fat compositions tended to be higher among cow's fed millet silages than GS. But, milk protein concentration was higher ($P < 0.05$) among cows fed GS than EM or MM. Milk lactose, TS and SNF concentrations were not different between dietary treatments. Milk urea nitrogen was greater ($P < 0.05$) for cows fed MM than those fed GS. However, MUN levels were not different between cows fed GS and EM or between cows fed EM and MM.

5.4.5. Nutrient digestibility and ruminal fermentation

Apparent total-tract digestibility of DM, OM, CP, NDF, and GE were not affected by silage type or maturity stage of millet (Table 5.5). No time x treatment interaction were observed for ruminal fermentation; therefore, only main effects were reported (Table 14). Ruminal pH and total VFA levels were not affected by dietary treatments. Ruminal NH_3-N concentration was greater ($P < 0.05$) for cows fed EM than cows fed GS, however not different between cows fed GS and MM or between cows fed GS and EM. Feeding GS or MM compared with EM increased ($P < 0.05$) molar proportions of butyrate. In contrast, molar proportion of acetate and propionate were not affected by dietary treatments.

5.5. DISCUSSION

In this experiment, we studied the effects of feeding EM or MM relative to GS to lactating dairy cows. In order to obtain high quality grass silages, grasses (>90%) were harvested and ensiled at the early boot or immature stage. In contrast, forage sweet millet offers flexible harvest dates. Therefore, we targeted harvesting EM and MM at the vegetative and heading stages, respectively. However, due to unfavourable rainy weather conditions, millet silages were harvested at early boot stage (EM; 65 d, 22 % DM) and dough to ripe seed stage (MM; 108 d, 27 % DM) for EM and MM, respectively. Experimental diets were formulated to contain a high forage to concentrate ratio (60:40) and equal proportions of CP, NDF and NE_L. Forages were added in equal proportions in all dietary treatments. Due to shortage of alfalfa silage at the experimental farm, rations were formulated without alfalfa silage. Consequently, the forage portion of diets consisted of test silages, corn silage and dry alfalfa hay only (Table 5.2). Alfalfa hay was the principal forage source of dietary CP. Relative to GS, EM and MM silages contained 41 % and 65 % less CP (Table 5.1). Therefore, to balance for CP across treatments, soybean meal and slow release urea were

added in greater quantities into the EM and MM diets. Calcium salts of palm oil (Megalac; Church & Dwight Co. Inc., Princeton, NJ) were added in all diets to balance for NE_L whereas soybean hulls were added into GS and EM rations to balance for fiber. Nevertheless, in contrast to EM and MM, the GS diet contained higher NE_L (1.69 *vs.* 1.64, Mcal/kg) and DE (3.0 *vs.* 2.7 Mcal/kg), and lower NDF (35.2% *vs.* 38.6%) and ADL (2.2 *vs.* 2.6%).

In general, advancement in maturity of grasses is usually associated with reduced CP, and higher NDF and ADF contents (Rinne et al., 1997; Cone et al., 1999; Holtshausen et al., 2012). However, despite advancement in maturity, the NDF and ADF contents were not different between EM and MM. But, CP contents of millet silages were significantly reduced with maturity. Hassanat et al. (2006) also reported similar NDF and ADF levels, higher ADL but lower CP when millet silages were harvested at the vegetative and heading stage. Our CP, NDF, and ADF data for EM and MM were in good agreement with millet silages harvested at the flowering (65 d) and dough stage (102 d), respectively (Morales et al., 2011). In contrast to our previous study with high WSC millet (vegetative stage, 53 d) (Brunette et al., 2014), EM (early boot stage, 65 d) and MM (dough to ripe seed stage, 108 d) had lower CP levels but higher NDF, ADF and ADL levels. These differences may be explained by differences in maturity stages at which millet was harvested and ensiled. But, MM silage contained higher WSC (3.9 *vs.* 1.6%) and starch (2.7 *vs.* 0.5%) than EM. Our results were expected given that WSC deposition in millet stems increases with maturity, whereas higher starch was due to presence of millet seeds in MM. However, during the process of grain filling, starch increased at the expense of WSC content. This may explain the higher WSC content of pre-ensiled EM (15%) relative to MM (13.8%). Similar effects of maturity on WSC concentration was reported by Filya (2004). Nevertheless, the forage millet cultivar used in this study is expected to contain less seeds (starch) than typically grain millet cultivars. The greater residual WSC concentration of MM and GS relative to EM is likely due to the lower utilization of WSC by lactic acid bacteria. On the other hand, the higher concentration of acetic acid in EM relative to other silages suggested that prolonged heterolactic fermentation of WSC (i.e. further conversion of lactic acid into acetic acid) may have occurred during ensiling. Our results for organic acids and pH of forage millet silages were similar to those reported by Messman et al. (1992), Ward et al. (2001), and Amer and Mustafa (2010).

