EVALUATION OF PEARL MILLET FORAGE

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Montreal, Quebec

November , 2007

A Thesis

Submitted for the Faculty of Graduate and Postdoctoral Studies in partial

fulfilment of the requirements of the degree of Doctor in Philosophy

ABSTRACT

This research evaluated millet as forage source for ruminants. Four studies were conducted using two cultivars of forage millet [i.e. brown midrib (BM) and regular (RM)]. The first investigated the effect of seeding rate on yield, chemical composition and in vitro degradability of the two forage millet cultivars. Yield of RM was 56% more than BM due to taller plants and more tillers m^{-2}. A 25% increase in yield as seeding rate increased from 5 to 10 or from 10 to 15 kg ha^{-1} was observed for two millet cultivars. Brown midrib millet contained 15% more CP, and 4, 13 and 31% less NDF, ADF and ADL than RM cultivar. In vitro DM digestibility was 10% higher in BM due to differences in chemical composition.

The second study determined the effect of stage of development at harvest [i.e. vegetative (VS) and heading stage (HS)] on the two millet cultivars yield and cell wall composition in leaves and stems. Yield of BM was lower than that of RM at both stages of development. Concentrations of NDF, ADF and ADL were reduced in BM stems by 8, 16, and 58%, respectively, compared to RM stems. Leaves ADF and ADL concentrations were 6 and 49% less in BM than RM. Increase in fibre fractions with advancing stage of development was most pronounced in RM stems. Brown midrib trait affected cell wall structure of BM leaves and stems by increasing arabinose and xylose proportion as well as concentrations of ester- linked *p*-coumaric acid and ether-linked ferulic acids. Cell

wall content of arabinose, xylose and glucose in leaves and stems, and phenolics in stems was higher at VS than at HS. This effect was more pronounced for stems of RM than BM. In situ DM and NDF disappearances were higher in leaves and stems of BM than RM and were higher in leaves and stems of millet harvested at VS than at HS. Lignin concentration had negative linear impact on in situ DM and NDF degradability.

Ensilability of forage millet as affected by cultivar and stage of development at harvest was investigated in the third study. Both millet cultivars at VS and HS contained sufficient WSC to produce silage with a pH less than 4.2 after 45 days of ensiling. During ensiling, extensive proteolysis occurred in the first 8 days of ensiling as almost 50% of the TP was degraded to NPN. Millet Silages harvested at HS were more aerobically stable than those harvested at VS. Differences in yield, chemical composition, and in vitro DM and NDF degradability between RM and BM at VS and HS were consistent with what was observed in the first two studies.

Effects of inoculating RM and BM with homofermentative lactic acid bacteria on ensiling characteristics and aerobic stability of millet silages were examined in the fourth study. Inoculation caused a fast reduction in silage pH, increased lactic acid but reduced acetic acid concentrations for RM and BM silages. No effect of inoculation on proteolysis during ensiling was observed. However, aerobic stability of inoculated silages was less than untreated silages

by 40 h. Chemical composition and in vitro degradability of the two millet cultivars were not affected by inoculation.

In conclusion, the two forage millet cultivars grow successfully under southern Quebec conditions. The brown midrib trait in forage millet reduced yield, but increased forage quality. As stage of development advanced for the two millet cultivars, yield increased, but forage quality was negatively affected. The brown midrib trait reduced the negative impact of advancing stage of development at harvest on forage millet quality. Both millet cultivars at VS and HS produced good quality silage. Inoculating RM and BM with homofermentative lactic acid bacteria reduced aerobic stability, and thus, is not recommended.

RÉSUMÉ

Cette recherche constitue a évalue millet comme une source de fourrage pour les ruminants. Quatre études ont été effectuées en utilisant deux variétés de millet [par exemple midrib brun (BM) et régulier (RM)]. La première étude constituait une évaluation de l'effet de la vitesse de semer les graines de millet sur le rendement, la composition chimique et la degradabilité in vitro des deux variétés de millet. Le rendement de RM était 56% plus haut en comparaison de BM en raison des plus grandes plantes et plus de feuillage par m^{-2}. Une augmentation de 25% dans le rendement a été observée quand en augmentant la vitesse de semer les graines de millet de 5 à 10 ou de 10 à 15 kg ha^{-1} pour deux variétés. Le midrib brun de millet contenait 15% plus de CP, et 4, 13 et 31% moins de NDF, ADF et ADL que la variété RM. La digestibilité in vitro de DM était 10% plus haut chez BM en raison de ces différences.

La deuxième étude avait pour objectif de déterminer l'effet du niveau de développement à la moisson [par exemple végétal (VS) et niveau d'en-tête (HS)] sur le rendement des deux variétés de millet et la composition de membrane cellulaire dans les feuilles et les tiges. Le rendement de BM était plus bas en comparaison de RM aux deux niveaux de développement. Les concentrations de NDF, ADF et ADL ont été réduits dans les tiges de BM par 8, 16, et 58%, respectivement, en comparaison des tiges de RM. Les concentrations de ADF et ADL dans les feuilles étaient 6 et 49% moins dans BM que RM. Les contenus des différentes fibres ont augmenté avec le niveau de développement, mais

l'augmentation été plus prononcée dans les tiges de RM. Le millet brun a eu un effet sur les membranes cellulaires des feuilles et tiges de BM en augmentant la concentration d'arabinose et xylose ainsi que les concentrations d'ester- acides de p-coumaric et d'ester- acides de ferulic. Les contenus d'arabinose, xylose et le glucose dans les membranes cellulaires chez les feuilles et les tiges, et de phenolics dans les tiges était plus haut à VS qu'à HS. Cet effet été plus prononcé pour les tiges de RM que BM. In situ DM et NDF disparaissaient plus vite dans les feuilles et les tiges de BM que RM et étaient plus haut dans les feuilles et les tiges de millet moissonné à VS qu'à HS. La concentration de la lignine avait un impact linéaire négatif sur la degradabilité de DM et NDF in situ.

La troisième étude constituait a examiné l'effet de la variété et niveau de développement du millet sur l'ensilage. Les deux variétés de millet à VS et HS contenaient suffisamment de carbohydrate hydrosoluble pour produire de l'ensilage avec un pH moins que 4.2 après 45 jours d'ensilage. Pendant ensilage, la dégradation de protéine était élevée pendant les 8 premiers jours d'ensilage le fait que presque 50% du TP été dégradé à NPN. Les ensilages de millet moissonnés à HS étaient aérobiquement plus stables que ceux moissonné à VS. Les différences dans le rendement, la composition chimique, et la degradabilité de DM et NDF in vitro entre RM et BM à VS et HS étaient conforme aux observations dans les deux première études.

Les effets d'inoculer RM et BM avec des bactéries acides lactiques homofermentative sur les caractéristiques d'ensilage et la stabilité aérobic

d'ensilages de millet ont été examinées dans la quatrième étude. L'inoculation a causé une réduction rapide dans le pH d'ensilage, a augmenté la concentration d'acide lactique mais les concentrations d'acides acétiques ont été réduites pour les ensilages de RM et BM. Aucun effet d'inoculation sur la dégradation de protéine n'a été observé pendant l'ensilage. Cependant, l'ensilage inoculé était aérobiquement moins stable que les ensilages non traité par 40 h. La composition chimique et degradabilité in vitro des deux variétés de millet n'ont pas été affectés par l'inoculation.

En conclusion, les deux variétés de millet peuvent être cultivées avec succès sous les conditions de la partie sud du Québec. Le midrib brun de millet a réduit le rendement, mais la qualité du fourrage était meilleure. Avec une progression du niveau de développement pour les deux variétés de millet, le rendement a augmenté, mais la qualité du fourrage était négativement affectée. Le midrib brun a réduit l'impact négatif du niveau de développement sur la qualité de millet à la moisson. Les deux variétés de millet à VS et HS ont produit un ensilage de bonne qualité. En inoculant RM et BM avec des bactéries acides lactiques homofermentative, la stabilité aérobic a été réduite, et en conséquence, n'est pas recommandable.

ACKNOWLEDGEMENT

Success of this thesis would not be possible without the academic, financial, and moral support from my supervisors, committee members, my family and friends. I would like to acknowledge my supervisor Dr. Mustafa. I thank him for the continuous guidance, valuable directions and advise through out the study. I also acknowledge Dr. Seguin supervision, precious help and encouragement during planning, working on the field and writing the papers. I extend my appreciation for Dr. Cue for his valuable advice on statistical analysis. I express my thanks for Dr. Phillip for his valuable counselling and encouragement. I'm very grateful to David Meek and Dr. Idziak for their help during the microbiological lab work. My graditude goes out to Elizabeth Woonton for her contniouse encouragement. I thank my friends and collegues; Atef, Audrey, Dana, Kebba, Malik, Marsha and Shyam for their encouragement and help. The financial support of Balqa University during the years of study is greatly appreciated.

CONTRIBUTION TO KNOWLEDGE

This research studied the impact of agronomical and non agronomical factors on yield, chemical composition and ensilability of millet forage. Emphasis on the impact of brown midrib trait on yield and chemical composition as interacted with changes in seeding rate, stage of development at harvest and inoculation contributed significantly to the importance of these studies.

In the first study, the outcome of changing seeding rate on agronomical characteristics, chemical composition, and ensiling characteristics of regular and brown midrib forage millet was determined. This is the first time that the combination of brown midrib trait and changing seeding rate in pearl millet was examined.

The effect of the brown midrib trait and stage of development at harvest on millet leaves and stems cell wall concentration, composition, cross link and degradability was examined for the second study. Studying the combined effect of cultivar (regular and brown midrib) and stage of development on forage millet yield, agronomical characteristics, and cell wall characteristics provided distinctive understanding of the nature of brown midrib trait in millet.

The third study determined the effect of cultivar and advancing stage of development at harvest on chemical composition and ensiling characteristics of forage pearl millet cultivars (normal and brown midrib) harvested at two stages of development was. Differences in silage quality between these two millet cultivars at two stages of development were not investigated before. Limited information is available on the ensilability of brown midrib forages.

Changes in ensiling characteristics and chemical composition two millet cultivars (regular and brown midrib) after addition of lactic acid bacteria was investigated in the fourth study. Impact of inoculation on silages of brown midrib forages was not studied before.

CONTRIBUTION OF AUTHORS

From this research, four co-authered manuscripts were submitted for publication. The study "**Chemical composition and ensiling characteristics of normal and brown midrib pearl millet harvested at two stages of development in South-western Québec**" was published in Can. J. Anim. Sci. 2006. 86: 71-80. The study "**Effect of the brown midrib trait and stage of development at harvest on cell wall composition and degradability of forage pearl millet leaves and stems**" was published in Can. J. Anim. Sci. 2007. 87: 421-429. The study "**Effects of inoculation on ensiling characteristics, chemical composition and aerobic stability of regular and brown midrib millet silages**" was published in Anim. Feed Sci. Technol. 2007. 139. 125-140. The study "**Evaluation of seeding rate effects on agronomical characteristics, chemical composition, and ensiling of regular and brown midrib forage millet**" will be submitted to Forage and Grazinglands Journal. Experiment, lab work and data analysis was performed by Fadi Hassanat. Dr. A. Mustafa and Dr. P. Seguin provided assistance in experimental design, field and lab work, and statistical analysis. All the manuscripts were written by Fadi Hassanat, and were revised by Dr. A. Mustafa and Dr. P. Seguin.

TABLE OF CONTENT

LIST OF ABREVIATION

ADF……acid detergent fiber

ADICP……acid detergent insoluble crude protein

ADL……acid detergent lignin

BM……..brown midrib millet;

CFU……colony forming units

CP……..crude protein

DM…….dry matter

HS……..heading stage

IVDMD……in vitro dry matter digestibility

IVNDFD……..in vitro neutral detergent fiber digestibility

LAB…….lactic acid bacteria

NDF……neutral detergent fiber

NDICP……neutral detergent insoluble crude protein

NEL…….net energy of lactation

NPN……non protein nitrogen

RM……..regular millet

SCP……soluble crude protein

TDN…….total digestible nutrients

TP……true protein

VS……vegetative stage

WSC……water soluble carbohydrates

LIST OF TABLES

LIST OF FIGURES

CHAPTER I.

GENERAL INTRODUCTION AND LITERATURE REVIEW

GENERAL INTRODUCTION

Cattle and sheep raised in Canada is estimated to be 15 million heads, about 10% of which are in Quebec. These animals produced $11.6 billion value of milk, meat, and replacement animals in 2006 (Statistics Canada 2006). To these animals, forages are valuable and low-cost source of energy, CP and minerals despite their low net energy content. Forage proportion in their diets are usually 40-60% for dairy cattle, 10-60% for feed lot beef and sheep, 90-100% for grazing beef and sheep (Galyean and Goetsch 1993).

Forages are vital for ruminants diet to optimize milk production, maintain rumination, rumen buffering, prevent displaced abomasums, acidosis, and lameness (NRC 2001; Van Soest 1994). Between 1999-2001, annual forage demand in Canada was estimated to be 44.6 million ton DM, 4.8 million ton for Quebec (Statistics Canada 2003). Supplying high quality forage for grazing, hay and silage making is an important factor in the success of any feeding program. Selection of proper forage to do that depend on available cultivars, soil and climate conditions, and intended use.

About 7.6 million hectares are used to grow forages in Canada to produce 27 million ton of forage with an estimated value of $140 million, 11% of which are

in Quebec (Statistics Canada 2001; Statistics Canada 2006). Large percentages of these lands are used to grow perennial forages such as timothy, orchardgrass and alfalfa. However, these forages are susceptible to winterkill due to exposure to low temperature with insufficient snow cover, soil freezing and accompanying diseases (Belanger et al. 2002; Belanger et al. 2006). Areas of southern Quebec and south eastern Ontario are most influenced as days of winterkill are above 14; subfreezing air temperature, above 0 temperature which cause loss of hardiness, soil heaving and ice encasement which cause root damage (Belanger et al. 2006). Annual forages would provide a potentially good alternatives for perennial forages in areas exposed to these conditions.

Out of all annual grasses, corn was given most attention since it provides high quality and yield combination. In Canada, corn is grown in 236,000 ha (52,000 ha in Quebec) to produce 1.3 million ton of silage annually (Statistics Canada 2001; Bagg 2005). Preference of corn is attributed to its high DM content, ease of harvest, stable quality, and use flexibility for grain or forage (Allen et al. 2003) Water requirement of corn varies considerably between cultivars, soil type and climatic conditions between 260 and 590 mm. During summers (June to October) of 2001, and 2002, the Montreal area received 108 and 151 mm respectively, which is not suitable for growing corn. Other forages, such as sorghum, sudangrass and millet could be used for the same purpose

and provides higher yield under drought or low moisture conditions (Jaster et al. 1985).

LITERATURE REVIEW

1. Pearl millet classification and importance

Pearl millet (*Pennisetum glacum*) belongs to the family Poaceae, subfamily Panicoideae which also includes maize, sorghum and sudangrass. These annual tropical forages belong to C4 grasses that fix CO_2 in oxalacetate rather than phosphglyceric acid in C3 grasses. They are characterized by higher vascular tissues proportion, higher photosynthetic efficiency, more drought and heat resistant than C3 grasses, and are vulnerable to cold temperature. Due to more compacted structure and low intercellular space in leaves and stems, C4 plants are generally less digestible than C3 plants (Nelsen 1995; Wilson 1993; Balasako and Nelsen 2003). Corn is the most studied plant in this subfamily, thus observations and findings could be generalized, to a limited extent, to other family members.

Pearl millet (*Pennisetum glacum* (L.)) also recognized as bulrush cattail millet, was domesticated 5000 years ago in west Nile area. Pearl millet could be grown for grain, forage or dual purpose. About 26 million hectares are utilized to grow millet world wide, mainly in Africa and India where it originated (CGIAR 2004). These areas are characterized by drought, erratic rain and saline soil, and

therefore unsuitable for corn production. Most of these areas are used to grow millet to produce grains, which is the main cereal for about 90 million people (Andrews and Kumar 1992; Andrews et al. 1993). Grain pearl millet cultivars produce around 3 ton ha^{-1}. The grains are expected to contain 8 to 24% crude protein, 3 to 7% fat and 70% nonstructural carbohydrates (Andrews et al. 1993). Incorporating millet grain in poultry, pigs and beef cattle diets was successful and produced results (weight gain, eggs) equal or better than corn and sorghum grains (Andrews et al. 1993; Collins et al. 1997).

1.1. Pearl millet as a forage crop

Forage pearl millet is popular in USA and Australia, utilizing over 10 hybrids for different attributes; higher yield, multiple cuts, higher quality, diseases and drought tolerance (Andrews and Kumar 1992; Hanna and Gupta 1999). In USA, 500,000 hectares are planted with millet annually, mainly with Tifleaf and Ghai cultivars. About 10% of the dairy cattle in some states in USA are fed millet forage, while 40 to 83% of the cows are fed forage corn. For these cows, millet contributes about 30% of the whole diet and 50 to 60% of the forage fed (Fribourg 1995; Mowrey and Spain 1999).

Millet is expected to grow better than other forages under drought conditions. Jaster et al. (1985) reported that pearl millet yielded 4.6 ton ha^{-1} dry matter while sorghum yielded 3.6 ton ha^{-1} under drought conditions (150 mm

rain). Singh and Singh (1995) reported that millet out yielded corn under moderate and sever drought conditions. Millet forage production was similar to sorghum under low rain conditions (350 to 450 mm), but grain yield was higher for sorghum (Maman et al. 2004). In another study, grain yield of sorghum and millet under drought conditions was similar, while sorghum had higher yields in more favorable conditions (Christensen et al. 1987). The highest forage millet yield reported was 55 ton ha^{-1}, 45% higher than sorghum-sudangrass, grain sorghum and forage sorghum (Bishnoi et al. 1993).

Millet grows best in light soils with pH 6.2 to 7.7 and optimum temperature of 33 ºC (range of 12 to 45 ºC). Seedling death reported when day or night temperature drops below 12 ºC (Andrews and Kumar 1992; Fribourg 1995). Plants can grow on annual variable precipitation (250 to 450 mm) and may tolerate as low precipitation as 150 mm. Drought and heat resistance characters of millet originate from the deep spreading root system (1.8 m), high water use efficiency, rapid recovery after drought, and less defined critical water use period (Andrews and Kumar 1992; Fribourg 1995; Maiti and Wesche-Ebeling 1997).

Germination of millet seedlings is ideal when soil temperature is 20 to 30 ºC and when planting depth is 1.5 to 5 cm. Seeding rate required to achieve optimum stand varies between 5 to 33 kg ha^{-1} for different cultivars, growing purposes, field conditions, and harvest method (Fribourg 1995; Maiti and

Wesche-Ebeling 1997; Bidinger and Raju 2000). Increasing seeding rate increases millet yield up to a level when plant competition for light, moisture and nutrients become critical (Carberry et al. 1985; Horrocks and Vallentine 1999; Bidinger and Raju 2000). However, increasing seeding rate is expected to reduce tillering and IVDMD and increase NDF concentration (Cusicanqui and Lauer 1999; Widdicombe and Thelen 2002).

Rust, leaf spot, armyworm, corn earworm are the most pests affecting millet. Diseases reduce forage yield, protein content, digestibility and palatability up to 50%. However, millet is resistant to root lesions nematodes, smut and other disease that affect corn and other crops. These characters give millet advantage over corn and sorghum (Andrews and Kumar 1992; Kumar and Andrews 1993).

1.2. Utilization of forage pearl millet

Forage millet could be grazed or mechanically harvested for green chops, hay or silage making. Suitability of millet for different harvest methods depends on cultivar, expected re-growth, and stage of development at harvest (Andrews and Kumar 1992; Fribourg 1995). Harvesting millet around heading stage with stubble height above 15 cm produces the highest digestible and palatable yield and fastest re-growth (Andrews and Kumar 1992). This would also prevent increase in lignin concentration, reduction in CP content and digestibility (Andrews and Kumar 1992), which occurs at later stages of development.

Yields of pearl millet forage vary between 3 to 55 ton DM ha^{-1} according site, cultivar, stage of development at harvest, climatic and soil conditions (Bishnoi et al. 1993; Gray et al. 2000). In a study by Messman et al. (1992), millet yielded 6.9 ton ha^{-1} in a cool damp season, while Banks and Stewart (2003) reported a yield of 12 ton ha^{-1} DM.

1.3 Pearl millet silage

Under North America's conditions, ensiling is the best method to conserve millet forage and ensures year round forage supply. This is because ensiling is less weather dependent, conserve large quantities in short time with minimum losses, and does not need to bring the plant to a late maturity stage as in hay making (Charmley 2001; Wilkinson et al. 2003). Forage corn, sorghum and sudangrass produced high quality silage (Fribourg 1995, Horrocks and Vallentine 1999), and thus, forage millet is not expected to be any different. High quality silage is characterized by low pH, high organic acids and low ammonia concentration, and high aerobic stability. Development of these qualities in silage depends on the initial forage conditions which include dry matter and water soluble carbohydrates concentrations, microbial count, and interaction of these factors (Buxton and O'Kiely 2003; McDonald et al. 1991; Muck et al. 2003b).

Recommended forage dry matter content at ensiling ranges between 250-450 g kg^{-1} (Bolsen 1996). Lower DM concentration increase the extent of

fermentation as well as effluents and respiration losses, and reduce aerobic stability (McDonald et al. 1991; Muck et al. 2003a). Millet forage dry matter content at harvest is usually low (less than 250 g kg^{-1}) and varies widely depending on stage of maturity (Maiti and Wesche-Ebeling 1997; Hanna and Gupta 1999). Welting, conditioning, windrowing or any method that could remove moisture and increase DM content of millet forage before ensiling is recommended and could bring DM content above 300 g kg^{-1} (Collins and Moore 1995; Han et al. 2006).

Water soluble carbohydrates are the lactic acid bacteria substrate to produce organic acids during ensiling. Presence of adequate water soluble carbohydrates concentration to produce enough acids that would reduce silage pH to a stable point is an imperative part of successful ensiling. Millet water soluble carbohydrates concentration averages 100 g kg^{-1} DM (Fisher and Burns 1987; Ward et al. 2001; Han et al. 2006) and varies with cultivar, stage of maturity and time of the day at harvest (Buxton and O'Kiely 2003). According to Lunden Pettersson and Lindgren (1990), water soluble carbohydrates concentration of 25 g kg^{-1} (on as ensiling basis) is expected to be sufficient to produce enough acids to make good silage. This means that millet forage ensiled at 250 g kg^{-1} DM would have a marginal water soluble carbohydrate concentration to produce good silage. Hill et al. (1999) recommended addition of

corn to millet before ensiling in order to increase water soluble carbohydrate content and establish good ensiling conditions. Chopping before ensiling is also recommended to increase water soluble carbohydrates availability and lactic acid bacteria count (Lin et al. 1992). Lactic acid bacteria count before ensiling is not affected by forage type, stage of development at harvest or number of cut (Lin et al. 1992, Muck et al. 2003).

Table 1-1 shows chemical characteristics of millet silage. Millet silage pH (4.3) was higher than that observed in forage sorghum (3.9), grain sorghum (4.0), and sorghum-sudangrass (4.0) silages (Bishnoi et al. 1993). Compared to tropical corn and forage sorghum, pearl millet forage had 50 and 30% lower water soluble carbohydrates concentration before ensiling. Thus, millet silage contained 35% lower lactic acid concentration and a pH 0.5 unit higher than the other silages (Ward et al. 2001). Pearl millet silage had a pH of 4.6, 0.2 unit higher than that of sorghum (Jaster et al. 1985).

Compared to pea with triticale silage, pearl millet silage had lower pH (4.2 vs. 5.3) and higher lactic acid (42 vs. 28 g kg^{-1}) (Messman et al. 1992). In another study by Weinberg et al. (1995), pearl millet and corn ensiled at 200 and 350 g kg^{-1} DM with 97 and 59 g kg^{-1} water soluble carbohydrates, respectively, had similar pH (3.7-3.8) and lactic acid (44-50 g kg^{-1}). In one location, Fisher and Burns (1987) reported that millet silage pH was 3.8, similar to corn silage, while

in the other location, it was 4.4, 0.8 unites higher than corn silage. Variations between locations and cultivars in silage quality were observed when millet silage was compared to those of corn, sorghum and sudangrass (Fisher and Burns 1987).

Table 1-1 Ensiling characteristics of millet in comparison with corn.

	Pearl millet	Forage corn
DM content g kg^{-1}	189-417	305-380
Water soluble carbohydrates g kg^{-1} DM	47-99	80-210
pH	3.7-4.6	3.6-4.0
lactic acid g kg^{-1} DM	26-82	44-109
acetic acid g kg^{-1} DM	2-42	17-39

Adapted from Jaster et al. 1985; Fisher and Burns 1987; Bishnoi et al. 1993; Weinberg et al. 1995; Hill et al 1999; Ward et al. 2001; Han et al. 2006).

1.4. Nutritive value of forage pearl millet

Pearl millet forage has comparable chemical composition to corn and sorghum, except that forage corn accumulates more starch in grains than forage millet and sorghum (Table 1-1). Chemical composition of millet varies between cultivars, stage of development at harvest and growing conditions (Table 1-1). Unlike sorghum and sudangrass, pearl millet produces no prussic acid, with nitrate, oxalic acid and alkaloids concentrations below levels that would affect

forage palatability, animal health or performance (Karejsa et al. 1984; Andrews and Kumar 1992).

Table 1-2. Chemical composition of forage pearl millet relative to annual tropical forages.

Chemical composition (g kg^{-1} DM)	Pearl millet	corn	Sorghum
Neutral detergent fiber	580 - 685	540	605
Aid detergent fiber	313 - 425	295	379
Lignin	35 - 63	49	83
Crude protein	90 - 180	86	133
Degradability	640 - 690	730	630
Net energy of lactation Mcal kg^{-1}	1.3-1.5	2.3	1.1

(Adapted from Banks and Stewart 2003; Fisher and Burns 1987; Grant et al. 1995b; Messman et al. 1992a; Ward et al. 2001; NRC 2001).

Neutral detergent fiber is the main component of pearl millet forage making with CP almost 80% of the forage dry matter. Minor components are water soluble carbohydrates, starch, ash and fat. Thus, most of the digestible energy is derived from NDF fermentation, which depends on NDF composition, concentration and rumen environment.

Lignin and cell wall phenolics are the main factor affecting NDF degradation (Jung and Deetze 1993; Jung and Casler 2006). These compounds are more prevalent in stems than leaves. Cultivar and stage of development at harvest are the main factors affecting lignin concentration by influencing leaf:stem ratio, cell wall thickness and lignification (Aman 1993, Wilson and Hatfield 1997). Lignin concentration in forage millet is similar to that of sorghum and corn when harvested at flowering stage, while phenolics estrified to cell wall were lower in millet than sorghum or corn (Cherney et al. 1988). Stems of millet contained similar lignin content to sorghum and less than maize when harvested at seed formation stage (Lam et al. 1996). Relative to stems of sorghum and corn, stems of forage millet contained the lowest ferulic:*p*-coumaric and highest etherified cell wall phenolics content (Lam et al. 1996).

In vitro studies showed that potentially digestible NDF of pearl millet harvested at full flower stage was 520 to 650 g kg^{-1} NDF (Cherney et al. 1988) and for stems harvested at dough stage in vitro NDF digestibility was 420 to 530 g kg^{-1} of NDF (Thorstensson et al. 1992). Neutral detergent fiber digestibility of pearl millet harvested at vegetative stage for dairy heifers was similar to that of sorghum and corn and averaged 510 g kg^{-1} (Ward et al. 2001), while when harvested at booting stage and fed to sheep, NDF digestibility averaged 700 g kg^{-1} (Cherney et al. 1990).

1.5. Pearl millet forage and animal performance

Various studies reported performance of animals fed forage pearl millet compared with other forages. Hill et al. (1999) compared the feeding value of forage millet for heifers to that of corn silage. The concentrations of CP, NDF and ADF observed were 40, 35 and 47% higher in millet silage while non-structural carbohydrates concentration where 50 % less than that observed in corn silage. The low energy concentration of millet compared with corn led to 20% increase in DM intake of millet silage. Average daily gain was 0.5 kg higher for the heifers fed corn silage compared to those fed millet silage (Hill et al. 1999). In another study, Ward et al. (2001) observed that Holstein heifers consumed 1 kg DM day^{-1} more compared to heifers fed sorghum silage with DM digestibility slightly, but significantly higher for sorghum silage. In their study, pearl millet silage contained slightly more CP and less NDF than sorghum silage.

