Effects of Processing and Moisture Content on the Fermentation and Intake of Alfalfa-grass Ensiled as Big Bales

A Thesis Submitted to

The Faculty of Graduate Studies

The University of Manitoba

By

Lynne Pinder

In partial fulfillment of requirements for the degree of

Master of Science

Department of Animal Science

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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Effects of Processing and Moisture Content on the Fermentation and Intake of Alfalfa-Grass Ensiled as Big Bales

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ABSTRACT

Advances in baling technology over the last decade have enabled producers to process forage by chopping it at the time of baling. This technology may be attractive to beef cattle producers as it may provide an opportunity to improve forage quality and add value to their calves by feeding high quality big bale silage (BBS) in backgrounding systems. To examine the merit of such a procedure, the effects of processing and moisture content on the fermentation patterns and intake of an alfalfa-grass stand, ensiled as BBS, were examined. Alfalfa-grass forage was baled at dry matter (DM) levels ranging from 22 to 67% DM. Both long-fibre and short-fibre bales were manufactured. Representative grab samples were collected throughout the trial to estimate the particle size of the chopped diets. Core samples were collected immediately after baling and at 24 and 48 h post-wrapping for bacterial analysis. Samples were also collected immediately after baling and on days 1, 2, 7, 21, 42 and 70 of storage for nutrient analysis.

Processing had a significant effect on particle size as the diets chopped at baling had a higher proportion of particles > 19mm compared to diets that were chopped at baling or left as long fibre. There was a significant DM*Time (T) interaction ($P<0.05$) for lactic acid producing bacteria populations. At baling, DM had no effect but after 48 h, the low DM bales had significantly higher counts with 9 $\log_{10}$ colony forming units (CFU) g^{-1} compared to 8 $\log_{10}$ CFU g^{-1} in the medium DM bales and 6 $\log_{10}$ CFU g^{-1} in the high DM bales. This corresponded with a lower pH of 5.75 in the low DM bales during the 70 days of storage, compared to the medium and high DM bales, which had pH levels at 6.14 and 6.11, respectively. However, at the end of 70 days of storage, the effect of the higher number of lactic acid producing bacteria after the first 48 h of storage was no

longer evident in the low DM bales as there was no longer a significant difference in pH values across the three DM treatments. Moisture content also had an effect on the WSCHO profile, with the high DM bales having a significantly higher WSCHO level over the 70 days of storage. Lactic acid levels were significantly ($P < 0.05$) lower in the high DM bales after 21 days of storage. This corresponded with higher lactic acid producing bacteria count and lower pH in the low DM during 70 days of storage. However, even though lactic acid levels continued to be lower in the high DM bales at 70 days, this did not result in a significantly higher pH level in the high DM bales at 70 days of storage. At feeding, lactic acid, propionic, isobutyric, butyric and ethanol levels were all greater in the low DM bales. Processing had no effect on the fermentation end products.

Effects of chopping and DM on intake were determined by subsequently feeding BBS at low (55.5%) and high (66.4%) DM to 24 crossbred steers, body weight (BW) 260 SE $\pm$ 20.3 kg, for four 28-day periods. Feed delivered samples were taken daily on an individual basis for the chopped diets and weekly for the long fibre diets and analyzed for DM, crude protein (CP) and neutral detergent fibre (NDF). Total feed offered and refused was recorded daily for the chopped diets and weekly for the long fibre diets. Intake levels of low DM bales (3.27 % % $BW^{.75}$) were lower ($P<0.05$) than intake levels of high DM bales (3.56 % $BW^{.75}$).

Overall, the high CP and intake levels that were achieved indicate that BBS can be produced to meet CP requirements. Results of this study indicate that processing did not have a significant effect on the fermentation patterns or intake of alfalfa-grass forage ensiled as BBS. Dry matter had an effect on silage quality and intake of alfalfa-grass

ensiled as BBS. Since successful fermentation did not occur in any of the treatments in this trial the optimum DM range is unclear. However, based on a combination of fermentation parameters, labor requirements and intake values, a targeted DM level between 55 and 60% DM is suggested.

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ABBREVIATIONS

ADF	=	acid detergent fibre
ADL	=	acid detergent lignin
BA	=	butyric acid
BBS	=	big bale silage
BSE	=	bovine spongiform encephalopathy
BW	=	body weight
CC	=	chopped at baling
CFU	=	colony forming unit
CHO	=	carbohydrate
CO_2	=	carbon dioxide
CP	=	crude protein
DM	=	dry matter
DMI	=	dry matter intake
DON	=	deoxynivalenol
HCl	=	hydrogen chloride
H_2O	=	water
KHO	=	potassium hydroxide
LA	=	lactic acid
LC	=	chopped at feeding
LL	=	long fibre
ME	=	metabolizable energy
mL	=	milliliter
NDF	=	neutral detergent fibre
NH_3	=	ammonia
NH_3-N	=	ammonia nitrogen
NO_3	=	nitrate
NPN	=	non-protein nitrogen
O_2	=	oxygen
PC	=	precision chopped
peNDF	=	physically effective NDF
RFV	=	relative feed value
T	=	time
TDN	=	total digestible nutrients
TMR	=	total mixed ration
WSCHO	=	water-soluble carbohydrate
VFA	=	volatile fatty acid

1.0. INTRODUCTION

The high equipment costs and labor requirements of conventional silage systems are not suitable for many smaller-scale livestock producers in Western Canada. Although big bale silage (BBS) is a relatively popular alternative to conventional silage, there is limited information available to producers that clearly define optimum ensiling conditions for production of high quality forage, as well as optimum intake and performance of cattle who consume it.

A variety of factors influence silage quality at the time of ensiling. Two major factors are forage dry matter (DM) content (Nicholson et al. 1992, Wright et al. 2000, Han et al. 2004) and particle size at time of ensiling (Charmley et al. 1999). Since the ensiling conditions in BBS systems are different than those in conventional systems, it is thought that optimum moisture level and chop length also differ. Various sources have reported optimum forage DM values, but the range is from 20 to 65 % moisture (Muck 1988, Beaulieu et al. 1993, Nicholson et al. 1992, Huhnke et al. 1997) and as such requires further examination such that production of high quality silage using BBS systems may be achieved.

Big bale silage systems have been shown to have reduced fermentation and higher pH levels compared to traditional chopped silage (Nicholson et al. 1991). As such, the recent introduction of baling equipment which possesses the ability to chop forage at the time of baling may offer significant advantages to that produced using traditional production strategies where little, if any, processing occurs during the harvest period. However, the advantages and disadvantages of chopping high quality forage at the time

of baling compared to that which is chopped at the time of feeding or fed as long fibre have not been fully explored.

This study was conducted to assess the effect of moisture content and processing on the fermentation process and intake of alfalfa-grass ensiled as BBS. Information regarding optimum moisture and particle size will be valuable to producers looking for an alternative to traditional silage systems when high quality feed and optimum intake levels are required.

2.0. LITERATURE REVIEW

2.1 INTRODUCTION

2.1.1 Manitoba cattle production systems

Manitoba's cattle industry is comprised of approximately 9,900 beef cattle farms, of which 98% are cow-calf operations (Honey 2007). Traditionally, significant portions of the calves produced by these operations have been shipped to Alberta or the United States for backgrounding, finishing and slaughtering (Honey 2007). However, the closure of the United States-Canadian border in 2003 as a consequence of the identification of bovine spongiform encephalopathy (BSE, CFIA 2005) had a considerable impact on the cow-calf sector. The border was closed to live cattle for over two years. During this time, not only did Manitoba witness a significant increase in cow numbers, but also federal compensation programs coupled with low cattle prices served to encourage producers to retain ownership of their calves in the attempt to increase their value. Hence cow calf operators have gained experience and interest in backgrounding calves.

Backgrounding allows producers to add weight and, consequently value to young beef animals. This process involves raising weaned calves on a lower-energy, mainly forage-based diet for a period of time before the animal is placed on a high-energy ration for fattening (Vaage et al. 1998). Backgrounding has shown to be an effective method of increasing the carcass weight of light steers without negatively affecting carcass composition (Vaage et al. 1998). Given the large number of calves in Manitoba, the

potential to produce high quality forage in Western Canada and the dramatic increase in grain prices since mid-2006, adding value to beef animals by backgrounding calves on all- or high forage diets has become a very attractive alternative for many producers.

Traditionally, all-forage diets have been viewed to be most suited for animals at low production levels, such as pregnant beef cows. However, research conducted in the Western Canadian production environment (Moshtaghi-Nia and Wittenberg 1997) has suggested that there may be potential for all-forage diets to be used for other classes of cattle, which require a higher level of nutrition, such as backgrounded steers. A consistent supply of high quality forage is also important for lactating cows and replacement heifers in the dairy industry. Therefore, both beef and dairy producers would benefit from knowledge related to production and utilization of high quality forage.

Producers are currently utilizing a number of management techniques to ensure that the forage is high quality and that intake of the forage is optimal. These techniques include forage preservation as BBS and processing of forages prior to feeding. Cost and efficiency are also important criteria for feed preservation given that cost of production has been a key factor limiting the profitability of beef operations in the province (Small and McCaughey 1999). Therefore, available technology, coupled with the advantages listed above, such as the potential to produce high quality forages, gives producers in Western Canada a tremendous opportunity to add value to their animals before they are shipped for finishing.

2.1.2 Manitoba forage preservation systems

The only account of forage preservation systems utilized in Manitoba was published almost a decade ago. Survey results reported by Small and McCaughey (1999) showed that the winter feeding period in Manitoba covers more than 50% of the calendar year (approximately 220 days or longer for most farms). On a large scale basis, haymaking and ensiling are the two main options available to producers in Western Canada who wish to preserve forage for winter-feeding. Small and McCaughey (1999) reported that hay was the predominant winter forage for 97% of the survey respondents. Additionally, 77% of respondents reported also using straw and 2% reported using stockpiled pasture or swath grazing. Only 13% reported using silage as part of their winter feeding program. Of the respondents utilizing silage, 49% of the systems were bunker, 30% round bales, 20% stacked, 4% upright and 6% were bagged systems. Some respondents also reported using more than one type of forage preservation system in their operation. The authors of this study speculate that greater quantities of hay are produced relative to silage in Western Canada because hay is easier to transport and requires relatively low capital costs to produce.

Regardless of the forage preservation system used, the primary goal when making high quality feed is to preserve the original nutrients in the forage so that they are available for feeding at a later date (Charmley 2001). The highest quality feed is produced when the composition of the original forage is least altered during the various processes that take place between the time that the forage is standing in the field until the time that the feed is being consumed by the animal. Hay production is associated with higher field losses and lower storage losses compared to traditional silage production (McDonald et al. 1991). This is because one of the major differences between silage and

hay is the water (H_2O) content in the two types of feed. Haymaking involves the removal of the majority of the H_2O in the forage. Removal of H_2O is critical because the hay must be dry enough to prevent spoilage by microorganisms during the storage period since the forage is exposed to air and is not stored in a sealed silo (McDonald et al. 1991). During the wilting process that is required to reach the desired DM level, there is always a risk of DM and quality losses related to respiration, leaching and leaf shatter (McDonald et al. 1991). McDonald et al. (1995) conducted a study examining six commercial farms in Northeast England over a three-year-period. They measured losses of nutrients between hay harvest and feeding. This study found that total DM losses associated with hay production averaged 19.3%, with 13.7% related to field losses and 5.6% related to losses within the bale. Similarly, Collins (1991) reported that respiration and leaf shatter accounted for almost 80% of the DM loss from alfalfa that was dried without rainfall. If the forage is baled before it is adequately dried, there is an increased risk of mould developing and also the potential for spontaneous combustion (Coblentz et al. 2000).

To minimize the field losses associated with hay production, producers have the option of preserving forage as silage. Any crop that has sufficient water-soluble carbohydrates (WSCHOs) and moisture levels has the potential to be ensiled. The most popular silage crops in Manitoba include cereal grains, corn and alfalfa (MAFRI 2006). Silage is stored at a higher moisture level than hay, with moisture levels ranging from 20 to 80% (Charmley 2001). These moisture levels can generally be achieved within a relatively short period of wilting time, often within 24 hours (Nicholson et al. 1991, Nicholson et al. 1992). Therefore, silage production allows the producer to preserve plant

nutrients while being less dependent on weather conditions. When forage is stored at these high moisture levels, it must first go through a successful fermentation process to prevent spoilage. As discussed in the following section, successful fermentation relies on rapid exclusion of oxygen (O_2), production of acid and the ability of the forage to achieve a stable state (McDonald et al. 1991).

2.2 CONVENTIONAL SILAGE PRODUCTION

2.2.1 Benefits and limitations of conventional silage production

There are benefits and limitations to conventional silage production. Two of the main drawbacks associated with silage production are the required capital investment for silage harvesting equipment and for storage and feeding facilities (MAFRI 2006). There is also limited market potential because silage cannot be transported for long distances since it is heavy and deteriorates when exposed to air.

Furthermore, as a consequence of the utilization of WSCHO and proteolysis during the ensiling process, silage typically possesses lower levels of readily fermentable WSCHO and true proteins compared to fresh forage (McDonald et al. 1991). There are increased levels of organic acids and non-protein nitrogen (NPN) when conventional silage is compared to fresh forage. Furthermore, the pH of silage is lower than hay and, with the exception of big bale silage (BBS), the particle size is usually smaller than fresh or dried forage.

2.2.2 Silage storage systems

There are a variety of different silage storage systems available. However, each has the same objective, which is to provide an anaerobic environment to allow for fermentation of fresh forage to take place with minimum loss in feed value. The main silage storage systems include tower silos, bunker silos, pit silos, pile silage and BBS systems. All silos rely on compaction of the forage material to achieve O_2 removal and to establish an anaerobic environment.

The tower silo is a vertical structure with a cylindrical shape used to store a wide variety of livestock feed including whole-plant annual crops such as corn and barley, as well as perennial grasses and legumes and is well suited for mechanical feeding systems (Savoie and Jofriet 2003). Bunker silos require lower capital cost compared to tower silos, although increased labour is required during the time of feeding (Savoie and Marcoux 1985). Bunker silos consist of a flat area enclosed on two or three sides by vertical walls. Alternatively, in pile silage, the chopped forage is unloaded on the ground and compressed by a tractor driving over the forage material. In both cases, the compressed forage is then covered with a plastic film to exclude O_2 and prevent spoilage. The most common forage types used in bunker silos in North America are very similar to the forages ensiled in tower silos, as listed above.

Another type of system, which does not rely on a separate compaction process for O_2 exclusion, is BBS. Although these systems were once considered as an emergency alternative for storing surplus forage, they have become a mainstream conservation technique (Haigh et al. 1996) and will be discussed in detail in subsequent sections.

2.2.3 The ensiling process

Generally, silage can be defined as livestock feed that has been preserved through acidification. The acidic environment required for proper ensiling to take place results via fermentation in the absence of O_2 (Hancock and Collins 2006).

The three main criteria required to produce high quality silage include (1) rapid exclusion of O_2 (2) rapid growth of desirable lactic acid producing bacteria and (3) limited secondary fermentation throughout storage and feed-out (McDonald et al. 1995). There are many factors, including dry matter (DM) level, WSCHO level and the length of cut of the forage being ensiled, that can affect the efficiency of the fermentation process and the production of lactic acid producing bacteria in conventional chopped silage systems (Van Soest 1994; Huhnke et al. 1997; Charmley 2001). The stages of the fermentation process have been well summarized and documented by McDonald et al. (1991), Weinberg and Muck (1996), Pahlow et al. (2003) and Rooke and Hatfield (2003) as the 1) aerobic phase 2) fermentation phase 3) stable phase 4) feed-out phase. These phases, as described by the authors cited above, can be summarized as follows:

Phase I is the initial aerobic phase. This phase begins at the time of ensiling and continues until residual O_2 has been used up in the silo. Oxygen that is trapped within the forage is used for respiration of the plant and the microorganisms, which results in the generation of heat. During this first phase, plant proteases begin the breakdown of proteins to amino acids and carbohydrases increase the amount of WSCHO available for fermentation. Opportunistic organisms such as mold, yeasts and some bacteria, which require O_2, may also utilize these breakdown products for growth. If the silage is appropriately chopped and well compacted, and the silo is sealed as quickly as possible, the length of the aerobic phase can be minimized. A short aerobic phase leads to a

greater availability of WSCHO for lactic acid producing bacteria, whose presence is critical for a rapid drop in pH to occur.