The higher in vitro disappearances and in situ degradability of DM for GS than millet silages may be explained by its higher CP and ether extracts. Likewise, corn silage that contained lower NDF and ADF but higher starch content also had higher in situ DM degradability than regular and high water WSC millet silages (Amer, and Mustafa, 2010;

Brunette et al., 2014). However, in situ effective degradability of NDF was similar between GS and EM. The similarity in NDF degradability occurred despite the fact that EM contained higher NDF (+34%), ADF (+32%) and ADL (+26%) contents than GS. The lower NDF degradability of MM than EM is attributable to its higher ADL concentration (+48%) as a result of advanced maturity. We previously showed higher in situ NDF degradability of millet silages than corn silage (Brunette et al., 2014).

Total-tract digestibility of DM, OM, CP, NDF and GE were not affected by dietary treatments (Table 5.5). However, cows fed GS consumed higher DM than MM-fed cows which agreed with our results for higher effective rumen DM degradability of GS silage, and higher dietary intakes of CP and NE_L. Despite the higher NDF digestibility of GS and EM than MM silage, cow's NDF intake was not different across treatments probably due to soybean hulls addition in the GS and EM diets to balance for fibre. But, NDF degradability of experimental silages may also explain DMI differences. Forages with greater NDF digestibility may allow for greater DMI because of faster disappearance from the rumen. Indeed, rumen degradability of NDF was higher in GS than MM. In agreement with our results, Oba and Allen (1999) reported that diets with improved in vitro or in situ NDF digestibility showed greater DMI and performance of lactating dairy cows. However, although rumen NDF degradability was higher for millet silages than corn silages, cows fed corn silage did consume higher DMI as a result of higher starch intake (Brunette et al., 2014).

Under the conditions of this study, milk yield was greater for cows fed GS than MM likely due to higher intakes of DM and NE_L (Table 5.4). A reduction in milk yield among cows fed regular millet silage than corn silage has previously been associated with lower DM (starch) and NEL intakes (Brunette et al., 2014). Cows fed EM also consumed less NE_L. However, milk yield was similar between GS- and EM-fed cows. In our previous study, cows fed high WSC millet silage had lower DM and NE_L intakes but milk yields were not different when compared with corn silage (Brunette et al., 2014). On the other hand, despite equivalent DM and NE_L intakes, the lower milk yield obtained among MM- than EM-fed cows is difficult to explain at this time. Data regarding the performance of cows fed millet silages are inconsistent. Whereas Kochapakdee et al. (2002) reported a 12% reduction in milk yield as a result of feeding cows pearl millet silage than corn silage, Amer and Mustafa (2010) observed no difference in milk yield between cows fed high-WSC pearl millet silage and corn silage.