A study comparing dairy cattle performance on corn or millet silage based diet showed cows fed forage millet silage lost 0.8 kg body weight d^{-1} had 18% lower DM intake than those fed corn silage. This was mainly due to 22% higher NDF and 4% less net energy of lactation in the millet diet (Messman et al. 1992). However, cows fed diet with millet silages produced 23.3 kg d^{-1} milk that contained 3.7% fat and 3.2% protein, not significantly different from the group fed

corn silage. However, when diets are iso-caloric, animal performance on millet or corn silages is expected to be similar.

2. Forage cell wall

Forage cell wall constitutes 500 to 800 g kg^{-1} of most forage. It is chemically extracted as neutral detergent fiber, which is defined as the portion of feed sample insoluble in EDTA and sodium lauryl sulfate neutral solution (Van Soest, 1994). Ruminants have the ability to utilize NDF as an energy source due to the microbiological fermentation in the rumen. Neutral pH (6-7), constant osmotic pressure and anaerobic conditions are maintained in the rumen to provide microbes ideal fermentation conditions. Buffering effect of urea and bicarbonate in saliva, and constant removal of fermentation products keeps the pH values 6-7. Ruminal bacteria (*Ruminococcus Sp.* and *Fibrobacter Sp.*) and protozoa (*Epidinium Sp.*) digest forage cell wall either by attaching to cell wall or by secreting extracellular enzymes. Cellulase and hemicellulases produced in the rumen degrade cell wall polysaccharides to volatile fatty acids; acetate, propionate, and butyrate (Fig x). These metabolites are absorbed through the rumen wall, and are used to synthesize glucose and fatty acids for different functions in the animal (Van Soest 1994; Perry et al. 2003). Proportion of the cell wall polysaccharides available for degradation in the diet determines the amount of organic acids produced.

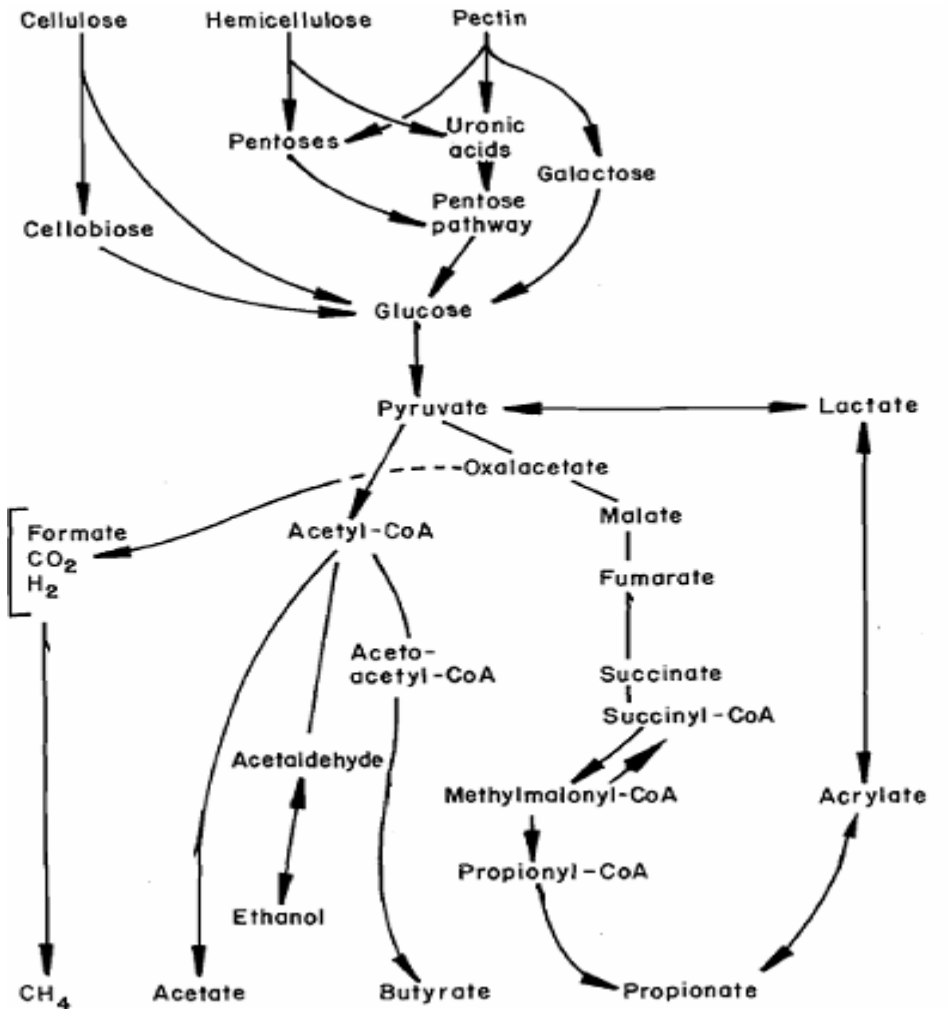


Fig 1-1. Cell wall degradation in the rumen, adapted from Van Soest 1994

Dietary NDF partial and slow digestibility reduces the energy concentration in the diet and DM intake, increase heat increment and reduces productivity (Casler and Jung 2006; Jung and Allen 1995; NRC 2001; Van Soest 1994). However, these effects vary according to NDF digestibility. It is reported that one unit enhancement in NDF digestibility would increase DM intake by 0.17 kg and increase milk yield by 0.25 kg (Oba and Allen 1999). Degradability of NDF

depends on components proportion in the cell wall and their structural interaction, affected mainly by cultivar, stage of development at harvest, growing and harvesting conditions (Mechin et al. 2000; Moore and Jung 2001 Jung and Casler 2006).

2.1. Structural polysaccharides

Structural polysaccharides are the main components of cell wall material. Minor components include lignin, phenolics, structural proteins, silica and cutin (Aman 1993; Carpita 1996). Cellulose and hemicellulose are the major polysaccharides in the cell wall of grasses, while pectins have significant contribution to cell wall of legumes.

2.1.1. Cellulose

Cellulose is the most homogeneous cell wall polysaccharides, made up of linear chain of β 1-4 glucose. However, 15% of the cellulose could be other cell wall sugars such as arabinose and xylose, contributing to its structure (Van Soest 1994; Harris and Smith 2006). Cellulose is usually combined with hemicellulose, lignin, cutin and minerals.

Cellulose is the skeleton of the cell wall and contributes the fibrous character of leaf or stem tissue, making up 20 to 40% of whole forage plant DM (Aman 1993; Harris and Smith 2006b; Van Soest 1994). Concentration of cellulose is higher in stems than in leaves due to structural differences. It

increases with advancing plant maturity and varies widely according to forage species and cultivar (Fritz et al. 1990; Goto et al. 1994; Vogel and Jung 2001; Abedon et al. 2006).

Cellulose is considered a slow degrading component as its linearity, lignin shielding and structural features in the cell wall create major degradation constrains (Hatfield 1993). Yet, there is no evidence of a cross link between lignin and cellulose, however cellulose properties are greatly influenced by lignin shielding (Van Soest 1994; Ishii 1997; Grabber et al. 2004).

2.1.2. Hemicellulose

Hemicellulose is a heterogeneous group of polysaccharides that include xyloglucans, glucourono-arabinoxylan and glucomannan. Xylan chain (β 1, 4) with arabinose, glucouronic acid, or both sugars as a side chain (Carpita 1996; Harris and Smith 2006) is more abundant in C4 grasses (Carpita 1996; Harris and Smith 2006; Taiz and Zeiger 2002). Other minor sugars contributing to hemicellulose structure are galactose, and rhamannos.

Hemicellulose is held in place by lignin crosslink (Van Soest 1994). Delignification makes hemicellulose soluble, and based on that solubility, it could be classified as branched (insoluble in acetic acid) or linear (soluble in acetic acid) xylan (Van Soest 1994; Aman 1993). Hemicellulose also binds cellulose with hydrogen bonds, which contributes to cell wall rigidity. Arabinose:xylose ratio

determines hemicellulose linearity and cross link to other cell wall polymers, contributing to cell wall rigidity. This ratio varies between 1:2 at early stages of plant growth to 1:15 in mature tissues (Goto et al. 1991; Wilson et al. 1997; Jung 2003). Concentration of hemicullose is higher in forage leaves than stems, vary widely between cultivars, and increase with advancing development until seed formation, then stabilize (Fritz et al. 1990; Carpita 1996; Vogel and Jung 2001).

Hemicellulose monosaccharide composition is most heterogeneous in the primary cell wall, which change in the secondary wall to xylose dominant structure, with less side chain substitution (Aman 1993; Scobbie et al. 1993; Abedon et al. 2006). Cell wall structure and concentration in forages vary according to plant part, plant type and cultivar, and stage of development at harvest.

2.2 Variations in cell wall due to plant parts

Plant cells design their cell wall according to their functional requirements. Leaf cells such as mesophyll and paranchyma have functions of photosynthesis and transportation and therefore, their cell walls are mainly primary cell walls with minimum lignification (Aman 1993). Stem cells main function is nutrient transport and structural support, which requires thick lignified cell wall as in xylem and scleranchyma cells (Akin 1989; Aman 1993). Therefore, it is expected that concentration and composition of cellulose and hemicelluloses would be different

between leaves and stems. In table (1-3), it is observed that leaves are composed of tissues that have thinner and more degradable cell walls than stem tissues.

Proportion of leaves and stems in the whole plant is a main factor determining the nutritional value of forages. Factors affecting leaf:stem ratio include stage of development, forage type and cultivars, and environmental conditions (Wilson and Hatfield 1997; Wivstad 1997; Balasako and Nelsen 2003; Collins and Fritz 2003; Volenec and Nelsen 2003;).

2. 3 Variations due to forage type

On average, contribution of glucose, xylose and arabinose to cell wall polysaccharides in grasses, is 52, 30, and 8%, respectively. The corresponding values for legumes are 45, 14, and 5, respectively (Aman 1993; Harris 2005). Cell wall concentration in grasses is higher than legumes, but properties are different due to higher concentration, but less spread of lignin in legumes cell wall (Buxton and Redfern 1997).

Within grasses, commelinated monocots have high concentrations of glucouronoarabioxylans in their cell wall, while non commelinated monocots, have more xyloglucans in their cell wall (Carpita 1996; Harris 2005). Cell wall content is almost 20% higher in C4 grasses than C3 grasses due to higher

proportion of mesophyle and less vascular tissues in C3 grasses (Buxton and Redfern 1997).

Table 1-3: Tissue composition (%) and characteristics in leaves and stems grasses.

	Leaf	Stem	Cell wall type	Cell wall Degradability
Epidermis	22	2	Secondary	Partial
Mesophyll	30	2	Primary	Complete
Paranchyma	14	75	Secondary	Partial
Paranchyma bundle sheath	24	0	Secondary	Partial
Scleranchyma	2	8	Secondary	Indigestible
Vascular tissues	6	12	Secondary	Indigestible

(Adapted from Akin 1989; Buxton and Redfern 1997; Varaga and Kolver 1997; Wilson 1993)

Among warm season annual grasses in north America, cell wall concentration in pearl millet is lower than sorghum, but similar or higher than corn (Cherney et al. 1988; Lam et al. 1996; Ward et al. 2001). Cell wall concentration is expected to vary considerably between cultivars of con (Abedon et al. 2006; Mechin et al. 2000), sorghum and sudangrass (Casler et al. 2003; Fritz 1990), and millet (Zerbini et al. 1999; Zerbini et al. 2002).

2.4 Variations due to stage of development

Impact of advancing stage of development on cell wall concentration and composition is two overlapping factors; increasing cell wall thickness and cross

linking, and increasing stem proportion in the plant (Galyean and Goetsch 1993; Wilson and Hatfield 1997).

Cell walls in most plant tissue develop in three distinctive layers; middle lamella, primary, and secondary cell wall (Jung and Lamb 2003; Khan 2001). Middle lamella is the material filling the intercellular space between the cells, composed of pectic substances. Primary cell wall (0.1 to 0.2 μm thickness) is made up from branched heterogeneous hemicellulose, cellulose, lignin, and structural proteins. Secondary cell wall (1 to 3 μm thickness) develops in certain tissues toward the center of the cell. In grasses, it is composed of cellulose, linear xylan, and lignin (Aman 1993; Buxton and Redfern 1997; Jung 2003; Wilson 1993).

Primary cell wall develops at cell division. Its flexible structure allows the cell to elongate. After the cease of elongation some tissues (such as xylem, sclarenchyma, and stem parenchyma) develop secondary cell wall (Table 1-2). Cell wall thickness might increase 15 fold, which increase the cell wall proportion in the plant on the expense of other components such as moisture and protein (Jung and Lamb 2003). Chemical properties of cell wall also change due to change in monomers proportion and cross linking.

It was shown that in leaves and stems of corn, arabinose concentration is highest in young tissues then declined, while xylose and glucose concentrations

are lowest in young tissues then increase. This means that the arabinose:xylose ratio increase, which indicates the presence of linear xylan with fewer side chains, a characteristic of secondary cell wall development. Concentration of other minor cell wall sugars such as galactose, mannose, and uronic acid decreases, since they exist only in the middle lamella and primary cell wall (Abedon et al. 2006; Jung 2003; Scobbie et al. 1993; Sudekum 1994). Further more, ferulic acid estrified to arabinoxylan in primary and secondary cell walls would become ether linked to lignin, which change properties of cell wall considerably.

Increased cell wall thickness will reduce rate and extent of structural polysaccharides degradability (Mertens 1993). This is due to an increase in concentration of less degradable cellulose and linear xylan and a decrease in concentration of the rapidly degradable branched hemicellulose (Fritz et al. 1990; Jung 2003; Sudekum 1994). The cross linkage with lignin reduces microbial attachment and reduce the ability of enzymes to attack structural polysaccharides, which reduces the extent of cell wall degradability (Jung and Deetze 1993; Buxton and Redfern 1997; Jung and Casler 2006).

These changes are more prevalent in stems than leaves with advancing development. Before flower development, leaves and stems tissue have almost comparable degradability. However, during and after flowering, stem tissues

elongate and increase their cell wall thickness through deposition and cross linkage of linear structural polysaccharides and lignin. This will increase stem and reduce leaf proportion in the whole plant, leading to a reduction in plant cell degradability (Galyean and Goetsch 1993; Jung 2003; Wilson and Hatfield 1997).

2.5 Lignin in forage

A minor component of cell wall, but with greatest effect on cell wall properties and degradability is lignin. It is an amorphous polymer of monolignols (hydroxycinnamyl (H), guaiacyl (G), and syringyl (S) units) formed at the plant cell wall (Baucher et al. 1998; Vogel and Jung 2001). Phenolic acids such as *p*-coumaric and ferulic acids attached to lignin and other cell wall polymers, which contributes to Lignin characteristics. Lignin deposition in the cell wall is part of the maturation process to support cell structure. It provides structural integrity for important plant tissues such as the epidermis, sclarenchyma and xylem. Furthermore, it provides a water proof coat of the cell that would limit fluid movement horizontally (Baucher et al. 1998; Moore and Jung 2001). Physical and chemical properties of lignin, along with cross link to phenolic acids have significant impact on cell wall physical and chemical properties, which prevent ruminal microbial enzymes from degrading cell wall, which reduces forage quality (Hatfield et al. 1999; Boerjan et al. 2003; Grabber 2005).

2.5.1. Lignin synthesis

Lignin is a product of polymerization of *p*-coumaryl, coniferyl, and sinapyl alcohol (Figure 1-1) the shikimic acid pathway (Figure 1-2). The pathway starts by the deamination of phenylalanine to produce *p*-coumaric acid, ferulic acid and sinapic acid (Ishii 1997, Boerjan et al. 2003).This step utilize the enzymes phenylalanine ammonia-layse (PAL), cinnamate-4-hydroxylase (C4H), 4-coumarate-3-hydroxylase(C3H), 0-methyltransferase (OMT), ferulate-5-hydroxylase (F5H). Theses cinnamic acids will form cinnamoyl CoA by hydroxycinnamate CoA ligase (4CL) then cinnamic aldehydes by cinnamoyl CoA reductase (CCR) and finally cinnamic alcohols by cinnamyl alcohol dehydrogenase (CAD. Oxidation of 4-hydroxycinamyl alcohol, coniferyl alcohol, and sinapyl alcohol will form monomers (H units, G units and S unit, respectively) that polymerize to form lignin (1-1) (Baucher et al. 1998).

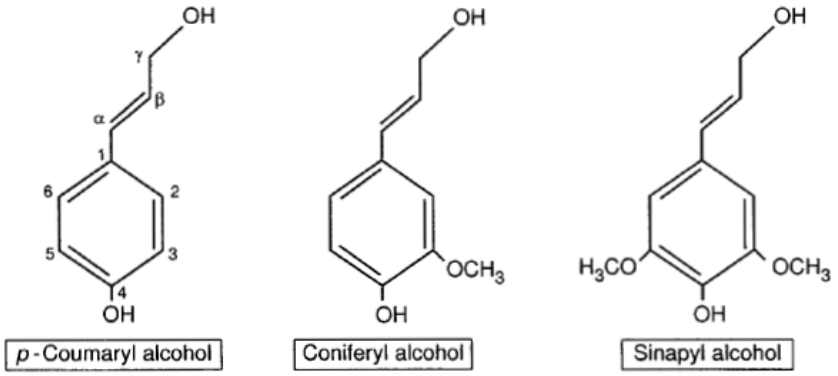


Figure 1-2. Main phenolic monomers contributing to lignin formation (Baucher et al. 1998)

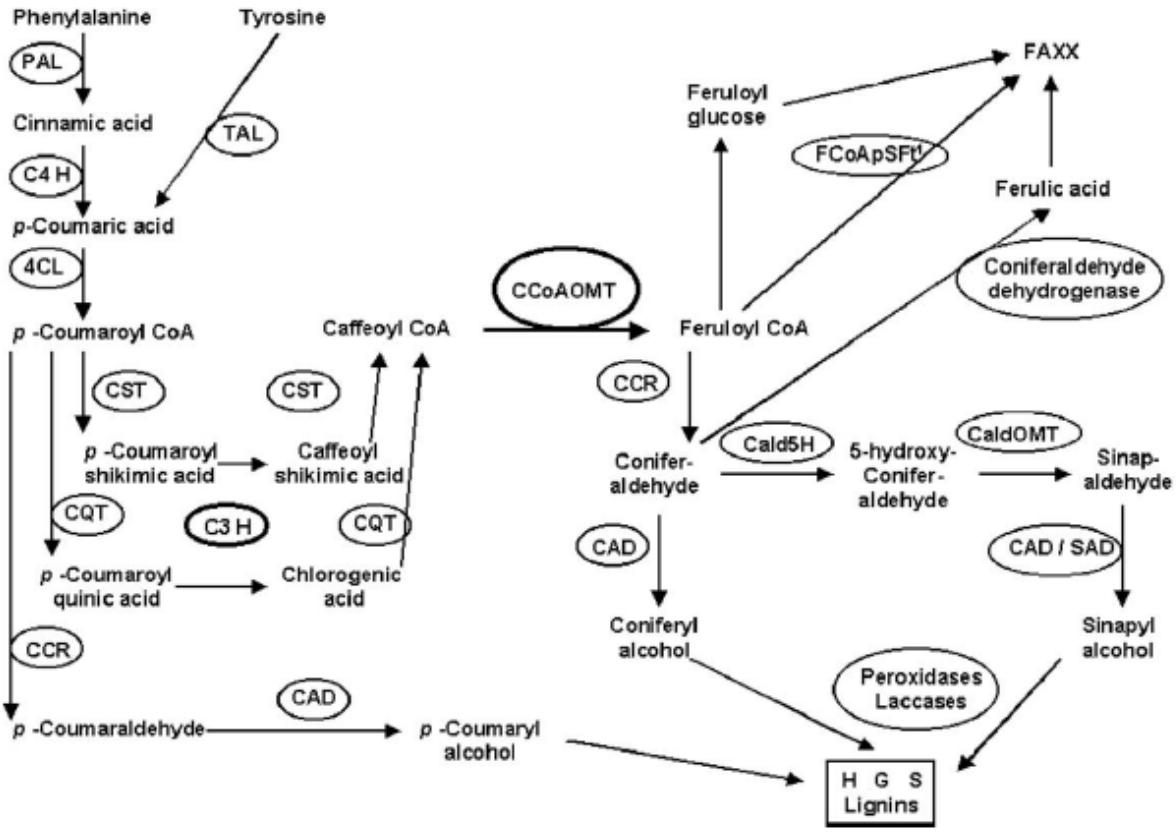


Figure 1-3. Lignin synthesis pathway (Barriere 2003).

2.5.2. Variation in lignin composition and concentration

Lignin synthesis is a web rather than a linear pathway (Moore and Jung 2001). In different cultivars, morphological parts, stages of cell growth or environmental conditions, enzymes activities would change the pathway and eventually affect lignin structure or concentration (Table 1-4) (Moore and Jung 2001; Van Soest 1994). For example, corn lignin has more S and less G unites than wheat lignin, proportion of S units in lignin increase with advancing

development. Lignin rich in S units is linear and more frequent in internodes, while lignin rich in G units is branched and more frequent in leaves, and S:G units vary with season (Mechin et al. 2000; Moore and Jung 2001; Grabber et al. 2004; Grabber 2005).

Modifying lignin concentration or composition, and thus its impact on nutritive value, results from manipulating these factors. Genetics and breeding play an important role in manipulating lignin synthesis (Casler 2000; Ralph et al. 2004; Grabber 2005). Manipulating lignin structure does not always produce positive outcome. It was reported that lignin enriched with coniferyldehyde, rather than coniferyl alcohol, was found to be more hydrophobic, and have more detrimental effect on cell wall degradability than lignin made with coniferyl alcohol (Grabber et al. 1998). Negative impact on vigor and reproductive ability of plants was observed after manipulating shikimic acid pathway enzymes (Casler 2000; Casler et al. 2003).

2.6. Cell wall phenolics

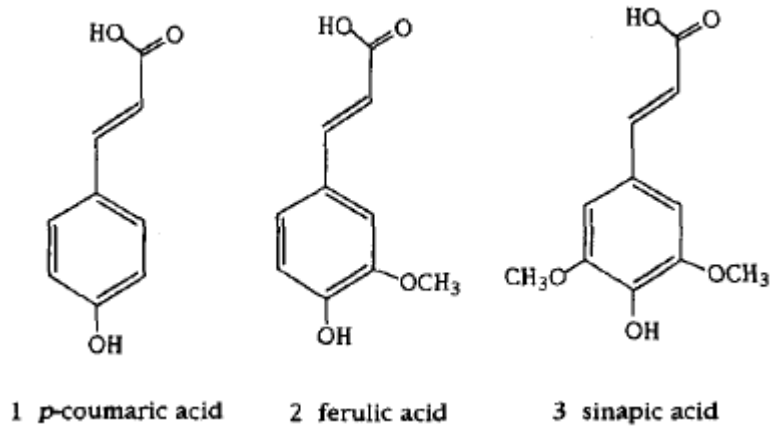
Cell wall phenolics play an important role in determining the relationship between lignin and other cell wall polymers. Ferulic and *p*-coumaric acids (Figure 1-3) are the major phenolics attached to cell wall polymers through ester or ether

Table 1-4. Concentration of lignin and cell wall phenolics concentrations in various forages (g kg^{-1} of NDF).

Forage	Lignin	Ester-*p*-coumaric acid	Ester-ferulic acid	Ether-ferulic acid	Reference
Corn Stems	56-251 [b]	0.8-68.2	1.2-11.8	0.4-2.3	Goto et al. (1994), Méchin et al. (2000), Abedon et al. (2006), Jung and Casler (2006)
Corn Leaves	143-162 [b]	1.3-13.0	3.0-8.2	1.0-1.8	
Sorghum, Sudangrass Sorghum X Sudangrass	62-174[a]	5.4-16.0	2.8-9.9	2.0-3.8	Cherney et al. (1988), Fritz et al. (1990), Grant et al. (1995), Lam et al. (1996) Morin et al. (2005)
Millet	63-75	2.0-26.0	3.0-7.0	3.5-7.3	Cherney et al. (1988), Hartley et al. (1992), Lam et al. (1996)
Barley straw	108-135[b] 34-43 [a]	3.3-3.4	1.0-2.3	2.43	Hartley and Keene (1984), Jung et al. (1997), Sun at al. (2002).
Alfalfa	208-417[b] 80-200[a]	0.01-5.3	0.01-2.4	0.04-0.06	Jung et al. (1997), Jung et al. (2000)
Broom grass	132-158 [b]	1.6-2.7	3.6-5.0	3.7-5.4	Casler and Jung (2006)

[a] Acid detergent lignin, [b] Klason Lignin

link (Jung and Allen 1995; Sun et al. 2002; Grabber 2004). Other phenolics that could be found in the cell wall; sinapic acid, p-hydroxybenzoic acid, p-hydroxybenzaldehyde, vanillic acid, syringic acid, vanillin, syringaldehyde, acetovanillone, acetosyringone and sinapic acid (Ostrander et al. 1999; Grabber et al. 2004). Links of some of these phenolics and cell wall polymers is not fully described yet.



1 *p*-coumaric acid 2 ferulic acid 3 sinapic acid

Figure 1-4. Phenolic acids attached to cell wall (Jung and Allen 1995).

2.6.1. Ferulic acid

Ferulic acid is estrified to the C_5 of the arabinoxylan terminal during cell wall elongation (Figure 1-4). After the cease of cell wall elongation, lignin will deposited on ferulic acid in an ether link, making it the nucleation site for lignin polymerization (Figure 1-5) (Hatfileld et al. 1999; Grabber 2004). Ferulic acid recovered through breaking the ester bond indicates the frequency of potential

lignin nucleation sites, while the one recovered after breaking the ether bond indicates the association of lignin with cell wall polysaccharides, or polysaccharide cross link (Grabber 2004, Ishii 1997, Sun 2002). Ferulic acid esters reduce cell wall digestion rate by obstructing enzyme alignment, while etherified FA reduces extent of cell wall digestion by protecting the cell wall from enzymatic exposure (Jung and Allen 1995).

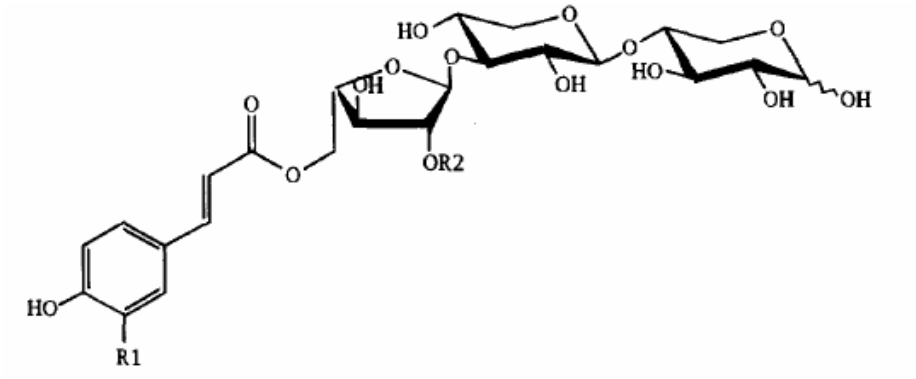


Figure 1-5. Ferulic acid estrified to arabinoxylan in cell wall (Ishii 1997).

2.6.2 *p*-Coumaric acid

Most of the *p*-coumaric acid is estrified to the S unit in lignin, serving as a good indicator of lignifications. It is also believed that *p*-coumaric acid enhances deposition of S units in lignin, making structure more linear. There is no direct relation between *p*-coumaric acid and digestion (Jung and Allen 1995; Sun et al 2002).

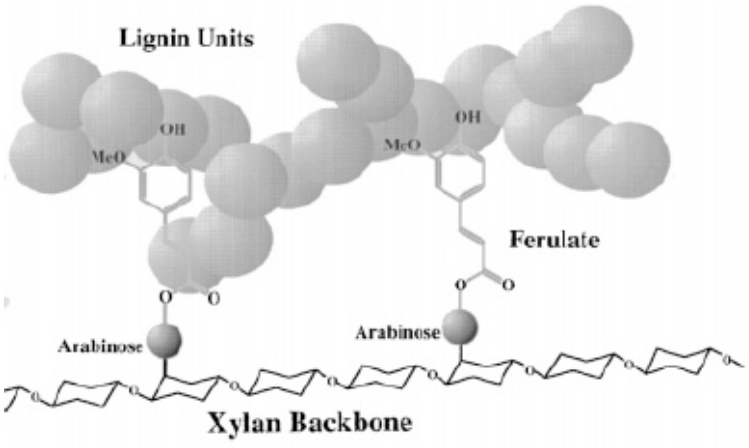


Figure 1-6. Deposition of lignin on ferulic acid crosslinked to arabinoxylan (Hatfield et al. 1999).