Phase II is the fermentation phase. Fermentation is the process where a substrate, usually a sugar, is converted into other products by microorganisms under anaerobic conditions (Savoie & Jofriet 2003). Therefore, this phase begins once all the O_2 within the forage has been depleted. The length of this phase is influenced by the properties of the crop being ensiled and the ensiling conditions. Legumes, for example, are more difficult to ensile because of their higher buffering capabilities, compared to grasses (McDonald et al. 1995). Also, if the ensiling conditions are such that insufficient amounts of WSCHO are available, the fermentation process will be unable to successfully drop the pH level quickly enough to establish a stable storage environment. During the fermentation phase, facultative and obligate anaerobic microorganisms such as Enterobacteriaceae, clostridia, some types of bacilli and yeasts may compete with the lactic acid producing bacteria for the nutrients, which are being released as plant cells and tissues collapse. Under ideal fermentation conditions, the enterobacteria disappear, which leads to the development of a dominant lactic acid producing bacteria population. The speed of this transition to a lactic acid producing bacteria dominated population has a significant impact on the rate of lactic acid (LA) production and the resulting pH decline.

Once all of the O_2 is utilized within the forage, lactic acid producing bacteria and other anaerobic microorganisms continue to multiply, leading to the production of organic acids such as LA and acetic acid and a further reduction in pH. In conventional systems, the pH of well-preserved grass silage usually ranges between 3.7 and 4.2 (McDonald et al. 1995). In this pH range, the growth of undesirable microorganisms will

be restricted. Phase II lasts until the fermentable sugars are used up or when the low pH level inhibits further lactic acid producing bacteria growth.

Phase III is referred to as the stable phase. There is little activity during this phase if anaerobic conditions can be maintained. The pH during this phase is approximately 4.5 or lower depending on the buffering capacity of the forage. This phase may be shortened for a number of reasons which include: O_2 entering the forage, moisture infiltration, or if the pH level was never low enough to stop metabolic activity. Therefore, stability normally requires anaerobic conditions, low pH and/or freezing temperatures. During this phase, only acid-tolerant enzymes are active, causing a slow acid hydrolysis of structural and storage carbohydrate (CHOs). Proteases can also remain active and convert complex nitrogen compounds to ammonia (NH_3). Highly acid-tolerant yeast species often survive this period, along with bacilli and clostridia. These species are dormant as endospores and may become active once again if the proper conditions are created.

The fourth and final phase is referred to the feed-out phase. During this phase, O_2 has access to the silo face and to the silage approximately 10 to 15 cm behind the face (Tremblay 2008). As a result of air infiltration, acid-tolerant aerobic microorganisms, such a yeast and spores of certain molds, can proliferate. The microorganisms oxidize residual sugars, LA, acetic acid, and ethanol (Pahlow et al. 2003). The heat released by the oxidation process causes a rise in temperature and the pH begins to rise as the concentration of the acids decreases.

Overall, with sufficient LA, resulting in a low pH, spoilage during the feed-out phase is delayed. Conversely, presence of a heterogeneous microbial population

associated with secondary fermentation and a relatively high pH at feed-out can lead to rapid spoilage, especially if ambient temperatures are not low enough to prevent spoilage.

2.2.4 Factors affecting the ensiling process

Although silage has been produced for centuries, much research has been conducted in the last two decades to more fully understand the factors that impact the ensiling process. Further, the ability to evaluate the effects of ensiling on feed quality by measuring silage quality parameters has only recently been introduced through the Van Soest proximate analysis system (1994). Numerous factors can influence the chemical characteristics of the feedstuff being ensiled such as moisture, the nutrient profile, and the buffering capacity of the forage. Furthermore, the resulting pH levels contribute to the ensiling environment and the time that it takes to reach stable state. After a stable state has been achieved, there are further factors that affect silage quality. For instance, secondary fermentation can result in proteolysis and the establishment of undesirable microbial populations, such as yeast and clostridia. Fermentation losses are related to the more than 20 different reactions that can take place during the fermentation process depending on the substrate and microorganisms present (McDonald et al. 1991; McGechan 1990). Moisture level and temperature also affect microorganism activity (McDonald et al. 1991). High DM losses are usually due to a conversion of substrate into carbon dioxide (CO_2). The substrates available for fermentation, such as soluble sugars and hydrolysed cellulose, are usually 10 to 15% of the total DM. It has been estimated that the maximum fermentation loss is approximately 4% (McGechan 1990).

Silage quality is greatly affected by the moisture concentration of the crop being ensiled. The moisture level of the forage affects the physical, biological and chemical processes occurring in the silo (Muck et al. 2003). Wilting of forage before ensiling to reach the desired moisture content is a common practice throughout the world as a means of reducing the adverse microbial activity in silage, which then restricts the extent of the secondary fermentation processes (Marsh 1979; Charmley 2001). Another advantage to wilting is that the drier conditions tend to favor lactic acid producing bacteria over other types of bacteria; this reduces the risk of more harmful microbial pathways dominating the ensilage process (McDonald et al. 1991). The amount and extent of wilting that takes place affects the final DM content of silage.

Silages can be produced at a range of moisture levels depending on the type of silage system used. Generally, conventionally chopped silage should be produced at less than 70% moisture (McDonald et al. 1995). Although higher moisture levels in forage are advantageous for optimum compaction of the resulting silage, excess moisture can create both effluent problems and the risk that clostridia and other undesirable bacteria will proliferate (Collins and Owens 2003). Optimum forage moisture for grass and alfalfa stored in a tower silo can be as low as 40% (McDonald et al. 1991). An optimum moisture level for bunker, pit or pile silos tends to range from 50 to 75% depending on the type of storage system, additives used and the crop being ensiled (Rotz et al. 2003). Provincial recommendations state that the optimum moisture levels for most silos is 65% and 50 to 60% for BBS (MAFRI 2006). Savoie & Jofriet (2003) report that fermentation losses in Eastern Canada are mainly a function of moisture content and the possibility of clostridia fermentation, which produces butyric acid (BA).

The buffering capacity of the crop to be ensiled can also impact the ensiling process. The expected pH ranges for alfalfa preserved in conventional silage systems are usually at 4.0 to 5.0 (Whiter and Kung 2001). The increased pH associated with fermentation of legumes such as alfalfa, compared to that observed in grasses, may be attributed to their buffering capacity, which refers to the plants' ability to minimize changes in pH by accepting hydrogen (H) ions when they are in excess and donating H ions when they have been depleted (Campbell 1996). The high protein concentration of alfalfa is thought to contribute to the higher buffering capacity of legumes, such as alfalfa, compared to grass (Charmley 2001). Therefore, alfalfa requires more H ions to be produced than grasses, making it more difficult to achieve a rapid drop in pH. Fast acidification is necessary to control the growth of enterobacteria and clostridia (Pahow et al. 2003). As such, buffering capacity is even more of a concern in BBS production systems where there may be a decrease in DM density leading to prolonged aerobic phase and a slower pH reduction.

Good quality silage can be characterized by dominant lactic acid producing bacteria-populations resulting in high concentrations of LA. Acetic acid producing enterobacteria are usually the second most numerous groups of bacteria in silage and are often the most important lactic acid producing bacteria competitor. An indicator of silage quality is the number of and the rate at which enterobacteria decline in silage, that is partially influenced by anaerobiosis (Pahlow et al. 2003). The reduction in enterobacteria reflects the availability of nutrients and H_2O, an efficient conversion of those nutrients to fermentation products and a low pH. Furthermore, enterobacteria play an important role by degrading nitrate (NO_3) to nitrite and nitric oxide, which inhibit clostridial growth.

Despite this positive effect of enterobacteria, rapid suppression is desirable to reduce their fermentation of sugars to acetic acids (Pahlow et al. 2003)

Clostridia, which proliferate if the silage is aerobically unstable, usually derive their energy from the fermentation of CHOs and proteins (Pahlow et al. 2003). A crop is also prone to the presence of clostridia if the sugar concentration of the substrate is insufficient for the lactic acid producing bacteria to produce a low pH environment, which will inhibit clostridial growth in silage. The required sugar concentration will be dependent on the buffering capacity of the crop. Clostridia catabolize amino acids resulting in a mixture of NH_3, organic acids and other products such as ethanol (McDonald et al. 1991). The acids formed include the volatile fatty acids (VFAs) acetic, propionic and BA, which, depending on the combination and concentration, can be associated with decreases in dry matter intake (DMI) (Dulphy and Van Os 1996). *Clostridium tyrobutyricum* is the most studied clostridial species in silage, which contributes to BA fermentation. This involves two moles of LA being converted one mole of the weaker BA and H and CO_2, resulting in increase in pH, energy and DM losses (Pahlow et al. 2003). As such, in traditional silage systems, clostridial silages have a high pH (> 5), contain little or no LA and have high BA (>5 g kg^{-1} DM) and ammonia N (NH_3-N) (>120 g kg^{-1} total N) concentrations (McPherson and Violante 1966). As described in a later section, BA levels have been negatively correlated with intake by beef cattle, but it is difficult to define a precise range at which BA will affect intake because it is speculated that intake is dependent on a combination of a number of factors, including moisture, VFAs, NH_3, amine and BA levels. McDonald et al. (1991) reported that typical BA levels of conventional ryegrass and maize silages were 1 g kg^{-1} DM.

These same authors also reported BA levels range from 8 to 36 g kg^{-1} DM in badly preserved silage, in which either enterobacteria or clostridia or both have dominated the fermentation process.

Under aerobic conditions many species of yeast can breakdown sugars into ethanol and CO_2 and fungal populations may develop. The concentration of ethanol in silage is approximately 10 g kg^{-1} of DM but has been reported as high as 50 g kg^{-1} DM (McDonald et al. 1991). Fungal populations may also develop under aerobic and high moisture conditions. Some species of fungi may produce secondary metabolites called mycotoxins, including alfatoxin, deoxynivalenol (DON or vomitoxin), T-2 toxin, fumonisin and ochratoxin (Whitlow & Hagler 1997). Some mycotoxins can be destroyed by the ensiling process (Lyon 1985) while others remain intact (Clark et al. 1996). There has been a great deal of research conducted on mycotoxins in general but few studies identify silage as a significant source of mycotoxins (Cherney and Cherney 2003). This may be attributed to the challenge of collecting a representative sample and accurately determining mycotoxin levels in silage, as the fungal contamination is not usually distributed uniformly throughout the silage.

Silage protein quality is an important factor influencing the nutritive value of silage from an economic perspective. Degradation of plant protein during wilting and ensiling is unavoidable. Hydrolysis of peptide bonds (proteolysis) results in the formation of free amino acids and peptides which are then degraded to a variety of end-products including NH_3, organic acids and amines (Rooke and Hatfield 2003). Rapid acidification reduces proteolysis in the silo (Carpintero et al. 1979). Conditions favourable for growth of clostridia, such as high pH and excess moisture, support

proteolysis by the clostridia bacteria. The proteolytic clostridia ferment free amino acids to a variety of end products including organic acids, such as acetic and BAs, and NH_3. Extensive proteolysis can occur during the fermentation process of well-preserved silage. This can result in non-protein nitrogen (NPN) levels of 500 to 700 g kg^{-1} of total N (McDonald et al. 1991). The NPN found in silages is rapidly broken down in the rumen. Peak concentrations of NH_3 in the rumen have been closely correlated with the NPN concentration of the silage (McDonald and Edwards 1976). Silage NH_3 concentration itself may not reduce intake, but ruminal NH_3 levels may have a direct impact and are related to the amount and solubility of crude protein (CP) in the silage. As such, excessive solubility of protein has been linked to reduced voluntary intake of silages through mild NH_3 toxicosis (Choung et al. 1990). This can be a particular concern when ensiling alfalfa at early maturity when CP content and solubility are high (Tamminga et al. 1991).

It is evident that many factors may influence the ensiling process, thereby significantly affecting the resulting forage quality. Overall, the criteria for evaluating the success of fermentation of high moisture silage (> 50%) should include: pH < 4.2, LA > 30 g kg^{-1} DM, BA < 1 g kg^{-1} DM, NH_3-N < 111 g kg^{-1} of total N and very low anaerobic spore counts (<10^2 colony forming units (CFU) g^{-1}, Cherney and Cherney, 2003). Criteria for evaluating fermentation of low moisture silage (< 50%) should include: pH < 5.2, LA > 10 g kg^{-1} DM, BA < 5 g kg^{-1} DM, NH_3-N < 240 g kg^{-1} of total N, and low anaerobic spore counts (<10^3 CFU g^{-1}) (Cherney and Cherney, 2003).

2.2.5 Quality losses during storage

The four phases previously discussed refer to the ideal silage preservation process. However, there are conditions that can disrupt the normal preservation process. For example, if the level of fermentable CHOs in the forage is too low or if they are inefficiently utilized by heterolactic rather than homolactic bacteria, a rapid drop in pH will not occur, leading to elevated pH during phase II, and subsequent secondary fermentation during phase III and/or IV. During secondary fermentation, residual plant sugars as well as lactic and acetic acids may be fermented to BA (by clostridia) and to ethanol (by yeasts), causing a further rise in pH. At the same time, amino acids may be fermented to amines and NH_3-N (Pahlow et al. 2003). In Western Canada, the two main causes of quality loss during the storage phase include air infiltration and low available WSCHO (MAFRI 2006). However, in the wetter areas of Eastern Canada, effluent production also becomes a problem (Sullivan and McKinlay 1998).

Air infiltration occurs any time that the silage is exposed to air. This can occur at filling, during storage and at feed-out. As described above, respiration losses also occur as long as O_2 is present. Plant cells will continue to respire after cutting as long as there is adequate substrate (i.e. sugars), moisture and O_2 present (McDonald et al. 1991). As a consequence of loss of the anaerobic environment, there are often shifts in the silage microbial populations. The presence of O_2 may promote the numbers of enterobacteria and decrease the lactic acid producing bacteria population resulting in an increase in pH levels (Muck et al. 2003).

Quality loss due to sugars being oxidized and converted into H_2O and CO_2 because of air filtration is dependent on many factors including the length of time between harvesting and sealing, forage density as have already been discussed, as well as

permeability of the silo walls or plastic film and the amount of time the feed is exposed at feed-out. In the production of BBS permeability of the plastic film and the number of layers of plastic may greatly affect air filtration and can be major concerns. Recent advances in plastic technology will be addressed in a subsequent section.

Effluent production can also result in nutrient losses during storage. Effluent is produced when there is excess forage moisture and pressure applied to the silage, or when Phase I and II are extended, causing excess H_2O production during storage. Effluent production is mainly dependent on the moisture content of the crop being ensiled. Other factors that influence effluent production include the type of silo, the chop length, and weather conditions during the harvest period (Savoie & Jofriet 2003). Silage effluent losses include a loss of soluble nutrients and a potential pollutant. Effluent losses can be reduced by wilting the crop in the field long enough to bring moisture content below a critical point and by ensuring that anaerobic conditions are achieved rapidly.