The reductions in milk protein yields and milk protein concentrations among cows fed the millet diets relative to those fed GS (Table 5.4) may be explained by lower NE_L and CP

(i.e., MP from soybean meal) intakes (Broderick, 2003; Jenkins and McGuire, 2006; Brunette et al., 2014). Our explanation about lower MP intake or amino acid supply is consistent with our results about higher ruminal NH3-N concentrations (Table 5.5). Lower milk protein levels when feeding cows pearl millet silage than with CS has previously been reported (Messman et al., 1992; Kochapakdee et al., 2002). In contrast, milk protein yield and milk protein concentration were not different between cows fed high WSC millet and corn silage (Amer and Mustafa, 2010). Our findings indicate significantly lower ruminal NH_3-N concentration among cows fed GS than EM, but only numerically lower when cows were fed the MM diet. In the rumen, NH_3-N utilization is mainly affected by carbohydrate availability (Russell et al., 1992). In agreement with Hristov et al. (2005), we believe that higher intakes of fermentable carbohydrates (starch) by cows fed the GS and MM diets might have reduced NH_3-N synthesis in the rumen (by reducing the deamination of AA or enhancing microbial capture of released AA) or increased NH_3-N utilization by the rumen microbes. Moreover, a reduction in ruminal NH_3-N concentration due to higher starch intake or increased proportions of corn silage in the diets, have previously been associated with reduced urinary N losses (Brito and Broderick, 2006; Hassanat et al., 2013). We previously observed a reduction in ruminal NH_3-N for cows fed corn silage (higher starch) relative to those fed millet silages (Brunette et al., 2014).

In the current study, feeding cows GS or millet silages had no significant effect on ruminal pH, VFA pattern, and molar proportions of acetate, propionate and acetate:propionate ratio (Table 5.5). Our findings occurred despite higher starch intakes by cows fed the GS or MM diet. Higher starch intakes are usually associated with a more acidic ruminal environment and VFA pattern shifted toward proportionally more propionate at the expense of acetate (Bradford et al., 2006; Brunette et al., 2014). According to Heldt et al. (1999), ruminal fermentation of starch produces more propionate than fermentation of other carbohydrates such as glucose, fructose, and sucrose. Data investigating the effects of millet silages with grass silages on ruminal fermentation are lacking. When millet silages were compared with corn silage (higher starch intake), molar proportions of acetate and acetate:propionate ratio were significantly increased at the expense of propionate proportions (Messman et al., 1992; Brunette et al., 2014).

Taken together, EM and GS were comparable given that cows had similar DM and NDF intakes, and produced equivalent milk yields. All of these effects occurred despite the lower CP and NE_L intakes of EM-fed cows. Our findings about inferior milk yield of MM than GS and EM indicate clearly that MM was harvested at too extreme maturity stage.

Indeed, MM suffered from lodging, seed formation occurred at the expense of its WSC content (149.7 *vs.* 138.5 g/kg in pre-ensiled EM and MM, respectively), and lignin content was considerably increased (+48%) which consequently reduced NDF digestibility. The high WSC contents of sweet millet make it extremely suitable as forage for cows. Unfortunately, unfavourable weather conditions delayed the harvesting of millet. Nevertheless, we believe that forage millet may be used as an emergency forage or routinely as a new forage option by dairy producers. On the majority of dairy farms, grasses are seeded together with legume forages (i.e. alfalfa). However, if grasses were to be seeded as pure stands, yields would have been too low in the first year of cultivation in order to sustain on-farm forage needs. Unlike grasses (pure stands), forage pearl millet (annual gramineae) is high yielding and offers flexible harvest dates from the vegetative (i.e., 24% DM) to more mature (i.e., 32% DM) stages, thus making it suitable for the cold regions.

5.7. CONCLUSIONS

Findings of this study indicate equivalent performance between EM and GS, but lower milk yields among MM-fed cows. In contrast to GS, cows fed MM consumed less of dietary DM and NE_L probably due to higher lignin contents (+87%) of MM silage. Advanced maturity of millet (MM vs. EM) did not affect NDF and ADF levels, but increased lignin (+48%) and reduced CP (-68%) contents of millet silages. Milk CP yield and milk CP concentrations were higher cows fed GS than millet silages because of higher dietary CP intakes. Finally, based on findings of this study, forage millet silages may be an alternative to GS, especially in the more temperate regions. However, millet should be harvested prior to seed setting for better nutritive values for dairy cows.