2.6.3. *p*-coumaric: ferulic acids

Ratio of *p*-coumaric:ferulic acid has a big influence on cell wall degradability. At a certain concentration of lignin, this ratio affect digestibility in two different scenarios. High *p*-coumaric:ferulic acid ratio indicates uniform distribution of lignin in the plant tissue, while low *p*-coumaric:ferulic acid ratio indicates localization of lignin at certain parts of the tissue (Grabber et al. 2004). Concentration of these phenolics along with their ratio will further explain the degree of lignification as lignified plant tissues such as xylem and schlerncyma contain more cell wall phenolics and higher *p*-coumaric:ferulic acid ratio than parenchyma tissues (Chesson et al. 1997; Hatfield et al. 1999). Figure 1-6 show

the complexity of the known crosslinks between cell wall polysaccharides, lignin, and cell wall phenolics (Baucher et al. 1998).

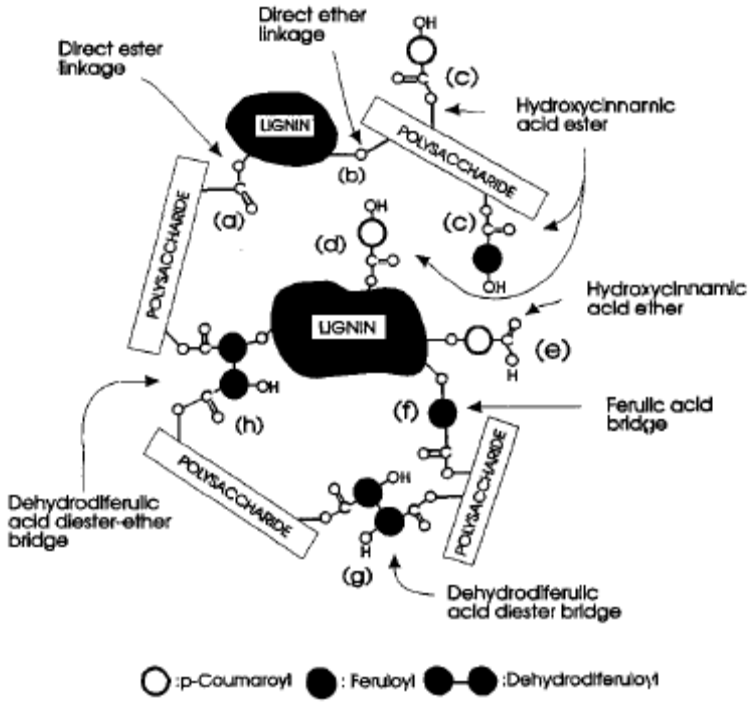


Figure 1-7. Complexity of crosslinks between cell wall polysaccharides, lignin, and phenolics (Baucher et al. 1998).

2.7 Lignin and cell wall degradability

Physical and chemical properties, complexity, cross-linking and various interactions with cell wall polysaccharides and cell wall phenolics (Figure 1-6) explain lignin's impact on cell wall degradability. These effects were highlighted and summarized by Buxton and Redfearn (1997), Jung and Allen (1995), Jung

and Deetze (1993), Jung et al. (2000), Moore and Jung (2001), Oba and Allen(2000a) and VanSoest (1994) in the following points:

1. Lignin is a hydrophobic complex that resists degradation.
2. Lignin acts as a physical barrier between enzymes and cell wall polysaccharides.
3. Lignin reduces bacterial attachment and enzyme alignment to cell wall polysaccharides.
4. Lignin increases cell wall rigidity, which reduced brittleness of plants tissues.
5. Lignin reduces microbial accessibility to digestible parts of the cell wall.

All these effects will increase the percentage of indigestible fiber, reduce the concentration of digestible energy in the diet, increase rumen filling and eventually reduces feed intake. Lignin and ether linked ferulic acid concentrations are highly correlated with DM intake, in vitro DM and NDF digestibility (Cherney 1991; Marvin et al. 1995; Jung et al. 1997; Mechin et al. 2000).

Reducing lignin concentration or modifying structure would produce more digestible NDF digestibility. It is reported that one unit increase in NDF digestibility would increase DM intake by 0.17 kg and increase milk yield by 0.25 kg (Oba and Allen 1999). Choosing forage cultivars with lower lignin concentration, or modified structure an effective way to achieve higher NDF

degradability. Brown Midrib mutants of corn, sorghum, or millet produce less lignified and more digestible fiber.

3. Brown midrib trait

Brown Midrib trait was first discovered in corn plants by Jorgenson (1931). The name came from the brownish coloration of the central vein in the leaf of corn. The recessive homozygous state of this trait results in a better cell wall composition, which made this mutation interesting for forage producers and animal nutritionists. In 1975, brown midrib mutation was introduced into sorghum, and later in 1988, it was introduced into pearl millet. Further transfer of this trait to other crops was not successful (Cherney et al. 1991).

No botanical differences were noticed between brown midrib and normal corn, sorghum or pearl millet (Akin 1989; Cherney et al. 1991). The modification in lignin concentration and composition seem to be in less lignified tissues of the plant. Brown midrib plants seem to have reduced secondary wall development compared with normal cultivars (Wilson and Hatfield 1997).

3.1. Types of brown midrib mutation

Influence of the brown midrib mutation on lignin concentration and structure depends on the genetic background of the cultivar on which the mutagenesis has been applied, and on the enzymes affected by mutation. Four types of brown midrib mutations in corn, 19 in sorghum have been fully described

(Barriere et al. 2004; Cherney et al. 1991). The most popular enzymatic modification is down regulation of caffeic acid O-mytheltransferase (COMT), which is wide spread in different brown midrib corn and sorghum. Reducing the activity of this enzyme reduces lignin concentration and S:G units ratio in lignin polymer. A less frequent modification in lignin synthesis enzymes is down regulation of cinnamyl alcohol dehydrogenase (CAD). Reduced CAD activity also reduces lignin concentration, incorporate cinnamyl aldehyde unites in lignin and reduce S:G ratio (Vogel and Jung 2001; Casler et al. 2003). Usually, lignin content of the brown midrib mutants is 5 to 50% less than there counterparts (Cherney et al. 1991). Concentration of *p*-coumaric acid and other phenolics were reduced in brown midrib mutants of corn and sorghum as well as the *p*-coumaric:ferulic acid ratio. However, ester and ether linked ferulic acid concentration were reduced or did not change in different brown midrib mutants compared with normal (Grant et al. 1995; Wilson and Hatfield 1997; Ostrander et al. 1999; Mechin et al. 2000).

3.2. Impact of nrown midrib trait on forage nutritive value

3.2.1. Impact on chemical composition

Several studies reported significant impact of brown midrib trait on cell wall concentration and structure, and to less extent, CP concentration. Concentrations of NDF, ADF and ADL in brown midrib corn, sorghum, sudangrass and millet

were less than the concentrations observed in normal counterparts (Casler et al. 2003; Cherney et al. 1991; Goto et al. 1994; Morin et al. 2005; Oliver et al. 2003; Ostrander 1999). However, other studies reported no differences in NDF and ADF concentrations (Aydin et al. 1999; Grant et al. 1995; Greenfield et al. 2001). Cellulose concentration was reduced while hemicellulose fraction increased in brown midrib corn compared to normal cultivar (Goto et al. 1994). However, this effect was not consistent, and varied between cultivars (Wedig et al. 1988). Effect of brown midrib trait on proportion of sugars in plant cell wall was not consistent across studies. Fritz et al (1991) reported no effect of the brown midrib trait on sugars proportions in the cell wall, while Cherney et al. (1986) and Goto et al (1994) reported increased xylose and arabinose proportion in the cell wall.

The brown midrib trait had the most noticeable impact on lignin concentration, which was reported to be 5-50% less than the concentration observed in the normal cultivar of corn, sorghum, sudangrass and millet (Casler et al. 2003; Cherney 1990; Grant et al. 1995; Oba and Allen 2000). The Structure of the lignin is affected by the brown midrib trait. Ratio of syringyl: guaiacyl monomers were reduced by the brown midrib trait in corn and sorghum stems, but not in millet (Lam et al. 1996; Mechin et al. 2000), which indicate that brown midrib lignin is less linear. The brown midrib trait also reduces concentration of phenolic acids estrified or etherified to cell wall polymers (Barriere et al. 2004;

Cherney et al. 1988; Mechin et al. 2000). In general, brown midrib trait reduced ester linked *p*_coumaric acid concentration and ether linked ferulic acid, but the effect on ester-ferulic acid concentration was not consistent between studies. Reduction in ester linked ferulic acid concentration indicates less lignin nucleation sites on the cell wall, while reduction in ether linked ferulic acid indicates reduced crosslink between lignin and hemicellulose. When concentration of ester linked *p*_coumaric acid is reduced, it indicate less frequency of syringyl unit in lignin deposited in the cell wall.

Decline in concentration, cross link, and change in structure of lignin in brown midrib forages explain the increased in vitro DM and NDF degradability when compared with the normal counterparts. It is reported that brown midrib corn, sorghum, and sudangrass had respectively 110, 20, and 40 g kg^{-1} higher NDF degradability than normal cultivars (Oba and Allen 1999; Casler et al. 2003; Lewis et al. 2004; Oliver et al. 2005). This is mainly due to higher proportion of digestible tissues in leaves and stems of the brown midrib plants (Akin et al. 1993; Cherney et al. 1991). Increase in stems NDF degradability was most noticed because of the higher proportion of lignified tissues that are influenced by the brown midrib trait (Mechin et al. 2000). Degradation of cell wall sugars is higher in brown midrib plants than normal specially for glucose and xylose (Goto et al. 1994) as correlated with lower lignin concentration (Jung and Buxton 1994).

Due to increase in NDF degradability, animals fed brown midrib forages are expected to have higher DM intake, whole improvement of diet digestibility, or both, which would lead to better animal performance (Cherney et al. 1991).

3.2.2. Impact on animal performance

Improvement in animal performance on brown midrib forages depends on the type of cell wall modification that occurred, which varies with the type of mutation in the forage. The effect of the brown midrib trait on lignin concentration and structure is the main factor that would affect animal performance on these forages. Lignin has a strong negative impact on forage intake and digestibility (Cherney et al. 1991; Jung and Allen 1995; Van Soest 1994). Dry matter intake is expected to increase when animals are fed low lignin forages, such as those affected by the brown midrib trait.

Ruminal NDF disappearance increased when lignin concnetartion was reduced. It was observed that the extent of NDF digestion was 5-10% higher in brown midrib compared to normal sorghum cultivars (Fritz et al. 1988). Similar observations were made on two brown midrib and normal sorghum x Sudangrass cultivars leaves and stems (Fritz et al. 1990). The reduced lignin concentration is expected to allow more bacterial attachment and enzyme alignment to digest cell wall polysaccharides, and reduce cell wall hydrphobicity. Therefore, acetate, propionate and butyrate concentrations produced from fermenting NDF from

brown midrib forages would be higher than that of normal forages. This results in higher digestible energy harvest from NDF in forages affected by the brown midrib trait, and greater energy utilization for production from the whole diet (Oba and Allen 2000a).

Reduction in lignin concentration also reduces the rigidity of the cell wall (Jung and Moore 2001), which enhance particle size reduction which in turn will facilitate digestion, and increase passage rate (Oba and Allen 2000 c). The increased passage rate is expected to reduce rumen fill and allow higher feed intake.

Because of the high NDF disappearance, and enhanced particle size reduction of brown midrib forages, they are considered to have less rumination stimulating effect on animals. This may slightly reduce, but consistent rumen pH, and thus affecting methane production, without impacting acetate: propionate ratio (Oba and Allen 2000a). This means that brown midrib forages might increase metabolisable energy supply to the animal. Further more, the efficient fermentation of NDF, and the high passage rate observed when cows feed on brown midrib forages would increase microbial flow to the duedinum, rumen nitrogen utilization, as well as bypass of nutrients that are better utilized in the duodenum, such as starch.

Cows fed brown midrib corn silage (35% less lignin and 20% more in vitro NDF degradability) consumed one kg d^{-1} more than control group. This was due to higher passage rate (3.34% h^{-1} vs. 3.06% h^{-1}) which was caused by enhanced particle size reduction. Increased passage rate increased protein and starch bypass and microbial flow from the rumen to the fore stomach, which eventually increased milk yield in brown midrib corn silage group by 4 kg d^{-1} and increase 3.5% fat corrected milk and solid corrected milk (Oba and Allen 2000a, b, c). The authors reported that total tract digestibility for both types of corn silage were similar between the two groups. The increased passage rate in cows fed the brown midrib corn diet offset the higher digestibility between the two groups. Greenfield et al. (2001) explained the enhanced animal performance on brown midrib corn diet by better DM digestion, duodenal starch flow, and reduced nitrogen excretion.

Performance of cows fed brown midrib sorghum silage was also better than those fed normal cultivar in terms of higher DM intake, milk production and milk fat yield (Aydin et al. 1999). However, this increase in animal performance was sue to higher NDF digestibility, rather than increased passage rate, and shift of nutrient digestion to the hind gut observed in Oba and Allen (2000 a,b,c). Long term milk production (10 weeks) was increased by the brown midrib sorghum diet compared to the normal one (Aydin et al. 1999). The increase in

NDF degradability due to the brown midrib trait would increase either passage rate and feed intake, or NDF digestibility. The increase in either or both of these factors would have a positive impact on milk yield.

In another study with sorghum, Oliver et al (2004) showed that brown midrib trait type 6 had a positive impact on total tract NDF digestion, but not the 18 brown midrib trait type. Cows fed brown midrib sorghum type 6 had similar performance to those fed corn, which was higher than that of brown midrib 18 and conventional sorghum diets. This indicates that not all brown midrib trait types would have an impact on animal performance.

3.3.3. Impact on forage agronomics and economics

Although brown midrib mutants produce better quality forage, yield is negatively affected by this trait. Reduced plant height, tillering, and higher leaf:stem ratio are the main reasons for yield depression in brown midrib compared with normal forages (Casler et al. 2003; Oliver et al. 2005). The brown midrib locus negatively interacted with other loci that control tillering, height, and environmental sensitivity. Miron et al. (2006) showed that reduction in brown midrib sorghum yield was due to lower DM content and lodging of plants compared to normal sorghum cultivar. The severity of yield reduction depend on the type of mutation and other field factors. Casler et al. (2003) reported yield depression between 60 to 80%, while Oliver et al. (2005) showed that brown

midrib-6 and brown midrid-12 yielded 85% and 90%, respectively of the normal cultivar yield, which also varied according to the original cultivar genetic background. However, Morin et al. (2005) showed no differences between brown midrib and other sorghum cultivars in DM yield and morphological characteristics.

Net economical return resulting from higher brown midrib forage quality but less quantity was shown to be equal or less than normal cultivar in sorghum x sudangrass (Casler et al. 2003). Cox et al. (2001) reported that brown midrib corn had inconsistent impact on predicted milk yield ha^{-1}. This character varied with stage of maturity and cutting height of plants (Lewis et al. 2004). However, when difference between normal and brown midrib cultivars yield are small, superior net return from brown midrib cultivars is expected.

4. Objective and hypothesis of this research

After reviewing the literature, information about millet forage adaptability to southern Quebec conditions is required. Numerous studies were conducted on annual forages such as sorghum and sudangrass in north-eastern US and south eastern Canada area, but studies on millet forage are scarce. Millet would provide alternative source of forage during drought summer years, something that occurred during summers of 2001 and 2002 in areas of southern Quebec, and southeastern Ontario. Yield and agronomical characteristics of millet vary widely between cultivars, and are very dependent on location and environmental

conditions. Changes in yield and agronomical characteristics of millet forage when seeding rate or stage of development at harvest change need to be assessed.

Forage cultivar and development stage at harvest are the main factors affecting chemical composition and degradability of forages. Brown midrib trait impact on forage quality is well documented, and would vary according to the type of mutation, which was not characterized for millet. Impact of stage of development on chemical composition changes in regular millet or millet with brown midrib trait was not previously investigated. Such information is vital to make decision to manage and harvest the forage for best yield-quality combination.

Changes in leaves and stems cell wall structure and degradability in millet affected by brown midrib trait and stage of development at harvest was not previously studied. This knowledge would provide knowledge on how the brown midrib trait and advancing maturity affect cell wall neutral sugar and phenolics concentration and cross linkage, and therefore, how availability of cell wall polysaccharides for rumen microbes would be affected. This would contribute to understanding the type of brown midrib mutation in millet, which was not previously characterized.

Previous studies reported poor ensilability of millet. Effect of lactic acid bacteria addition to millet forage before ensiling should be investigated. This would determine the need for this practice and its impact on silage acidification, proteolysis, aerobic stability, and nutritive value. Studies on the impact of inoculation on brown midrib millet are scarce.

This research has the following objectives:

1. Evaluate the impact of seeding rate on yield, chemical composition, ensiling characteristics and in vitro degradability of regular and brown midrib millet.
2. Investigate the effect of the brown midrib trait and stage of development at harvest on cell wall concentration, composition, structure, and degradability in millet leaves and stems.
3. Determine the impact of brown midrib trait, and stage of development at harvest on yield, ensilability, chemical composition and in vitro degradability.
4. Study the effects of adding lactic acid bacteria to regular and brown midrib millet forage on ensiling characteristics, chemical composition, and in vitro degradability.

Hypotheses of the research are:

1. Cultivar and seeding rate will impact yield, agronomical characteristics and chemical composition of forage millet. Reducing seeding rate will increase tillering, and thus have no or small negative effect millet yield.

2. Brown midrib trait will reduce fiber concentration and increase degradability in millet forage. Lignin and cell wall phenolics concentrations are expected to be reduced in brown midrib stems, and to a less extent, in leaves. These changes are expected to increase cell wall degradability in brown midrib millet. However, brown midrib trait have negative influence on forage yield.

3. Advancing stage of development at harvest will have significant impact on yield and chemical composition of RM and BM by increasing fiber component and reduce CP concentration. This is expected to reduce DM and NDF degradability. However, advancing stage of development is expected to impact quality of RM and BM differently.

5. Ensiling millet forage is expected to be difficult due to it's high moisture content. Inoculating millet forage will improve fermentation and aerobic stability of the two millet cultivar.

CHAPTER II.

EVALUATION OF SEEDING RATE EFFECTS ON AGRONOMICAL CHARACTERISTICS, CHEMICAL COMPOSITION, AND ENSILING OF REGULAR AND BROWN MIDRIB FORAGE MILLET

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Will be Submitted to Forage and Grazingland Journal

Abstract

A study was conducted to evaluate the impact of seeding rate on forage yield, agronomical characteristics and chemical composition of regular and brown midrib millet. Regular (RM) and brown midrib (BM) millet were seeded at a rate of 5, 10, and 15 kg ha^{-1} at 2 sites in southwestern Quebec. Forage was harvested at the vegetative stage, and ensiled for 28 d in laboratory silos. Forage yield increased with increasing seeding rate for both cultivars, mainly due to increase in number of plants m^{-2}, and increase in tillers m^{-2}. Regular millet yielded 56% more (P <0.05) than BM, mainly due to lower (P < 0.05) plant height, and tillers m^{-2} in BM. Leaf: stem ratio increased (P <0.05) with increasing seeding rate for both cultivars, with BM having a higher ratio than RM. Impact of seeding rate on forage quality was minimal. Concentrations of CP, NDF, ADF and ADL averaged 136, 604, 387, and 29 g kg^{-1} for RM and 157, 580, 336, and 20 g kg^{-1} for BM, respectively. Differences in chemical composition caused the in vitro dry matter digestibility of BM to be 10% higher (P <0.05) than that of RM. Seeding rate or millet cultivar had minimal effect on ensiling characteristics. All silages ensiled well with an average pH of 4.2, 120 g kg^{-1} lactic acid, and 4.5 days of aerobic stability. Increasing seeding rate is the main option to increase yield of RM and BM.

Introduction

Most forage fed to dairy cattle in northeastern US and eastern Canada are perennials. Due to the recurrence of winterkill of perennial species, annual forages are gaining importance. Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is an annual grass that can be used as an emergency forage species in early- mid summer, when corn is not ready to harvest. It tolerates drought, resists most diseases affecting corn [*Zea mays* (L.)], and unlike sorghum [*Sorghum bicolor* (L.)] does not produce prussic acid (Kumar and Andrews 1993). About 0.5 million hectares of millet are grown in the US (Gray et al. 2000), mainly in the south, where 5% of the dairy cattle are fed forage millet. This species is not commonly used in eastern Canada. However, the recent development of regular and brown midrib cultivars adapted to local conditions, making it possible to grow this species in northern areas, should result in an increase in use locally (AERC 1998).

The brown midrib trait in corn, sorghum, sudangrass, and millet affects forage yield and quality, when compared to normal cultivars (Barriere et al. 2004; Hassanat et al. 2006). Forages with the brown midrib trait have low activity of lignin synthesis enzymes, mainly caffeic acid O-mytheltransferase and cinnamyl alcohol dehydrogenase (Vogel and Jung 2001; Barriere et al. 2004) This changes lignin structure and concentration, and might cause up to a 50% reduction in

lignin concentration (Hassanat et al. 2006; Lam et al. 1996), which has a positive impact on animal performance (Aydin 1999). The drawback of the brown midrib trait is that it reduces forage yield (Casler et al. 2003; Hassanat et al. 2006).

Seeding rate is also a factor affecting forage yield and quality. Grasses response to varying seeding rates depending on their ability to tiller, as tillers might contribute to 75% of the total plant biomass (Barriere et al. 2004; Bidinger and Raju 2000). Seeding rates optimizing forage yield and quality can vary depending on the species. Nonetheless, there is a general agreement on the positive impact of increasing plant density on forage yield (Cox et al. 1998; Graybill et al. 1991). The extent of improvement in yield varies between cultivars, year, and location. Some cultivars require less plant per area to achieve maximum yield, while others might suffer from yield loss at very high plant densities (Cox et al. 1998; Graybill et al. 1991; Subedi et al. 2006). For example, in high tillering cultivars, tillers number and contribution to plant yield is high at low plant densities, which is reduced severely with increasing plant densities (Bidinger and Raju 2000, Carberry et al. 1985). The AERC (1998) which developed new forage millet cultivars for eastern Canada, currently recommends seeding regular and BM at rates of 16 and 18 kg ha^{-1} respectively. Lower seeding rates might be preferable due to the current relatively high cost of seeds;

information on the impact on forage yield of lower seeding in eastern Canada is currently limited.

Forage quality may be reduced at high seeding rates. Some studies showed that CP and IVTD is often reduced, while NDF concentration is increased with increasing plant density (Cusicanqui and Lauer 1999; Widdicombe and Thelen 2002), but others showed no effect of plant density on forage quality (Cuomo et al. 1998). Recommended seeding rate of millet varies between 5-33 kg ha^{-1} depending on growing purpose (i.e. grain or forage), cultivar, tillering ability, seedbed conditions, and sowing method (Bidinger and Raju 2000, Maiti and Wesche-Ebeling 1997). The objective of this study was to evaluate the impact of seeding rate on yield, chemical composition, ensiling characteristics and in vitro degradability of regular and BM grown in southwestern Quebec.

Materials and methods

Field condition and ensiling

An experiment was established in 2005 at 2 sites in Sainte-Anne-de-Bellevue, QC, Canada (45°25′N, 73°56′W). Total rain and average temperature for the two sites during the 2005 growing season (June, July and August) was 288 mm and 21.8 ºC, respectively compared to values of 268 mm and 19.4 ºC for the 30-yrs average for the same time period. Seeding of the two forage millet cultivars: regular (CFPM101) and brown midrib (CFPBMR) was done at 5, 10 and

15 kg ha^{-1} pure germinating seeds. Soil (sandy loam) was fertilized with 250 kg ha^{-1} of 19-19-19 (N-P_2O_5-K_2O) before seeding. Seeding was done on June 6 and 13 2005 at site A and B, respectively, using a disc-drill seeder (Fabro, Swift Current, SK, Canada) in 0.35 × 5 m plots. Weeds were controlled using Pardner (Hydroxybenzonitrile) at a rate of 1 L ha^{-1}. Harvesting was done manually at a 7 cm stubble height when plants had an average of 8 fully developed leaves (i.e., in the vegetative stage). Yield, number of plants and tillers m^{-2}, plant height, and leaf: stem ratio were recorded. Plants from each plot were then chopped using a forage chopper (Pern-Ohio Machinery Company, Erie, PA) to a theoretical length of 2.5 cm. Chopped material was either sampled (500 g) and dried for 48 hour at 55°C in a forced-air oven, stored at –20°C for later analyses, or packed into polyvinyl chloride laboratory silos (7.6-cm diameter × 25-cm height, (Sebastian et al. 1996)). A manual pestle was used to pack laboratory silos, which were sealed after packing with a plastic cap fitted with a small valve to permit gas release with no exchange. Laboratory silos were stored at room temperature and weighed before and after the 28 d ensiling period to estimate dry matter recovery.

Chemical analyses.

After 28-d of ensiling, silos were opened and the ensiled forage was mixed thoroughly. Samples of fresh and ensiled forage (50 g) were homogenized with 500 mL of distilled water for 5 min to obtain water soluble extract. Forage extracts

where used to determine water soluble carbohydrates concentration (Dubois et al. 1956). The pH of silage extracts were immediately determined using an Accumet pH meter (Denver Instrument Company, Mansfield, TX). One milliliter of silage extract was combined with 200 µL of 25% metaphosphoric acid containing 2-ethyl butyric acid as an internal standard. Samples were centrifuged for 15 min at 10 000 × g and analyzed for lactic, acetic, propionic, and butyric acid by gas chromatography (Hewlett Packard model 5890 series II, equipped with flame ionization detector and model 7673 auto injector; Hewlett Packard, Palo Alto, CA) equipped with 15 m Nukol fused capillary column (Supelco Inc., Bellefonte, PA). Column temperature was fixed at 150°C for a run time of 8 min. Injector and detector temperatures were 180 and 200°C, respectively. Gas flows were 30, 300, and 30 mL min–1 for He, air, and H_2, respectively.

Five hundred g of fresh and ensiled forages were also dried in a forced-air oven at 55°C for 48 hour and then ground through a 1-mm screen using a Wiley mill (A. H. Thomas, Philadelphia, PA). Ground samples were then used to determine concentrations of neutral (NDF), acid (ADF) detergent fiber and acid detergent lignin (ADL) by incubating samples in neutral (Van Soest et al. 1991), acid (AOAC 1991) detergent solution, and 20 N H_2SO_4 (AOAC 1991), respectively, using an Ankom Fiber Analyser (Ankom Technology Corporation, Fairport, NY). Crude protein (CP = N × 6.25) was analyzed using a Leco Nitrogen

Analyser (Truspec Nitrogen Determinator System, Leco Corporation, MI). Dry matter concentration of samples was determined by oven drying (AOAC1991). In vitro DM digestibility was determined using the DAISY system (Ankom Technology, Fairport, NY) following a two-stage procedure (i.e., a 48 hour incubation with rumen fluid followed by a 24 hour incubation with 6 N HCl and pepsin) (Holden 1999). Aerobic stability of ensiled material was defined as time required to raise temperature by 2°C (Kung et al. 2000) and measured as described in Hassanat et al. (2006).

Statistical analyses

Plots were assigned to a 2 × 3 factorial (i.e., two millet cultivars and three seeding rates) in a randomized complete block design with four replications at each of the two sites. Data were analyzed using an analysis combined over sites as described by McIntosh (1983) using PROC GLM of SAS (1989) in the following model:

$$Y_{ijkl} = \mu + M_i + R_j + S_k + M_i \times R_j + M_i \times S_k + R_j \times S_k + M_i \times R_j \times S_k + e_{ijkl}$$

Y_{ijkl} represents the observations for dependent variables, μ is the least square mean, M_i is the fixed effect of millet cultivar i, R_j is the fixed effect of seeding rate j, $M_i \times R_j$ is the interaction between millet cultivar i and seeding rate j, $M_i \times S_k$ is the interaction between millet cultivar i and site k, $R_j \times S_k$ is the interaction between seeding rate j and site k, $M_i \times R_j \times S_k$ is the three way

interaction between millet cultivar i, seeding rate j and site k,e_{ijkl} is the residual error. When significant effects were detected ($P < 0.05$), means were separated using Scheffe's test (SAS 1989).