2.3 BIG BALE SILAGE PRODUCTION

Big bale silage provides a more flexible alternative to the traditional precision chopped methods of silage production; however, the fermentation process and factors affecting feed quality are not as thoroughly documented for this system compared to conventional chopped silage systems. Production of BBS involves drying the forage to approximately 35-65% moisture (Rhein et al. 2005; Borreani and Tobacco 2006) baling in a round (or large square) baler and enclosing the bale in a plastic cover to create an O_2-free environment.

The criteria required to produce high quality BBS are similar to those conditions that are required to produce conventional silage. The goal is essentially to mimic the conditions that are produced in a conventional silage system - only on a smaller scale. Therefore, like conventional silage production, establishing anaerobic conditions and the desired pH level as quickly as possible is also important in BBS production.

2.3.1 Benefits and limitations

Similar to any forage conservation system, there are advantages and disadvantages related to BBS systems. Two major advantages are the lower capital costs and increased flexibility, compared to traditional silage systems. The equipment needed to produce BBS is similar to that which is used in hay production, allowing producers the flexibility to alternate easily between the two systems according to changes in weather conditions and/or crop conditions (Rhein et al. 2005). Also, large investments into storage systems, such as upright or bunker silos, are not required in BBS systems. Furthermore, the specialized equipment that is required for BBS production, such as wrapping equipment, which is not needed for hay production, is often available on a rental or custom basis allowing for gradual adoption of this system.

The reduced curing time required for BBS production makes it well suited for areas where weather conditions are typically not favorable for hay production. In Manitoba, this includes areas such as the Interlake region, which normally receives 275 mm of rainfall during the growing season (MAFRI, 2003). In comparison, the Manitoba Red River Valley region typically receives almost 30% less precipitation (or about 200 mm) during this same time period (MAFRI, 2003). Untimely amounts of precipitation

increase drying times and make it difficult to reach the levels of DM necessary for storage of hay. Big bale silage also has marketing advantages over traditional silage. If bales are wrapped individually or wrapped in a line and subsequently separated, transportation is possible. The fear of quality loss due to O_2 exposure can also be reduced if transported short distances or if transportation occurs during winter months when temperatures are low enough to reduce the risk of secondary fermentation. Rhein et al. (2005) reported that exposure to O_2 during the winter months in Arkansas (Upper South region of United States) when temperatures range from 0.6 to 19.4 °C was less of a concern. These authors examined well-sealed wheat and orchard grass preserved as BBS at moisture levels of 37.6 and 45.6% respectively, and found that the bales were stable for up to 32 days after being exposed to air according to DM losses, neutral detergent fibre (NDF), acid detergent fibre (ADF), and CP concentrations. This is a major advantage to BBS systems as a consequence of the increased flexibility associated with feeding, transporting and marketing BBS without significant aerobic deterioration at temperatures less than 20°C. Although BBS is easier to transport than conventional silage, there are also limitations, which are related to the bale weight. Since the bales are of higher moisture than hay, specialized equipment is required to transport the heavier bales. As such, opportunities for marketing BBS are more limited than those available for hay.

Increased experience of producers and equipment improvements have led to improvements in silage quality and greater use of BBS as a forage preservation technique. As a result, BBS shows great potential as a forage preservations system in areas where typical conditions are not suitable for hay production (Han et al. 2004). Although, BBS production does have unique challenges that must be overcome.

2.3.2 Factors affecting quality

The wide variations in quality are often a concern when producers are considering BBS. For many years, BBS systems were not considered to be a comparable alternative to traditional chopped silage systems or hay systems due to the lower quality of the final feed product. However, a more recent study by Han et al. (2004) reported that BBS has higher nutritive levels than hay and can be comparable to traditional chopped silage in terms of quality. Han et al. (2004) compared the effects of moisture concentration and crop density during fermentation on DM losses, fermentation end products and physical characteristics of alfalfa preserved as BBS or as dry round-bale hay stored outside. After eight months of storage, the DM recovery of hay was 81.5%, whereas the % recovery for the BBS bales was significantly higher, ranging from 98.6 for the high moisture (58.7%) to 98.3 for the low moisture (52.4%) bales. Storage DM losses are typically greater for silage compared to hay. However, in this study, the hay was stored outside and did increase in moisture content during the storage period, which may explain the higher than expected DM losses in the hay bales. The BBS also had lower concentrations of NDF ranging from 468 to 496 g kg^{-1} DM for the high and low DM bales, respectively compared to 592 g kg^{-1}DM level of the hay. Furthermore, the CP levels in the BBS were also higher at 208 to 206 g kg^{-1} DM compared to 160 g kg^{-1} DM for the hay bales. The authors speculated that the lower CP levels could be due to the increased leaf loss that is experienced during hay production.

The ideal moisture range for BBS has not been clearly defined. A number of studies have been conducted to determine the optimum moisture level to produce high

quality silage and optimum silage intake (Nicholson et al. 1992, Beaulieu et al. 1993, Huhnke et al. 1997, Han et al. 2004).

An examination of BBS samples, from four production sites in eastern Oklahoma was conducted by Huhnke et al. (1997) to examine moisture, DM loss, quality parameters, which included CP, ADF, NDF and total digestible nutrients (TDN), the products of fermentation (LA, acetic acid, succinic acid, formic acid, propionic acid, BA, ethanol, and 2, 3-butanediol), and pH when bales were wrapped at moisture levels ranging from 25 to over 65%. The bales represented forage typical of eastern Oklahoma with a mixture of grasses (Annual Ryegrass, Bermudagrass and Japanese Bromegrass) and clover (Arrow Leaf, Ladino, Red, Large Hop and Crimson) and had actual moisture levels range from 14 to 72% as a consequence of variable harvest conditions at the four sites. Dry matter losses, which ranged from 0 to 2%, were not influenced by initial moisture content.

Huhnke et al. (1997) also reported that although there were distinct differences in CP (11.4 vs. 14.7%), ADF (35.2 vs. 40.2%), and TDN (58.0 vs. 59.8%) between the initial vs. the final levels after six months of storage for three out of the four sites, and changes in these parameters were not significantly affected by initial moisture content. In this same study, moisture content did impact the fermentation process. Bales with greater than 50% moisture content had LA levels ranging from 0.57 to 2.51% of DM and acetic acid levels ranging from 0.19 to 0.47% of DM after six months of storage. These values are lower than those typically observed for chopped grass and legume silage by Muck (1990) who reported LA levels of approximately 1.3 to 6.5% of DM and acetic acid levels of approximately 0.5 to 2.0% of DM for chopped alfalfa when ensiled in mini silos

with a similar initial moisture content (36 to 73%). Nicholson et al. (1992) reported higher lactic and acetic acids concentrations with LA representing a higher proportion of total acids, higher lactic acid producing bacteria counts and lower clostridia counts for the bales with an initial moisture content of 60 to 65% compared to those with a moisture content of 50 to 55%. Conversely, Han et al. (2004) reported that LA concentrations were not affected by moisture treatments for alfalfa silage bales between 52.4 and 58.7% moisture.

In several studies, moisture content of the silage has also impacted the stability of silage, as measured by pH. Nicholson et al. (1991) observed a lower ($P < 0.01$) pH of 4.84 and 5.29 in high (35 to 40.5% DM) and low (46.5 to 50.6% DM) moisture alfalfa-grass bales, respectively, after 58 days of storage. Huhnke et al. (1997) reported that (17.9%) of the silage in the Oklahoma study had a pH higher than 6.5, which usually represents that spoilage has occurred (Borreani and Tabacco 2006). Overall, optimum moisture level recommendations are variable, ranging from less than 50% (Muck 1988), 20 to 50% (Huhnke et al. 1997), 50 to 60% (Beaulieu et al. 1993) and 59 to 65% (Nicholson et al. 1992). Therefore, it is clear that additional research is required in this area to determine a more accurate moisture recommendation for BBS.

In addition to moisture content, particle size may also affect BBS quality. Although considerable work has been done studying the effects of particle size on forage preserved as conventional silage (Charmley et al.1997; Kononoff et al. 2003; Beauchemin et al. 2003; Einarson et al. 2004) there are considerably fewer studies examining the effects in BBS since traditionally, there has been limited processing of BBS prior to ensiling. As a result of the larger particle size of BBS, compared to

conventional chopped silage, packing is more difficult and density is lower, resulting in slower removal of O_2, slower production of acetic and LA to decrease the pH and reduced stability of fermented silage (McDonald et al. 1991). Han et al. (2004) reported that increasing density of alfalfa silage bales, by increasing hydraulic pressure from 421 to 842 X 10^3 had some improvements in silage quality with the higher density bales having a lower pH (4.76) than the lower density bales (5.01). However, density did not affect LA concentration, CP or NDF values.

The absence of chopping is also thought to reduce the physical damage to the stems and leaves thereby potentially slowing the release of nutrients, such as WSCHO, which decreases the likelihood of a successful fermentation process (Jonsson et al. 1990). In long fibre bales, there is less broken forage surface area for lactic acid producing bacteria to multiply in the initial phases of fermentation (Savoie and Jofriet 2003). As a consequence of the lower concentration of lactic acid producing bacteria, pH declines more slowly and is ultimately higher and there are less organic acids and more residual sugars remaining in the silage (Van Soest 1994). As such, the pH of alfalfa ensiled as BBS is usually higher at 5.0 to 6.0 (Borreani and Tabacco 2006) in comparison to alfalfa which has been reported to range from 4.76 to 5.0 (Suwarno et al. 2000) or grass silage with a pH 4.01 to 4.97 (Krizsan et al. 2007) when ensiled as short fibre in tower silos. In a second experiment by Nicholson et al. (1991), it was demonstrated that after 60 days of storage, daily weight gain, DMI and efficiency of feed conversion of cattle fed long-fibre bale silage was less than that of cattle fed chopped forage stored in a plastic tube with silage compactor, referred to as bag silage. This difference in efficiency was attributed to either a difference in particle size (long vs. chopped) or to the difference in fermentation

patterns since the bale silage had significantly higher pH (5.1 vs. 4.4) and significantly lower LA concentrations (18.5 vs. 49.7 mg g^{-1} DM) compared to the bag silage.

2.3.3 Quality losses during storage and feed-out

Quality losses during storage are mainly related to improper O_2 exclusion. One reason for improper O_2 exclusion may be because BBS is often stored in long continuous rows that are wrapped in plastic. This storage method is convenient and efficient at harvest. However, this can lead to problems at feed-out because once the row of bales is opened, O_2 has access to one end of the row and aerobic deterioration can occur if the silage is not fed quickly.

A second reason for improper O_2 exclusion in BBS systems is the time, which elapses from production of the bales to the time that the bale is wrapped. Moshtaghi-Nia and Wittenberg (2000) compared the quality of whole crop barley forage ensiled as big bales when wrapping was delayed 2, 10 or 19 hours post-baling. They concluded that delaying wrapping for 19 hours, resulted in an increase in bale temperature and a decrease in protein quality. No differences were reported between the 2 and 10-hour treatments.

Aerobic conditions may also occur as a consequence of the permeability of the plastic used to wrap the bales. Recently, Hancock and Collins (2006) concluded that more than two layers of plastic film are required for alfalfa BBS bales to be preserved properly. This is an important consideration for producers since they must balance the costs with the risks associated with spoilage if anaerobic conditions are not maintained. Furthermore, there is a risk that the plastic wrap can be damaged by wildlife or

unfavorable weather conditions during storage, which would also affect the quality of BBS.

2.3.4 Advances in big bale silage production

Despite these challenges, there are now several strategies that may be used, such as chopping at the time of baling, using additives and improvements in quality of the plastic wrap, which help to create BBS that is comparable in quality to traditionally chopped silage. For instance, Charmley (2001) has suggested that performance of cattle fed BBS silage may be comparable to that of cattle fed conventional silage because of the increased use of silage additives. These factors will be discussed further in the following sections.

Borreani and Tabacco (2006) performed three trials in Italy examining the effects of chopping forage prior to ensiling on DM density. The three trials (two with forage harvested from first cut and one from second cut) were conducted in two different alfalfa fields with different stages of maturity to provide a range of drying conditions, moisture concentrations and fibre concentrations. Forage was harvested using a baler with knives spaced 93 mm which led to an increase in the percentage of stems that were shorter than 10 cm from 14% to 38% (on a DM basis). Bale DM densities ranged from 163 to 204 kg m^{-3}, with a 4% increase in bale density observed in the chopped bales compared to the non-chopped bales. These densities were comparable to those measured in a survey of primarily corn and alfalfa bunker silage by Muck and Holmes (2000) who reported that DM densities ranged from 106 to 434 kg m^{-3}. Huhnke et al. (1997) reported that the

average round bale silage density was 159 kg m^{-3} and that there was significant variation due to the type of baler that was used.

In this same study, Borreani and Tabacco (2006) considered the effects of chopping prior to baling on silage quality and fermentation parameters. They predicted that higher DM densities in BBS may result in less O_2 availability, faster establishment of anaerobic conditions and higher lactic acid producing bacteria levels available for LA production. Dry matter losses, pH, LA, acetic acid, propionic acid, BA and NH_3-N did not show consistent improvements over the three trials. Crude protein concentration was also not different between the chopped and un-chopped bales. As such, Borreani and Tabacco (2006) concluded that chopping forage at the time of baling did not improve the fermentation quality of alfalfa silage.

At present, research examining the use of forage additives in BBS systems is limited. Jonsson et al. (1990) inoculated silage bales with either lactic acid producing bacteria (10^6 lactic acid producing bacteria g^{-1} DM) or treated with formic acid (4 or 8 l t^{-1} DM) and then added spores of *Clostridium tyrobutyricum* and *Aspergillus flavus*. Although, both additives reduced pH and decreased NH_3-N, BA and clostridial spores levels, the lactic acid producing bacteria treatment had significantly lower pH values and less growth of moulds on the surface of the bales. However, the authors concluded that additives cannot compensate for poor anaerobic conditions. Other factors such as permeability of plastic wrap and adequate chopping were deemed to be more important than the benefits garnered from silage additives.

As described above, to reduce quality losses it is ideal that there is immediate O_2 exclusion within the silage bale. In a review examining the effects of air on silage,

Woolford (1990) stated that the most important factor influencing the efficiency of the ensiling process is the degree of anaerobiosis achieved. If the silage is not sealed properly, air is able to penetrate the silage and allows for microorganisms to multiply, which can lead to aerobic deterioration. Advances in plastic technology have been explored as another avenue by which silage quality may be improved. The most common material used to seal bunker silos and piled silage is 100-um polyethylene film. However, Lindgren et al. (1985) reported that the use of this film was not sufficient to prevent oxygen diffusion in bunker silages and resulted in the production of yeast. Borreani et al. (2007) compared the O_2 permeability of a new black-on-white coextruded oxygen barrier (OB) film of Italy (Bromostop® Industria Plastica Monregalese) with standard polyethylene (ST) film. The OB film is 4-metre wide and has 20-fold lower O_2 permeability than polyethylene film (Borreani and Tobacco 2008). The patent description for this product indicates that this film is a multilayer polyamide-containing barrier with excellent tear strength (World Intellectual Property Organization 2006). Borreani et al. (2007) set up two bunkers (on separate farms) with half of the feed-out face covered with the OB film and half with the ST film. The silos were opened in the summer, after more than eight months of storage. Samples were collected and analyzed for DM concentration, fermentation profile and yeast and mold counts. These authors found that when used to ensile whole-crop corn in bunker silos, the new film reduced DM losses by 3.7 times. There was also a film type effect for pH, LA and BA, with the OB film having more favorable levels for each of these parameters. The silage covered with the OB film had lower mold counts (1.55 $\log_{10}$ cfu/g) compared to the ST treatment

(5.07 $\log_{10}$ cfu/g) and the authors concluded that the OB film had the potential to produce higher quality silage by deceasing O_2 permeability.