Table 5.1: Fermentation characteristics and chemical composition (mean ± SD) of grass and millet silages (DM basis)

	Experimental silage[1]		
	GS	EM	MM
Chemical composition, %			
DM	30.1 ± 1.08	22.0 ± 0.87	27.0 ± 0.19
Ash	8.2 ± 0.15	8.9 ± 0.42	5.2 ± 0.23
NDF	47.2 ± 1.04	63.3 ± 2.79	63.4 ± 1.05
ADF	29.7 ± 0.21	39.1 ± 1.26	39.6 ± 0.56
ADL	2.3 ± 0.24	2.9 ± 0.05	4.3 ± 0.18
CP	17.7 ± 0.24	10.4 ± 0.58	6.2 ± 0.59
Soluble protein, % of CP	66.0 ± 2.26	70.8 ± 2.59	76.4 ± 3.26
Neutral detergent insoluble CP, % of CP	11.3 ± 0.88	11.7 ± 1.04	10.6 ± 0.37
Acid detergent insoluble CP, % of CP	3.5 ± 0.56	4.0 ± 0.60	4.8 ± 0.38
Starch	0.2 ± 0.09	0.5 ± 0.04	2.7 ± 0.21
Ether extract	4.7 ± 0.19	2.7 ± 0.06	1.7 ± 0.19
In vitro DM disappearance (48h)	84.7 ± 0.84	76.7 ± 1.95	70.0 ± 1.01
In vitro true DM disappearance (48h)	87.1 ± 0.73	79.3 ± 1.81	72.4 ± 1.11
In vitro NDF disappearance (48h), % of NDF	72.7 ± 1.56	67.3 ± 2.33	56.6 ± 1.68
NE_L[2], Mcal/kg	1.62 ± 0.010	1.39 ± 0.031	1.38 ± 0.006
Fermentation characteristics			
pH	4.0 ± 0.04	4.4 ± 0.15	3.7 ± 0.18
Water soluble carbohydrates, %	3.0 ± 0.06	1.6 ± 0.29	3.9 ± 0.74
Lactic acid, %	8.0 ± 0.05	4.3 ± 3.24	8.5 ± 0.07
Acetic acid, %	1.7 ± 0.05	5.4 ± 3.14	1.4 ± 0.05
Butyric acid, %	0.3 ± 0.00	0.3 ± 0.11	0.1 ± 0.04

[1] GS = mixed grass silage; EM = high water-soluble carbohydrates millet silage harvested at 65 d (early millet; early boot stage); MM = high water-soluble carbohydrates millet silage harvested at 108 d (mature millet; dough to ripe seed stage).

[2] Estimated according to Weiss et al. (1992).

Table 5.2: Ingredients and chemical composition (mean ± SD) of experimental diets