Results and discussion

Agronomical characteristics

Three-way interactions between cultivar, seeding rate, and site were not observed for any of the agronomical or chemical composition parameters. Site had significant ($P < 0.05$) effects on several agronomical characteristics including forage yield, number of plants m^{-2}, tillers m^{-2}, and leaf: stem ratio (data not shown). The magnitude of differences between the two sites was slightly greater for RM compared to BM, which caused a cultivar × site interaction for these variables. The interaction was directional, not crossover. Thus Data of agronomical characteristics were pooled for the two sites. On the other hand, no site main effects or interactions with site were observed for chemical composition variables. In general, increasing seeding rate increased ($P < 0.05$) forage yield linearly in both regular and brown midrib plants (Figure 2-1). Corn and millet forage yield were also reported to increase with increasing seeding rates in other regions (Carberry et al. 1985; Cox et al. 1998; Cusicanqui and Lauer 1999; Bidinger and Raju 2000; Subedi 2006). The increase in these cases was quadratic (Cuomo et al. 1998; Cusicanqui and Lauer 1999) or linear (Graybill et

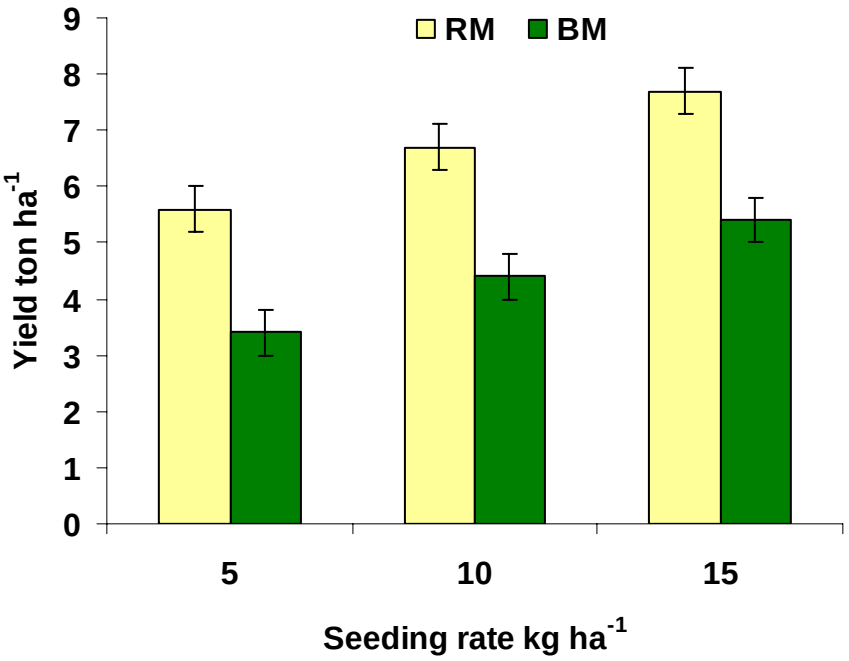

Figure 2-1. Effect of seeding rate on forage dry matter yield of two millet cultivars (BM, brown midrib millet; RM, regular millet). Vertical bars indicate Scheffe's 0.95 confidence interval.

al. 1991; Cox et al. 1998; Widdicombe et al. 2002). Response of forages to increasing seeding rate varied with cultivar and growing season conditions (Sanderson et al. 1995; Cox et al. 1998). At low plant densities, yield per plant is maximized, but yield per unit area is reduced. With increasing plant density, yield per unit area increase, but plant competition increase also, which reduces yield per plant (Horrocks and Vallentine 1999; Bidinger and Raju 2000). Very high seeding rates may compromise plant survivability, reduce yield, and result in lodging (Horrocks and Vallentine 1999). Furthermore, very high seeding rates

increases production costs. Optimum seeding rate is the one that produces maximum yield with minimum seeds per unit area (Horrocks and Vallentine 1999; Bidinger and Raju 2000). The range of seeding rates evaluated in our study is below the seeding rate that will result in higher forage yield (Hassanat et al. 2006).

The observed yield increase is in part due to a 20% increase (P <0.05) in number of plants m^{-2} for both millet cultivars with increasing seeding rate (Table 2-1). Increase in plants m^{-2} for RM was more pronounced for RM than BM, which resulted in a cultivar by seeding rate interaction. Number of tillers m^{-2} increased in RM with increasing seeding rate, while no differences were observed in BM, which again resulted in a cultivar by seeding rate interaction. One of the desirable qualities in any forage crops is the ability to adjust to a wide range of plant densities and maintain satisfactory yield. Tillering could partially compensate for fluctuations in number of plants m^{-2} as their contribution to plant biomass could reach up to 75% (Carberry et al. 1985; Bidinger and Raju 2000). Tillers per plant usually respond negatively, while tillers per unit area responds positively to increasing seeding rate (Bidinger and Raju 2000; Smith et al. 1999). In our study, seeding rate impact on yield was due to an increase in plants per unit area for RM and BM, and to increasing tillers per unit area in RM.

Leaf: stem ratio increased (P <0.05) with increasing seeding rate (Table 2-1) for both millet cultivars, but the increase was greater for RM than BM, which caused a cultivar by seeding rate interaction. Increasing seeding rate reduces stem diameter (Horrocks and Vallentine 1999), and produces leafy tillers (Carberry et al. 1985), which reduces stems contribution to whole plant composition, which was also observed in our study. Opposite to our findings, Cuomo et al. (1998) reported an increase in stem proportion of whole plant forage corn, when plant density was increased from 44 to 73 plants m^{-2}, with no effect on leaf proportion, which reduced leaf to stem ratio. This observation was made with high seeding rates, which we have not been evaluated in our study, where the maximum number of plants we observed was 37 plants m^{-2}. Finally, plant height was not affected by changes in seeding rate.

On average, RM produced 56% more forage (P < 0.05) than BM at any seeding rate. Plant height averaged 230 cm for RM, 30 % more (P <0.05) than BM (Table 2-1). Tillers m^{-2} was 30% higher (P <0.05) for RM than BM plants at seeding rates of 10 and 15 kg ha^{-1}, but not at the lowest seeding rate, which caused a cultivar by seeding rate interaction (Table 2-1, 2-2). Leaf: stem ratio and DM content of BM was higher (P < 0.05) than RM at all seeding rate (Table 2-1). The negative impact of the brown midrib trait on forage yield has been previously reported in a range of species (Casler et al. 2003; Hassanat et al.

2006). Reduction in plant height, tillering, and stem proportion in whole plant explain in part the lower yield of brown midrib forages compared to normal cultivars (Casler et al. 2003; Cherney et al. 1988).

Table 2-1. Effect of cultivar and seeding rate on agronomical characteristics of millet.

	Seeding rate (kg ha^{-1})				Millet cultivar		
	5	10	15	SE	Regular	Brown midrib	SE
Plant height (cm)	201	212	201	4	233a	179 b	4
Plants m^{-2}	25 c	35 b	43 a	1	39 a	31 b	1
Tillers m^{-2}	117 b	138 a	142 a	4	143 a	121b	3
Leaf: stem ratio	0.91 c	1.04 b	1.17 a	0.01	1.0 a	1.1 b	0.01

Means with different letters for a given factor within the row are significantly different (P <0.05).

SE: Pooled standard error of the means

Forage quality

Seeding rate had limited effects on quality of fresh forage (Table 2-2). Seeding rate had no effect on forage DM, CP, NDF, ADF or water soluble carbohydrate concentrations. Regular millet produced at a seeding rate 5 kg ha^{-1} contained slightly, but significantly less (P <0.05) ADL than at the other two seeding rates, while ADL concentration in BM was not affected by seeding rate, causing a cultivar by seeding rate interaction. Similar to our findings, increasing seeding rate had small or no effect on chemical composition and in vitro

digestibility of forage corn (Graybill et al. 1991; Cusicanqui and Lauer 1999; Widdicombe and Thelen 2002). At higher plant densities than those used in our study, Cuomo et al. (1998), and Wibbicombe et al. (2002) observed that as plant density increased, NDF and ADF concentrations increased while CP concentration and in vitro NDF digestibility were reduced. They observed that stem proportion increases with increasing seeding rate. Stem contains less CP and more NDF and ADF than the leaves due to structural differences (Maiti and Wesche-Ebeling 1997), which may explain their findings. We observed an increase in leaf: stem ratio with increasing seeding rate, and because the differences in chemical composition between millet leaves and stems at vegetative stage are small (Hassanat et al. in press), it did not impact chemical composition and in vitro dry matter digestibility.

Brown midrib had 16% greater (P <0.05) CP concentration and 4, 13 and 29% lower (P <0.05) NDF, ADF, and ADL concentrations, respectively than RM (Table 2-2). These differences in chemical composition may explain the 10% greater (P <0.05) in vitro dry matter digestibility of BM compared to RM. Similar differences between regular and BM have been previously reported (Hassanat et al. 2006).

Table 2-2. Effect of seeding rate and millet cultivar on chemical composition (g kg^{-1}) of fresh forage millet.

	Seeding rate (kg ha^{-1})				Millet Cultivar		
	5	10	15	SE	Regular	Brown midrib	SE
Dry Matter	125	128	126	2.3	117b	136a	1.9
Crude protein	143	147	149	3.3	136b	157a	2.8
Neutral detergent fiber	595	589	592	4.2	604a	580b	3.2
Acid detergent fiber	364	361	359	3.4	387a	336b	2.7
Acid detergent lignin	23b	27a	25ab	0.6	28a	21b	0.5
Ash	121	126	124	2.5	125	122	2.0
Water soluble carbohydrates	129	133	142	4.0	132	137	3.1
In vitro dry matter digestibility	710	698	714	4.1	672b	743a	3.2

Means with different letters for a given factor within the row are significantly different (P <0.05).

SE: Pooled standard error of the means

Dry matter content of silage averaged 174 g kg^{-1} and did not vary significantly between the different treatments. All forages ensiled well, with an average pH of 4.2, and 117 g kg^{-1} of lactic acid, with very small concentrations of acetic acid (Table 2-3). Dry matter losses during ensiling were negligible for all treatments (Table 2-3), and silage remained aerobically stable for 4.5 days after aerobic exposure. Silage parameters observed in our study are similar to those previously reported for millet silage (Hassanat et al. 2006). There were significant effects of seeding rate and millet type on pH, lactic and acetic acid concentration, but these differences were numerical.

Chemical composition of silage was similar to that of fresh forage (Table 2-2 and Table 2-3). Seeding rate had no effect on chemical composition of silage. Silage of BM contained 5, 10 and 30% less (P <0.05) NDF, ADF and ADL concentrations than RM, which resulted in a 10% greater (P <0.05) in vitro dry matter digestibility. Previous studies showed that the brown midrib trait in millet improved in vitro dry matter digestibility by reducing NDF, ADF, and ADL, concentrations (Cherney et al. 1990; Hassanat et al. 2006).

Table 2-3. Effect of seeding rate and millet cultivar on chemical composition (g kg^{-1}) of forage silage.

	Seeding rate (kg ha^{-1})				Millet Cultivar		
	5	10	15	SE	Regular	Brown midrib	SE
Dry Matter	177	167	176	3.7	175	174	2.9
Crude protein	125	131	136	3.4	132	130	2.8
Neutral detergent fiber	590	584	589	4.6	604 a	572 b	3.7
Acid detergent fiber	379	375	379	3.3	399 a	357 b	2.7
Acid detergent lignin	26	26	27	0.8	31 a	21 b	0.7
pH	4.2	4.2	4.2	0.03	4.1 b	4.3 a	0.02
Lactic acid	114	124	114	3.3	121 a	113 b	2.6
Acetic acid	4	3	3	0.1	3	3	0.1
DM recovery	99.5	99.4	99.8	0.13	99.6	99.6	0.1
In vitro dry matter digestibility	743	733	742	6.9	706 b	773 a	5.5
Aerobic stability	116	112	107	4.0	108	114	3.5

Means with different letters for a given factor within the row are significantly different (P <0.05).

SE: Pooled standard error of the means

Conclusion

Forage yield of RM and BM linearly with increasing seeding rate; consequently the seeding rate maximizing forage yield could not be identified in the present study. Number of tillers produced per m^{-2} increased with increasing seeding rate, but did not produce enough DM to compensate for loss in number of plants per unit area. Reductions in plant height and tillering ability were the main reasons for the lower forage yield of BM when compared to RM. However, the brown midrib trait reduced fiber concentration and increased CP concentration, which had a positive impact on in vitro dry matter digestibility. Effect of seeding rate on forage and silage quality was negligible.

CONNECTION STATEMENT 1

In the previous study, reduction in yield, but improvement in forage millet quality was observed in brown midrib millet compared to the normal cultivar. The improvement in forage yield was mainly attributed to reduction in lignin concentration. Investigation of the causes of yield reduction in brown midrib millet at vegetative and heading stage is required. Furthermore, it is necessary to determine the changes in cell wall concentration and composition in normal and brown midrib millet morphological parts with advancing development. This will help us understand changes in cell wall structure that lead to the higher DM degradability observed in the previous study. The second study was designed to look into cell wall components, concentration, interaction and impact on cell wall degradability of regular and brown midrib millet harvested at vegetative and heading stage. The study will also show the causes of yield reduction in brown midrib millet compared to the normal cultivar as affected by stage of development at harvest.

CHAPTER III.

EFFECT OF THE BROWN MIDRIB TRAIT AND STAGE OF DEVELOPMENT AT HARVEST ON CELL WALL COMPOSITION AND DEGRADABILITY OF FORAGE PEARL MILLET LEAVES AND STEMS

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Can. J. Anim. Sci. 2007. 87:421-429

Abstract

This study was conducted to determine the effect of the brown midrib trait and stage of development [vegetative (VS) vs. heading (HS) stage] on chemical composition and in situ rumen disappearance of forage millet leaves and stems. Forage yield of brown midrib millet was 80 and 50% of that of regular millet at VS and HS, respectively. The reduction in brown midrib millet yield was mainly due to reduction in plant height and tillers m^{-2}. The brown midrib trait reduced concentrations of neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) in stems by 8, 16, and 58%, respectively and concentrations of ADF and ADL in leaves by 6 and 49%, respectively. Effects of stage of development on fiber fractions were more pronounced in stems than in leaves. Millet stems harvested at HS had greater concentration of NDF, ADF and ADL than at VS, while only ADL concentration in millet leaves increased with advancing development. Leaves and stems of brown midrib millet (BM) contained more arabinose and xylose than those of regular millet (RM), while glucose concentration was not affected by millet type. Concentrations of xylose and arabinose were higher in leaves, while those of glucose and arabinose were higher in stems of millet harvested at HS than at VS. The brown midrib trait reduced concentrations of ester- linked *p*-coumaric acid and ether-linked *p*-coumaric and ferulic acids in both leaves and stems. However, concentration of ester-linked ferulic acid was only reduced in stems. Concentrations of all phenolic acids were higher in stems of millet harvested at HS than at VS. However, the effects of stage of development for

most phenolic acids were more pronounced for stems of RM than BM. In situ DM and NDF disappearances were higher in leaves and stems of BM than RM and were higher in leaves and stems of millet harvested at VS than at HS. The brown midrib trait reduced the negative impact of increasing maturity on in situ DM and NDF disappearance in leaves and in situ DM disappearance in stems. It was concluded that the brown midrib trait caused significant changes in cell wall composition of both leaf and stem fractions which resulted in improved in situ nutrient disappearances. The trait also reduced the negative effect of advanced maturity on nutrient digestibility.

Introduction

Cell wall digestibility is a key determinate of forage quality. Lignin is the cell wall polymer most resistant to ruminal microbial digestion, and its interaction with other cell wall polymers has a large impact on forage utilization by ruminant animals (Casler and Jung 1999; Grabber et al. 2004; Casler and Jung 2006). Cell walls of grass forages consist of polysaccharides and phenolics (i.e. lignin and hydroxycinnamic acids). Hydroxycinnamic acids [i.e., ferulic and *p*-coumaric acids] are the main phenolic acids attached to the cell wall polysaccharides (Grabber et al. 2004; Casler and Jung 2006). Lignin is attached to cell wall polysaccharides by covalent bonds through ether-ester linkages with phenolic acids (Ishii 1997; Jung 2003). Increased concentrations of phenolic acids is usually an indication of more frequent cross linkages between lignin and structural carbohydrates, which reduces access of ruminal microbes to cell wall polymers (Casler and Jung 2006). Brown midrib trait has resulted in significant reduction in concentrations and structure of lignin and phenolic acids in cell walls of corn, sorghum and millet (Cherney et al. 1988; Fritz et al. 1990; Lam et al 1996; Barrière 2004). The trait improve cell wall digestibility by reducing lignin concentration and substantially decreasing *p*-coumaric acid ester- and ferulic acid ether-linkages (Cherney et al. 1988; Goto et al. 1994; Mèchin 2000). The effects of brown midrib traits on cell wall composition varies according to species, stage of development at harvest and plant fraction (i.e., leaves vs. stems) (Cherney et al. 1988; Jung and Deetz 1993; Barrière 2004).

The importance of millet as a forage crop is growing in many regions of the world due to its ability to tolerate drought and its resistance to several diseases affecting corn (Kumar and Andrew 1993). In Canada, millet offers a good alternative for perennial forages that are lost to winter kill. Recently, new forage millet types that are suitable for growth in eastern Canada and the northeastern USA have been introduced including brown midrib (BM) and regular (RM) types. In a previous study, Mustafa et al. (2004) reported higher first cut yield, NDF, ADF and ADL concentrations, but lower in situ ruminal degradability's in RM compared to the values observed in BM. Data regarding cell wall composition and nutrient digestibilities of these types as affected by stage of development at harvest are not available. The objectives of this study were to compare the effect of the brown midrib trait and stage of development at harvest on cell wall fiber, neutral sugar and phenolic acid concentrations of leaf and stem fractions and their effect on in situ DM and NDF disappearances of forage pearl millet.

Materials and methods

General description and management.

Forage pearl millet developed by Agriculture Environmental Renewal Canada (AERC 2004) for forage production in eastern Canada was used in this experiment. The two types, regular (RM, CFPM101) and brown midrib (BM, CFPBMR) were seeded in a sandy loam soil in Sainte-Anne-de-Bellevue, QC, Canada (45°25′ 45″ N, 73°56′ 00″ W) on June 6, 2004. This location has a seasonal rainfall of 278 mm and an average

temperature of 19.4 ºC from June to September. In 2004, the total rainfall for the same period was 282 mm and the average temperature was 18.3 ºC. Before seeding, plots were fertilized with 250 kg ha^{-1} of (19-19-19) N-P_2O_5-K_2O fertilizer. Plots (1.35 x 5 m) were seeded using a disc-drill seeder (Fabro, Swift Current, SK, Ca) with 7 rows per plot (row spacing of 18 cm). The seeding rate was 16 and 18 kg ha^{-1} for RM and BM respectively. Differences in seeding rate reflected unequal seed size. Weeds were controlled using herbicide (i.e., Pardner at a rate of 1 L ha^{-1}). Harvesting was done manually (to a 7 cm stubble height) 8 (an average of 7 fully developed leaves in the vegetative stage; VS) or 12 weeks after seeding (50% of the plants at heading stage; HS) between 1 and 3 pm on the harvest day. Harvesting beyond heading stage was avoided to maintain reasonable NDF degradability as cultivars used in the present study were developed for forage production and were not multipurpose ones. Yield data, plant height, and number of tillers per m^2 were recorded at each harvest. After harvest, 10 plants from each plots (i.e., 2 millet types × 2 stages of maturity × 4 replicates) were separated into leaves and stems, dried at 55 °C in an a forced air oven for 48 h and then ground through a 2-mm screen using a Wiley mill (A. H. Thomas, Philadelphia, PA).

Chemical analyses

All samples were reground to 1-mm for chemical analysis. Dry matter content of ground samples was determined by oven drying according to the Association of Official Analytical Chemists (AOAC 1990, method no. 934.01). Ground samples were analyzed

for neutral (NDF) and acid (ADF) detergent fiber using an Ankom Fiber Analyzer (Ankom Technology Corporation, Macedon, NY) by incubating the samples in neutral (Van Soest et al. 1991) and acid detergent solution (AOAC 1990, method no. 9738.18), respectively. Acid detergent lignin (ADL) was determined by washing ADF residues with 20 N H_2SO_4 (AOAC 1990, method no. 9738.18).

Cell wall sugars were released by treating NDF residues of leaves and stems samples with 72% H_2SO_4 and then converted to their alditol acetate forms as described by Englyst and Cummings (1984). The extract was injected into a gas chromatograph (Hewlett Packard model 5890 series II, equipped with flame ionization detector and model 7673 auto injector; Hewlett Packard, Palo Alto, Ca) equipped with a 30 m fused silica capillary column (SP 2330, Supelco, Bellefonte, PA). Column temperature was set at 200 ˚C for 2 min, then increased by 4 ˚C min^{-1} to 250 ˚C and maintained for 3 min. Injector and detector temperature were both set at 300 ˚C. Gas flows were 5.3, 300, and 30 mL min^{-1} for He, air, and H_2, respectively.

Determination of cell wall phenolics was carried out according to the procedure of Jung (2003) with some modifications. From the NDF residue, 100 mg were incubated in 10 mL of 2 M NaOH for 24 h at room temperature to extract ester linked phenolic acids, or in 10 mL of 4 M NaOH for 2 h at 170 ºC to extract total phenolic acids. Samples were kept under N_2 and dark conditions and centrifuged (2500 x *g,* 30 min), the supernatant was obtained, acidified to pH >2.0, and 5 μg trans-cinnamic acid was added as an internal

standard. Samples were freeze-dried, then extracted with 10 mL methanol three times and re-centrifuged (1000 g, 30 min). The supernatant was transferred into a round bottom flask, evaporated under N_2 and dark conditions then eluted in 1 mL methanol. Samples (50 μL) were injected in a Gold System Beckman HPLC (Beckman, San Ramon, Ca) equipped with a reversed phase column (protein and peptide Zorbax, pore size 5 μm, 250 x 4.6 mm SB-C18, Agilent Technologies, Santa Clara, Ca) operated at room temperature, and a UV detector set at 280 nm. The gradient system consisted of the following two-buffers:solvent A, 0.2% trifluoroacetic acid in water (V V^{-1}); solvent B, 100% methanol, with a linear gradient starting at 5% to 80% methanol in 50 min at a flow rate of 0.75 mL min^{-1}. Purified chemical standards (p-coumaric acid, ferulic acid cinnamic acid; Sigma-Aldrich, Oakville, ON, Canada) were used to identify and determine the concentration of phenolic acids. Ether linked phenolics were determined as the difference between total and ester linked phenolics.

In situ DM and NDF disappearances

Samples of the leaf and the stem (2-mm size) replicates from all treatments were weighed (5 g each) in duplicates into nylon bags (20 × 10 cm, 50 µm pore size, ANKOM Technology, Fairport, NY). The nylon bags were incubated for 48 h in the ventral sac of two lactating Holstein cows fitted with flexible ruminal cannulas. Cows were fed 50:50 forage:concentrate diet as total mixed ration that contained 180 g kg^{-1} CP, 350 g kg^{-1} NDF, 170 g kg^{-1} ADF, and 25 g kg^{-1} ether extract. Following incubation, the nylon bags

were removed, washed with tap water until the runoff was clear and then were dried at 55 ºC for 48 h. Dried residues were analyzed for DM, NDF and cell wall sugars as previously described. In situ disappearance was calculated from nutrient concentrations in the original samples and the ruminal residues.

Statistical analyses

Forage yield data, agronomical characteristics, and chemical composition for each plant part were analyzed as a 2 × 2 factorial design (i.e., 2 millet types and 2 stage of maturity) using PROC MIXED of SAS (1989) in the following model

$$Y_{ijk} = \mu + M_i + S_j + Mi \times S_j \_ + e_{ijk}$$

Y_{ijk} represents the observations for dependent variables, μ is the least square mean, M_i is the fixed effect of millet cultivar i, S_j is the fixed effect of stage of development at harvest j, Mi x S_j is the interaction between millet cultivar i and stage of development j, e_{ijk} is the residual error.

Data of in situ DM, NDF and cell wall sugars disappearance were analyzed as a 2 × 2 factorial design (i.e., 2 types and 2 stages of maturity) in a randomized complete block design using cows as blocks in the following model

$$Y_{ijkl} = \mu + M_i + S_j + Mi \times S_j + C_k + e_{ijkl}$$

Y_{ijkl} represents the observations for dependent variables, μ is the least square mean, M_i is the fixed effect of millet cultivar i, S_j is the fixed effect of stage of development at harvest j, Mi x S_j is the interaction between millet cultivar i and stage of development j, C_k is the random effect of cow l (block l), e_{ijkl} is the residual error. When significant effects

were detected ($P < 0.05$), least significant difference was used to determine differences among means. Data of cell wall phenolics and lignin concentrations in leaves and stems were correlated using PROC CORR of SAS (1989). Analysis of covariance was performed on non-correlated cell wall phenolics and ADL and multiple regressions were used to estimate regression coefficients and values of R^2 using PROC GLM of SAS (1989).

Results and discussion

Yield and agronomical characteristics

Dry matter yield was higher ($P < 0.05$) for RM than BM and for forages harvested at HS than VS for both millet types (Table 3-1). There was a millet type × stage of development interaction indicating that the effect of maturity was larger for RM than BM. The brown midrib trait in the present study reduced forage DM yield by 18 and 52% for VS and HS, respectively. Similar negative effects of the brown midrib trait on forage DM yield have been reported by Casler et al. (2003) and Hassanat et al. (2006).

Regular millet plants were 44.0% taller ($P < 0.05$) and produced 18.6% more tillers than BM plants. Plants harvested at HS tended ($P = 0.09$) to be taller than those harvested at VS. Furthermore, number of tillers tended ($P = 0.06$) to decrease for RM but not for BM as stage of development advanced. The reduction in forage DM yield due to the brown midrib trait is been attributed to lower plant height and reduced stalk mass per unit length (Casler 2003). In the present study, advancing stage of development tended to

increase plant height without affecting number of tillers. According to Mangat et al. (1999), maximum growth occurs around the flag leaf stage (pre-heading), however, growth may continue after flowering, which might be the case in millet as they can reach a height of 5 m (Fribourg 1995).

Leaf:stem ratio was higher ($P < 0.05$) for BM than RM and decreased ($P < 0.05$) for both types as the stage of development advanced from VS to HS (Table 3-1). In accordance with our findings, Cherney et al. (1988) and Morin et al. (2005) found that sorghum types with brown midrib traits had more leaves and less stems per plant than their normal counterparts. However, the authors found no effect of the brown midrib trait on leaf:stem ratio of pearl millet or corn. It is well documented that leaf:stem ratio declines as forages mature (Wilson 1997), mainly due to the increase in stem weight as a result of increased cell wall content.

Fiber fractions

Concentration of NDF in leaves was not affected by brown midrib trait or stage of development and averaged 653 g kg^{-1} of DM (Table 3-2). However, concentration of ADF was higher ($P < 0.05$) in leaves of RM than BM and was not influenced by stage of development. Acid detergent lignin was low in leaves of both millet types but was higher ($P < 0.05$) in leaves of RM than BM. The brown midrib trait reduced ADL content in leaves

Table 3-1. Effect of millet type and stage of development at harvest on yield and agronomical characteristics of forage millet.

	Regular millet		Brown midrib millet			Treatment effect (*P* value)		
	Vegetative	Heading	Vegetative	Heading	SEM[z]	Millet type	Stage of development	Millet type x stage of development
DM yield (ton ha^{-1})	10.8	22.3	8.9	10.6	0.67	< 0.01	< 0.01	< 0.01
DM (g kg^{-1})	146	202	152	178	3.0	0.03	< 0.01	< 0.01
Plant height (cm)	250	270	175	185	10.2	< 0.01	0.09	0.51
Number of tillers (m^{-2})	151	123	119	104	11.5	0.03	0.53	0.06
Leaf:stem ratio	1.0	0.4	1.1	0.5	0.03	0.01	< 0.01	0.49

[z] Pooled standard error of the mean for the four treatments.

by 50% at both stages of development. For both millet types, concentration of ADL in leaves increased ($P < 0.05$) as stage of development progressed from VS to HS. However, the increase was more pronounced for RM than BM as indicated by a millet type × stage of development interaction (Table 3-2). In agreement with our findings, Goto et al. (1994) found that the brown midrib trait in corn decreased ADL content in leaves without affecting NDF content. Similar results have also been reported for sorghum × sudangrass hybrids (Fritz et al.1990). Concentrations of NDF, ADF and ADL were all lower ($P < 0.05$) in stems of BM than RM and were higher ($P < 0.05$) for stems of millet harvested at HS than at VS (Table 3-2). At VS, stems of BM contained 9, and 51% less ADF, and ADL, respectively than RM.