2.4 SILAGE DRY MATTER INTAKE

2.4.1 Importance of optimum dry matter intake

Advances in the genetic potential of beef and dairy cattle, along with escalating feed costs, have made accurate DMI predictions and ration formulation especially important to the livestock industry. There are many factors, related to the animal, the environment and the feed, that influence DMI. This is particularly apparent for silage, in which end products of the fermentation process can potentially affect intake. As such, although DMI is a key factor in animal performance, predicting silage DMI is often very difficult (Rook and Gill 1990).

The nutritional value of fresh forages is often quite different than the nutritional value of preserved forage. As described previously, this is largely due to the loss of nutrients, such as WSCHO, that occurs during the fermentation process (McDonald et al. 1991). Prior to 1995, it was generally accepted that intake of silage was anticipated to be lower than that of the same forage that had not undergone the fermentation process (Cushnahan et al. 1995). Therefore, the combination of the lower nutrient value of silage and decreased intake led to a reduction in the overall feeding value of silage compared to that of forage preserved as hay. Since feeding value has a direct relationship on animal performance, the lower feeding value of silage has been one of the major disadvantages associated with silage making in the past.

However, in the last several decades, research has shown that the adverse impact of ensiling on animal performance has either decreased (Cushnahan and Mayne 1995) or that shortcomings in silage-based diets can be overcome by supplementation with dietary additives (Keady and Murphy 1998). For example, Keady and Murphy (1998) found that the decrease in animal performance, measured by milk yield that was caused by ensiling could be overcome by supplementing a silage-based diet with fishmeal, as a source of un-degraded dietary protein. Similarly, a review by Charmley (2001), studying improvements in silage quality over recent years, reported that the negative effects of ensiling on intake, protein quality and CHO fermentation can be minimized through wilting and the use of acid-type additives. Charmley also suggested that ensiling might become a method for increasing the nutritive value and voluntary intake of forages in the future.

2.4.2 Factors affecting silage dry matter intake

Generally, the major factors that impact silage DMI are related to the characteristics of the forage before fermentation and the resulting characteristics of the silage after fermentation. There are chemical factors related to plant maturity at harvest, DM concentration and particle size. There are also chemical factors that relate to the quality of the fermentation process and the concentration of the end products of fermentation. The main acids related to the fermentation process include lactic, acetic, butyric, isobutyric, propionic, isovaleric and valeric. The concentration of these acids impacts the pH of silage. Furthermore, other end products of fermentation, including ethanol and 2, 3-butanediol are also produced and may have an effect on silage DMI.

2.4.2.1 Effect of plant maturity at harvest

Silage quality is influenced by the maturity of the crop at harvest. To help improve silage intake predictions and gain a greater understanding of the factors impacting silage intake, Steen et al. (1998) conducted a large two-year study using 192 beef steers. In this study, 136 silage types containing different silage additive treatments and various ensiling techniques obtained from commercial farms in Ireland were examined and compared to hay. The silages were selected such that a range of pH, DM, NH_3 and metabolizable energy (ME) were represented with the aim to have the silages evenly distributed over these four parameters. The silages mainly consisted of perennial ryegrass with low or zero amounts of clover. Each of the silages was offered as the sole feed to ten animals for a period of 14 days. The mean DM, protein, fibre, fermentation products, WSCHO and digestibility of the 136 silages provided a wide range of chemical and biological compositions.

These authors concluded that DMI is more closely related to the chemical characteristics that are affected by plant maturity, such as CP and fibre fractions, than to the products of fermentation. The CP values in this study ranged from 77 to 212 g/kg DM. The researchers found a distinct curvilinear relationship between CP and DMI at low N concentrations (maximum intake at 160 g CP per kg DM) and a small decrease in silage DMI above 160 g CP per kg DM. The authors attributed the positive effect of protein concentration on intake to the fact that as forage matures, the protein concentration decreases while the fibre concentration increases and the digestibility decreases. Therefore, silages with low protein concentrations have a decreased rate of

disappearance from the gut resulting in lowered intake due to rumen fill effects (Steen et al. 1998).

A more recent study by Krizsan and Randby (2007) was conducted to separate the effect of fermentation quality on silage DMI from other factors affecting intake. Twenty-four silages plus hay were fed to 30 beef steers. Treatments included six types of tower silos (two harvested with a flair harvester and four with a precision chopper) and two types of stack silos, harvested with either a precision chopper or a self-loading wagon. There were also 16 types of BBS harvested with a round baler with either knives engaged or without knives engaged. The majority of these different harvesting treatments also had different combinations of additives included. As concluded by Steen et al. (1998), these authors demonstrated that silage DMI was closely ($P < 0.05$) related to maturity characteristics of the fresh crop, such as DM and acid detergent lignin (ADL). The authors speculated that the ensiling process influenced NDF concentrations, which ranged from 476 to 601 g/kg of DM. Concentrations of NDF in the silage that were lower than the original crop indicated that NDF was being broken down to hemicellulose during the ensiling process, which provided additional substrate for fermentation. McDonald et al. (1991) reported that an increase in NDF and ADL relative to the initial crop could be due to effluent losses of soluble nutrients or secondary fermentation resulting in DM losses.

Steen et al. (1998) observed that when ADF and NDF values ranged from 268 to 452 and 413 to 662 g/kg respectively, these parameters were similar in terms of R^2 values (0.31) when predicting silage DMI. Neutral detergent fibre has a greater filling effect than non-fibrous feed components and has been found to be the best single chemical

predictor of DMI (Allen 1996). However, there are many other factors which affect fill, including particle size, which will be discussed below.

2.4.2.2 Effect of dry matter content

Much of the initial work examining the effects of wilting was conducted in Europe or New Zealand where direct ensiling of fresh forage was common. As mentioned previously, wilting of forage before ensiling is now a common practice throughout the world. Research by Rook and Gill (1990), examined a European data set of predominantly perennial ryegrass silage. Silages were made using a precision-chop forage harvester with or without silage additives. Moisture levels ranged from 73.9 to 83.7%. These authors found that the relationship between intake and wilting was curvilinear with little benefit in terms of intake when moisture content was less than 75%. Wright et al. (2000) reported that wilting rate (from 0.01 to 0.122 kg H_2O loss kg^{-1} DM h^{-1}) and the extent of moisture loss (from 0.67 to 3.7 kg H_2O kg^{-1} DM) in wilted silages were two variables that were highly correlated with improved intake and performance when comparing wilted silages relative to unwilted silages with increases in both variables resulting in an increase in DMI. However, moisture levels can also impact fermentation if too low since the relationship between moisture and intake is likely to reflect the influence of the concentrations of fermentation products, such as lactic and acetic acids, that are positively related to moisture content (Dulphy & van Os 1996). Overall, Charmley (2001) suggested that benefits of wilting on animal performance are likely greatest under good wilting conditions, and are likely reduced when wilting conditions are not favorable. Warm weather with low humidity and no rainfall are ideal

wilting conditions, whereas rainy, cool, high humidity conditions are much less favorable.

2.4.2.3 Effect of particle size

Silage is traditionally chopped prior to being ensiled to assist the fermentation process by increasing the surface area exposed to microorganisms. However, chopping can also have negative effects by reducing the amount of time cows spend chewing which can lead to a reduction in saliva flow into the rumen. Over the past few years, a number of studies have been conducted examining the effect of forage chop length on the performance, behavior and health of dairy cattle. Kononoff et al. (2003) found that as particle size of corn silage decreased from 8.8 to 7.4 mm, DMI increased, which positively affected rumen fermentation, and reduced sorting behavior. Furthermore, since dairy cows require rations that have adequate particle size for healthy rumen function, Beauchemin and Yang (2005) studied the effects of particle size of corn silage by creating diets which ranged from 84% to 67% of particles retained on both the 19.0 and 8.0 mm sieves, when using a Penn State Particle Separator. From this, treatments with physically effective fiber ranging from 11.5 to 8.9% were created. These authors concluded that although increasing the physically effective NDF (peNDF) content of diets increased chewing time, it did not always result in a reduction of ruminal acidosis, which is benchmarked as occurring when ruminal pH is below 5.8 but above 5.0. Differences in milk yield and milk composition of dairy cows has been related to particle length of silage (Grant et al. 1990). Krause et al. (2002) reported that milk fat percentage was correlated to mean ruminal pH (r^2=0.41), which decreased as forage particle size of

alfalfa and corn silage decreased from 6.3 to 2.8 mm. Conversely, Armentano et al. (1988) reported that decreasing particle size of 80% alfalfa silage diets from 5.63 to 3.12 mm did not result in any changes in feed intake, feed digestibility, or milk production. All of these studies were conducted on conventionally processed silage.

Particle size has not been evaluated to the same extent under BBS conditions, but it is thought that the same concepts can be applied. One study by Nicholson et al. (1991) reported that calves that were fed bag silage, which had been chopped prior to being stored in a plastic tube with a silage compactor, had higher DMI levels ($P < 0.01$), gained weight faster and had higher feed efficiencies compared to calves that were fed long fibre baled silage. As described in a subsequent section, ensiling characteristics such as pH, LA and acetic acids levels were also measured in this study.

In a study by Petit et al. (1993), forage DM and harvesting/conservation technique were assessed. Forage was harvested at 33% moisture by a cylinder-type forage harvester, at 33% moisture by a self-loading forage harvester and at 54.3% moisture by a round baler. The chopped material, grass heap silage cut with the cylinder-type harvest and grass heap silage cut with the self-loading forage harvester was ensiled as a long heap on a concrete pad. The forage was then sealed with one polyethylene sheet. The forage for the grass BBS treatment was ensiled as big bales and wrapped with plastic film. The authors reported that total DMI was lower for the BBS and suggested this may be due the intake of fine or coarse chopped silage being greater than that of long fibre (>100mm), or due to a 50% reduction in the LA content of the BBS treatment compared to the chopped material.

In addition to chopping, maceration is another type of processing may be used in silage production. This process involves conditioning forage at the time of cutting by using variable speed rollers that act to split plant stems longitudinally. It differs from traditional conditioners, which break the plan stems diagonally. When macerated forage has been ensiled and fed to ruminants, the effect on intake, digestibility and animal performance has been variable. Frost et al. (1995) compared silage DMI of forage mats to unconditioned grass at 76.6, 78.7 and 83.7% moisture in sheep. The process to create the forage mats involved macerating the grass and then pressing it to squeeze out the juice. Squeezing the forage also helped to bind it together to help prevent mechanical losses. These authors reported that the forage mat treatment did not significantly impact intake in sheep compared to the three long fibre unconditioned treatments. In the above study, the macerating technique involved laboratory scale macerating equipment which had two rollers located with a 0.5 mm gap between their surfaces. Each roller surface had small grooves measuring 1.5 mm deep by 2.0 mm pitch. This equipment was similar to the macerating equipment used by Charmley et al. (1997) who found no effect of maceration on intake or digestibility in sheep compared to forage that was mowed and conditioned conventionally. In a subsequent study, Charmley et al. (1999) examined the effects of maceration at mowing on silage fermentation, intake, digestibility and growth rate of steers fed precision chopped (PC) or BBS that had been mowed with either a regular mower conditioner or a mower macerator. The PC treatment involved harvesting with a PC forage harvester with knives set for a chop length of 1.9 cm as opposed to the BBS treatment, which involved long-fibre forage being harvested with a round baler. These authors concluded that maceration reduced intake of precision-chopped orchard

grass/white clover but did not affect intake of the same forage harvested as BBS. It has been suggested that this variation in response to maceration may reflect the variation in the degree of maceration and the degree of improvement in drying rate achieved (Suwarno et al. 1999; Agbossamey et al. 2000).

2.4.2.4 Effect of fermentation end products

Butyric acid was first reported to be responsible for reducing silage intake by Harris and Raymond (1963). Other VFAs, such as acetic acid, have also been shown to influence silage DMI (Brown and Radcliffe 1971). To study the effects of fermentation products on intake, Buchanan-Smith and Phillip (1986) infused various extracts of fermentation products, acetic acid and BA, with or without additional acids (LA) and products of protein degradation (such as ammonium and BA) directly into the rumen and monitored intake. They concluded that although no single end product was primarily responsible for decreasing intake, soluble components in silage were shown to decrease intake. In a later study, Buchanan-Smith (1990) found that acetic acid alone would reduce intake when infused into the rumen, but other VFA did not have consistent effects. Subsequently, there have also been a number of studies conducted examining the effect of silage fermentation characteristics on DMI. Rook and Gill (1990) found that DM, NH_3-N and VFAs (acetic and butyric) all had important effects on silage DMI. Similarly, Cushnahan et al. 1995 reported that silage DMI decreased as the concentration of silage NH_3 and BA increased. Krizsan and Randby (2007) found that silage DMI was also closely related to fermentation end products such as VFAs (acetic, propionic and BAs), LA, total acids and NH_3-N. Specifically, they found that the two best models describing

silage DMI took into account the concentrations of propionic acid, BA and LA. These two models explained 75 and 84% of the variation in silage DMI. The difference between the two models was that one contained both the BA and BA^2 terms and had an r^2 = 0.73. The other model excluded the BA term and had an r^2 value of 0.51. The model including both BA and BA^2 had a smaller error term indicating that it was the better model for predicting silage DMI.

Conversely, the Steen et al. (1998) experiment described previously, reported that silage DMI was poorly correlated to such factors as total acidity and the concentrations of lactic, acetic and BAs. Similarly, work by Cushnahan and Mayne (1995) examined the effect of ensiling on feed intake and milk production in lactating dairy cows. Fresh forage was compared to silage that had either undergone restricted or extensive fermentation. Ensiling resulted in a decrease in forage pH and WSCHO levels and an increase in NH_3-nitrogen concentration. In this study, these differences in concentrations of end products of ensiling had no significant impact on DMI or milk yield. Charmley (2001) speculated that a low pH in silage is commonly associated with poor intake because is it thought that the low pH in the rumen is not in the optimum range for cellulase activity. Therefore, the celluloytic activity is reduced, which leads to a reduction in intake.

Silage NH_3-N, which is mainly a product of clostridial fermentation of amino acids, is often associated with reduced silage intake. Ammonia nitrogen levels are relatively easy to measure and often acts as a simple index of silage quality. Wright et al. (2000) reported that NH_3-N concentrations were correlated (r^2 = 0.24) with intake when

comparing wilted and unwilted (or directly ensiled) silages. Krizsan and Randby (2007) reported a significant quadratic relationship between NH_3-N and silage DMI ($r^2 = 0.33$). Overall, given the points mentioned above, it is difficult to predict how silage intake is related to fermentation end products. In a review of advances in silage (Charmley 2001) it was broadly speculated that when silages at different stages of plant maturity are compared, the chemical factors, such as CP and fibre concentrations, affecting digestibility are most important. However, when comparing silages of similar maturity, the factors related to fermentation (such as VFA, LA, NH_3 and pH) begin to play a more significant role. Given the literature citied above, it is difficult to predict how silage DMI is affected by the end products of fermentation. Furthermore, since these factors affecting intake have not been well established in conventional silage systems, even less is known about the factors affecting silage DMI in BBS systems.

2.4.2.5 Impact of protein solubility and rumen ammonia

As indicated above, although silage NH_3-N *per se* may not have a direct impact on silage DMI, it is thought that ruminal NH_3 levels may affect intake (Charmley 2001). Levels of rumen NH_3 are related to the amount and solubility of the CP in silage. It is thought that high levels of ruminal NH_3, (above 475 g kg^{-1} total N) could lead to mild NH_3 toxicosis which could then decrease feed intake (Choung et al. 1990). This is supported by work done by Charmley and Veira (1990 a, b) when they used heat treatments to denature the plant protease enzymes in alfalfa, leading to a decrease in the solubility of CP and an increase in intake.