	Dietary treatment[1]		
	GS	EM	MM
Ingredients, %			
Pearl millet silage		24.25	
Pearl millet silage			24.38
Grass silage	24.49		
Corn silage	20.63	20.60	20.60
Dry hay	14.52	14.49	14.50
High moisture corn	24.39	24.63	24.36
Soybean meal	8.56	10.41	12.64
Soybean hulls	4.89	2.26	
Mineral premix[2]	1.70	1.70	1.70
Megalac[3]	0.81	1.42	1.21
Maxiflex[4]		0.53	0.61
DM, %	45.7 ± 1.69	40.1 ± 1.46	42.5 ± 1.72
Chemical Composition, % of DM			
Ash	6.7 ± 0.71	6.9 ± 0.61	5.9 ± 0.65
Ether extract	3.4 ± 0.12	3.2 ± 0.16	2.7 ± 0.22
NDF	35.2 ± 2.07	39.6 ± 3.29	37.7 ± 3.84
ADF	22.1 ± 1.42	24.3 ± 0.59	23.5 ± 3.49
ADL	2.2 ± 0.26	2.4 ± 0.16	2.7 ± 0.10
CP	14.5 ± 0.75	14.4 ± 0.65	14.1 ± 0.64
SCP, % of CP	38.0 ± 1.34	39.2 ± 2.31	38.8 ± 0.93
Neutral detergent insoluble CP, % of CP	12.2 ± 0.64	12.2 ± 0.71	11.4 ± 0.79
Acid detergent insoluble CP, % of CP	3.8 ± 0.39	3.7 ± 0.55	3.6 ± 0.39
Starch	21.4 ± 0.82	20.6 ± 2.59	22.8 ± 3.05
NDF:Starch	1.6 ± 0.11	2.0 ± 0.40	1.7 ± 0.40
DE, Mcal/kg	3.0 ± 0.16	2.7 ± 0.30	2.7 ± 0.07
NE_L[5], Mcal/kg	1.69 ± 0.029	1.64 ± 0.031	1.65 ± 0.031
In vitro disappearance, % of DM			
DM (48h)	86.2 ± 2.31	82.0 ± 1.49	81.7 ± 2.49
True DM (48h)	89.2 ± 1.65	85.5 ± 0.74	85.4 ± 1.85
NDF (48h), % of NDF	69.5 ± 3.94	63.1 ± 3.30	60.8 ± 6.85

[1] Experimental diets (60:40 forage:concentrate ratio; DM basis) contained a mixture of grass silage (GS), vegetative stage of high water-soluble carbohydrates millet silage (EM; 65 d; early boot stage), or mature stage of high water-soluble carbohydrates millet silage (MM; 108 d; dough to ripe seed stage).
[2] Contained 38.84% sodium bicarbonate, 25.07% dicalcium phosphate, 15.10% NaCl, 5.35% Mg, 4.57% K, 1.56% Ca, 2.04% Na, 0.63% Zn, 0.54% Mn, 0.22% Cu, 0.02% Co, 0.01% I, 0.01% sodium selenite, 1.38% mineral oil, 3.63% canola meal, 2,200 kIU of vitamin E/kg, 2,900 kIU of vitamin A/kg, and 1,450 kIU of vitamin D/kg.
[3] Manufactured by Church & Dwight Co., Inc. Princeton, NJ, USA.

[4] Manufactured by Belisle Solution Nutrition Inc. (Saint Mathias sur Richelieu, Quebec, Canada).
[5] Estimated according to Weiss et al. (1992).

Table 5.3: *In situ* ruminal degradability of grass and millet silages

	Experimental silage[1]				
Item	GS	EM	MM	SEM[2]	*P*-value[3]
DM					
Soluble fraction, %	45.3[a]	40.6[b]	39.6[b]	0.64	0.0015
Slowly degradable fraction, %	36.9	35.9	32.1	3.63	0.6416
Degradable rate, % / h	2.2	1.6	1.3	0.37	0.2849
Lag time, h	4.5	3.8	2.8	0.60	0.2148
Effective degradability	54.7[a]	47.7[b]	44.2[b]	0.95	0.0006
NDF					
Soluble fraction, %	7.8	7.3	4.1	1.24	0.1519
Slowly degradable fraction, %	74.2	70.8	59.0	3.55	0.0509
Degradable rate, % / h	2.8	2.5	1.7	0.34	0.1185
Lag time, h	4.5	3.7	3.4	0.39	0.1654
Effective degradability	30.7[a]	27.2[a]	16.7[b]	2.30	0.0121

[a, b] Values with different superscript within the same row are different ($P < 0.05$).
[1] GS = mixed grass silage; EM = high water-soluble carbohydrates millet silage harvested at 65 d (early millet; early boot stage); MM = high water-soluble carbohydrates millet silage harvested at 108 d (mature millet; dough to ripe seed stage).
[2] Pooled SEM.
[3] *P*-value for treatment effects.