The corresponding values at HS were 12, 21 and 52% for NDF, ADF and ADL, respectively. Concentrations of NDF, ADF and ADL were consistently lower in stems of brown midrib sorghum × sudangrass lines harvested at three different stages of development (Fritz et al.1990). Similar results have also been reported for corn (Goto et al. 1994; Mechin et al. 2000). Acid detergent lignin in stems of the brown midrib sorghum, corn and millet is at least 50% less than that in stems of normal counterparts (Lam et al. 1996).

Table 3-2. Effects of millet type and stage of development at harvest on fiber fractions of leaves and stems of forage millet (g kg^{-1} of DM).

	Regular millet		Brown midrib millet			Treatment effect (*P* value)		
	Vegetative	Heading	Vegetative	Heading	SEM[z]	Millet type	Stage of development	Millet type x stage of development
Leaves								
Neutral detergent fiber	649	662	652	649	8.3	0.56	0.56	0.37
Acid detergent fiber	370	366	343	348	5.8	< 0.01	0.97	0.41
Acid detergent lignin	21	30	10	16	0.9	< 0.01	0.01	< 0.01
Acid detergent lignin[y]	32	46	16	25	0.8	< 0.01	< 0.01	0.01
Hemicellulose	278	294	316	306	2.8	< 0.01	0.33	< 0.01
Cellulose	342	336	333	332	5.8	0.26	0.54	0.65
Stems								
Neutral detergent fiber	659	736	673	647	9.2	< 0.01	0.02	< 0.01
Acid detergent fiber	415	472	378	370	6.8	< 0.01	< 0.01	< 0.01
Acid detergent lignin	29	56	14	24	1.3	< 0.01	< 0.01	< 0.01
Acid detergent lignin[y]	44	76	21	37	1.8	< 0.01	< 0.01	< 0.01
Hemicellulose	249	263	290	277	4.7	< 0.01	0.99	0.01
Cellulose	392	411	356	347	4.8	< 0.01	0.33	0.02

[z] Pooled standard error of the mean for the four treatments.

[y] g kg^{-1} of neutral detergent fiber

Significant millet type × stage of development interactions of the various fiber fractions indicated that the effects of stage of development on fiber fractions of stems were greater for RM than BM (Table 3-2). This can be explained by the fact that the brown midrib trait down regulates lignin synthesis and thus reduces rate of lignification, especially at early stages of plant development (Cherney et al. 1991; Ishii 1997).

The effect of the brown midrib trait was more pronounced on stems than leaves at both stages of development (Table 3-2) which was also observed in previous studies (Fritz et al. 1990; Goto et al. 1994). Due to the structural functions of stems, the effect of the brown midrib trait is expected to be more evident on the cell wall composition of stems than that of leaves. The effects of stage of development on fiber fractions were also more pronounced in stems than in leaves for both millet types (Table 3-2). Leaves are rich in non-lignified mesophyll cells while lignified parenchymal, sclerenchymal, and vascular tissues are more abundant in stems (Maiti and Wesche-Ebeling 1997). Similar effects of maturity on fiber composition of stems and leaves have been reported for normal forages and forages with the brown midrib traits (Fritz et al. 1990).

Cell wall sugars

The concentration of total neutral sugars in leaves was higher ($P < 0.05$) in BM than RM and was not influenced by stage of development (Table 3-3).

Concentrations of arabinose and xylose were higher ($P < 0.05$) in leaves of BM than RM and were higher ($P < 0.05$) in leaves of millet harvested at HS than at VS. Glucose content in leaves was not affected by millet type or stage of development and averaged 439 g kg^{-1} of NDF. Concentrations of total sugars and glucose in stems were similar for both millet types but were higher ($P < 0.05$) in stems of millet harvested at HS than at VS (Table 3-3). Xylose concentration was higher ($P < 0.05$) in stems of BM than RM and was not influenced by stage of development (Table 3-3). Differences in arabinose concentrations in stems were similar to those observed for leaves. Concentration of galactose and mannose in leaves and stems of the two millet types was low.

Effects of the brown midrib trait on cell wall neutral sugar composition are inconsistent. Fritz et al. (1990) found no effects of the brown midrib trait on concentrations of neutral sugar monomers of leaves and stems of sorghum × sudangrass hybrids. However, Cherney et al. (1986) reported higher xylose and arabinose and lower glucose concentration in the brown midrib genotypes of sudangrass relative to their normal counterparts. Inconsistencies between various studies can in part be explained by differences in stage of development of plant material used (Reid 1997).

Table 3-3. Effects of millet type and stage of development at harvest on cell wall neutral sugar and protein concentrations of leaves and stems of forage millet (g kg^{-1} of NDF).

	Regular millet		Brown midrib millet			Treatment effect (*P* value)		
	Vegetative	Heading	Vegetative	Heading	SEM[z]	Millet type	Stage of development	Millet type x stage of development
Leaves								
Arabinose	34	38	40	46	0.6	<0.01	<0.01	0.20
Xylose	188	225	264	302	7.7	<0.01	<0.01	0.98
Galactose	4	8	8	ND[y]	0.1	<0.01	<0.01	<0.01
Mannose	6	12	16	ND[y]	0.3	<0.01	<0.01	<0.01
Glucose	428	423	446	460	12.0	0.11	0.52	0.67
Arabinose:xylose	0.10	0.12	0.14	0.16	0.006	<0.01	0.02	0.38
Total sugars	659	707	775	808	19.0	0.01	0.62	0.14
Cell wall proteins	59	48	63	51	3.1	0.82	<0.01	0.09
Stems								
Arabinose	24	31	38	43	1.4	< 0.01	0.03	0.41
Xylose	237	252	265	279	9.2	0.01	0.14	0.95
Galactose	7	6	ND	3	0.4	< 0.01	0.06	< 0.01
Mannose	9	12	9	7	0.7	< 0.01	0.82	< 0.01
Glucose	475	509	470	518	13.6	0.86	0.01	0.63
Arabinose:xylose	0.19	0.16	0.15	0.15	0.004	< 0.01	< 0.01	< 0.01
Total sugars	751	810	782	851	19.2	0.09	0.01	0.98
Cell wall proteins	32	18	35	27	8.3	0.42	0.83	0.11

[z] Pooled standard error of the mean for the four treatments.

[y] not detected

Concentration of xylose in leaves, and glucose in stems were higher ($P < 0.05$) for millet harvest at HS than at VS. In general, concentrations of neutral sugar monomers in plant cell wall increase as plants mature (Aman 1993). Secondary thickening of the cell wall involves increase in glucose and xylose concentrations (Wilson 1997; Aman 1993). Goto et al. (1990) reported that cell wall content of glucose and xylose increased as sorghum plants advanced in development from the 4-leaves to the heading stage, while concentrations of arabinose and other sugars were similar. Aman (1993) reported a consistent increase in arabinose, xylose and glucose content in timothy grass cell wall as maturity advanced.

The arabinose:xylose ratio was greater ($P < 0.05$) for leaves of BM than RM and was higher ($P < 0.05$) for leaves of millet harvested at HS than at VS (Table 3-3). The Arabinose:xylose ratio was higher ($P < 0.05$) for RM than BM and decreased ($P < 0.05$) in stems as stage of development advanced from VS to HS in RM only as indicated by millet type x stage of development interaction. The arabinose:xylose ratio has been used as an indicator of cell wall development. The ratio is usually higher in primary than secondary cell walls and as forages mature, more xylose and less arabinose are deposited in secondary cell walls (Wilson Hatfield 1997; Jung 2003). Primary cell walls are rich in ararabinoxylan and cellulose, while secondary cell walls are richer in xylose and

lignin (Aman 1993; Reid 1997). Thus, increased concentrations of glucose and arabinose are mostly related to continual development of primary rather than secondary cell walls. Our results suggest that development of secondary cell walls was only observed in stems of RM harvested at HS. For leaves, the observed increase in the arabinose:xylose ratio as stage of development advanced suggest that the two millet types are still in the primary cell wall development phase and contained more branched xylan than stems.

Cell wall phenolics

About 95% of *p*-coumaric acid and 80% ferulic acid in leaves and stems of both millet types were ester-linked to the cell wall (Table 3-4). In leaves, concentration of *p*-coumaric acid was 26% lower ($P < 0.05$) in BM than RM and was not influenced by stage of development (Table 3-4). Concentration of ester-linked ferulic acid in leaves were similar for both millet types and was higher ($P < 0.05$) in leaves of millet harvested at HS than at VS. However, concentration of ether-linked ferulic acid was higher ($P < 0.05$) in leaves of RM than BM and was higher ($P < 0.05$) for leaves of millet harvested at HS than at VS. In stems, ester-linked *p*-coumaric acid concentration was 50% higher ($P < 0.05$) in RM than BM and was 24% greater ($P < 0.05$) for stems of millet harvested at HS than at VS. Both ester- and ether-linked ferulic acid were greater ($P < 0.05$) for stems

Table 3-4. Effects of type and stage of development at harvest on concentration of cell wall phenolics of leaves and stems of forage millet (g kg^{-1} of NDF).

	Regular millet		Brown midrib millet			Treatment effect (*P* value)		
	Vegetative	Heading	Vegetative	Heading	SEM[z]	Millet type	Stage of development	Millet type x stage of development
Leaves								
Ester *p*-coumaric acid	3.0	3.1	2.2	2.3	0.06	0.01	0.20	0.80
Ether *p*-coumaric acid	0.1	0.0	0.1	0.2	0.04	0.04	0.16	0.10
Ester ferulic acid	3.8	5.2	3.8	5.2	0.12	0.80	< 0.01	0.98
Ether ferulic acid	1.1	1.5	0.7	1.0	0.08	< 0.01	0.04	0.11
Ratio[x]	0.76	0.61	0.58	0.45	0.013	< 0.01	< 0.01	0.33
Stems								
Ester *p*-coumaric acid	5.3	6.0	2.4	3.3	0.20	< 0.01	< 0.01	0.53
Ether *p*-coumaric acid	0.0	0.9	0.0	0.4	0.04	< 0.01	< 0.01	< 0.01
Ester ferulic acid	3.9	5.5	3.5	4.0	0.20	< 0.01	< 0.01	< 0.01
Ether ferulic acid	1.1	1.9	0.8	0.9	0.11	< 0.01	< 0.01	< 0.01
Ratio[x]	1.34	1.05	0.70	0.83	0.025	< 0.01	< 0.01	< 0.01

[z] Pooled standard error of the mean for the four treatments.

[x] *p*-coumaric acid:ferulic acid ratio.

of RM than BM and were higher ($P < 0.05$) for stems of millet harvested at HS than at VS. Similar effects of the brown midrib trait on phenolic acid concentrations of corn stems have been reported by Méchin et al. (2000) and Barrière et al. (2004). Significant millet type x stage of development interactions were observed for all phenolic acids indicating that differences between VS and HS were greater for RM than BM (Table 3-4). Our results suggest that the brown midrib trait reduced the impact the stage of development on the accumulation of phenolic acids in stems of forage millet.

Ferulic acid is estrified to arabinose branch in xylan during cell wall formation, while 90% of the *p*-coumaric acid is attached to lignin (Ishii 1997; Grabber et al. 2004). Thus ester-ferulic acid is associated with cell wall elongation and development while *p*-coumaric acid is more linked to lignin deposition. As cell wall development and lignification proceed, ferulic acid becomes incorporated into lignin and would only be recovered as ether-linked ferulic acid (Moore and Jung 2001; Jung 2003). The higher concentrations of ester-linked *p*-coumaric acid and ether-linked ferulic acid in RM compared with BM indicated more lignificantion at both stages of development. This is consistent with the higher ADF and ADL concentrations of RM relative to BM (Table 3-2).

In the present study, the brown midrib trait reduced ester-linked *p*-coumaric acid in leaves by 26% and ester-linked *p*-coumaric acid and ether-

linked ferulic acid in stems by 50 and 26%, respectively. These results suggest that the effect of the brown midrib trait was greater on stems than leaves. In agreement with our findings, Goto et al. (1994) reported small reduction in p-coumaric acid concentration (1.7 g kg^{-1}) in brown midrib corn leaves while the stems of the brown midrib corn contained 13.6 g kg^{-1} less ester-linked p-coumaric acid than the normal counterpart. The reduction in ether-linked ferulic acid in leaves and stems of forage millet caused by the brown midrib trait in our study is similar to responses reported for corn, sorghum, millet and sorghum x sudangrass (Fritz et al. 1990; Lam et al. 1996; Ostrander et al. 1999; Mechin et al 2000). The effects of stage of development on phenolic acid concentrations were more pronounced for RM than BM and were more apparent for stems than leaves suggesting that the brown midrib trait reduced the accumulation of phenolic acids as stems of forage millet matured (Table 3-4).

The effects of stage of development on phenolic acid concentrations seem to differ between leaves and stems. Similar to our findings, others have reported consistent increase in *p*-coumaric acid and ferulic acid concentrations in stems of grasses with advancing development (Wilson et al. 1997; Jung 2003). In a study with corn, concentration of both ester-linked *p*-coumaric acid and ferulic acid in leaves increased as corn advanced from juvenile to adult vegetative stage, while ether-linked *p*-coumaric acid and ether-linked ferulic acid showed minimal

changes throughout development (Abedon et al. 2006). The significant increase in ferulic acid as the development of forage millet advanced from VS to HS can be attributed to the fact that ferulic acid deposition is not restricted to the primary cell wall and that cross linkages between lignin and arabinoxylan mediated by ferulic acid occur in the secondary wall (Jung 2003).

Ratio of *p*-coumaric acid:ferulic acid was higher ($P < 0.05$) in leaves and stems of RM than BM and was lower ($P < 0.05$) for leaves and stems of millet harvested at HS than at VS (Table 3-4). A significant millet type x stage of development was noted for stems indicating that the reduction caused by the stage of development was only significant for RM. The brown midrib trait reduced *p*-coumaric acid:ferulic acid ratio in corn, sorghum and millet (Cherney et al. 1991; Goto et al. 1994). Ester-linked *p*-coumaric acid is bound to the syringyl unit in lignin and is considered an indication of lignification (Grabber et al. 2004). However, ester-linked ferulic acid is bound to arabinoxylose in the cell wall and not lignin. Once ester-linked ferulic acid becomes incorporated in lignin, it would only be recovered as ether-linked phenolics (Ishii 1997; Grabber et al. 2004). Therefore, low ester *p*-coumaric acid:ferulic acid ratio indicates that ferulic acid is present in the cell wall without being cross linked to lignin. On the other hand, high ester *p*-coumaric acid:ferulic acid suggests cross linkage between ferulic acid and lignin. Our results suggest that the degree of lignin cell wall cross

linkages through ferulic acid bridges in leaves and stems of RM are higher than that in BM. Furthermore, the degree of cross-linkage increases in leaves and stems of RM and leaves of BM as stage of development advances.

Data on the effects of maturity on *p*-coumaric acid:ferulic acid ratio are limited. Ratio of *p*-coumaric acid:ferulic acid in sorghum increase then stabilize with advancing cell development (Goto et al. 1991). The increased *p*-coumaric acid:ferulic acid ratio is mainly related to increase in *p*-coumaric acid concentration. Reduction in *p*-coumaric acid:ferulic acid concentration observed in leaves and stems of RM and leaves of BM is mainly related to increase in ester-linked ferulic acid concentration.

In situ disappearance

In situ DM and NDF disappearances were higher ($P < 0.05$) for leaves of BM than for those of RM and were higher ($P < 0.05$) for leaves of millet harvested at VS than at HS (Table 3-5). Significant millet type x stage of development interactions for these parameters indicated that the negative impact of stage of development on in situ 48 h DM and NDF disappearances were greater ($P < 0.05$) for RM than BM. In situ disappearance of leaves glucose and xylose was higher ($P < 0.05$) for BM than RM and was greater ($P < 0.05$) for leaves of millet harvested at VS than at HS (Table 3-5). Millet type x stage of development

Table 3-5. Effects of type and stage of development at harvest on in situ disappearances of leaves and stems of forage millet (g kg^{-1}).

	Regular millet		Brown midrib millet			Treatment effect (p value)		
	Vegetative	Heading	Vegetative	Heading	SEM [z]	Millet type	Stage of development	Millet type x stage of development
Leaves								
DM	693	622	723	703	10.2	<0.01	<0.01	0.05
Neutral detergent fiber	603	497	765	744	16.6	<0.01	<0.01	<0.01
Arabinose	567	473	646	554	21.7	<0.01	0.98	<0.71
Xylose	535	449	676	652	8.1	<0.01	<0.01	<0.01
Glucose	552	342	600	426	12.5	<0.01	<0.01	<0.01
Stems								
DM	660	452	687	571	8.3	<0.01	<0.01	<0.01
Neutral detergent fiber	537	321	595	388	13.0	<0.01	<0.01	0.77
Arabinose	638	466	764	618	10.1	<0.01	<0.01	0.40
Xylose	508	427	672	440	17.1	0.04	<0.01	0.68
Glucose	418	351	568	382	22.6	0.19	0.04	0.08

[z] Pooled standard error of the mean.

interactions for glucose and xylose in situ disappearances indicated that differences between VS and HS were greater for RM than BM. The effects of millet type and stage of development on DM and NDF disappearance of stems were similar to those reported for leaves (Table 3-5). A significant millet type x stage of development interaction was observed for DM disappearance but not for NDF, again indicating that differences in DM disappearance between VS and HS were greater for RM than BM. In situ disappearance of arabinose and xylose were greater ($P < 0.05$) for stems of BM than RM and were higher ($P < 0.05$) for stems of millet harvested at VS than HS. In situ disappearance of glucose was not influenced by millet type and was higher ($P < 0.05$) for stems of millet harvested at VS than HS. Our results suggest that the brown midrib trait reduced the negative impact of maturity on in situ DM disappearance in both leaves and stems and in situ NDF disappearance in leaves.

Several studies reported improved in vitro and in situ DM and NDF disappearances of stems, leaves and whole plant of corn, millet, and sorghum as a result of the brown midrib traits (Cherney et al. 1988; Goto et al. 1994; Lam et al. 1996). The improved nutrient disappearances were associated with reduced ADL, ester-linked-*p*-coumaric acid and ether-linked ferulic acid concentrations (Goto et al. 1994; Marvin et al. 1995*)*. Ether-linked ferulic acid concentration had a negative impact on 24 and 96 h in vitro NDF disappearance in perennial grasses (Casler and Jung 2006). Ether-linked ferulic acid covalently cross-links arabinose in the arabino-

xylan complex, reducing its digestibility (Goto et al. 1994; Ishii 1997). Furthermore, ferulic acid linked to arabino-xylose is considered the site of nucleation of lignin in the cell wall (Iiyama et al 1994; Ishii 1997). Therefore, reducing ferulate bridges between cell wall and lignin significantly increased cell wall digestibility (Fritx et al. 1990; Lam et al 1996).

Advancing maturity reduced in vitro and in situ DM and NDF disappearances in regular and the brown midrib forages (Ostrander et al. 1999; Mustafa et al. 2004; Hassanat et al. 2006). In our study, the decline in situ disappearance with advancing maturity was associated with increases in concentrations of lignin and cell wall phenolics especially for stems. Stems are rich in lignified tissues such as xylem which will increase the proportion of indigestible cell wall (Jung and Engels 2002). Furthermore, lignin produced after the vegetative stage have more detrimental effect on NDF digestibility compared with that produced during the vegetative growth (Jung and Deetz, 1993; Moore and Jung 2001). This would explain the greater impact of stage of development on in situ DM and NDF disappearance of stems than leaves.

Cell wall phenolics and lignin in leaves and stems were highly correlated ($P < 0.05$), except for ester-ferulic acid and ester *p*-coumaric acid in leaves (Table 3-6). These results agree with the finding of Marvin et al. (1995) and Méchin et al. (2000). The high correlation indicates that for the two millet types, and at these two stages of development, the deposition of these substances is equal, and would have similar

impact on cell wall degradability. Thus, it is rational look at regression of digestibility values on lignin concentration only, since it is highly correlated with all cell wall phenolics (Table 3-6).

Table 3-6. Correlation coefficients between cell wall phenolics and lignin in leaves and stems of regular and brown midrib millet harvested at two stages of development.

	Ester-ferulic acid	Ether-ferulic acid	Lignin
Ester-Pcoumaric acid	0.45[z]	0.74 [z]	0.86 [z]
Ester-ferulic acid		0.78 [z]	0.67 [z]
Ether-ferulic acid			0.90 [z]

[z] Significant at $p < 0.05$

Regression analysis over and above classification effect of millet type, stage of development at harvest or plant part showed no interaction between the fixed effect (i.e., millet type, stage of development at harvest, plant part, and their interactions) and regression parameter of lignin. This indicates that the impact of lignin concentration on digestibility would not vary between the different fixed effects. Regression analysis showed significant ($P < 0.05$) quadratic effect of lignin concentration on xylose and glucose digestibility (R^2 value 0.74 and 0.72, respectively) and a linear effect of lignin concentration on DM, NDF and arabinose digestibility (R^2 value 0.74, 0.72, 0.36, respectively) (Table 3-7).

Table 3-7. Regression values of lignin concentrations on in situ nutrient disappearance.

	Regression equation	R^2	SE [z] Linear	SE [z] Quadratic
DM	786.6 - 5.7 ADL	0.75 [y]	0.60	-
Neutral detergent fiber	777.9 - 8.6 ADL	0.62 [y]	1.22	-
Arabinose	696.9 - 4.2 ADL	0.36 [y]	1.03	-
Xylose	819.1 - 15.2 ADL + 0.1 ADL^2	0.74 [y]	3.06	0.06
Glucose	796.4 - 20.2 ADL + 0.2 ADL^2	0.72 [y]	3.93	0.04

[z] Standard error

[y] significant at $p < 0.05$

The impact of ADL on DM and NDF digestion of forages is well documented (Casler and Jung 2006, Jung 2003, Mèchin et al. 2000). Covalent linkages between ADL and arabinoxylan through ferulic acid are considered a major factor affecting digestion of forage grasses (Jung and Deetz 1993; Casler and Jung 2003). Lignin is thought to affect the extent rather than the rate of digestion. This is because rapidly degradable cell wall tissues do not lignify while slowly degradable tissues are partially lignified (Jung and Engels 2001; Jung and Engels 2002).

Conclusions

Results of the present study showed that the brown midrib trait reduced DM yield of forage millet by reducing plant height and number of tillers and by increasing the leaf:stem ratio. As expected, the trait reduced fiber and phenolic acid concentrations in leaves and stems of forage millet with greater effects on stem than leaf fractions. Advancing stage of development at harvest increased fiber and phenolic acid

contents for both millet types particularly for the stem fractions. However, the brown midrib trait reduced the impact of advancing development on stems, and to a lesser extent on leaves. The reduction in lignin and phenolic acid concentrations as a result of the brown midrib trait was reflected in higher 48 h in situ nutrient disappearances for leaves and stems of BM relative to those of RM. The increase in forage quality of brown midrib millet is related to alternation in cell wall structure, which showed little change with advancing stage of development.

CONNECTION STATEMENT 2

Results of the previous study explained how brown midrib trait reduced DM yield of forage millet at VS and HS through affecting, plant height, number of tillers, and leaf:stem ratio. The study also showed the impact of the brown midrib trait and advancing stage of development at harvest on cell wall composition and nutritive value of forage millet. The chemical composition and ensilability of millet forage as affected by cultivar and stage of development at harvest need to be determined. The third study was designed to investigate the effect of advancing stage of development on chemical composition and ensilability of regular and brown midrib millet. The study will determine the effect of these two factors on proteolysis and aerobic stability of millet silages

CHAPTER IV.

CHEMICAL COMPOSITION AND ENSILING CHARACTERISTICS OF NORMAL AND BROWN MIDRIB PEARL MILLET HARVESTED AT TWO STAGES OF DEVELOPMENT IN SOUTHWESTERN QUÉBEC

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Can. J. Anim. Sci. 2006. 86:71-80.

Abstract

The interest in annual forages in eastern Canada has increased in recent years. This study was conducted to determine the effects of cultivar and stage of development at harvest on ensiling characteristics, chemical composition, in vitro digestibilities, microbial population, and aerobic stability of forage pearl millet. Regular (RM) and brown midrib (BM) millet were harvested at vegetative (VS) or heading (HS) stage, then ensiled in laboratory silos for 0, 2, 4, 8, 16, and 45 d. Regular millet yielded twice as much as BM. Forage dry matter (DM) yield was also greater when pearl millet was harvested at VS than HS. Both millet cultivars were well ensiled and had a pH less than 4.2 after 45 d of ensiling. Compared with millet harvested at VS, millet harvested at HS contained 45% more water soluble carbohydrates at ensiling time and had a lower pH after 45 d of ensiling. Most proteolysis occurred between day 0 and day 8 post-ensiling, when 40 to 50% of true protein was lost to non-protein N for the two millet cultivars at any stage of development. Silages made from BM contained 2% less neutral detergent fiber (NDF), 6% less acid detergent fiber (ADF), 27% less acid detergent lignin (ADL), and 15% more crude protein (CP) than silages made from RM. For the two millet cultivars, silages harvested at HS contained more ADF and ADL (5 and 80%, respectively) and 30% less CP than silages harvested at VS. In vitro DM and NDF digestibilities were greater for BM than RM silages by 4 and 10%, respectively, and were higher for silages harvested at VS than for those

harvested at HS by 5 and 8%, respectively. Changes in the microbial population during ensiling were similar for the four treatments. There was an increase in the lactic acid bacteria population in the first 2 d of ensiling. Enterobacteria population decreased as ensiling progressed and enterobacteria were not detected from day 8 post-ensiling. Yeast and mold populations followed the same trend, but stayed at detectable level up to day 45 post-ensiling. Silage made from millet harvested at VS had more yeast and mold at 45 d post-ensiling (2.9 log CFU g^{-1}) than that harvested at HS (< 2.0 log CFU g^{-1}). Aerobic stability (time required for the silage temperature to rise by 2°C) ranged between 80 to 168 h, with silages harvested at HS being more aerobically stable than those harvested at VS. It was concluded that silages made from BM pearl millet had a higher nutritive value than those made from RM pearl millet. For both types of pearl millet, forages harvested at VS produced a better quality silages that those harvested at HS. The improved quality of BM silages was offset by its low DM yield.

Introduction

The interest in annual forage species in eastern Canada is increasing with the recurrence of drought and winterkill of traditionally used perennial species. Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is an erect tropical annual grass that can be utilized for forage or grain production. It is highly drought tolerant and it is resistant to many diseases affecting corn (Kumar and Andrews 1993). Unlike sorghum, it produces no prussic acid that can endanger animals. Millet forage DM yield varies between 3 and 27 t ha^{-1}, depending on geographical location, cultivar, and soil and climatic conditions (Gray et al. 2000). It frequently yields more than other annual warm season grasses under drought conditions (Jaster et al. 1985). While pearl millet has generally been used in tropical environments, its use in North America has been mainly concentrated in the southern USA. Forage cultivars adapted to eastern Canadian conditions have recently been developed, including a brown midrib cultivar (Agricultural Environmental Renewal Canada 2004).Brown midrib cultivars of sorghum, corn, or millet produce less lignified fiber, and are thus often more digestible. Lignin concentration of the brown midrib mutants is 5 to 50% lower than their regular counterparts due to a mutation in genes controlling lignin synthesis (Cherney et al. 1991; Oba and Allen 1999). The rate of lignin synthesis in brown midrib mutants is very low in the early stage of growth, but it becomes similar to rates observed in normal cultivars as plants mature (Cherney et al. 1991). Due to a

lesser degree of lignification and consequently of a greater digestibility, brown midrib mutants generally improve animal performance when compared with regular cultivars. For example, Oba and Allen (2000) reported that cows fed brown midrib corn silage have a higher DM intake than cows fed regular corn (22.8 vs. 21.7 kg d^{-1}), resulting in a greater milk production (35.3 vs. 31.5 kg d^{-1}). Several studies were conducted to determine the nutritive value of brown midrib corn (Oba and Allen 1999) and sorghum (Casler et al. 2003) silages for ruminants but few studies on brown midrib millet silage have been conducted. The objective of this study was to determine the effects of cultivar and stage of development at harvest on ensiling characteristics, chemical composition, in vitro digestibility, microbial population, and aerobic stability of pearl millet grown under south-western Québec conditions.