2.5 SUMMARY

Although preserving forage as BBS has become popular by Canadian dairy and beef producers because of its lower capital costs for machinery, feeding and storage facilities and reduced labour requirements compared to traditional silage systems, there are a number of challenges which may potentially prevent optimum quality when producing BBS. This is because the basic conditions required for effective preservation of silage are often difficult to achieve in BBS (Jonsson et al. 1990). Furthermore, these conditions have not been well defined for BBS and more research is required to ensure that optimal intake and quality are achieved.

Some of the major challenges associated with BBS production include limited options for processing prior to ensiling, appropriate moisture content at the time of harvest and conditions which facilitate an anaerobic environment, including adequate bale density and the time which elapses prior to wrapping. Achieving anaerobic conditions quickly is important so that the microbial populations required to initially drop pH can dominate. The continued drop in pH will then limit microbial activity such as steady state conditions can be achieved. Furthermore, since BBS production is a relatively new technology compared to conventional silage production, a number of areas require further research, including the effects of moisture level and processing at the time of baling on fermentation patterns and intake of BBS.

3.0. RESEARCH HYPOTHESES AND OBJECTIVES

3.1 Hypothesis

The optimum range of moisture content has been extensively studied for conventional silage, but the ideal moisture level for optimum fermentation resulting in high quality silage and optimum intake of BBS has not been clearly defined. Recommended moisture contents, which range from 20% (Huhnke et al. 1997) to 65% (Nicholson et al. 1992) have been cited in the literature. Bales harvested at 50 to 60% moisture are expected to provide an environment favorable for fermentation, as determined by higher lactic acid-producing bacterial populations, lower pH, with less potential for secondary fermentation as measured by lactic acid, ethanol and butyric acid concentrations as well as increased intake when fed to backgrounded steers.

Furthermore, use of baler technology that is capable of chopping forage at the time of baling is expected to lead to a reduction in particle size, an increase in bale density, and higher WSCHO content, leading to a more effective fermentation process compared to long-fibre bales, as measured by increased lactic acid bacterial populations and reduced pH. As such, it is anticipated that chopping will improve the fermentation pattern of BBS, leading to higher silage quality and improved animal intake compared to long-fibre bales.

3.2. Objectives

The overall objective of the first trial was to assess the effect of moisture content and processing on the fermentation process of alfalfa-grass ensiled as BBS. Big bale silage bales were produced at three targeted DM levels, ranging from 35 to 65%. These bales were either processed (chopped) or remained as long fibre. Bale weights were recorded and the nutrient profile of the resulting silage was determined. The fermentation process, as measured by pH, lactic acid, VFA and ethanol concentrations, were also measured throughout the storage period to determine the optimum DM level and processing technique to produce the highest quality silage.

To study the effects of processing and moisture content on animal intake, BBS bales were produced at two targeted DM levels. At each DM level, bales were chopped at baling, chopped at feeding or remained as long fibre. Particle size was determined and forage and fermentation parameters, such as pH, lactic acid, VFA and ethanol concentrations, were measured and examined together with daily intake values from a four-month feeding trial to determine the optimum DM level and processing technique when targeting optimum intake.

4.0 MANUSCRIPT I

Effects of processing and moisture content on the fermentation of alfalfa-grass ensiled as big bales

4.1. ABSTRACT

Advances in baling technology over the last five years have enabled producers to process forage by chopping at the time of baling. To examine the merit of such a procedure, the effects of processing and moisture content on the fermentation patterns of alfalfa-grass, cut at 10% bloom and ensiled as big bale silage (BBS), were examined. Alfalfa-grass forage was baled at low (22.1-29.75), medium (36.7-44.1) and high (58.9-67.3) % dry matter (DM). Both long-fibre and short-fibre bales were manufactured using a round baler equipped with knives spaced 7 cm apart. Bales, weighing 407 $\pm$ 74 kg, wet basis, were wrapped within five h of baling, with five layers of plastic film. During wrapping, two thermocouple wires were inserted into each bale to monitor bale temperature. Core samples were collected at baling and on days 1, 2, 7, 21, 42 and 70 of storage and analyzed for pH, crude protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF), ammonia nitrogen (NH_3-N) and water-soluble carbohydrate (WSCHO) levels as well as lactic acid (LA), ethanol and volatile fatty acid (VFA) concentrations. Processing did not have a significant effect on the temperature, pH, CP, ADF, NDF, NH_3-N or WSCHO profiles during the 70 days of storage. Moisture content did not have a significant effect on the ADF, NDF or NH_3-N profiles during the 70 days of storage but did result in significantly higher effluent production, CP loss and WSCHO loss in low DM bales. Although LA concentrations were significantly higher in the low DM bales, pH was not significantly different at the end of the storage period. Ethanol concentrations were however, higher in the low DM treatment, suggesting the presence of undesirable heterofermentative processes. Results of this study indicate that processing did not have a significant effect on the fermentation patterns of alfalfa-grass forage

ensiled as BBS. Moisture content of the forage at the time of ensiling affected nutrient profile of the forage, as well as the fermentation process.

Abbreviations: ADF, acid detergent fibre; **BBS**, big bale silage; **CP**, crude protein; **DM,** dry matter; **LA**, lactic acid: **NDF**, neutral detergent fibre; **NH_3-N**, ammonia nitrogen; **VFA**, volatile fatty acid; **WSCHO**, water soluble carbohydrate

Keywords: silage, bale, alfalfa, grass, fermentation, processing, moisture

4.2 INTRODUCTION

Conventional, precision-chopped silage systems are an effective way to conserve forage. However, the high equipment costs and labor requirements of this system make it cost prohibitive for many smaller-scale livestock producers in Western Canada. Big bale silage (BBS) systems, in which partially wilted forage is baled at a higher moisture content than hay and wrapped in plastic to exclude oxygen, are characterized by lower infrastructure costs and high labor efficiency (Holmes 1997) and, therefore, are an alternative to hay and conventional silage. Big bale silage systems are especially attractive in areas which receive high amounts of rainfall during the harvest season. In these areas, significant field losses may occur if long curing times are required. Further, high quality forage may rapidly lose quality if adequate wilting is not achieved. Since equipment needs for hay and BBS are similar, producers may opt for silage production when crop maturity or weather forecasts dictate a change in harvest strategy. Although popularity of BBS has increased in many parts of North America over the past two decades (MAFRI 2006), research to validate the frequently recommended optimum ensiling techniques and conditions is limited.

Chopping forage prior to ensiling is required in conventional silage systems. Smaller particle size improves packing of forage material, resulting in more rapid removal of oxygen, more efficient fermentation and higher quality forage as compared to forage that is not chopped (Muck et al. 2003). Generally, forage ensiled as BBS is not chopped prior to baling or ensiling. Nicholson et al. (1991) have shown that cattle gained less weight and had poorer feed efficiency when fed unchopped, long fibre bale silage compared to forage chopped with a roller-type conditioner and packed in a plastic bag.

These authors suggested a slower, less extensive microbial fermentation on the basis of a slower decline and higher overall pH as the major reason for reduced animal performance. Several studies have speculated that chopping forage prior to baling would improve fermentation efficiency and resulting silage quality in BBS systems (Nicholson et al. 1991, Nicholson et al. 1992, Charmley et al. 1999). However, this has not been demonstrated to date.

Today, there are a variety of balers available (Claas Variant 180 © RC; John Deere 582 MaxiCut © precutter; New Holland CropCutter™ BR740) with knives that can be engaged or disengaged to alter the fibre length of the forage. This technology may provide an affordable and reliable technique to improve silage quality in BBS systems.

Forage moisture content at the time of ensiling can influence silage quality (Marsh 1979, Charmley and Thomas 1987, Muck 1989, Nicholson et al. 1992, Beaulieu et al. 1993, Wright et al. 2000, Han et al. 2004). Optimum moisture levels recommendations for BBS are variable, ranging from less than 50% (Muck 1988), 20 to 50% (Huhnke et al. 1997), 50 to 60% (Beaulieu et al. 1993; Place and Heinrichs) and 59 to 65% (Nicholson et al. 1992). Research is required to identify optimum forage moisture levels for the major forages used in BBS production.

The purpose of this study was to identify optimum forage moisture content, as well as potential benefits associated with chopping forage prior to baling on silage nutrient and fermentation profile. As alfalfa-grass stands are commonly used in Manitoba hay production systems, the research was conducted with alfalfa-grass forage.

4.3 MATERIALS AND METHODS

4.3.1 Experimental design

A four year-old, first cut alfalfa-grass stand was divided into two equal 5.11 ha (335.3 x 152.4 m) blocks and cut at 10% bloom. Botanical composition of the stand biomass consisted of 72% legumes, 21% grass and 7% weeds, dry matter (DM) basis. The field was cut and windrowed. Windrows within each block were randomly assigned to six ensiling treatments reflecting a combination of three forage moisture levels and two baler settings. Moisture levels at baling were targeted to be 35, 50 and 65% moisture for the low, medium and high moisture treatments, respectively. The baler was set to produce 1.22 m x 1.22 m bales with (chopped fibre) and with out (long fibre) knives engaged at the time of baling.

Forage was wilted in windrows until the desired DM was achieved. Forage DM content was assessed on a regular basis using a microwave and balance. Preweighed forage samples weighing approximately 250 grams were placed in a microwave oven for one min, heated, and weighed. This step was repeated until a weight change of less than one gram was observed at which time moisture content was calculated. A Claas Variant 180 Roto-cut baler was used in the study. To chop the forage, a row of knives (spaced 7 cm apart) located inside the pick-up of the baler was engaged.

Two bales per windrow were weighed and ensiled for each treatment, yielding a total of 24 bales. Bales were tagged and core-sampled (8-10 cores per bale, approximately 350 g each) immediately after baling using a Penn State core sampler (38 x 3 cm). Bales were weighed and wrapped, within five h of baling, with five layers of plastic film (25.4 um thickness and 750 mm width, Max Tech Superior Cling Silage Film

™ Tyco Plastics). During wrapping, two thermocouple wires were inserted approximately 40 and 80 cm into each bale to monitor bale temperature. All wires were tested using a water bath before being inserted into the bale. Bale temperature was monitored daily for the first 21 days and then on days 28, 35, 42, 49, 56, 63 and 70, post ensiling.

Bales were elevated and plastic funnels and tubing with removable rubber stoppers were attached to facilitate effluent collection. If present, effluent was collected and measured using the same sampling schedule. Two core samples (approximately 350 g each) were collected from each bale at baling on days 1, 2, 7, 21, 42 and 70 of storage. These samples were taken to identify forage changes during the ensiling period. Samples were returned to the laboratory and frozen immediately at -20°C for subsequent nutrient, pH, volatile fatty acid (VFA) and ethanol analysis. Core samples were also collected for bacterial analysis at the time of baling and at 24 and 48 hours post-wrapping. To avoid cross-contamination, the sampling core, plunger and the operator's gloves were washed with 95% ethanol before sampling each bale. Samples were placed in sterile bags, stored on ice immediately and transported to the laboratory. All bales were flushed with carbon dioxide (CO_2) after each sampling and resealed with tape ("Tuck Tape", Canadian Technical Tape Ltd. Montreal, QC) to ensure anaerobic conditions were maintained inside the bale. At 70 days of storage, final weight of the wrapped bales were recorded while.

4.3.2 Bacterial analysis

Samples for total and lactic acid producing bacteria populations were analyzed immediately. Ten grams of fresh forage was placed into a sterile disposable stomacher bag containing 90 milliliters (mL) of sterile wash solution (King et al. 1979). The wash solution consisted of 0.1 mL Tween80 (CAS No 9005-65-6 Mallinckrodt, Mississauga, ON) in 100 mL distilled water, stored at -60°C and then thawed over night prior to use. The stomacher bag was placed into a Stomacher 400 lab blender (Seward Medical, London, UK) and set at normal speed for two minutes. One mL of the resulting solution was used for serial dilutions. Plates were prepared by dropping 0.1 mL of the diluted solution on two media types: nutrient agar (MacFaddin 1985) and media formulated by de Man, Rogosa and Sharpe (1960), known as MRS media (BBL MRS 95617 Becton Dickinson Systems, Cockeysville, MD) to determine total bacteria and lactic acid producing bacteria, respectively. Cyclohexamide antibiotic (100 mg/L) was added to the both agar to prevent yeast and mold growth, which was an alteration of the original protocol. Spreading of the inoculum was done by using a turntable and a bent glass rod. The rod was flamed with 95% ethanol between every plate to avoid contamination between plates. The original protocol was also altered such that each plate was inverted, with the lid down, when placed in the incubator to avoid condensation build up. Plates were counted after 48 and 72 hours of incubation at 25°C.

To confirm the presence of lactic acid producing bacteria after 72 h of incubation the colonies were visually evaluated and the morphology of the colonies was recorded. If the colony was pink, circular, convex and entire it was classified as likely being yeast. If the colony was white, circular, convex and entire it was classified as likely being bacterial. To confirm whether the white colonies were *Lactobacillus* spp., three

confirmation tests were needed to narrow down the different classes of possible colonies. For example, there are a number of gram-positive bacteria, but only certain colonies (such as *Lactobacillus* spp.) do not produce catalase. The three confirmation tests conducted were the gram test, the catalase test and the oxidase test (Falkow et al. 1992). If the three tests gave conflicting results, those particular colonies were not counted as *Lactobacillus* spp.

The gram test was conducted by dropping one drop of 3% potassium hydroxide (KOH) on the colony to be identified using a wire loop. The wire loop was then flamed and allowed to cool. The same colony was then picked up using the wire loop. If the colony was runny it was gram positive, which indicated *Lactobacillus* spp. (Falkow et al. 1992). If the colony was thick and stringy it was gram negative, which indicated that the colony was not *Lactobacillus* spp.

The catalase test (Falkow et al. 1992) involved sterilizing the glass rod by dipping it in ethanol and flaming it. A colony was then picked up and put on a sterile plate. A few drops of 3% hydrogen peroxide were added to the colony. Appearance of bubbles indicated a positive result with catalase present, suggesting it was not *Lactobacillus* spp. If no bubbles appeared it was a negative result, no catalase was present and the colony was likely *Lactobacillus* spp.

The oxidase test (Falkow et al. 1992) used 1% tetramethyl-1,4-phenylenediamine dihydrochloride 98% which was poured on filter paper onto which a colony was then placed. If no color change appeared the colony was considered *Lactobacillus* spp. (oxidase negative). If the filter paper colour changed to blue the colony was not *Lactobacillus* spp.

4.3.3 Nutrient analysis

One of the two frozen forage core samples taken at baling on days 1, 2, 7, 21, 42 and 70 of storage was dried and processed for DM, crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF) and water-soluble carbohydrates (WSCHOs). The other frozen sample was thawed at room temperature and then analyzed for pH, VFA, lactic acid (LA), ethanol, 2,3-butanediol and ammonia nitrogen (NH_3-N).