Table 5.4: Performance of lactating dairy cows fed grass and millet silage treatments

Item	Dietary treatment[1]			SEM[2]	*P*-value[3]
	GS	EM	MM		
Intake					
DM, kg/d	22.94[a]	22.06[ab]	21.65[b]	1.022	0.0117
DM, % of BW	3.71[a]	3.55[ab]	3.50[b]	0.162	0.0142
NDF, kg/d	8.06	8.43	8.02	0.272	0.0997
NDF, % of BW	1.31	1.37	1.30	0.044	0.1145
CP, kg/d	3.27[a]	3.10[b]	3.10[b]	0.192	0.0139
CP, % of BW	0.54[a]	0.51[b]	0.50[b]	0.032	0.0127
Starch intake, kg/d	4.85[a]	4.55[b]	4.86[a]	0.393	0.0016
NE_L intake, Mcal/d	38.52[a]	35.89[b]	35.27[b]	1.915	0.0001
Yield, kg/d					
Milk	29.1[a]	28.4[a]	26.1[b]	0.79	<.0001
Fat	1.11	1.13	1.10	0.044	0.1824
Protein	0.96[a]	0.89[b]	0.83[c]	0.028	<.0001
Lactose	1.34[a]	1.32[a]	1.21[b]	0.041	<.0001
TS	3.88[a]	3.73[ab]	3.50[b]	0.110	0.0036
SNF	2.77[a]	2.60[ab]	2.43[b]	0.096	0.0074
ECM	30.45[a]	30.04[a]	28.04[b]	0.915	0.0001
SCM	29.61[a]	28.86[ab]	27.10[b]	0.829	0.0063
4% FCM	28.25[a]	28.30[a]	26.45[b]	0.917	0.0031
Milk Efficiency[4]	1.30[a]	1.33[ab]	1.23[b]	0.055	0.0092
Composition, %					
Fat	3.85	4.02	4.13	0.109	0.0750
Protein	3.32[a]	3.17[b]	3.20[b]	0.067	<.0001
Lactose	4.63	4.65	4.65	0.055	0.9671
TS	13.35	13.21	13.50	0.263	0.5411
SNF	9.50	9.19	9.38	0.282	0.4778
MUN, mg/dL	10.43[b]	11.43[ab]	12.33[a]	1.075	0.0053

[a-c]Values with different superscript within the same row are different ($P < 0.05$).

[1] Experimental diets (60:40 forage:concentrate ratio; DM basis) contained a mixture of grass silage (GS), vegetative stage of high water-soluble carbohydrates millet silage (EM; 65 d; early boot stage), or mature stage of high water-soluble carbohydrates millet silage (MM; 108 d; dough to ripe seed stage).

[2] Pooled SEM.

[3]*P*-value for treatment effects.

[4]Milk yield/DMI.

Table 5.5: Total-tract nutrient digestibility and ruminal fermentation of lactating dairy cows fed grass or millet silages diet

	Dietary treatment[1]				
	GS	EM	MM	SEM[2]	*P*-value[3]
Digestibility, %					
DM	69.14	66.05	63.07	3.339	0.2242
OM	71.64	70.30	66.23	2.253	0.1627
CP	65.30	65.24	60.40	4.152	0.4127
NDF	58.12	59.40	47.64	2.403	0.0783
GE	68.12	62.31	63.11	2.636	0.4122
Fermentation					
pH	6.14	6.12	6.29	0.122	0.2743
NH3-N, mg/dL	10.33[b]	13.73[a]	11.79[ab]	0.591	0.0010
VFA, mM	114.09	122.10	113.15	4.686	0.3427
Molar proportion, %					
Acetate	63.57	66.58	64.69	2.071	0.4197
Propionate	24.17	23.68	22.12	1.709	0.4155
Butyrate	12.26[a]	9.73[b]	13.19[a]	0.610	0.0007
Acetate:proprionate	2.77	2.83	2.93	0.259	0.7957

[a, b] Values with different superscript within the same row are different ($P < 0.05$).