Materials and methods

Forage material and ensiling

Two forage pearl millet cultivars, developed for forage production in eastern Canada, were evaluated:(i) regular (RM, CFPM101) and (ii) brown midrib (BM, CFPBMR). Seeding was done on 2003 Jun. 03 in Sainte-Anne-de-Bellevue, QC, Canada (45°25′N, 73°56′W), on a Saint Bernard clay loam soil that was in fallow the previous year. The area received 207 mm of rain during the growing season (2005 Jun. 01 to Aug. 30) with an average temperature of 20.7°C. The 30-yr average precipitation and temperature for this location are 278 mm and 19.4ºC, respectively,

for the same period. Plots were fertilized at seeding with 120 kg N ha^{-1} as urea and with 22 kg P ha^{-1} and 41 kg K ha^{-1} in a mixed fertilizer (0- 20-20). Seeding was done in 1.35 × 5 m plots at a rate of 16 and 18 kg ha^{-1} for RM and BM, respectively, with seven rows per plot (18 cm between rows) using a forage seeder (Fabro, Swift Current, SK). Differences in seeding rate were due to differences in seed size and germination percentage between the two millet cultivars. Treatments (i.e. cultivar × stage of development combinations) were arranged spatially in a randomized complete block design with three replicates. Weeding was done manually throughout the season. Each millet cultivar was harvested manually (at a 5-cm cutting height) at the vegetative stage (VS, 8 wk after seeding, average height of 75 cm) or at the heading stage (HS, 12 wk after seeding, average height of 90 cm) and weighed. Plants from each plot were then chopped to a theoretical length of 25 mm using a forage chopper (Pern-Ohio Machinery Company, Erie, PA). Representative 500-g samples of harvested chopped forage were obtained from each plot, dried in a forced-air oven at 60°C for 48 h, and weighed to express yield on a DM basis. Sample of fresh chopped forage from each field plot were also stored at –20°C for later analyses. Harvested forage from plots of the same treatment (i.e., combination of millet cultivar and stage of development) was then pooled and packed in triplicates into polyvinyl chloride laboratory silos (7.6-cm diameter × 25-cm height) and allowed to ensile for 2, 4, 8, 16, and 45 d (Sebastian et al. 1996). Chopped

forages were packed into the laboratory silos using a manual pestle (packing density approximately 658 kg m^{-3}). Laboratory silos were then sealed with a plastic cap fitted with a small valve to permit gas release with no exchange, and stored at room temperature. Three extra laboratory silos were prepared from each treatment, ensiled for 45 d, and used for aerobic stability determination. Dry matter recovery after ensiling was estimated by weighing the laboratory silos before and after the 45 d ensiling period.

Chemical analyses

After the designated ensiling period, silos were opened and the ensiled forage was mixed thoroughly. Fifty grams of ensiled and thawed fresh forages were homogenized with 500 mL of distilled water for 5 min. The pH of the water extract was immediately determined using an Accumet pH meter (Denver Instrument Company, Mansfield, TX). A portion of the extract was stored at –20°C before further analysis. One millilitre of water extract was combined with 200 μL of 25% metaphosphoric acid containing 2-ethyl butyric acid as an internal standard. Samples were centrifuged for 15 min at 10 000 × g and analyzed for acetic, propionic, and butyric acid by gas chromatography (Hewlett Packard model 5890 series II, equipped with flame ionization detector and model 7673 auto injector; Hewlett Packard, Palo Alto, CA) equipped with 15 m Nukol fused capillary column (Supelco Inc., Bellefonte, PA). Column temperature was fixed at 150°C for a run

time of 8 min. Injector and detector temperatures were 180 and 200°C, respectively. Gas flows were 30, 300, and 30 mL min^{-1} for He, air, and H_2, respectively. Water soluble carbohydrates (WSC) and lactic acid concentrations were determined by colorimetric methods as described by Dubois et al. (1956) and Barker and Summerson (1941), respectively.

Sub-samples (500 g) of chopped fresh and ensiled forages were dried in a forced-air oven at 55°C for 48 h and then ground through a 1-mm screen using a Wiley mill (A. H. Thomas, Philadelphia, PA). Ground samples were analyzed for crude protein (CP = N × 6.25) using a Leco Nitrogen Analyser (FP-428, Nitrogen Determinator System, Leco Corporation, MI). Neutral (NDF) and acid (ADF) detergent fiber were determined using an Ankom Fiber Analyser (Ankom Technology Corporation, Fairport, NY) by incubating the samples in neutral (Van Soest et al. 1991) and acid [Association of Official Analytical Chemists (AOAC) 1990] detergent solution, respectively. Dry matter concentration of samples was determined by oven drying (AOAC 1990). Buffer-soluble crude protein (SCP) and non-protein N (NPN) were determined according to Licitra et al. (1996). Neutral (NDICP) and acid (ADICP) detergent insoluble crude protein were determined by analyzing NDF and ADF residues, respectively, for total N (Licitra et al. 1996). Buffer insoluble crude protein minus the NDICP was used to estimate the true protein (TP) as described by Sniffen et al. (1992).

Dried and ground samples of fresh forages and 45-d silages were analyzed for ash and ether extract (AOAC 1990) and acid detergent lignin (ADL, Ankom Fiber Analyzer, Ankom Technology Corporation, Fairport, NY) by washing samples with 20 N H2SO4 (AOAC 1990). Starch was determined colorimetrically according to McCleary et al. (1997). In vitro DM digestibility (IVDMD) was determined using the DAISY (Ankom Technology, Fairport, NY) following the two-stage procedure (i.e., a 48- h incubation with rumen fluid followed by a 24-h incubation with 6 N HCl and pepsin) as described by Holden (1999). In vitro NDF digestibility (IVNDFD) was determined by analyzing for NDF residue in samples before and after 48 h incubation with rumen fluid. Estimates of total digestible nutrients (TDN) and net energy of lactation (NEL) were calculated according to the equations of Weiss et al. (1992).

Microbial population analyses

Samples of pre-ensiled forages and silages were pooled for each treatment and ensiling period (i.e. 0, 2, 4, 8, 16 and 45 d) and stored at –20°C for determining lactic acid bacteria, enterobacteria, yeast and mold count according to Kung et al. (2000) with some modification. The sample was homogenized with 0.1% peptone water in a blender and used for culturing. Lactic acid bacteria were cultured using pour plating with Rogosa SL media and incubated for 60 h at 30°C. Cycloheximide (0.01%) was added to the media before pouring to prevent mold growth.

Enterobacteria were pour plated on a Violet Red Bile agar, and incubated for 18 h at 38°C. Yeast and mold were counted after spread plating on malt extract agar acidified with 0.25% lactic acid (media final pH was 3.5) and incubated for 60 h at 30°C.

Aerobic stability

Laboratory silos used for aerobic stability assessment were opened at day 45 post-ensiling, and aerobic stability was determined according to Kung et al. (2000) with some modifications. Representative samples (80 g) from each laboratory silo were thoroughly agitated to ensure air exposure, then packed loosely in 500-mL plastic containers covered with double-layered cheesecloth. Four holes were made on the top and bottom of each container to permit air exchange. Thermal insulator was wrapped around the sides of each container to prevent heat dissipation. Samples were incubated for 6 d at 25°C and then analyzed for pH, lactic acid, yeast and mold count as mentioned above. Dry matter losses were determined by weighing silage samples before and after aerobic exposure. Aerobic stability was measured using representative samples (80 g) from each laboratory silo by inserting thermocouple probes in the core of each similar plastic containers to detect temperature difference from the environment. Aerobic stability was defined as time required to raise temperature by 2°C (Kung et al. 2000). Temperature was measured

using a Hotmux data logger (DDC Corporation, Pennsauken, NJ) with temperature recorded every 5 min and averaged every 2 h.

Statistical analyses

Forage yield and chemical composition data of the fresh forage were analyzed as a 2 × 2 factorial design (i.e., two cultivars and two stages of development) using PROC MIXED of SAS (SAS Institute, Inc. 1989) with the model

$Y_{ijk} = \mu + M_i + S_j + Mi \times S_j + e_{ijk}$

Y_{ijk} represents the observations for dependent variables, μ is the least square mean, M_i is the fixed effect of millet cultivar i, S_j is the fixed effect of stage of development at harvest j, $M_i \times S_j$ is the interaction between millet cultivar i and stage of development j, e_{ijk} is the residual error.

Data of the changes in the chemical composition throughout ensiling were analyzed separately for each ensiling period as a completely randomized design with three replications (i.e., laboratory silos) using PROC MIXED of SAS (SAS Institute, Inc. 1989) using the model

$Y_{ij} = \mu + D_i + e_{ij}.$

Y_{ij} represents the observations for dependent variables, μ is the least square mean, D_i is the effect of day I, e_{ij} is the residual error.

Data were also analyzed similarly to determine differences between ensiling periods for a given treatment. Finally, data of aerobic exposure were analyzed as completely randomized design with three replications (i.e., laboratory silos) using the

previously mentioned model SAS (SAS Institute, Inc. 1989). When significant effects were detected ($P < 0.05$), least significant difference was used for mean separation (Gomez and Gomez 1984).

Results and discussion

Forage yield

Regular millet yielded more ($P < 0.05$) than BM at the two stages of development (Table 4-1). On average, the yield of RM was twice that of BM. Delaying harvest from VS to HS tripled the DM yield of RM and doubled it for BM. The effect of the brown midrib trait on forage yield is well documented (Cherney et al. 1991; Casler et al. 2003). The reduction in yield due to the brown midrib trait is related to lower plant height and lower stalk mass per unit length (Casler et al. 2003). In their study, Casler et al. (2003) reported lower yield and number of tillers, and reduced height of brown midrib Sudangrass compared with a regular cultivar. The DM yield of pearl millet in this study is within the range reported by Gray et al. (2000).

Table 4-1. Effect of cultivar and stage of development at harvest on chemical composition of fresh pearl millet forage (DM basis).

	Regular millet		Brown midrib millet			Treatment effect (P value)		
	Vegetative	Heading	Vegetative	Heading	SEM[z]	Millet type	Stage of development	Millet Cultivar × Stage of development
DM yield (ton ha^{-1})	3.65	9.03	2.50	5.67	0.234	<0.01	<0.01	<0.01
DM (g kg^{-1})	240	279	209	296	4.2	0.07	<0.01	<0.01
Ash (g kg^{-1})	75	61	77	66	1.6	0.06	<0.01	0.20
Ether Extract (g kg^{-1})	20	18	18	18	0.7	0.28	0.14	0.31
Neutral detergent fiber (g kg^{-1})	654	635	628	640	8.3	0.21	0.68	0.11
Acid detergent fiber (g kg^{-1})	333	339	302	327	4.3	<0.01	<0.01	0.07
Acid detergent lignin (g kg^{-1})	13	21	8	17	0.7	<0.01	<0.01	0.23
Starch (g kg^{-1})	14	19	19	9	0.6	<0.01	<0.01	<0.01
Crude protein (CP, g kg^{-1})	98	65	107	83	3.1	<0.01	<0.01	0.18
Water soluble carbohydrates (g kg^{-1})	111	160	123	178	4.2	<0.01	<0.01	0.87
Soluble protein (g kg^{-1} CP)	224	330	369	294	20.4	0.03	0.47	<0.01
NPN (g kg^{-1} CP)[z]	205	202	330	209	10.2	<0.01	<0.01	<0.01
NDICP (g kg^{-1} CP)[y]	358	415	306	390	10.3	<0.01	<0.01	0.21
ADICP (g kg^{-1} CP)[x]	107	142	76	122	9.0	0.02	<0.01	0.59
IVDMD (g kg^{-1})[w]	727	703	772	723	1.2	<0.01	<0.01	0.76
IVNDFD (g kg^{-1})[v]	614	566	682	619	1.2	<0.01	<0.01	0.46

[z]Pooled standard error of the means from four treatments

Ensiling characteristics

The pH declined rapidly in the first 2 d of ensiling for all treatments (Figure 4-1). The pH continued to decline up to day 8 post-ensiling for both cultivars of forage millet harvested at the VS, and up to day 45 post-ensiling for those harvested at HS. Brown midrib and RM cultivars harvested at the VS had higher ($P < 0.05$) pH than when harvested at the HS at any ensiling period. Millet cultivars had no effect on the pH of the 45-d silages and all had a pH less than 4.2. The pH values reported in our study for the 45-d silages are within the range reported for well-ensiled millet silages (Fisher and Burns 1987; Messman et al. 1992). Lactic and acetic acids were the main fermentation acids in the four silage treatments, regardless of the ensiling period. Butyric and propionic acids were generally undetectable (data not shown). The concentration of lactic acid increased ($P < 0.05$) rapidly in all treatments between day 0 and day 4 post ensiling, then increased slightly up to day 45 post-ensiling for VS but stabilized between days 4 and 8 for HS (Figure 4-1). Differences in lactic acid concentration between the four treatments for the 45-d silages were minimal and values were in good agreement with those reported by Messman et al. (1992) and Fisher and Burns (1987) for millet silage. The presence of acetic acid in the silages suggests that hetero-fermentative bacteria were active during ensiling (McDonald et al. 1991). Acetic acid is produced from fermentation of WSC or further fermentation of lactic

acid. The concentration of acetic acid at any given ensiling period was lower than that of lactic acid. Concentration was low until day 2, and then increased rapidly in all treatments until day 16 post-ensiling (Figure 4-1). Beyond d 16, concentrations stabilized for HS but continued to increase for VS. For the 45-d silages, acetic acid concentration was higher ($P < 0.05$) for BM than RM silages and was higher ($P < 0.05$) for silages harvested at VS than for those harvested at HS. In agreement with our results, Meeske et al. (1993) and Fraser et al. (2001) reported that acetic acid concentration of silages declines as forage maturity progresses. Concentrations of lactic and acetic acid suggest that millet silages were well preserved. The patterns of increase in lactic and acetic acid were similar to those reported for legume silages (Mustafa and Seguin 2003b) and sorghum silage (Meeske et al. 1993). Pre-ensiled BM contained more ($P < 0.05$) WSC than RM (Table 4-1). Both forage millet cultivars harvested at HS also had higher ($P < 0.05$) WSC concentrations than those harvested at VS. Water-soluble carbohydrate concentrations for the four pre-ensiled treatments (25 g kg^{-1} fresh matter) were over the minimum level recommended for optimum ensiling (Lunden Pettersson and Lindgrin 1990). Reports on changes in WSC in grasses with advancing maturity are conflicting. In general, WSC concentration of grass forages increases as forages mature (Fraser et al. 2001; Buxton and O'Kiely 2003). However, during grain formation, WSC is converted to starch causing a

reduction in WSC concentrations in forages such as corn and sorghum harvested after grain formation (Meeske et al. 1993; Johnson et al. 2003). In the present study, forages were harvested before the start of grain formation. For all treatments, the concentration of WSC declined ($P < 0.05$) rapidly between day 0 and day 4 post ensiling and stabilized between day 16 and day 45 post ensiling (Figure 4-1). Residual WSC (day 45 post-ensiling) was not affected by forage millet cultivar or stage of development. The sharp drop in WSC between day 0 and day 4 is likely due to the utilization of WSC by lactic acid bacteria that resulted in a sharp increase in lactic acid concentration and a rapid decline in pH (Figure 4-1). Similar changes in WSC concentration during ensiling have been reported by Mustafa and Seguin (2003b).

Change in protein fractions during ensiling

Buffer-soluble crude protein and NPN of all treatments increased ($P < 0.05$) sharply during the first 2 d of ensiling, continued to increase slowly up to day 8 post-ensiling, and then stabilized (Figure 4-2). At day 45 post-ensiling, SCP averaged 62% of CP for all the treatments, 90% of which was NPN (Table 4-2). Differences in SCP and NPN between treatments at any given ensiling period were minimal. Neutral detergent insoluble crude protein of all treatments declined ($P < 0.05$) as ensiling progressed (Figure 4-2). Most of the decline in NDICP occurred between day 0 and day 8 post-ensiling with no further decline between

day 16 and day 45 post-ensiling except for RM harvested at HS, where NDICP continued to decline up to day 45 post-ensiling. At any given ensiling period,

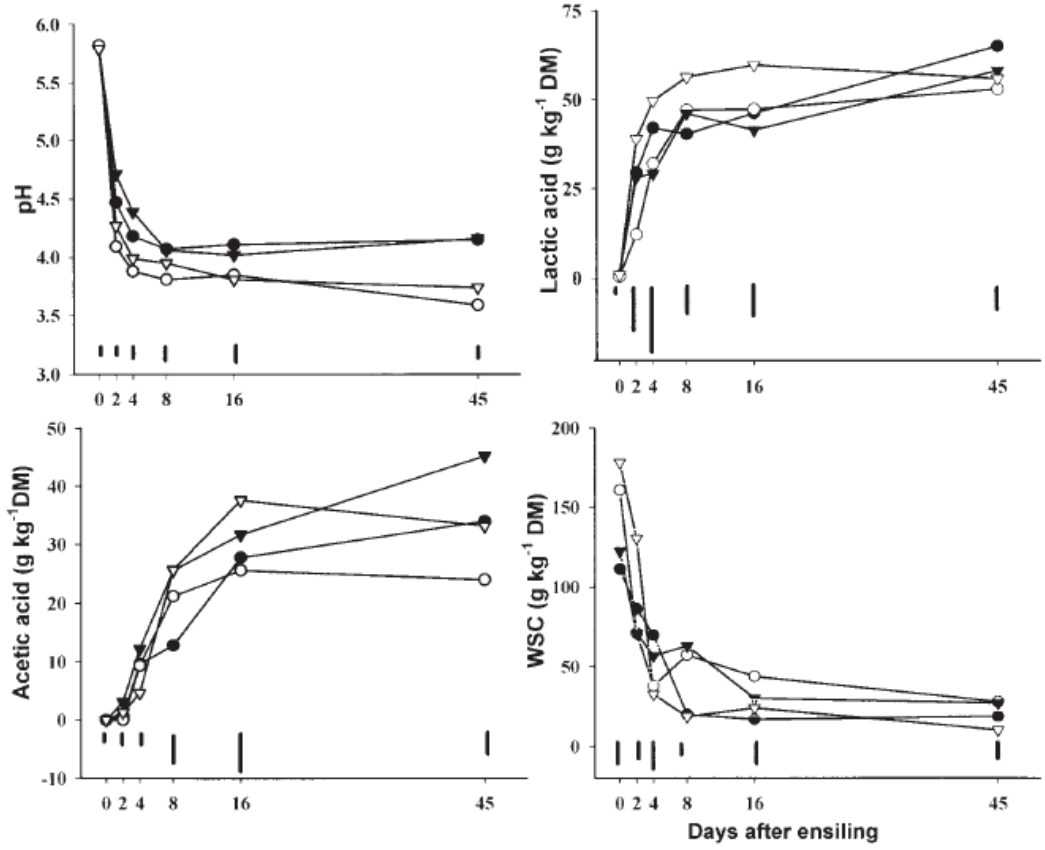

Figure 4-1. Changes in pH and concentrations of lactic acid, acetic acid, and water-soluble carbohydrates (WSC) during ensiling of regular (RM)and brown midrib (BM) pearl millet harvested at the vegetative (VS) or heading (HS) stage (● RMVS, ○RMHS, ▼BMVS, ▽ BMHS) Vertical bars represent ± SEM (pooled standard error of the mean for the four treatments).

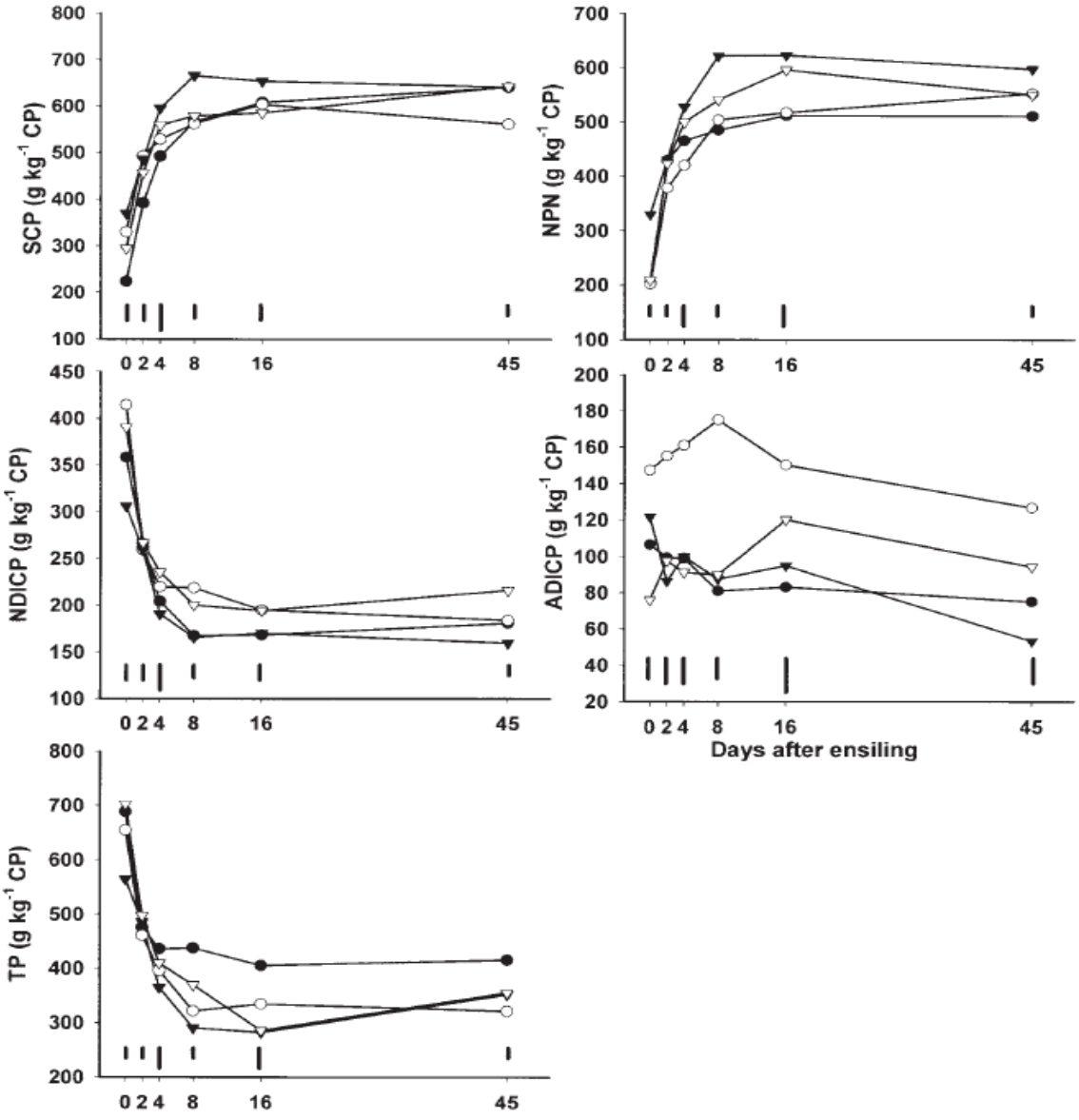

Figure 4-2. Changes in soluble crude protein (SCP), non protein N (NPN), neutral detergent insoluble crude protein (NDICP), acid detergentinsoluble crude protein (ADICP), and true protein (TP) during ensiling of regular (RM) and brown midrib (BM) pearl millet harvested at the vegetative (VS) or heading (HS) stage (● RMVS, ○RMHS, ▼BMVS, ▽ BMHS). Vertical bars represent ± SEM (pooled standard errorof the mean for the four treatments).

NDICP was higher ($P < 0.05$) for ensiled forages harvested at HS compared with those harvested at VS, except for RM silage 45-d post-ensiling. Changes in ADICP during ensiling were small with RM harvested at HS containing higher ($P < 0.05$) ADICP than the other treatments at all ensiling periods. True protein concentration decreased ($P < 0.05$) rapidly between day 0 and day 4 for all treatments. The sharpest decrease was observed during the first 2 d of ensiling, and there were no significant changes after day 8 post-ensiling. Regular millet harvested at VS had the highest ($P < 0.05$) concentration of TP at any given ensiling period. On average, 50% of the TP was degraded during ensiling. Proteolysis of forage proteins is a natural outcome of the ensiling process. Proteolysis is due to the action of plant proteases during ensiling where plant proteins are rapidly converted to peptides, amino acids, and other nitrogenous compounds (Papadopoulos and Mckersie 1983). Optimum pH for plant proteases activities is between 5 and 6, and the faster the decline in pH, the less proteolysis would be expected (Rooke and Hatfield 2003). In our study, pH was reduced below 5.0 after 2 d of ensiling for all treatments. The end of proteolysis after 8 d of ensiling indicated adverse conditions for the activities of proteases after that time. Changes in protein fractions during ensiling as a result of proteolysis observed in the present study are similar to those observed for other cereal and legumes silages (Mustafa et al. 2002; Mustafa and Seguin 2003a,b).

Chemical composition of the 45-d silages

The DM concentration of silages was low for all treatments and was higher ($P < 0.05$) for silages harvested at HS than for those harvested at VS. It is well documented that increasing the stage of development at harvest increases DM concentration of silages (Meeske et al. 1993; Dawson et al. 2002; Johnson et al. 2003). Hill et al. (1999) reported that millet silage harvested at dough stage contains 180 to 260 g DM kg^{-1} fresh matter. It is usually recommended to ensile forages at a DM concentration of 30% (Bolsen et al. 1996). In our study, although the DM concentration was less than 30%, the pH dropped below 4.6 4 d post-ensiling for all treatments likely due to the high initial WSC concentrations of the millet.

Except for protein fractions (i.e., SCP, NPN and NDICP) and WSC concentration, the composition of pre-ensiled forages (Table 4-1) was similar to that of the corresponding 45-d silages (Table 4-2). Concentrations of NDF, ADF, and ADL were greater ($P < 0.05$) for RM than BM silages (Table 4-2). With increasing maturity, the ADL concentration increased ($P < 0.05$) in both millet cultivars, while NDF and ADF concentrations did not change (Table 4-2). It is well documented that brown midrib millet, corn, and sorghum have lower concentrations of fiber fractions than their normal counterparts (Cherney et al. 1988; Oba and Allen 1999; Casler et al. 2003). The mutation of the lignin

Table 4-2. Effect of cultivar and stage of development at harvest on chemical composition of pearl millet silage after 45-d ensiling.

	Regular millet		Brown midrib millet			Treatment effect (P value)		
	Vegetative	Heading	Vegetative	Heading	SEM[z]	Millet type	Stage of development	Millet Cultivar × Stage of development
Dry matter (g kg^{-1})	201	248	190	241	4.0	0.05	<0.01	0.60
Ash (g kg^{-1})	85	66	88	70	2.0	0.18	<0.01	0.87
Ether Extract (g kg^{-1})	33	22	34	22	0.6	0.28	<0.01	0.20
Neutral detergent fiber (g kg^{-1})	674	689	631	660	11.4	0.01	0.09	0.57
Acid detergent fiber (g kg^{-1})	365	358	336	346	4.4	0.05	0.90	0.37
Acid detergent lignin (g kg^{-1})	14	25	06	19	0.3	<0.01	<0.01	0.03
Starch (g kg^{-1})	16	12	22	11	0.8	0.84	<0.01	<0.01
Crude protein (CP, g kg^{-1})	96	71	118	83	1.8	<0.01	<0.01	0.02
Soluble protein (g kg^{-1} CP)	640	561	641	631	22.5	0.13	0.14	0.13
NPN (g kg^{-1} CP)	510	552	597	548	10.5	<0.01	0.80	<0.01
NDICP (g kg^{-1} CP)	181	184	159	216	5.2	0.33	<0.01	<0.01
ADICP (g kg^{-1} CP)	75	127	64	96	7.4	0.22	<0.01	0.23
True protein (g kg^{-1} CP)	414	321	352	355	11.7	<0.01	0.26	<0.01
Total digestible nutrients (g kg^{-1})[y]	652	621	681	640	3.6	<0.01	<0.01	0.20
IVDMD (g kg^{-1})	737	670	772	696	1.2	<0.01	<0.01	0.57
IVNDFD (g kg^{-1})	597	548	661	561	1.2	<0.01	<0.01	<0.01
N_{EL} (MJ kg^{-1})[y]	6.57	6.23	6.86	6.40	0.04	<0.01	<0.01	0.20
Dry matter recovery	99.2	98.6	98.8	98.2	0.3	0.86	0.94	0.98

zPooled standard error of the mean for the four treatments.

yEstimated according to Weiss et al. (1992).

synthesis genes in the brown midrib genotypes reduces lignin concentration by 5 to 50% when compared with normal genotypes (Cherney et al. 1991). In our study, BM silage contained 57 and 24% less ADL than RM silage at VS and HS, respectively. Rate of lignin synthesis in brown midrib genotypes is low in the early stage of growth, but becomes similar to rates observed in normal genotypes at later stages of development (Cherney et al. 1991). In the first characterization of the brown midrib trait in millet, Cherney et al. (1988) reported similar hemicellulose concentration, but lower concentrations of NDF, cellulose, and ADL for brown midrib forage millet compared with normal forage millet. In contrast, Cherney et al. (1990) found no differences in NDF or ADF concentrations between brown midrib and normal forage millet harvested at the boot to early heading stage. Our NDF, ADF, and ADL concentrations for millet silages are similar to those of Cherney et al. (1990), Hill et al. (1999), and Ward et al. (2001) for the same stage of development. A significant cultivar × stage of development interaction ($P < 0.05$) was observed for CP concentration (Table 4-2) and was due to CP concentration of BM silage being higher ($P < 0.05$) than that of RM silage at VS but not at HS. The difference in CP concentration between the two stages of development was greater for BM than RM silage. In agreement with our study, Cherney et al. (1988) and Mustafa et al. (2004) reported higher CP concentration for brown midrib than for normal forage millet.