Prior to nutrient analysis, forage DM was determined by drying at 60°C for 48 h in a forced-air oven. Dried samples were ground through a 1-mm screen (Tecator Cylotec 1093). Crude protein was analysed via the Kjeldahl method (AOAC. 1990; method no. 984.13) using a Kjeldahl analyser to determine the nitrogen content of the sample, which was then multiplied by 6.25 to estimate the CP content of the forage sample. Water-soluble carbohydrate content of forages was determined using the procedure described by Slominski et al. (1993). Acid detergent fibre and NDF (Komarek et al. 1994) were analysed using the ANKOM Fiber Analyzer #F200 (Fairport, NY). Relative feed value (RFV) was then calculated as:

Digestible dry matter (DDM) = 88.9 – (0.779 * % ADF)

Dry matter intake (DMI, % of body weight) = 120 / (% NDF)

RFV = (DDM * DMI) / 1.29

Forage pH was determined by immersing a 12.5 gram sample in 50 mL of distilled water and soaking for two hours. An Accumet pH meter, model 810 (Fisher Scientific) with a gel-filled combination electrode (Orion model 91-05) was used to determine pH of the resulting solution. Volatile fatty acids, LA, ethanol, 2,3 butanediol

and NH_3-N were extracted by placing a 10-gram sample into a 125 mL Erlenmeyer flask and adding 50 mL of 0.1 M hydrogen chloride (HCl). The contents were shaken at 180 rpm at room temperature overnight using a Controlled Environment Incubator Shaker (New Brunswick Scientific Co. Inc., Edison, NJ). Volatile fatty acid, LA, ethanol and 2,3-butanediol analyses were performed by adding 1 mL of 25 % metaphosphoric acid and 5 mL of sample solution to a 10 mL centrifuge tube (Erwin et al. 1961). The tube was vortexed and frozen until ready for analysis. Before analysis, the solution was thawed at room temperature, 0.4 mL 25% NaOH was added and the solution was vortexed. Then 0.64 mL of 0.3 M oxalic acid was added and the solution was again vortexed and then centrifuged at 3000 rpm for 20 minutes (Erwin et al., 1961). One to two milliliters of the supernatant were drawn off using a Pasteur pipette and put into an auto sampler vial to be injected directly into the gas chromatograph for VFA and 2,3 butanediol determination as described by Di Corca and Samperi (1974). A 1-uL sample was injected onto a 2 m x 2 mm ID glass column packed with 80/120 Carbopack™ B-DA/4% CARBOWAX® 20M (Supelco1-1889, 15g) installed in a gas chromatograph (Varian gas chromatograph Model 3400), with flame ionization detector and 8100 auto sampler. Injector and detector temperatures were 200°C. The column temperature was held at 175°C for 20 min and then increased to 215°C for 2 min. The flow rate of the helium carrier gas was 24 mL min^{-1}. Lactic acid and ethanol analyses were completed by diluting the original sample concentration by one half and the column temperature was decreased to 165°C. Forage NH_3-N analysis was conducted as described by Novozamsky et al. (1974).

4.3.4 Statistical analysis

The experiment design used to determine the effect of forage moisture level at baling, forage fibre length at baling and their interaction on bacterial populations, temperature over time and ensiled forage quality characteristics (CP, NH_3-N, ADF, NDF, WSCHO, fermentation end products, pH and relative feed value (RFV)) from day 0 to 70 of storage was a split plot. Dry matter level and processing technique were the main plot effect and sampling day was the subplot effect using the following model:

$$Y_{ijkl} = \mu + P_i + D_j + PD_{ij} + b_k + bPD_{ijk} + T_m + TP_{im} + TD_{jm} + TPD_{ijm} + \varepsilon_{jklm}$$

where, Y_{ijkl} = an observation resulting from the application of the i^{th} processing technique (i = chopped, not chopped), the j^{th} moisture level (j = low, medium or high) and the k^{th} block (k =1 to 2) in the m^{th} sampling day (m =1 to 5), μ = population mean, P_i = the deviation from μ that results from the i^{th} processing technique, D_j = the deviation from μ that results from the j^{th} DM level, PD_{ij} = the deviation from μ that results from the interaction between P and D, T_m = the effect of the m^{th} sampling day, TP_{im} = the deviation from μ that results from the interaction between T and P, TD_{jm} = the deviation from μ that results from the interaction between T and D, TPR_{ijm} = the deviation from μ that results from the interaction between T, P and D, ε_{jklm} = the error deviation. Data was analyzed using the Proc Mixed procedure of the Statistical Analysis System Institute, Inc. (1999). Interactions including the time variable were conducted using a simple T-test.

Effect of forage moisture level at baling, forage fibre length at baling and their interaction on bale weight into and out of storage, storage losses, maximum bale

temperature, days to reach maximum temperature and ensiled forage quality characteristics (CP, NH_3-N, ADF, NDF, WSCHO, fermentation end products, pH and RFV) on day 70 of storage were assessed using a split plot factorial analysis using the following model:

$$Y_{ijkl} = \mu + P_i + D_j + PD_{ij} + b_k + bPD_{ijk} + e_{ijkl}$$

where, Y_{ijkl} = an observation resulting from the application of the i^{th} processing technique (i = chopped, not chopped), the j^{th} moisture level (j = low, medium or high) and the k^{th} block (k =1 to 2) in the l^{th} bale (l=1 to 24), μ = population mean, P_i = the deviation from u that results from the i^{th} processing technique, D_j = the deviation from μ that results from the j^{th} DM level, PD_{ij} = the deviation from μ that results from the interaction between P and D, b_k = the effect of the k^{th} block (random effect), bPD_{ijk} = the deviation from μ that results from the interaction between b, P and D, e_{ijkl} = the error deviation. Contrast statements were used to elicit differences when treatment differences were observed ($P < 0.05$). The main effects for DM and processing were reported if DM x processing interactions were not significant.

4.4 RESULTS AND DISCUSSION

4.4.1 Forage harvesting conditions

Forage harvest occurred in late June, with daily maximum and minimum temperatures during the 4-d harvesting period, from June 25 to 28 2002 ranging from 26.5 to 32.5°C and from 13 to 20°C, respectively, with averages of 30.1 and 16.1°C, respectively. No precipitation was detected during the wilting period or baling (Environment Canada, Glenlea Station, MB). Wilting time for the high, medium and low moisture hay treatments averaged 24, 48 and 72 h, respectively. The high moisture treatment was baled on June 26, 2002 starting at 10:25 h, the medium moisture forage on June 27, 2002 starting at 09:50 h and baling for the low moisture treatment started on June 28, 2002 at 10:20 h.

Bale dimensions were 1.2 m wide by 1.2 m in diameter. Processing technique did not have an effect on bale weight into storage (P=0.49). However, there was a significant difference between initial bale weight for the low, medium and high DM bales with an average weight of 493 $\pm$ 14, 407 $\pm$ 22 and 321 $\pm$ 18 kg wet basis, respectively. There was a linear relationship between moisture level and wet bale weight (R = 0.98) with wet bale weight increasing 4.7 kg for every 1% increase in bale moisture content, from 38 to 74% moisture. These bale weights are lower than alfalfa silage bales of the same dimensions reported by Borreani and Tabacco (2006) who recorded weights between 679 and 706 kg wet basis for 35% DM bales and between 548 and 571 kg wet basis for 50% DM bales.

4.4.2 Bale dry matter

In western Canada, the recommended DM level for proper fermentation to occur in BBS ranges from 40 to 60 % DM (MAFRI 2008). Therefore, the initial targeted DM levels for this trial were 35, 50 and 65%. The actual bale DM ranges were 22.1 – 29.8 36.7 – 44.1 and 58.9 – 67.3 % for the low, medium and high DM bales, respectively. Sensitivity of the microwave method and in-field variability regarding the ratio of grass and legumes and biomass yield may have contributed to variations between the actual and targeted moisture levels of forage at baling. The average DM content per treatment group (n=8) was 26.4, 40.2 and 62.4 $\pm$ 1.7 %, for the low, medium and high DM bales, respectively at the onset of the trial.

4.4.3 Bacteria populations

The types and numbers of bacteria on the forage effect silage fermentation because preservation of forage as silage relies on the presence of lactic acid producing bacteria during the ensiling process. In the current study, lactic acid producing bacteria populations differed between treatments during the 48 h period in which they were monitored, as indicated by the significant DM*Time (T) interaction (Table 1). For all three DM levels lactic acid producing bacteria increased over the first two days of storage (Figure 1). The increase was greatest in the low DM bales and lowest in the high DM bales. The medium DM bales had intermediate levels between the low and high DM groups. Therefore, the low DM bales had the most favorable DM level for lactic acid producing bacteria growth after one day of storage. Nicholson et al. (1991) reported lactobacilli counts from 6.99 to 9.20 log CFU g^{-1} DM in 35-40.5% DM

TABLE 1. Lactic acid producing and total bacteria (Log10 CFU g^{-1} wet forage) in alfalfa-grass at 0, 1 and 2 days post-ensiling at 26.4 (L), 40.0 (M) and 62.4 (H) %DM (n=8) using two processing techniques (chopped or not chopped) (n=12)

Day	Dry matter level (DM)				Processing technique (P)			P-value					
	L	M	H	SE	Chopped	Not chopped	SE	DM	P	T	DM x P	DM x T	P x T
						Lactic acid producing bacteria							
0	5.94	6.25	4.68	0.27	5.59	5.65	0.22						
1	8.10	6.89	5.97	0.27	7.11	6.87	0.22						
2	8.59	7.81	5.53	0.27	7.24	7.38	0.22						
Mean	7.54	6.98	5.40	0.20	6.65	6.63	0.17	<0.01	0.95	<0.01	0.47	<0.01	0.53
						Total bacteria							
0	7.77	8.29	8.25	0.12	8.08	8.12	0.10						
1	8.06	7.80	8.26	0.12	8.03	8.04	0.10						
2	8.50	8.24	8.06	0.12	8.22	8.32	0.10						
Mean	8.11	8.11	8.19	0.07	8.11	8.16	0.06	0.63	0.53	0.08	0.85	<0.01	0.90

T=time

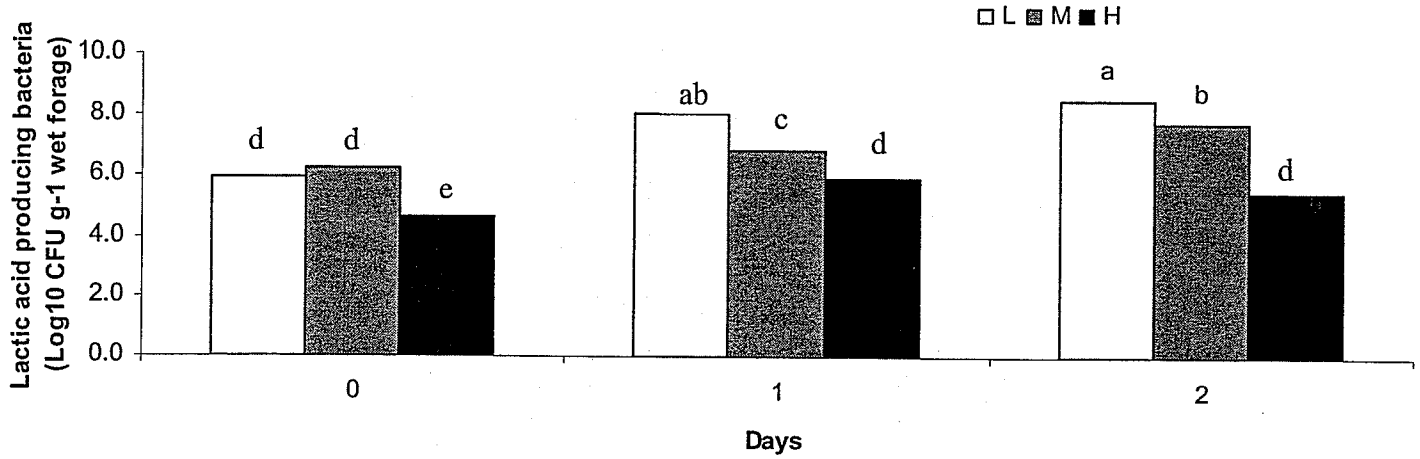

FIGURE 1. Lactic acid producing bacteria (Log10 CFU g^{-1} wet forage) in alfalfa-grass ensiled as big bales %DM (n=8, SE=0.27) at 26.4 (L), 40.0 (M) and 62.4 (H)

a,b values within the same day are different ($P < 0.05$)

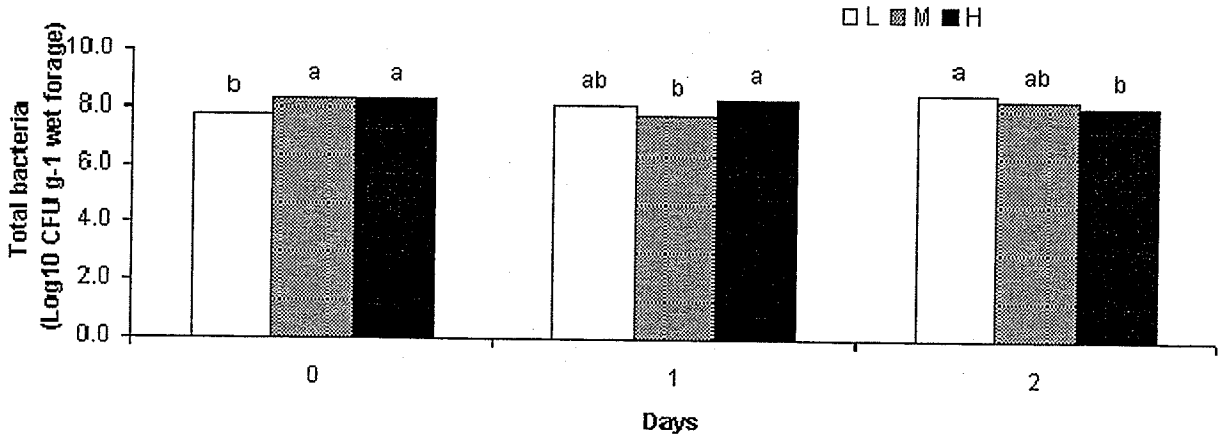

FIGURE 2. Total bacteria (Log10 CFU g^{-1} wet forage) in alfalfa-grass ensiled as big bales at 26.4 (L), 40.0 (M) and 62.4 (H) % DM (n=8, SE=0.12)

a,b values within the same day are different ($P < 0.05$)

alfalfa-grass ensiled as round bales. The low DM bales had significantly higher counts compared to the higher DM bales (46.5 to 50.6% DM), which ranged from 6.10 to 8.83 log CFU g^{-1} DM during the first three days of storage. These authors concluded that the low DM bales had a more desirable pattern of fermentation than the high DM bales since the pH decreased faster and further, lactic acid and acetic acid contents were high, and clostridia counts were lower. However, overall these authors reported that satisfactory silage was produced in all bales ranging from 35 to 50% DM. Since the initial lactic acid producing bacteria levels in both the high and low DM bales in this study were similar to the satisfactory silage that was reported by Nicholson et al. (1992), it is expected that the levels of lactic acid producing bacteria would also be sufficient for optimum fermentation at the start of the ensiling process

At baling, lactic acid producing bacteria made up 1.5, 0.9 and less than 0.5 % of total bacteria. At day one and day two post baling, lactic acid producing bacteria made up 100% of the total bacteria compared to only 12 to 37% in the medium DM bales and less than 0.5% in the high DM bales. Although there was not a clear advantage to the low DM bales when examining the total bacteria during two days of storage, a similar trend was seen for the total bacteria compared to the lactic acid producing bacteria (Figure 2). The total bacteria numbers in the low DM bales was lower than the medium and high DM treatments at baling (day 0). However, by day two the low DM bales had higher total bacteria compared to the high DM bales and the same levels as the medium DM bales. Overall, this indicates that the low DM bales had the most favorable conditions during the first two days of storage.

Processing did not have an effect on lactic acid producing bacteria counts in the current study (Table 1).

4.4.4 Initial nutrient content

The initial levels of WSCHO at harvest ranged from 78.8 to 84.8 g kg^{-1} DM (Appendix 1). Haigh (1990) suggested a minimum WSCHO level of 35 g kg^{-1} DM was necessary to prevent clostridial fermentation in low DM grass silages. These findings were further supported by Nicholson et al. (1992) who reported WSCHO levels at harvest ranging from 18.1 – 18.8 g kg^{-1} DM resulted in poor quality alfalfa bale silage, indicated by increasing numbers of clostridia and yeast organisms and high levels of NH_3-N (> 100 g kg^{-1} total N) and non-protein nitrogen (NPN) (> 400 g kg^{-1} total N). Therefore, both initial lactic acid producing bacteria and WSCHO levels in all bales would exceed the minimum required for optimum fermentation to occur.