[1] Experimental diets (60:40 forage:concentrate ratio; DM basis) contained a mixture of grass silage (GS), vegetative stage of high water-soluble carbohydrates millet silage (EM; 65 d; early boot stage), or mature stage of high water-soluble carbohydrates millet silage (MM; 108 d; dough to ripe seed stage).

[2] Pooled SEM.

[3] *P*-value for treatment effects.

CHAPTER V. GENERAL DISCUSSION AND CONCLUSION

Two studies were conducted in order to investigate the utilization of forage pearl millet in the nutrition of lactating dairy cows. In experiment 1, we examined the effects of replacing CS with a regular or a high-WSC forage millet silage in the diets of lactating cows on milk production, rumen fermentation, and total tract nutrient digestibility. In Experiment 2, based on findings of the first study, we further evaluated the high-WSC forage millet. In Experiment 2, we studied the effects of stage of maturity of forage millet silages on performance of lactating cows. Early (early boot stage, 65 day harvest) and late mature (dough to ripe seed stage, 105 day harvest) forage millet silages were compared with a grass silage diet. The parameters evaluated were same as in Experiment 1. Forage to concentration ratio was of 68:32 and 60:40 for Experiment 1 and Experiment 2, respectively.

Chemical compositions of experimental silages varied according to millet cultivars and stages of maturity at harvest. Compared with CS, RM and SM contained 66% more NDF, 88% more ADF, and 36% more CP. However, the chemical composition of both millet cultivars were similar. In Experiment 2, compared to GS, EM and MM contained 34% more NDF and 24% more ADF, whereas MM contained less CP (6.2 vs. 10.4%), similar NDF (average = 63.4%), and more ADL (4.3 vs. 2.9%) than EM. Advanced maturity of grasses is usually associated with reduced CP contents, increased NDF, and increased lignification of NDF (Rhine et al., 1997; Cone et al., 1999; Holtshausen et al., 2012; Kammes and Allen, 2012). Our results for NDF and CP were in good agreement with Messman et al. (1992) and Ward et al. (2001) for Experiment 1, and Morale et al. (2011) for Experiment 2. The greater residual WSC content of SM than RM (Experiment 1) and MM than EM (Experiment 2) is likely due to lower utilization of WSC by lactic acid bacteria. The greater proportion of acetic acid of CS and SM may explain greater aerobic stability relative to RM. Moreover, the low lactic and high acetic acids proportions of EM silage suggested that heterolactic fermentation occurred during ensiling. The low butyric acid concentration in all experimental silages indicated that all silages were well-preserved.

In situ NDF degradability was higher for RM and SM than CS (Experiment 1) and higher in GS and EM than MM (Experiment 2). Our results were somewhat

expected considering that advanced maturity of forages is negatively correlated with fiber degradability (Rinne et al., 2002; Holtshausen et al., 2012). Both CS and MM were harvested at the mature stage. However, in contrast to millet silages, in situ DM degradability was higher for CS (Experiment 1) due to its higher starch content, and GS (Experiment 2) as a result of its higher contents of CP, and digestible NDF. In agreement with our results, Amer and Mustafa (2010) reported higher in vitro DM degradability but lower in vitro NDF degradability for CS than millet. Moreover, Hassanat et al. (2006) reported lower in vitro NDF degradability for millet harvested at heading stage relative to vegetative stage.