A similar effect of the brown midrib trait has also been reported for sorghum (Fritz et al. 1988) and sorghum × Sudangrass hybrids (Weidg et al. 1987). Differences in the CP concentration between the two stages of development in our study agree with others who reported a decline in CP concentration of forages and silages with advancing maturity (Mustafa and Seguin 2003a; Dawson et al. 2002). Gupta and Pradhan (1975) reported a 50% reduction in CP concentration as pearl millet stage of development advanced from pre- to post-flowering stage. Soluble crude protein was not affected by millet cultivar or stage of development and averaged 618 g kg^{-1} of CP (Table 4-2). However, NPN was greater ($P < 0.05$) for BM than RM silage and was not affected by stage of development. Neutral detergent insoluble crude protein and ADICP concentrations were not affected by millet cultivar and were higher ($P < 0.05$) for silages harvested at HS than for those harvested at VS. The effect of stage of development on NDICP concentration was more pronounced for BM than RM silage. True protein concentration was higher ($P < 0.05$) for RM silage harvested at VS than other treatments.

Brown midrib millet silages had higher ($P < 0.05$) TDN and NEL contents than RM silages at both stages of development while silages harvested at VS had higher ($P < 0.05$) TDN and NEL contents than those harvested at HS (Table 4-2). Differences in estimated energy values between forages can be explained

by differences in CP fractions and cell wall contents (Weiss et al. 1992). Our NEL values for forage millet are on average similar to those reported by Messman et al. (1992) and Hill et al. (1999). In vitro DM and NDF digestibilities were higher ($P < 0.05$) for BM than RM silages, and were higher ($P < 0.05$) for silages harvested at VS than for those harvested at HS (Table 4-2). There was a significant ($P < 0.05$) cultivar × stage of development interaction for IVNDFD, which reflected a greater decline between VS and HS for BM than for RM silages. Cherney et al. (1988) reported higher IVDMD of brown midrib than for regular forage millet. Similar results have also been reported for corn silage (Goto et al. 1994). The improved IVDMD and IVNDFD of BM silages relative to RM silages can at least in part be attributed to their lower ADL concentrations. Concentration of ADL was 27% lower in BM than RM silages, and 80% lower in silages harvested at VS compared with those harvested at HS. The brown midrib trait reduces the amount of lignin and the extent of lignin cross linkage with cell wall carbohydrates through *p*-coumaric acid (Cherney et al. 1991). Goto et al. (1994) reported negative correlation between ester linked *p*coumaric acid, and cell wall degradability. Cherney et al. (1988) found that the brown midrib trait in forages is associated with low *p*-coumaric acid concentration and *p*coumaric:ferulic acid ratio. Our IVDMD and IVNDFD values for the two cultivars of forage millet are in good agreement with those reported by Cherney et al. (1990).

Changes in microbial population during ensiling

It was not possible to statistically analyze the microbial population data because samples were pooled prior to microbial analysis. In general, population of lactic acid bacteria increased from day 0 to day 4 post-ensiling then stabilized up to day 16 post-ensiling (Table 4-3). There was a decline in lactic acid bacteria population between day 16 and day 45 post-ensiling for all treatments. However, the decline in lactic acid bacteria was greater for silages harvested at VS than for those harvested at HS. Lack of substrate or accumulation of metabolites could explain this decline. A similar decline in lactic acid bacteria in high-moisture ear corn silage harvested at VS was reported by Sebastian et al. (1996) and Weinberg et al. (1999). The population of enterobacteria declined during ensiling and was undetected after day 4 post-ensiling. The decline was greater for silages harvested at HS compared with those harvested at VS. The population of yeast and molds also declined from day 0 up to day 45 post-ensiling, with the greatest decline occurring between day 0 and 2 post-ensiling. This might be related to a faster pH decline in millet harvested at HS. Changes in microbial populations in our study are similar to those reported by Kung et al. (2000) and Sebastian et al. (1996).

Table 4-3. Microbial population changes during ensiling of regular and brown midrib pearl millet harvested at different stages of maturity (log CFU g^{-1} sample).

	Days of ensiling					
	0	2	4	8	16	45
Lactic acid bacteria						
Regular millet-vegetative	2.93	6.14	7.63	7.56	7.74	5.92
Regular millet-heading	4.70	7.09	7.14	7.95	7.66	6.48
Brown midrib millet-vegetative	2.77	7.04	6.70	7.52	7.45	5.85
Brown midrib millet-heading	4.88	7.10	7.43	7.09	7.08	6.56
Enterobacteria						
Regular millet-vegetative	4.92	5.99	4.20	ND[z]	ND	ND
Regular millet-heading	5.06	4.56	2.54	ND	ND	ND
Brown midrib millet-vegetative	4.52	5.79	4.70	ND	ND	ND
Brown midrib millet-heading	5.42	5.70	2.77	ND	ND	ND
Yeast and molds						
Regular millet-vegetative	5.27	3.70	3.64	2.63	<2.00	2.92
Regular millet-heading	4.16	3.14	3.06	2.06	<2.00	<2.00
Brown midrib millet-vegetative	3.86	3.38	3.57	2.59	<2.00	2.96
Brown midrib millet-heading	4.09	3.11	2.98	2.08	<2.00	<2.00

[z]ND = not detected.

Aerobic stability

Aerobic stability, that is the time required for the silage temperature to rise by 2°C, ranged between 80 and 168 h, with silages harvested at HS being more ($P < 0.05$) aerobically stable than those harvested at VS. Millet cultivar had no effect on aerobic stability. Silage pH, yeast and mold count, and DM loss after 6 d of aerobic exposure were all higher ($P < 0.05$) for RM than BM silages, and

were higher ($P < 0.05$) for silages harvested at VS than for those harvested at HS (Table 4-4). A significant millet cultivar × stage of development interaction was observed for these parameters indicating that differences between VS and HS were greater for RM than for BM. It has been suggested that the deterioration of silages following aerobic exposure is likely due to a loss of lactic acid (McDonald et al. 1991; Weinberg et al. 1995). Yeast assimilates and degrades lactic acid in aerobically exposed silage. Therefore, silages with high lactic acid concentrations will deteriorate faster than those with low lactic acid concentrations (Johnson et al. 2002). However, in the present study, differences in lactic acid concentration between silages harvested at VS and RM were minimal and cannot explain differences observed in aerobic stability.

Table 4-4. Effect of aerobic exposure for 6 days on silages of regular and brown midrib pearl millet silage harvested at different stages of maturity.

	Regular millet		Brown midrib millet			Treatment effect (P value)		
	Vegetative	Heading	Vegetative	Heading	SEM[z]	Millet cultivar	Stage of development	Millet cultivar × Stage of development
pH	7.8	4.4	4.8	4.1	0.1	<0.01	<0.01	<0.01
Lactic acid (g kg^{-1} DM)	3	10	33	20	3.0	0.03	0.22	0.57
Yeast and mold (log CFU g^{-1})	7.0	5.2	5.8	5.4	0.1	<0.01	<0.01	<0.01
Dry Matter loss (%)	10.5	0.7	2.2	1.8	1.4	<0.01	<0.01	<0.01
Aerobic stavility[y]	80	156	130	168	22	0.26	0.04	0.42

[z]Pooled standard error of the mean for the four treatments.

[y]Time to heat ≤ 2°C (h)

Conclusions

Relative to regular millet, brown midrib millet has a higher nutritive value, which is offset by a significantly lower DM yield. Ensiling characteristics and microbial changes in general were not greatly affected by millet cultivar or stage of development. Reduction in in vitro digestibilities for silages harvested at the heading stage was likely due to increased lignin concentration. Millet silages made from forage harvested at the vegetative stage may be less stable than those made from forage harvested at the heading stage. Despite low DM concentration, forage millet can be utilized to produce good-quality silage. Results obtained from this study should be interpreted with caution due to the use of a single environment (i.e., one site and one year). Further studies from a greater number of environments are warranted to validate our findings.

CONNECTION STATEMENT 3

In the 3 previous studies, impact of field conditions (seeding rate, cultivar, maturity) on nutritive value of millet forage and silage were evaluated. Other non field factors have an impact on silage quality such as inoculation. Inoculation is a standard practice for some forage to improve forage ensilability and aerobic stability. However, inoculation may not give any additional advantage for other types of forages. This next study was conducted to evaluate the impact of inoculating regular and brown midrib millet with homofermentative lactic acid bacteria on ensiling characteristics, nutritive value, and aerobic stability.

CHAPTER V.

EFFECTS OF INOCULATION ON ENSILING CHARACTERISTICS, CHEMICAL COMPOSITION AND AEROBIC STABILITY OF REGULAR AND BROWN MIDRIB MILLET SILAGES

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Anim. Feed Sci and Technol. 2007. 139:125-140

Abstract

This study was conducted to determine the effects of inoculation on ensiling characteristics, chemical composition and aerobic stability of two forage millet cultivars in a 2×2 factorial experiment. Second cut regular and brown midrib forage millet were treated with a commercial inoculum containing *Lactobacillus plantarum* and *Enterococcus faecium* or left untreated. The forages were then ensiled in laboratory silos for 2, 4, 8, 16, and 45 days. The pH of treated silages was below 4.0 at day 2 post-ensiling, while untreated silages reached that pH at day 8 post-ensiling. Untreated and inoculated silages, however, had similar pH at 45 days post-ensiling with no cultivar×treatment interaction. Lactic acid concentration was higher ($P < 0.05$) while that of acetic acid was lower ($P < 0.05$) for inoculated than untreated silages at all ensiling times. Lactic acid bacteria were numerically higher for inoculated than untreated silages at day 0 and 2 post-ensiling. Proteolysis during ensiling was not affected by inoculation or forage millet cultivar. Inoculation reduced ($P < 0.05$) aerobic stability of forage millet silages by an average of 40 h. Inoculation also increased ($P < 0.05$) DM loss (in the laboratory silos) and yeast and mould populations in aerobically exposed millet silages by 4%.

Introduction

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is a tropical annual grass that can be utilized for grain or forage production. It is highly drought tolerant and resistant to many diseases affecting maize (Kumar and Andrews 1993). The interest in annual forages such as millet in Canada is increasing with the recurrence of drought and winterkill of traditionally used perennial species. The use of forage millet in North America has been mainly concentrated in southern parts of the USA. However, forage millet cultivars adapted to eastern Canadian condition have recently been developed including a brown midrib cultivar (Agriculture Environmental Renewal and Canada 1998). Several studies have shown that forages with the brown midrib trait have lower fibre and higher protein concentrations than their regular counterparts (Cherney et al. 1991; Mustafa et al. 2004). This resulted in increased nutrient digestibilities and improved performance by animals fed brown midrib forages compared with regular ones (Oba and Allen 1999, 2000). Interest in utilizing brown midrib maize, sorghum, or millet for silage has increased in recent years as more brown midrib cultivars become commercially available.

Ensiling is the main method of preserving forages in North America. A rapid drop in pH during ensiling and increase in the concentrations of non-dissociated organic acids will inhibit the growth of microorganism that causes

silage spoilage (Pahlow et al. 2003). Forages such as millet and sorghum are usually ensiled at lower dry matter (DM) content than maize due to the fact that they are harvested in a vegetative phase while maize is harvested after the grain development (Ward et al. 2001). Low DM silages are susceptible to saccharolytic and proteolytic clostridial fermentation (Leibensperger and Pitt 1987). Adding lactic acid bacteria (LAB) to ensiled forages ensures the presence of enough lactic acid bacteria to cause a rapid reduction in pH, which may contribute to eliminating undesirable fermentation and reduce proteolysis (Kung et al. 2003a). However, inoculation might reduce aerobic stability of silages during the feed out phase (Weinberg et al. 1993; Kung and Ranjit 2001). Studies on the effects of inoculation on forages with the brown midrib trait are limited. The objective of this study was to determine the effects of a commercial inoculant containing a *Lactobacillus plantarum* and *Enterococcus faecium* on ensiling characteristics, chemical composition, and aerobic stability of two cultivars of regular (RM) and brown midrib (BM) millet silages. We hypothesized that inoculation will improve the fermentation and the aerobic stability of millet silage regardless of cultivar.

Materials and methods

Forage preparation and ensiling

Two forage pearl millet (*Pennisetum glaucum* L.) cultivars (i.e. regular, CFPM101, and brown midrib, CFPBMR) developed for forage production in eastern Canada were seeded in a sandy soil in L'Acadie, QC, Canada on 4 June 2004. Two weeks before seeding, plots were fertilized with 304 kg ha^{-1} of 18.3:11.5:27.3 N–P_2O_5–K_2O. Each cultivar was seeded in 3m × 30m plots at a rate of 18 kg ha^{-1} with 18 cm row spacing using a Carter selfpropelled seeder (Carter Manufacturing Corporation Inc., Brookston, IN, USA). After the first harvest on 3 August 2004, plots were re-fertilized with 162 kg ha^{-1} of 27.5-0-0 N-P_2O_5-K_2O. Forage was cut a second time 5 weeks after the first cut (14 weeks after seeding), and used for the present study. Harvested forage was chopped to a theoretical length of 25mm using a forage chopper (Pern-Ohio Machinery Company, Erie, PA, USA) and wilted for 3 h. Half of the chopped forages were treated with a commercial homofermentative lactic acid inoculum (Pioneer® 1129 Sorghum Silage Inoculant, Johnston, IA, USA) containing *L. plantarum* and *E. faecium* at a rate of 2.5×10^6 colony forming unit (CFU) kg^{-1} fresh forage as recommended by the manufacturer. The rest of the forage was left untreated. Forage materials were then packed into PVC mini-silos (7.6 cm diameter×25 cm height) at a density of approximately 658 kg m^{-3} using a manual pestle as

described by Sebastian et al. (1996). Forages were allowed to ensile for 2, 4, 8, 16, and 45 days. Forages were ensiled in triplicates for each millet cultivar, inoculation treatment, and ensiling period. Mini-silos were then sealed with a plastic cap fitted with a small valve to permit gas expulsion with no exchange, and stored at room temperature. Triplicate samples of fresh forages of each treatment were also taken and stored at −20 ºC for subsequent analysis. Dry matter recovery was calculated from knowing the weight of the empty silos, the initial and final silo weights, and DM concentrations of the fresh and ensiled material.

Chemical analyses

Silos were opened after the designated ensiling period and the ensiled forage was mixed thoroughly. Pre-ensiled and ensiled forages (50 g wet basis) were homogenized in a blender with 500 ml of distilled water (20 ºC) for 5 min and the pH of the water extract was immediately determined using an Accumet pH meter (Denver Instrument Company, Mansfield, TX, USA). A portion of the extract was stored at −20 ºC before further analysis. One millilitre of water extract was combined with 200 µL of 25% metaphosphoric acid containing 2-ethyl butyric acid as an internal standard for volatile fatty acids. Samples were centrifuged for 15 min at 10,000×g and analyzed for acetic, propionic, and butyric acid by gas chromatograph (Hewlett Packard model 5890 series II, equipped with

flame ionization detector and model 7673 auto injector; Hewlett Packard, Palo Alto, CA, USA) fitted with a 15m Nukol fused capillary column (Supelco, Inc., Bellefonte, PA, USA). Column temperature was fixed at 150 ºC for a run time of 8 min. Injector and detector temperatures were 180 and 200 ºC, respectively. Gas flows were 30, 300, and 30 ml min^{-1} for He, air, and H_2, respectively. From the extract mentioned above, water soluble carbohydrates (WSC) and lactic acid were determined by colorimetric methods as described by Dubois et al. (1956) and Barker and Summerson (1941), respectively.

Sub-samples (500 g wet basis) of pre-ensiled and ensiled forages were dried in a forced air oven at 55 ºC for 48 h and then ground through a 1-mm screen using a Wiley mill (A.H. Thomas, Philadelphia, PA, USA). Ground samples were then analyzed for DM (method number 934.1) according to the Association of Official Analytical Chemists (AOAC 1999). Crude protein (N×6.25) was determined (AOAC 1999, method no. 990.03) by a Leco FP 428N Analyzer (Leco Corporation, MI, USA). Neutral (aNDF, Van Soest et al. 1991) and acid (ADF, AOAC 1999, method no. 973.18) detergent fibre were determined using an Ankom Fiber Analyser (Ankom Technology Corporation, Macedon, NY, USA). The aNDF was conducted using heat stable amylase without sodium sulfite. Both aNDF and ADF were expressed inclusive of ash. Buffer-soluble protein (SCP) and non-protein nitrogen (NPN) were determined according to Licitra et al.

(1996). Neutral (NDICP) and acid (ADICP) detergent insoluble protein were determined by analyzing aNDF and ADF residues, respectively, for total N (Licitra et al. 1996). Buffer insoluble protein and NDICP were used to estimate true protein (TP) content as described by Sniffen et al. (1992).

Dried and ground samples of the fresh forages and day 45 silages were also analyzed for lignin (lignin (sa); Ankom Fiber Analyzer, Ankom Technology Corporation) by washing samples with 20N H_2SO_4 (AOAC 1999, method no. 973.18). In vitro DM disappearance (IVDMD) was determined using the DAISY apparatus (Ankom Technology Corporation) and using the two-stage procedure (i.e. a 48-h incubation with rumen fluid followed by a 24-h incubation with 6N HCl and pepsin) as described by Holden (1999). Rumen fluid was obtained from a non-lactating Holstein cow 2 h after the morning feeding. The cow was fed a high forage diet consisted mainly of brome hay.

Microbial population

Samples of pre-ensiled forages and samples of silages for each forage millet cultivar, inoculation treatment, and ensiling period (i.e. 2, 4, 8, 16 and 45 days) were pooled across replicates (Kung et al. 2004) and stored at −20 ºC (Sebastian et al. 1996) for determining lactic acid bacteria, enterobacteria, yeast and mould count according to Kung et al. (2000) with some modifications. Samples were homogenized in 1 g kg^{-1} peptone water using a blender and were

cultured after adequate dilution. Lactic acid bacteria were cultured by pour-plating in Rogosa SL media (DIFCO, Becton, Dickinson and Company, Sparks MD, USA) containing 0.1 g kg^{-1} cycloheximide to prevent growth of mould, and incubated at 30 ºC for 60 h. Enterobacteria were pour-plated on Violet Red Bile agar (DIFCO, Becton, Dickinson and Company, Sparks MD, USA) and incubated at 38 ºC for 18 h. Yeast and mould were counted after spread plating on malt extract agar (DIFCO, Becton, Dickinson and Company, Sparks MD, USA) acidified with 250 g kg^{-1} lactic (media final pH 3.5) and incubation at 30 ºC for 60 h.

Aerobic exposure

Silage deterioration following aerobic exposure was assessed according to Kung et al. (2000) with some modifications. Representative samples (80 g wet basis) of day 45 silages from each mini-silo were thoroughly agitated to ensure air exposure, packed loosely in 500 ml plastic containers and covered with double-layered cheesecloth. Four holes (3mm diameter) were made on top and bottom of each container to permit air exchange. The containers were wrapped with thermal insulator to prevent heat dissipation. Samples were incubated for seven days at 25 ◦C and then analyzed for pH, lactic acid and acetic acid concentrations, yeast and mould count and DM loss as mentioned above. Aerobic stability was measured by inserting thermocouple probes in the core of

each container and temperature of each silage as well as ambient temperature were recorded using a Hotmux data logger (DDC Corporation, Pennsauken, NJ, USA). Temperature was recorded every 5 min and averaged every 2 h. Aerobic stability was defined as the time required to raise temperature of the silage by 2 ºC (Kung et al. 2003a).

Statistical analyses

Chemical composition data of the fresh forages were analyzed as a completely randomized design using PROC MIXED of SAS (SAS Institute Inc., 1989) using the following model

$Y_{ij} = \mu + M_i + e_{ij}$

Y_{ij} represents the observations for dependent variables, μ is the least square mean, M_i is the fixed effect of millet cultivar i, e_{ij} is the residual error.

Chemical composition and aerobic exposure data of day 45 silages were analyzed as 2×2 factorial design (two cultivars and two inoculation treatments) with three replications (mini-silos) using PROC MIXED of SAS (SAS Institute Inc., 1989) in the following model

$Y_{ijk} = \mu + M_i + T_j + Mi \times T_j + e_{ijk}$

Y_{ijk} represents the observations for dependent variables, μ is the least square mean, M_i is the fixed effect of millet cultivar i, T_j is the fixed effect of treatment with inoculant j, Mi x T_j is the interaction between millet cultivar i and treatment with inoculant j, e_{ijk} is the residual error.

Data of the changes in the chemical composition throughout ensiling were analyzed separately for each ensiling period as a completely randomized design with three replications (i.e., laboratory silos) using PROC MIXED of SAS (SAS Institute, Inc. 1989) useing the model

$Y_{ij} = \mu + D_i + e_{ij}$

Y_{ij} represents the observations for dependent variables, μ is the least square mean, D_i is the effect of day i, e_{ij} is the residual error.

The same data were also analyzed in a 2 x 2 factorial design as mentioned above to determine differences between ensiling periods for a given treatment. Least significant difference was used to separate means when significant effects ($P < 0.05$) were detected (Gomez and Gomez 1984). Data of microbial populations were transformed (log 10) but not statistically analyzed because samples were pooled for each forage treatment.

Results

Ensiling characteristics

There was a rapid decline in pH during the first four days of ensiling with no significant decline after day 8 post-ensiling for all treatments (Figure 5-1). Inoculated silages had lower ($P < 0.05$) pH than untreated silages at days 2, 4, and 8 post-ensiling. However the pH at day 45 was similar for all silage treatments. Forage millet cultivars had no effect on pH throughout the ensiling

periods. The pH of all the 45-day silages was below 4 suggesting that silages were well fermented.

The concentration of lactic acid increased ($P < 0.05$) between days 0 and 8 post-ensiling for inoculated silages and between days 0 and 16 post-ensiling for the untreated silages (Figure 5-1). Lactic acid concentration was higher ($P < 0.05$) for inoculated than untreated silages at all ensiling periods with minimal effect of forage millet cultivars. No cultivar×inoculation interactions were observed for pH and lactic acid concentration at any given ensiling period (Figure 5-1). Acetic acid concentration increased ($P < 0.05$) steadily throughout the ensiling period of all forage millet treatments and was higher ($P < 0.05$) for untreated than inoculated silages at days 8, 16 and 45 post-ensiling (Figure 5-1). Significant cultivar ×inoculation were noted for acetic acid concentration on days 8 and 45 post-ensiling which indicate that differences between untreated and inoculated silages were greater for BM than RM. There was a sharp decline in WSC for all ensiled forage treatments up to 4 days post ensiling (Figure 5-1), with minimal changes after day 8 post-ensiling. Inoculated silages had lower.

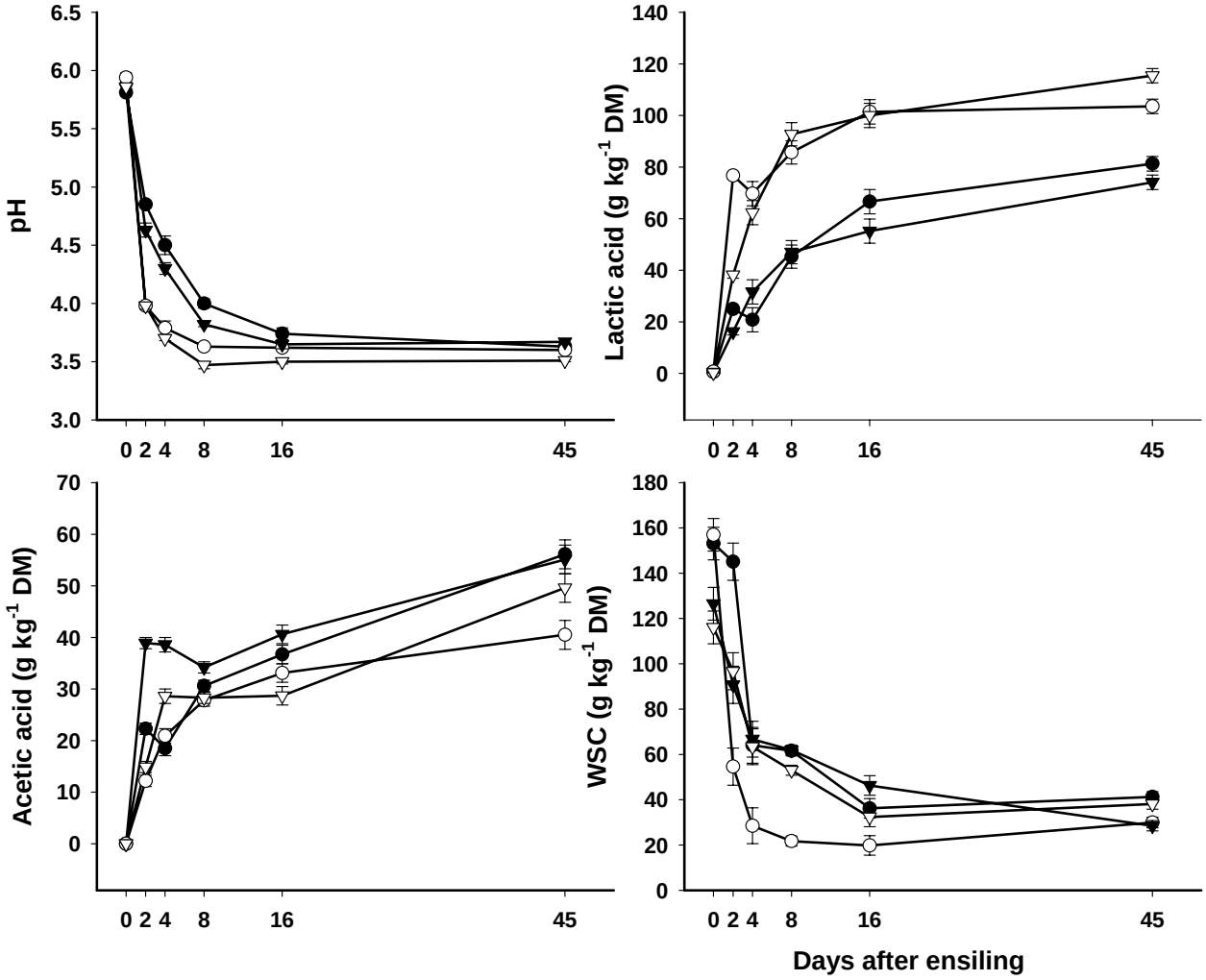

Figure 5-1: Changes in pH, lactic acid, acetic acid and water soluble carbohydrates (WSC) during ensiling of regular (RM), or brown midrib millet (BM) treated with inoculum (IN) or untreated (CT) (● RMCT, ○RMIN, ▼BMCT, ▽ BMIN). Vertical bar represent ± SEM

($P < 0.05$) WSC concentrations than untreated silages days 4, 8 and 16 post-ensiling (Figure 5-1)The decline in WSC during the first four days of ensiling was higher ($P < 0.05$) for inoculated than untreated silages. There were significant cultivar×inoculation interactions for WSC concentration on days 2 and 8 post-ensiling indicating that the effect of inoculation was greater for RM than BM. Dry matter recovery averaged 985 g kg^{-1} and was not affected by inoculation or forage millet cultivar (Table 5-2).

Protein fractions

Soluble protein and NPN increased ($P< 0.05$) rapidly between days 0 and 4 post-ensiling for all forage millet treatments suggesting extensive proteolysis during the early phases of ensiling (Figure 5-2). There were no changes in SCP or NPN content between days 16 and 45 post-ensiling for all forage treatments. Inoculation had no influence on SCP or NPN content throughout ensiling. However, RM silages contained less ($P< 0.05$) SCP than BM silages at days 8 and 45 post-ensiling. Neutral detergent insoluble protein declined ($P< 0.05$) rapidly between days 0 and 2 post-ensiling for all forage millet treatments with no further decline between days 16 and 45 post-ensiling (Figure 5-2). Changes in NDICP content during ensiling was not affected by inoculation or millet cultivar. Changes in TP content during ensiling were similar to those observed for NDICP.