4.4.5 Bale weights and density

Bale DM weights into storage ranged from 130 to 200 kg and were significantly different across treatments (Table 3). Bales lost on average 10 to 15 kg of DM after storage. Processing did not have a significant effect on initial or final bale weights. It is expected that since the high DM bales contained more DM and also had a lower initial bale weight, the cost per tonne of forage DM would decrease as DM increases since less tractor power would be required to produce and transport these bales. As bale volume is maintained constant, bale weight and bale DM levels into storage are major factors affecting packing density (Table 3). Packing density is one of major variables affecting

oxygen availability in silage (Van Soest 1994). Bale DM density in this study increased as DM level increased and were 96, 120 and 148 kg DM m^{-3} for the low, medium and high DM bales, respectively (Table 3). In comparison, Savoie and Jofriet (2003) reported DM density of alfalfa silage stored in tower silos (with a diameter of 3.7 m and a settled depth of 9.1 m) ranged from 200 to 234 kg DM m^{-3} as moisture level increased from 40 to 70% and in larger silos (9.1 m by 33.5 m) the DM density ranged from 319 to 356 kg DM m^{-3} as moisture level increased from 40 to 70%. Therefore, it is clear that the DM density values in BBS are much lower than those that can be achieved in tower silos. Furthermore, the density values reported in this trial were almost 90% lower than those reported by Borreani and Tabacco (2006) who saw DM density values for alfalfa bales ensiled as round bales between 182 and 204 kg DM m^{-3} for bales ranging from 35% to 49% DM, with DM density increasing as DM increased. However, this is contrary to a study by Han et al. (2004) who saw DM density of alfalfa BBS decrease as DM level increased from 42 to 47% DM. Therefore, these results suggest that there is not a consistent trend between DM and density and the effect will depend on the type of silage system used. Also, it is believed that the lower initial wet weights of the bales in this trial compared to those reported by Borreani and Tabacco (2006) contributed to the lower DM densities and would have been due to variation in baler types.

Chopping forage at the time of baling may help to overcome the low-density challenges associated with BBS systems (Nicholson et al. 1992). In this trial, the round baler had knives spaced 7 cm apart and dry matter density values were 104 and 101 kg DM $^{-3}$ for the chopped and not chopped bales, respectively (Table 3) which was an increase of 3%. Similarly, Borreani and Tabacco (2006) reported 4% higher DM density

values in the chopped bales compared to the unchopped bales. Collins and Owens (2003) also reported that finely chopped forages consolidate better and lead to increased silage density.

Although all bales experienced freezing, there were a greater proportion of frozen bales in the low treatment at feed-out, which made it difficult to remove the feed from the bale prior to delivery to the steers. In comparison, the long fibre bales that were fed in round bale feeders were not frozen to the same extent, which made it much easier and quicker to weigh and distribute the feed. In a commercial setting, more energy and time would be needed for these animals to consume the feed if it was frozen and forage was difficult to remove from the bale. This could also lead to increased labor by the producer if the silage had to be removed manually from the bale.

4.4.6 The fermentation process

4.4.6.1 Bale temperature

Bale temperatures in the first seven days of storage ranged from 32.6 °C in the low DM bales to 34.2 °C in the medium DM bales with no significant difference between treatments (Table 5). Peak temperatures occurred within seven days post wrapping for all treatments (Table 5). Dry matter had a significant effect on maximum bale temperature ($P = 0.02$) with the low DM bales having a lower maximum temperature (34.7 °C) compared to the medium and high DM bales (36.8 and 38.0 °C, respectively) (Figure 3). This difference in temperature may be attributed to the lower moisture content of the higher DM bales. For every gram of water, 4.184 joules of heat is required to raise the temperature one degree (Kotz and Treichel 1996). Therefore, it would have taken more

heat to raise the temperature in the low DM bales compared to the medium and high DM bales, which contained less water. As such, it is not surprising that the low DM bales had a lower maximum temperature and further, that it took the greatest number of days for these bales to reach maximum temperature ($P < 0.01$). Following the first week of ensiling, the main factor influencing bale temperature was ambient temperature (Figure 4).

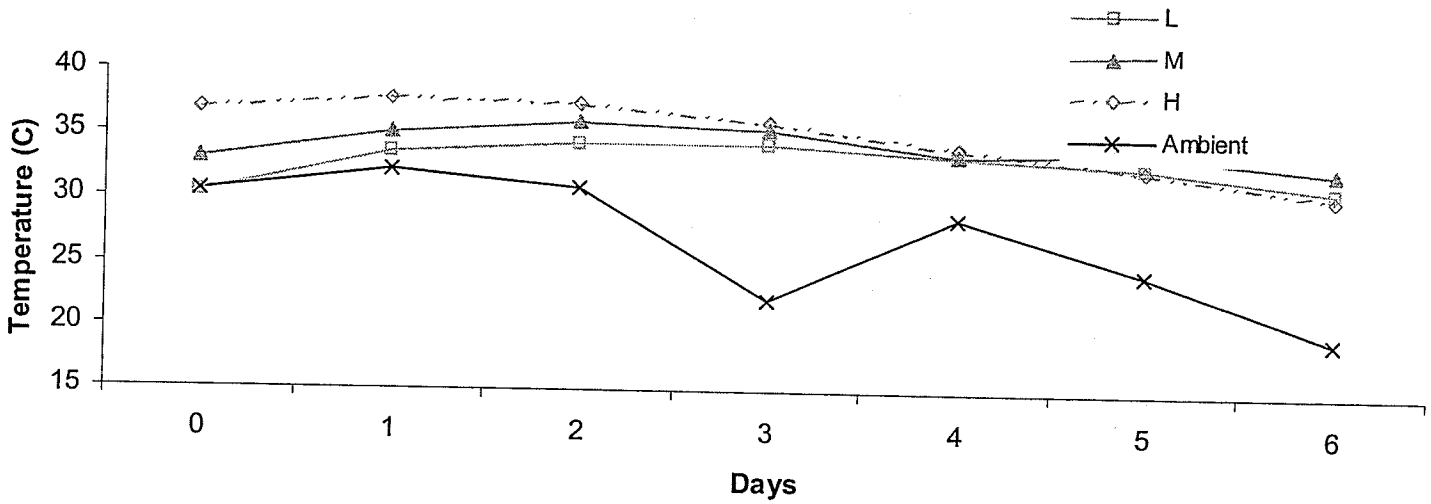

FIGURE 3. Mean bale temperatures during the first 7 days of storage in alfalfa-grass ensiled as big bales at 26.4 (L), 40.0 (M) and 62.4 (H) %DM (n=8) (SE = 1.0)

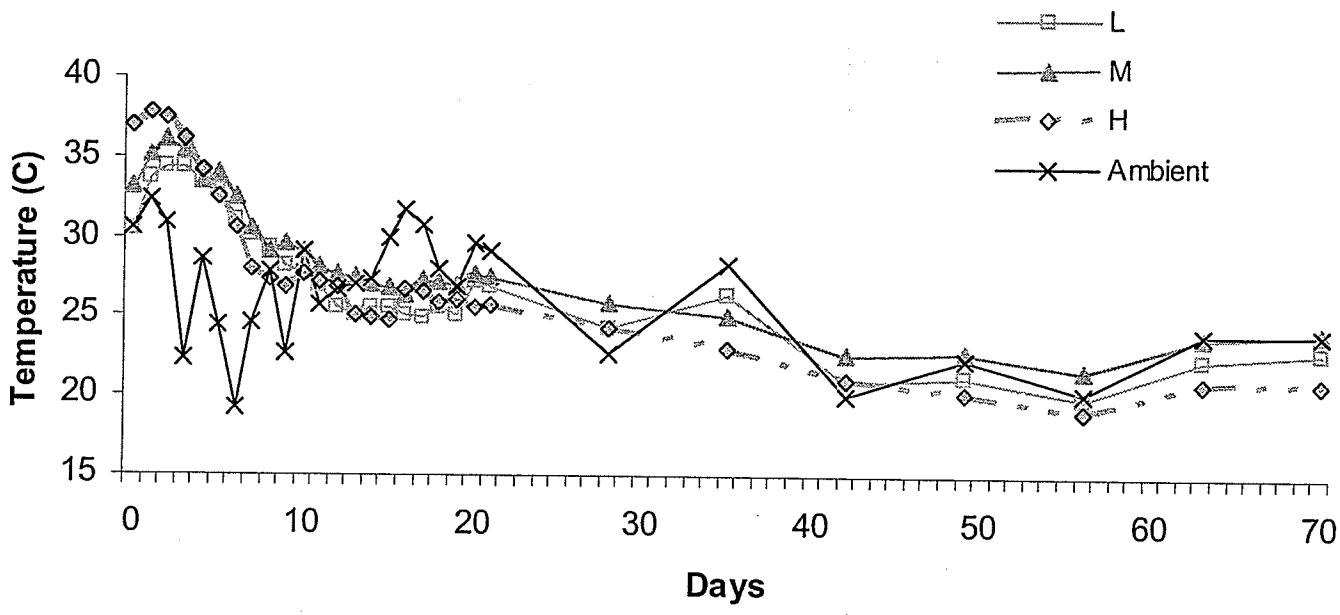

FIGURE 4. Mean bale temperatures during 70 days of storage in alfalfa-grass ensiled as big bales at 26.4 (L), 40.0 (M) and 62.4 (H) %DM (n=8) (SE = 0.4)

4.4.6.2 Fermentation end products

End products of silage fermentation are often measured and then compared to normal targeted levels in good quality silage. Typical levels of fermentation end products in good quality legume-grass BBS are: lactic (19.7 – 68.6 g kg $^{-1}$ DM), acetic (2.1 – 12.1 g kg $^{-1}$ DM), propionic (0.5 – 1.0 g kg $^{-1}$ DM) and butyric (0.6 – 7.2 g kg $^{-1}$ DM) (Nicholson et al. 1992; Charmley et al. 1999). High levels of ethanol, acetic acid and butyric acid (BA) are undesirable.

At 70 days of storage, the low DM bales continued to have higher levels of LA, at 31.9 g kg^{-1}DM, compared to the medium (16.0 g kg^{-1}DM) and high (2.3 g kg^{-1}DM) DM bales ($P < 0.05$) (Table 4).

The acetic levels reported in this study ranged from 3.3 g kg^{-1} DM at day 0 to 85.4 g kg^{-1} DM at day 70. Both values were observed in the low DM bales (Appendix 2). Although acetic acid is associated with undesirable heterofermentative fermentation it also has a positive role since it inhibits yeasts responsible for aerobic spoilage (Kung et al. 2000). After 21 days of storage all treatment groups were above the normal levels of acetic acid indicating that heterofermentative fermentation had begun. All treatments were also significantly above the ideal values at 70 days of storage with the low DM continuing to have the highest levels (Table 4). An important parameter used to determine the type of fermentation is the lactic acid to acetic acid ratio. The highest ratio was reported in the low DM bales throughout the storage period (Tables 3 & 7), demonstrating homofermentation and heterofermentation.

Initial levels of propionic acid ranged from 0.1 g kg^{-1} DM in the low DM bales to 12.3 kg^{-1} DM in the high DM bales (Appendix 2). At 70 days of storage this was reversed and the low DM bales had a significantly higher level of propionic acid of 7.8 kg^{-1} DM. These final levels are well above the recommended range of 0.47 - 0.95 kg^{-1} DM. Processing technique had a significant effect on valeric levels ($P = 0.04$) with the chopped bales having higher levels compared to the bales that were not chopped over the 70 days of storage. Dry matter and processing did not have significant effects on either isolvaleric or valeric concentrations at 70 days of storage.

Isobutyric levels were the highest in the medium DM bales at baling, throughout the 70 days of storage and after the 70 days of storage. At 70 days of storage isobutryic levels ranged from 5.9 g kg^{-1} DM in the low DM bales to 28.4 g kg^{-1} DM in the medium DM bales with significantly higher levels of isobutyric acid in the medium DM bales compared to the high DM bales ($P = 0.02$). These levels of isobuytric acid are much higher than those reported by Cushnahan and Mayne (1995) who saw levels ranging from 0.2 to 0.5 g kg^{-1} DM in grass silage. Isobuytryic acid in silage can be related to deamination of valine by clostridia (McDonald et al. 1991). Therefore, the high levels of isobuytric acid may be an indication of the presence of clostridia and proteolysis.

Butyric acid concentrations at the onset of the ensiling process were all very low (< 0.05 g kg^{-1} DM), but began to rise for all treatments after 21 days of storage (Appendix 2). At 70 days of storage, the low and medium DM bales contained higher than the recommended levels of butyric acid (7.2 g kg^{-1} DM) (Nicholson et al. 1992; Charmley et al. 1999). High levels of butyric acid are undesirable because butyric acid is associated with protein degradation and large DM and energy losses (Kung et al. 2000).

Bale moisture level at baling and processing did not have a significant effect on butyric concentrations.

Levels of 2,3-butanediol were very low at the onset of the ensiling process (< 0.1 g kg^{-1} DM) and increased slightly to 4.4 g kg^{-1} DM at 70 days of storage (Appendix 3). There was no significant effect of DM or processing. These levels of 2,3-butanediol are less than those reported by Muller et al. (2007) who saw values ranging from < 0.01 to 21.2 g kg^{-1} DM in grass silage. Enterobacteria ferment carbohydrates into a number of products including butanediol. Therefore, the relatively low concentrations of butanediol indicate that enterobacteria fermentation was not significant.

Ethanol concentrations of 3.0 g kg^{-1} DM and 5.8 g kg^{-1} DM have been reported in alfalfa bale silage (Charmley et al. 1999) and barley bale silage (Moshtaghi-Nia and Wittenberg 1999), respectively. Ethanol is an indicator of undesirable yeast fermentation and high DM losses (Kung et al. 2000). The initial ethanol level in the forage to be ensiled was 17.5, 1.3 and 5.9 g kg^{-1} DM in the low, medium and high DM bales, respectively. The high level of ethanol at baling in the low DM bales is not expected since ethanol is an end product of yeast or enterobacteria fermentation and requires anaerobic conditions. It is not expected that these microorganisms would produce ethanol at baling. A possible explanation for this is the wet weather conditions that occurred before the forage was cut. It is possible that the forage located on the underside of the windrow would have dried more slowly than the forage located in direct sunlight on the top of the windrow. Also, the ground was very wet so the forage near the ground could also have been taking in moisture while still in the field. These wet conditions may have created a micro-environment suitable for fungal or yeast growth to occur. These

ethanol producing microorganisms may have remained on the forage until baling and led to the high initial ethanol levels. The decrease in ethanol in the low DM bales could be explained by the high effluent production in the low DM bales.

4.4.6.3 pH

During 70 days of storage DM level had a significant effect (P = 0.05) on pH levels (Table 2) with the low DM bales having a significantly lower pH (5.8) compared to the medium (6.1) and high (6.1) DM bales. Charmley et al. (1999) reported pH levels in mower conditioned and mower macerated legume-grass big bale silage of 4.48 and 4.87, respectively. As referred to earlier, Nicholson et al. (1992) found a higher pH range of 5.38 – 5.69 in baled alfalfa silage at 27 and 40 % DM, respectively. The higher number of lactic acid bacteria reported in the low DM bales, compared to the medium and high DM bales, supports the finding of lower pH levels in the low DM bales during 70 days of storage. Nicholson et al. (1991) reported similar results stating that decreasing the DM content of alfalfa-grass bales ensiled as big bales caused a more rapid initiation of fermentation shown by a more rapid drop in pH and increase in concentration of lactic and acetic acids.