In both studies, the total-tract digestibility of DM, NDF, and CP were not influenced by dietary treatments. But, in Experiment 1, DMI was higher among cows fed CS than RM or SM, as a result of higher dietary starch intake. However, cows fed millet silage diet consumed more NDF likely due to greater fiber digestibility of RM and SM or due to higher NDF content of the millet diet. In Experiment 2, DMI was similar between cows fed EM and GS or MM. However, cows fed MM consumed less DM than cows fed GS, and this effect was attributed to lower dietary CP and NE_L intakes. Differences in DMI in the second study may also be linked with differences in dietary fiber digestibility and fiber contents. For instance, the higher rumen degradability of NDF among cows fed GS than MM may have allowed for greater DMI because of faster disappearance from the rumen (Oba and Allen, 1999). But, NDF intake was not affected by dietary treatments despite higher NDF degradability of GS and EM than MM. This is most likely due to soybean hulls addition in the GS and EM diets in order to balance for fibre across treatments. Previous studies have observed higher NDF intake for cows and heifers fed millet silage than CS (Amer and Mustafa, 2010; Ward et al., 2001). But, DMI was higher (Ward et al., 2001), similar (Amer and Mustafa, 2010), or less (Messman et al., 1992; Kochapakdee et al., 2002) when cows were fed millet silage than CS. Discrepancies among studies may be related to factors such forage:concentrate ratio, maturity stage of millet at harvest, and diet compositions.

Under the condition of our studies, milk yield was higher for cows fed CS than RM, but not different when cows were fed SM (Experiment 1). In Experiment 2, milk yield was similar between GS- and EM-fed cows whereas MM significantly reduced milk yield. The lower milk yields observed when feeding cows the RM (Experiment 1) and MM (Experiment 2) diets are likely due to lower DM and NE_L intakes. The

differences in DMI were due to starch (Experiment 1) and CP (Experiment 2). Despite lower DM and NE_L intakes among cows fed SM than CS in Experiment 1, the similarity in milk yields are difficult to explain. Likewise, in Experiment 2, EM-fed cows consumed lower NEL, but produced similar milk yields than MM-fed cows. Lower milk yield when feeding lactating cows with pearl millet silage than CS has previously been reported (Kochapakdee et al., 2002). In contrast, Amer and Mustafa et al. (2010) have reported similar milk yield when cows were fed a high-WSC millet compared to CS.

In both of our studies, milk fat concentrations were not influenced by dietary treatments. However, milk protein concentration was reduced as a result of feeding forage millet silage diets. Reduction in milk protein concentrations among cows fed millet relative to CS were also observed by Messman et al. (1992) and Kochapakdee et al. (2002). Lower starch, NE_L (Messman et al., 1992) and CP intakes may explain the depression of milk protein concentration.

Feeding forage millet silage diets altered the ruminal fermentation in both studies. In Experiment 1, feeding CS diet relative to forage millet diets reduced ruminal NH_3-N level. Reduced ruminal NH_3-N levels have been reported when cows were fed increasing amounts of CS in the diet (Brito and Broderick, 2006; Hassanat et al., 2013). In Experiment 2, NH_3-N level was lower for cows fed GS compared to EM, but similar between cows fed MM and GS or EM. In both of our studies, reduced ruminal NH_3-N levels were due to cow's higher starch intakes. It is well documented that higher intake of fermentable carbohydrates (i.e., starch) significantly reduces ruminal NH_3-N concentration or increases NH_3-N utilization by the rumen microbes (Russel et al., 1992; Hristov et al., 2005).

Molar proportion of volatile fatty acids was altered by feeding forage millet diets. However, the effects were more pronounced in experiment one than in experiment two. Feeding forage millet silage diets relative to CS diet, increased molar proportion acetate and decreased that of propionate. Messman et al. (1992) also observed lower propionate proportions, but greater molar proportions of acetate and acetate:propionate ratio when cows were fed pearl millet than CS diets. Cows fed the CS diet had lower ruminal pH than cows fed the RM or SM diets likely due to higher (+45%) dietary starch intake. Whereas in experiment two, ruminal pH, and acetate and propionate concentrations were not affected by feeding cows GS or millet silages.

Based on the results of Experiments 1 and 2, it can be concluded that forage pearl millet silages can be fed to lactating dairy cows with no major detrimental impacts on performance, ruminal fermentation and nutrient utilization. However, the negative effects of mature millet silage on cow's performance can be avoided by harvesting sweet millet prior to seed formation stage.

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