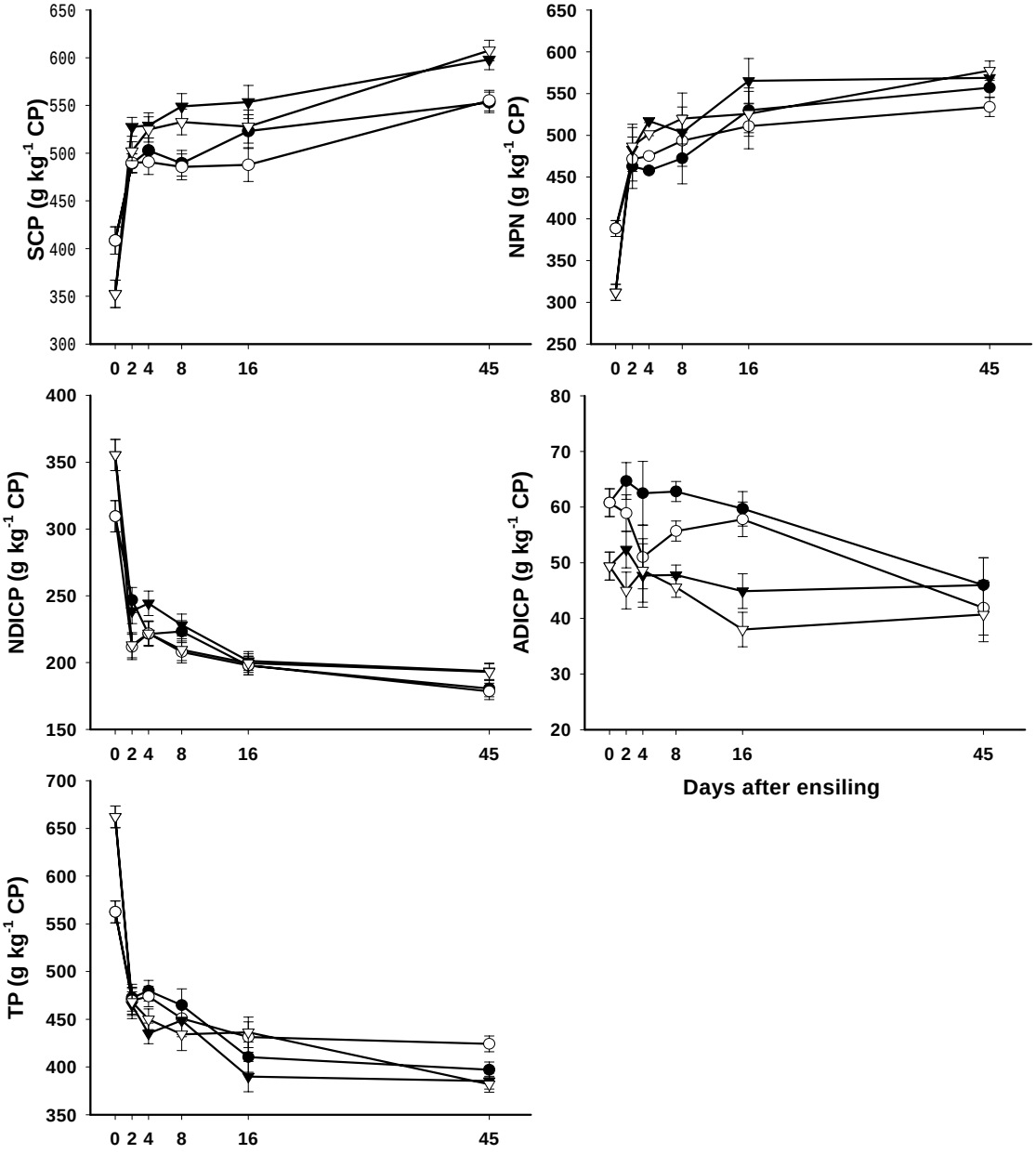

Figure 5-2:Changes in soluble crude protein (SCP), non protein nitrogen (NPN), neutral detergent insoluble crude protein (NDICP), acid detergent crude protein (ADICP), and true protein (TP) during ensiling of regular (RM), or brown midrib millet (BM) treated with inoculum (IN) or untreated (CT) (● RMCT, ○RMIN, ▼BMCT, ▽ BMIN). Vertical bar represent ± SEM.

In general, the increase in SCP and NPN was due to the degradation of NDICP and TP during ensiling. The ADICP concentration did not change significantly during ensiling for all forage treatments and averaged 55 and 44 g kg^{-1} in fresh forages and silages, respectively (Figure 5-2). No cultivar ×inoculation interaction was observed for any of the protein fractions at any given ensiling period. Chemical composition of the 45-day silages Chemical composition of pre-ensiled forages is presented in Table 5-1.

Relative to pre ensiled RM, pre-ensiled BM contained higher ($P< 0.05$) NDF, ADF, lignin (sa), and ADICP concentration but lower ($P< 0.05$) WSC concentration. Except for protein fractions (i.e. SCP, NPN, and NDICP) and WSC concentration, the composition of the 45 days silages (Table 5-2) was similar to that of the pre-ensiled forages. Inoculation had no effect on fibre or protein fractions of the 45-day silages. However, a tendency for lower ($P = 0.09$) aNDF concentration was observed for 45-day inoculated silages relative to untreated ones, whereas inoculation had no effect on protein fractions of the 45-day silages. Inoculation had no effect on IVDMD of the 45-day silages (Table 5-2). Concentrations of aNDF, ADF, and lignin (sa) were greater ($P < 0.05$) for RM than BM silages (Table 5-2). In vitro DM disappearance was also higher ($P < 0.05$) for BM than RM silages.

Table 5-1. Chemical composition of regular and brown midrib millet forage before ensiling.

	Regular millet	Brown midrib Millet	S.E.M
Dry matter (g kg^{-1})	244	261	5.1
Neutral detergent fiber (g kg^{-1} DM)	638a	595b	7.0
Acid detergent fiber (g kg^{-1} DM)	351a	308b	5.8
Acid detergent lignin (g kg^{-1} DM)	27a	14b	0.3
Water soluble carbohydrates (g kg^{-1} DM)	155a	121b	6.9
Crude protein (g kg^{-1} DM)	145	151	4.4
SCP (g kg^{-1} CP)	409	353	12.8
NPN (g kg^{-1} CP)	388	312	14.1
NDICP[y] (g kg^{-1} CP)	309	342	15.0
ADICP (g kg^{-1} CP)	61a	49b	1.6
TP (g kg^{-1} CP)	558a	639b	16.6
IVDMD	710b	771a	7.1

Means in the same row followed by different letter differ ($P < 0.05$).

Table 5-2. Effect of inoculation on chemical composition of regular and brown midrib millet silage.

	Regular millet		Brown midrib millet			Treatment effect (*P* value)		
	Untreated	Inoculated	Untreated	Inoculated	SEM[z]	Inoculation	Millet cultivar	Inoculation x millet cultivar
DM (g kg^{-1})	203	208	217	220	2.2	0.08	<0.01	0.58
NDF (g kg^{-1} DM)	632	607	598	588	9.6	0.09	0.03	0.47
ADF (g kg^{-1} DM)	368	368	329	334	3.3	0.46	<0.01	0.46
ADL (g kg^{-1} DM)	31	33	15	12	1.4	0.42	<0.01	0.12
CP (g kg^{-1} DM)	162	165	171	169	3.4	0.84	0.08	0.33
Soluble protein (g kg^{-1} CP)	553	555	598	608	1.07	0.62	<0.01	0.73
NPN (g kg^{-1} CP)	557	534	569	577	7.9	0.38	<0.01	0.08
NDICP (g kg^{-1} CP)	181	178	194	193	0.61	0.84	0.05	0.90
ADICP (g kg^{-1} CP)	46	42	46	41	4.1	0.29	0.88	0.89
TP (g kg^{-1} CP)	397	424	385	382	8.3	0.19	0.01	0.10
DM recovery %	98.3	99.4	98.0	98.3	0.40	0.17	0.51	0.81
IVDMD (g kg^{-1} DM)	736	755	791	774	7.0	0.90	<0.01	0.20

[z]Pooled standard error of the mean for the four treatments.

Changes in microbial population during ensiling

Even though no statistical comparisons could be made among treatments due to pooled samples of each treatment for microbial population, certain trends were observed. For all forage millet treatments, population of lactic acid bacteria increased numerically from days 0 to 4 post-ensiling then stabilized up to day 45 post-ensiling (Table 5-3). Population of lactic acid bacteria at days 0 and 2 post-ensiling were numerically greater for inoculated than untreated silages. However, differences in lactic acid bacteria between inoculated and untreated forages were minimal at later stages of ensiling (i.e. days 16 and 45 post ensiling). Population of enterobacteria declined to an undetectable level for all forage millet treatments by day 8 post-ensiling. However, the decline during the first 2 days of ensiling was numerically greater for inoculated than untreated silages. Total numbers of yeasts and moulds declined to less than 2.5 log CFU g^{-1} during the first 4 days of ensiling and remained at low levels up to day 45 post-ensiling. Inoculation and millet cultivars had no influence on yeast and mould counts.

Table 5-3. Microbial population changes during ensiling of control and inoculated regular and brown midrib millet (log CFU g^{-1} sample).

	Days of ensiling					
	0	2	4	8	16	45
Lactic acid bacteria						
Regular millet control	3.69	5.12	7.54	7.57	7.54	7.51
Regular millet inoculated	4.79	6.76	7.20	7.31	7.26	7.12
Brown midrib millet control	3.52	5.91	8.73	7.65	7.63	7.58
Brown midrib millet inoculated	5.10	7.93	8.86	7.98	7.56	7.52
Enterobacteria						
Regular millet control	5.67	2.74	ND	ND	ND	ND
Regular millet inoculated	5.80	<2.00	ND	ND	ND	ND
Brown midrib millet control	5.82	4.66	2.61	ND	ND	ND
Brown midrib millet inoculated	5.75	<2.00	ND	ND	ND	ND
Yeast and mould						
Regular millet control	4.15	3.25	<2.00	<2.00	<2.00	<2.00
Regular millet inoculated	3.10	3.42	<2.00	<2.00	<2.00	<2.00
Brown midrib millet control	4.63	2.77	<2.00	<2.00	<2.00	<2.00
Brown midrib millet inoculated	4.79	2.20	2.02	<2.00	<2.00	<2.00

ND :not detected.

Aerobic exposure and aerobic stability

All silages deteriorated following 7 days of exposure as indicated by high pH and low lactic acid concentration (Table 5-4). Inoculation had no effect on pH and lactic acid concentration following aerobic exposure. However, numbers of

yeasts and moulds and DM losses were higher ($P < 0.05$) for inoculated than untreated silages (Table 5-4). A cultivar×inoculation interaction was observed for yeast and mould counts reflecting that the effects of inoculation on yeast and mould counts were greater ($P < 0.05$) for BM than RM silages. Millet cultivar had no effect on aerobic deterioration (Table 5-4). The aerobic stability (i.e. time required for the silage temperature to rise by 2 ºC), ranged between 106 and 164 h with untreated silages being more ($P < 0.05$) aerobically stable than inoculated ones (Table 5-4). Millet cultivar had no effect on pH, yeast and mould counts, and DM loss, but BM decreased ($P < 0.05$) lactic acid compared with RM.

Table 5-4. Effect of aerobic exposure on quality and aerobic stability of control and inoculated regular and brown midrib silages.

	Regular millet		Brown midrib millet			Treatment effect (*P* value)		
	Untreated	Inoculated	Untreated	Inoculated	SEM[z]	Inoculation	Millet cultivar	Inoculation x millet cultivar
pH	7.4	7.5	6.9	8.1	0.37	0.12	0.82	0.18
Lactic acid (g kg^{-1} DM)	2.4	2.0	1.4	1.8	0.31	0.97	0.03	0.14
Yeast and mold (log CFU g^{-1})	6.9	7.6	6.7	8.1	0.10	<0.01	0.24	<0.01
Dry matter loss (%)	10.1	13.1	7.5	13.3	1.31	<0.01	0.38	0.30
Aerobic stability[y]	153	134	164	103	4.6	<0.01	0.07	<0.01

[z]Pooled standard error of the mean for the four treatments.

[y] Time to heat ≥2 °C (h).

Discussion

Our results for fermentation parameters agree with other studies which showed higher lactic acid concentrations and lower pH and acetic acid concentrations for inoculated than untreated silages (Fraser et al. 2001a; Kung et al. 2003a). The pool of naturally occurring lactic acid bacteria consists of both homo- and hetero-lactic acid bacteria (Pahlow et al. 2003). Homofermentative LAB produce two molecules of lactic acid from the fermentation of WSC, whereas heterofermentative LAB produce one molecule of lactic acid, and one molecule of other products (i.e. acetic acid, propionic acid, or ethanol; Kung and Ranjit 2001; Kung et al. 2003a,b). It is expected that inoculating forages with homofermentative LAB will increase the population of homofermentative LAB relative to that of heterofermentative ones which will drive the fermentation towards higher lactic acid and less acetic acid concentrations (Seal 1986; Hristov and McAllister 2002;Kung et al. 2003a). This will also accelerate the decline in pH during early stages of ensiling due to the faster production of lactic acid.

Concentrations of WSC in all pre-ensiled forages (121 to 155 g kg^{-1} of DM, Figure 5-1) were adequate for producing good quality silages (Lunden Petterson and Lindgren 1990). Pre-ensiled RM contained 30% more WSC than BM (Figure 5-1). However, in a previous study, pre-ensiled BM has been found to contain more WSC that pre-ensiled RM (Hassanat et al. 2006). Reasons for this

inconsistency are unclear. However, several differences existed between the two studies. Firstly, the forages were grown in two different locations and secondly, forages used by Hassanat et al. (2006) were first cut while the ones used in the present study were second cut. Factors such as forage type, stage of maturity, fertilization and environmental conditions have also been found to affect WSC of pre-ensiled forages (Buxton and O'Kiely, 2003). Differences in residual WSC concentration between inoculated and untreated silages are likely due to the increased population of LAB as a result of inoculation. Faster decline in WSC concentrations of inoculated relative to untreated silages have been reported for barley (Hristov and McAllister 2002) and alfalfa (Kung et al. 1991; Rizk et al. 2005) silages. Inoculation has been shown to reduce DM loss of alfalfa silage (Kung et al. 2003a). However, inoculation increased DM losses in maize silage (Kung et al. 2004) and produced variable results for barley silage (Kung and Ranjit 2001).

Proteolysis is an inevitable consequence of the ensiling process. Up to 75% of forage TP can be converted to NPN during the first few days of ensiling by the action of plant proteases (Rooke and Hatfield 2003; Hassanat et al. 2006). One of the anticipated benefits of inoculation is to reduce proteolysis during early phases of ensiling as a result of faster decline in pH (Weinberg and Muck 1996; Kung et al. 2003a). Controlling proteolysis is based on creating a low pH

environment that is unsuitable for the action of plant and microbial proteases (Rooke and Hatfield 2003). Nonetheless, data on the effects of inoculation on proteolysis are inconsistent. Inoculation reduced ammonia N concentrations in forage pea (Fraser et al. 2001a) and alfalfa (Sheperd et al. 1995; Whiter and Kung 2001) silages whereas it failed to reduce proteolysis in barley (Kung and Ranjit 2001; Hristov and McAllister 2002), wheat (Froetschel et al. 1991), maize (Ranjit et al. 2002) and kale (Fraser et al. 2001b) silages. Hristov and McAllister (2002) reported that only one out of three types of inoculants was able to reduce proteolysis in barley silage. Factors other than type of inoculant such as forage species, DM content and initial pH can significantly influence the extent of proteolysis (McKersie 1985). In the present study, despite the faster decline in pH, inoculation failed to reduce the extent of proteolysis. Our findings are in good agreement with those of Petit and Flipot (1990) who found no difference in SCP content between inoculated and untreated alfalfa silage. Muck (1989) reported that of the soluble N fractions, only ammonia was reduced by inoculation. In the present study, there was a rapid decline in pH of both untreated and inoculated silages despite statistical differences, which may help explain the lack of effect of inoculation on proteolysis.

The lack of effects of inoculation on chemical composition and IVDMD of the 45-day silages is in agreement with other studies that showed no significant

effects of inoculation of chemical composition of corn (Kung et al. 1993), alfalfa (Rizk et al. 2005) and forage peas and beans (Fraser et al. 2001a) silages. Inoculation also had no effect on in vivo digestibility of forage peas and beans (Fraser et al. 2001a, 2001b), or in situ digestibility of barley (Hristov and McAllister 2002; McAllister et al. 1995) and maize (Filya 2003) silages.

Differences in chemical composition and IVDMD between RM and BM silages are consistent with previous findings (Cherney et al. 1988; Oba and Allen 1999; Casler et al. 2003). Improvement in DM disappearance of brown midrib forages is strongly related to their lower lignin content relative to their normal counterparts (Cherney et al. 1991). In our study, BM silages contained 60% less lignin (sa) than RM silages.

It is expected that the rapid reduction in pH during early phases of the ensiling process will inhibit the growth of undesirable microbes such as enterobacteria and yeast. This was evident by the low yeast and mould numbers and the undetectable enterobacterial population by day 4 post-ensiling (Table 5-3). Growth of enterobacteria is usually inhibited at pH < 4.5 (Bolsen et al. 1996). Other factors in the silo such as competition for substrate from other microbial population and anaerobic environment may also contribute for the reduction in yeasts and moulds population in early stages of the ensiling process (Sebastian et al. 1996). The lack of effects of inoculation on yeast and mould numbers in the

present study is in agreement with the findings of Sebastian et al. (1996) and Kung and Ranjit (2001). However, inoculation of homofermentative LAB has been found to increase yeast and mould counts of maize and sorghum silages in some studies (Sanderson 1993; Filya 2003).

Effects of inoculation with homofermentative LAB on silage quality following aerobic exposure are well documented. Cereal silages inoculated with homofermentative LAB had higher pH, DM losses, and yeast and mould count than untreated silages following aerobic exposure (Weinberg et al. 1993; Weinberg et al. 1999; Filya 2003). It has been suggested that silages with high lactic acid concentrations will deteriorate faster than those with low lactic acid concentrations (Johnson et al. 2002). Our results in the present study support the findings of Johnson et al. (2002). Yeast and mould growing on aerobically exposed silages consume lactic acid and other nutrients, causing an increase in pH and DM loss. Heat generated from these metabolic activities causes a rise in silage temperature (McDonald et al. 1991; Pahlow et al. 2003).

Silages inoculated with homofermentative LAB are often less aerobically stable than those inoculated with heterofermentative LAB (Weinberg et al. 1993). This is likely due to the fact that more acetic acid is produced during hetero- than homo-fermentation. Acetic acid is stronger antimytotic than lactic acid (Weinberg and Muck 1996; Ranjit and Kung 2000). Furthermore, lactic acid can be used as

an energy source for yeasts and moulds under aerobic conditions, which is not the case with acetic acid (McDonald et al. 1991). Similar effects of inoculation on aerobic stability have also been reported for barley (Kung and Ranjit 2001) and high moisture ear maize (Sebastian et al. 1996) silages. The choice between homofermentative or heterofermentative inoculants depends of the purpose of inoculation. Homofermentative inoculants are more efficient in improving fermentation characteristics whereas heterofermentative inoculants are more powerful in maintaining aerobic stability of silages (Kung et al. 2003a).

Conclusions

Inoculating forage millet with homofermentative LAB caused a rapid decline in pH during the early stage of ensiling as a result of increased lactic acid concentration. Despite the fast decline in pH, inoculation failed to reduce the extent of proteolysis during ensiling and had no influence on chemical composition or in vitro disappearance of the 45-day silages. Differences in chemical composition and in vitro disappearance between the 45-day silage are mostly due to differences between the two forage millet cultivars. Inoculation reduced aerobic stability of forage millet silages by an average of 40 h. The two forage millet cultivars can produce good quality silage under low DM conditions without the addition of inoculants. Further studies are required to study the effects of other types of inoculants and silages additives on aerobic stability and fermentation characteristics of millet silages of various DM contents.

CHAPTER VI. GENERAL DISCUSSION AND CONCLUSION

Our results showed that millet can be grown under Southern Quebec conditions. Forage yield averaged 16.6 and 9.8 ton ha^{-1} respectively under conditions of sandy loam soil and 280 mm rain (Table 3-1). Regular millet and BM yielded 6.3 and 4.1 ton ha^{-1}, respectively, under conditions of clay loam soil and 207 mm rain (Table 4-1). Forage yield from brown midrib millet was 20-50% less than that obtained from RM. Brown midrib millet plants were 30% shorter, and 20% less tillering and more leafy than regular millet plants which is the reason behind yield reduction (Table 3-1), which is supported by the finding of Casler et al. (2003) on sudangrass.

The differences between RM and BM yield were small at VS (25%), compared with the differences at HS (45%). This suggests that a multiple cut approach at vegetative stage would maximize yield of high quality forage, and reduced differences between the two millet cultivars. Harvesting plants at heading rather than vegetative stage almost doubled millet yield. However, RM accumulated more DM with advancing development than BM. This increase in yield was due to increase in stem proportion and in DM content of the plant, which was also more pronounced for RM, compared with BM (Table 3-1). Increasing seeding rate from 5-15 kg ha^{-1} had positive impact on millet yield, (Table 2-1).

Because of high moisture and WSC content, millet was suitable for silage making. At harvest, moisture content varied between 800 to 880 g kg^{-1} (Table 4-1), which makes application of any moisture reducing procedure crucial for successful ensiling. Dry matter concentration increased with advancing development, but millet cultivar had inconsistent impact and no effect of seeding rate (Table 3-1, Table 4-1).Concentration of WSC averaged 140 g kg^{-1} DM and did not vary much between the two millet cultivars or affected by seeding rate (Table 2-2, 4-1), but increased by 45% with advancing development from vegetative to heading stage. According to Lunden Pettersson and Lindgrin (1990), and at this concentration of water soluble carbohydrate, the minimum DM content of millet at ensiling should not be less than 170 g kg^{-1} to ensure enough supply for lactic acid bacteria to acidify and preserve silage.

Lactic acid bacteria dominated ensiled material starting the first few days to the end of ensiling period as reached above 7 log CFU g^{-1} within the fourth day of ensiling (Table 4-3, Table 5-3). These bacteria produced increasing concentrations of lactic and acetic acids (Figure 4-1, Figure 5-1), which minimized activities of other microbes . Therefore, *Enterobacteria* population was reduced by day 4 and was not detected after day 8 of ensiling, while yeast and moulds count were reduced by day 2 of ensiling and was around 2 log CFU g^{-1} from day 8 of ensiling and thereafter (Table 4-3, Table 5-3). This explain the high

silage quality produced from the two millet cultivars; indicated by low pH (less than 4.2), high lactic and acetic acid concentration, and non detectable butyric or isovaleric acid concentration (Figure 4-1, Figure 5-1). Addition of lactic acid bacteria before millet ensiling did speed up the acidification process (Figure 5-1), and increased the lactic:acetic acid ratio, but did not have any considerable influence on millet silage preservation. Aerobic stability of millet silage averaged 5.6 days, and was lowest for inoculated silages, and for those with higher moisture content. Millet cultivar or seeding rate had no impact on millet silage aerobic stability (Table 4-4, Table 5-4).

Proteolysis is inevitable during ensiling, as plants true protein are degraded into non protein nitrogen, which reduces silage quality (Rooke and Hatfield 2003). About 40% of millet forage protein was solubleized and degraded into NPN during ensiling (Figure 4-2, Figure 5-2). There was no consistent trend for the impact of millet cultivar, stage of development at harvest on proteolysis. Inoculation failed to reduce rate or extent of proteolysis, which confirms the postulation that proteolysis is dependent on plant enzymes and not related to silage pH (Petit and Flipot 1990; Rooke and Hatfield 2003).

Nutritive value of millet forage and silage was most influenced by cultivar and stage of development at harvest, and not affected by variation in seeding rate or inoculation. Crude protein content did not vary much between the two

millet cultivars, ranging between 70 to 150 g kg^{-1} with millet forage harvested at vegetative stage contained 40% more CP than when harvested at heading stage. About 65 to 80% of plant nitrogen is part of the cell content, which decrease in proportion with advancing plant development. This agrees with Mustafa and Seguin (2003) and Gupta and Pradhan (1975) findings.

Fiber components were affected by millet cultivar and stage of development at harvest. Brown midrib millet forage contained significantly less NDF, ADF and ADL than RM by 5, 10 and 35% respectively across the studies (Table 2-2, Table 3-1, Table 4-1). It is well documented that brown midrib trait reduces lignin content in millet up to 50% (Cherney et al. 1991 Barriere 2004). Advancing stage of development increased ADF concentration by 5% and almost doubled ADL concentration in the two millet cultivars (Table 4-1).

These changes occurred mainly in the stem, rather than the leaf of the two millet cultivars during stages of development (Table 3-2). Neutral detergent fiber concentration in RM leaves was similar to leaves of BM, but was 5% less in stems of BM compared with RM stems. However, both leaves and stems of BM contained 6 and 15 % less ADF and 50% and 55% less ADL than leaves and stems of RM, respectively (Table 3-2). Millet leaves harvested at heading stage contained 48% more ADL than those harvested at VS, with no impact on NDF and ADF concentrations. In stems, NDF, ADF, and ADL concentration increased

by 4, 6 and 86%, respectively as stage of plant development advanced (Table 3-2). The impact of stage of development on NDF, ADF and ADL concentrations was more pronounced for RM than BM.

Millet forage cell wall mainly made of arabinose, xylose and glucose at concentrations of 40, 245 and 440 g kg^{-1} in leaves, and 35, 260, and 490 g kg^{-1} in stems respectively. Brown midrib trait increased arabinose and xylose concentration in millet leaves and stems but have no impact on glucose concentration (Table 3-3), which agree with the finding of Cherney et al. (1986). General increase in major cell wall sugars concentration was observed in leaves and stems, except for xylose in stems and glucose in leaves (Table 3-3), indicating an increase in cell wall proportion (Wilson 1997; Aman 1993). The increasing arabinose:xylose ratio observed in leaves indicated development of primary cell wall in BM, which continued with advancing plant development. However, in stems arabinose:xylose ration decreased in RM stems indicating secondary cell wall development, with no change in BM stems (Table 3-3).

Leaves and stem of RM contained higher concentration of ester linked *p*-coumaric acid and ether linked ferulic acid than those of BM plants, with lower lower *p*-coumaric acid:ferulic acid in BM leaves and stems (Table 3-4). This showed that degree of lignification was higher in RM plant parts. The concentration of these phenolics increased mainly in stems of the two millet

cultivars, not in leaves, with advancing development, and the increase was more pronounced in RM (Table 3-4) which indicated less lignification with advancing development from vegetative to heading stage in brown midrib stems. These differences are consistent with differences in ADL concentrations between the two millet cultivars at the two stages of development. The effect of the brown midrib trait was greater on stems than leaves, and reduced the impact the stage of development on the accumulation of phenolic acids in stems of forage millet.

In vitro and in situ degradability of millet forage were affected by cultivar and stage of development at harvest (Tables 2-2, 2-3, 4-1, 4-2, 5-1), but not influenced by seeding rate (Tables 4-2, 4-3) or inoculation (Table 5-2). Across all studies, in vitro DMD of BM forage was 8 to 10 % higher than RM (Tables 2-2, 2-3, 4-1, 4-2, 5-1). This increase in DM degradability resulted from 10% increase in NDF degradability in BM compared with RM (Tables 4-1, 4-2). Because the lignin concentration in BM plants was 50 to 70% of that in RM, we observed the higher DMD and NDFD in BM plants. The higher leaf proportion observed in BM (Tables 2-1, 3-1) further explained these differences, as leaves are more degradable than stems (Table 3-5). These differences in DM and NDF degradability were observed in RM and BM leaves as well as stems (Table 3-5). Brown midrib trait improved in vitro NDF degradability by increasing xylose and glucose degradability in and increasing xylose degradability in stems (Table 3-5).

Advancing stage of development reduced in situ DMD and NDFD in RM more severely than BM, especially in stems.

The high correlation between cell wall phenolic and lignin indicated that they were deposited in the cell wall equally (Table 3-6). Regression analysis showed that lignin concentration had negative linear effect on DM and NDF degradability, and quadratic negative effect on glucose and xylose degradability (Table 3-7). Lignin concentration could be used as indicative of forage millet degradability at vegetative and heading stages of development.

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