At 70 days of storage, pH was not significantly different between treatments (Table 4) with the low, medium and high DM bales having pH values of 5.7, 6.3 and 6.0, respectively. These values were all higher than those reported in the literature and indicate that successful fermentation did not occur in any of the treatments.

Although, the DM losses in the alfalfa-grass bales during the 70 days of storage in this trial was less than 10% for all treatments and was not affected by DM level (P = 0.47) or processing technique (P = 0.55), losses were higher than those reported by Huhnke et al. (1997) who found bale DM losses in alfalfa-grass ensiled as big bales to be less than 2% in a large Oklahama study which included bales that ranged from 25 to over 75 % moisture at baling. In comparison, reported losses of DM in bunker silos ranged from 10 to 20% (Muck and Rotz 1996), whereas tower silos losses ranged from 4 to 14% (Buckmaster et al. 1989). However, changes in wet weight will generally underestimate DM losses since approximately 60% of the respired carbohydrates will remain in the bale as water (Pitt 1990 as cited in Huhnke et al. 1997).

4.4.7 Silage quality

The goal of good silage making is to ensure forage quality does not decline during the ensiling process. For this to occur the forage must be of high quality at the beginning of the ensiling process and a stable state must be created at the onset of ensiling to result in a high quality product at feed-out. As such, the following discussion will examine changes in forage quality parameters from baling (day 0) throughout the 70-day storage based on the samples collected on days 1, 2, 7, 21, 42 and 70 of storage. These parameters are summarized in Table 2. However, the main discussion will be focused on the forage quality resulting at 70 days of storage, since this is the end product that will be fed and will ultimately affect animal performance. These parameters are summarized in Table 4.

TABLE 2. Forage quality parameters during 70 days of storage of alfalfa-grass ensiled as big bales at 26.4 (L), 40.0 (M) and 62.4 (H) %DM (n=8) using two processing techniques (chopped or not chopped) (n=12)

	Dry matter level (DM)				Processing technique (P)			P-value					
	L	M	H	SE	Chopped	Not chopped	SE	DM	P	T	DM x P	DM x T	P x T
DM (%)	26.0 ^{c}	41.3 ^{b}	62.4 ^{a}	1.4	43.9	42.5	1.2	<0.01	0.44	0.05	0.76	0.07	0.56
Temperature (^{0}C)	27.1	28.5	27.3	0.4	27.9	27.3	0.4	0.13	0.26	<0.01	0.31	<0.01	0.93
Nutrients													
CP (g kg^{-1}DM)	177.8	186.4	182.0	8.6	181.1	183.0	8.0	0.58	0.78	0.33	0.67	<0.01	0.43
Ammonia N (g kg^{-1}total N)	59.6 ^{a}	39.6 ^{b}	16.4 ^{c}	3.2	37.7	39.4	2.6	<0.01	0.66	<0.01	0.32	<0.01	0.51
ADF (g kg^{-1}DM)	334.3	335.8	325.7	7.8	334.0	329.8	7.2	0.42	0.53	<0.01	0.72	0.07	0.24
NDF (g kg^{-1}DM)	46.0	441.0	452.5	26.2	460.3	446.0	24.5	0.58	0.48	0.23	0.88	0.15	0.53
WSCHO (g kg^{-1}DM)	39.2 ^{b}	53.0 ^{a}	60.7 ^{a}	2.3	51.3	50.6	1.9	<0.01	0.80	<0.01	0.78	<0.01	0.12
Fermentation end products													
Lactic (g kg^{-1}DM)	24.3 ^{a}	10.8 ^{b}	2.6 ^{c}	2.2	12.3	18.9	1.9	<0.01	0.82	<0.01	0.81	<0.01	0.51
Acetic (g kg^{-1}DM)	33.0 ^{a}	28.6 ^{ab}	19.0 ^{b}	5.0	28.1	25.6	4.7	0.05	0.48	<0.01	0.84	<0.01	0.64
Lactic:Acetic	1.3 ^{a}	0.6 ^{b}	0.3 ^{b}	0.3	0.8	0.6	0.2	0.02	0.46	<0.01	0.67	<0.01	0.29
Propionic (g kg^{-1}DM)	2.6	2.2	4.1	0.8	2.6	3.3	0.7	0.30	0.46	0.10	0.21	0.00	0.72
Isovaleric (g kg^{-1}DM)	0.9	0.7	0.4	0.2	0.6	0.8	0.2	0.24	0.47	<0.01	0.41	0.09	0.12
Valeric (g kg^{-1}DM)	0.2	0.1	0.0	0.0	0.18 ^{a}	0.02 ^{b}	0.0	0.16	0.04	0.05	0.20	0.42	0.14
Isobutyric (g kg^{-1}DM)	8.9 ^{ab}	16.4 ^{a}	6.8 ^{b}	3.1	9.9	11.5	2.9	0.05	0.55	<0.01	0.46	0.03	0.80
Butyric (g kg^{-1}DM)	8.3 ^{a}	4.9 ^{ab}	1.0 ^{b}	1.9	6.0	3.6	1.5	0.10	0.32	<0.01	0.62	<0.01	0.13
2, 3 butanediol (g kg^{-1}DM)	0.2	1.0	0.6	0.2	0.8	0.5	0.2	0.17	0.35	<0.01	0.67	0.04	0.94
Ethanol (g kg^{-1}DM)	11.7	6.4	3.9	2.5	8.0	6.7	2.1	0.14	0.63	0.45	0.58	0.01	0.35
pH	5.75 ^{b}	6.14 ^{a}	6.11 ^{a}	0.1	6.0	6.0	0.1	0.05	0.81	<0.01	0.57	<0.01	0.12
RFV	128	133	132	8	129	133	7	0.76	0.51	<0.01	0.81	0.57	0.57

a-c Means in the same row followed by a different letter differ (P<0.05)
T = time

4.4.7.1 Crude protein

Crude protein, one of the most costly dietary ingredients, is one of the criteria in defining high quality silage. At 70 days of storage, silage CP content, which ranged from 170.9 – 186.3 g kg^{-1} DM, was not affected by DM level or processing technique (Table 4). These values were slightly higher than CP levels seen in alfalfa BBS reported by Nicholson et al. (1992) (161 – 170 g kg^{-1} DM) and similar to alfalfa ensiled in mini-silos (Agbossamey et al. 2000). Therefore, this clearly illustrates that it is possible to successfully produce high protein feed in BBS systems.

Rapid wilting can reduce the rate of protein breakdown (proteolysis) through inactivation of plant proteases in the forage (Rooke & Hatfield 2003). Low DM bales lost more CP ($P = 0.02$) compared to the medium and high DM bales (Table 3). Protein loss is not attributed to prolonged wilting as forage in low DM bales had the shortest wilting time. Nor is the CP loss attributed to reduced enzyme activity and inactivation of proteases associated with low pH (below 5.0) as although low DM bales did have a significantly lower pH compared to the medium and high DM bales ($P = 0.05$), they did not reach a pH below 5 during 70 days of storage. Proteolysis may have increased N migration to the bottom of the bale, which meant an error in the sampling technique because we could no longer assume homogeneity.

Crude protein loss associated with the low DM bales may be attributed to the increased volume of effluent ($P=0.01$) associated with the low DM bales. These bales lost more than five litres of effluent compared to the medium and high DM bales which lost less than one litre (Table 3). Weiss et al. (2003) reported that the CP content of

TABLE 3. Bale weights and storage losses in alfalfa-grass ensiled as big bales at 26.4 (L), 40.0 (M) and 62.4 (H) %DM (n=8) using two processing techniques (chopped or not chopped) (n=12)

	Dry matter level (DM)				Processing technique (P)			P-value		
	L	M	H	SE	Chopped	Not chopped	SE	DM	P	DM x P
Bale weight (kg DM)										
Into storage	129.6 ^{c}	162.3 ^{b}	200.3 ^{a}	6.4	166.9	161.2	5.4	<0.05	0.49	0.78
After storage	116.6 ^{c}	152.4 ^{b}	184.9 ^{a}	6.2	154.6	148.0	5.1	<0.05	0.40	0.49
*Bale volume (m^3)**	1.4	1.4	1.4		1.6	1.6				
Bale density (kg DM m^{-3})	96	120	148		104	101				
DM and storage losses, %[y]										
DM	10.0	5.4	7.6	2.4	6.8	8.6	2.0	0.47	0.55	0.21
CP	17.9 ^{a}	7.0 ^{b}	2.8 ^{b}	3.4	5.9	12.6	3.0	0.02	0.07	0.20
ADF	-4.7	-3.6	3.6	5.6	0.7	-3.8	4.6	0.56	0.52	0.96
NDF	6.5	1.3	0.6	4.7	3.50	2.08	3.8	0.65	0.80	0.98
WSCHO	76.1 ^{a}	56.8 ^{b}	39.3 ^{c}	4.2	59.4	55.3	3.4	0.00	0.44	0.15
Effluent production (L)	5.4 ^{a}	0.6 ^{b}	0.0 ^{b}	0.8	1.6	2.3	0.7	0.01	0.49	0.69

[y]Storage loss (%) = 100-(nutrient out/nutrient in) * 100.

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

* Assuming a bale's volume of 1.2 m long by 1.2 m diameter [1.2 m by pi $(1.2 \text{ m}/2)^2$]

effluent from grass silage could range from 200 to 300 grams per kilogram of DM. So, although the CP content of the effluent was not measured in this trial, an increase in the effluent collected from the low DM bales may explain the increased CP loss from these bales. Crude protein losses associated with processing approached significance as chopped bales lost 6.6% less CP ($P = 0.07$) compared to the bales that were not chopped at baling. Nicholson et al. (1991) reported that alfalfa chopped and compacted in a plastic tube had less extensive proteolysis compared to long, bale silage ensiled at similar DM levels. Apolant and Chestnut (1985) reported that the release of plant cell contents is mainly dependent on the collapse of the cell membrane when oxygen is removed and cell metabolism ceases. This release of plant cell contents occurs more rapidly in short-chopped forages since less air is trapped in the forage and it is delayed when air infiltrates the forage during early stages of fermentation (Marsh, 1979). Therefore, chopping silage tends to cause cell contents to be released more quickly, leading to increased rate of fermentation and decreased proteolysis. The results of the current trial support this theory since the chopped bales tended to have a lower loss of CP (Table 3).

4.4.7.2 Ammonia nitrogen

During 70 days of storage the NH_3-N levels were significantly higher ($P < 0.05$) in the low DM bales (59.6 g kg^{-1} total N) compared to the medium (39.6 g kg^{-1} total N) and high DM bales (16.4 g kg^{-1} total N) as indicated in Table 2. These levels of NH_3-N are less than those reported by Nicholson et al. (1992) who found extensive protein degradation in alfalfa ensiled at 27 and 40% DM as long forage in plastic-wrapped big

bales and chopped (to 1.25 cm) and compacted in plastic tubes. Higher levels of NH_3-N (Table 2) were combined with greater loss of CP in the low DM bales (Table 3).

Forage DM influenced NH_3-N produced in the bale during the course of the silage storage period ($P < 0.01$) (Table 2). Accumulation of NH_3-N occurred for a longer period of time in high moisture bales than for medium and low moisture bales (Table 2). This suggests that microbial activity or unstable storage conditions occurred for a longer period of time for the high moisture forage. At the end of 70 days of storage there was significant difference in ammonia nitrogen levels of 100.3, 56.9 and 28.7 g kg^{-1} total N for the low, medium and high DM bales, respectively (Table 4).

Processing technique did not have a significant affect on NH_3-N levels at 70 days of storage (Table 4). These results are contrary to those reported by Nicholson et al. (1992) who demonstrated higher levels of NH_3-N in long fibre bales than in bagged chopped forage ($P<0.01$). Chop length of the forage in the Nicholson study was not specified.

4.4.7.3 Acid and neutral detergent fibres

Dry matter level and processing technique did not significantly effect ADF or NDF levels at 70 days of storage (Table 4). Forage ADF and NDF concentrations often increase during silage production as WSCHO are being used up during plant cell and microbial respiration. Charmley et al. (1999) found that mower maceration increased fibre concentration in BBS with the greatest effect for ADF. Similarly, Simpson (1961) reported that fibre concentrations increased with maceration because of the increased post-cutting respiration following cellular damage. Differences in theoretical chop

TABLE 4. Forage quality parameters at 70 days of storage of alfalfa-grass ensiled as big bales at 26.4 (L), 40.0 (M) and 62.4 (H) %DM (n=8) using two processing techniques (chopped or not chopped) (n=12)

	Dry matter level (DM)				Processing technique (P)			P-value		
	L	M	H	SE	Choppped	Not chopped	SE	DM	P	DM x P
DM (%)	26.3 ^{c}	44.4 ^{b}	63.7 ^{a}	1.9	44.9	44.7	1.6	<0.01	0.92	0.47
Nutrients										
CP (g kg^{-1}DM)	170.9	184.7	186.3	10.9	181.9	179.3	10.0	0.36	0.78	0.78
Ammonia N (g kg^{-1}total N)	100.3 ^{a}	56.9 ^{b}	28.7 ^{c}	6.1	58.0	65.9	5.0	<0.01	0.32	0.14
ADF (g kg^{-1}DM)	349.8	344.7	328.2	11.5	337.4	344.4	10.4	0.28	0.52	0.50
NDF (g kg^{-1}DM)	474.6	452.6	468.2	34.0	465.0	465.3	31.9	0.75	0.99	0.63
WSCHO (g kg^{-1}DM)	20.8 ^{c}	37.7 ^{b}	51.8 ^{a}	3.3	36.7	36.8	2.7	0.00	0.98	0.75
Fermentation end products										
Lactic (g kg^{-1}DM)	31.9 ^{a}	16.0 ^{b}	2.3 ^{c}	3.5	16.9	16.6	2.9	<0.01	0.95	0.35
Acetic (g kg^{-1}DM)	85.4 ^{a}	47.7 ^{b}	26.5 ^{b}	7.5	56.9	49.5	6.1	0.01	0.43	0.81
Lactic:Acetic	0.4 ^{a}	0.4 ^{a}	0.1 ^{b}	0.1	0.3	0.3	0.1	0.04	0.75	0.60
Propionic (g kg^{-1}DM)	7.8 ^{a}	2.6 ^{ab}	1.1 ^{b}	1.8	4.5	3.2	1.5	0.10	0.55	0.55
Isovaleric (g kg^{-1}DM)	3.7	1.7	1.0	1.0	1.4	2.9	0.8	0.26	0.27	0.54
Valeric (g kg^{-1}DM)	0.5	0.2	0.0	0.2	0.5	0.0	0.2	0.34	0.12	0.32
Isobutyric (g kg^{-1}DM)	17.9 ^{ab}	28.4 ^{a}	5.9 ^{b}	3.6	17.5	17.3	3.0	0.02	0.98	0.92
Butyric (g kg^{-1}DM)	24.4	11.0	2.3	6.0	16.8	8.4	4.9	0.12	0.28	0.70
2, 3 butanediol (g kg^{-1}DM)	0.7	4.4	2.4	1.3	2.8	2.2	1.1	0.19	0.68	0.61
Ethanol (g kg^{-1}DM)	10.9 ^{a}	6.6 ^{b}	1.8 ^{c}	1.0	7.3	5.5	0.8	<0.01	0.17	0.59
pH	5.7	6.3	6.0	0.2	5.9	6.1	0.2	0.23	0.25	0.10
RFV	125	128	127	10	127	126	10	0.92	0.82	0.55

a-c Means in the same row followed by a different letter differ (P<0.05)

TABLE 5. Bale temperatures during 70 days of storage in alfalfa-grass ensiled as big bales at 26.4 (L), 40.0 (M) and 62.4 (H) %DM (n=8) using two processing techniques (chopped or not chopped) (n=12)

	Dry matter level (DM)				Processing technique (P)		
	L	M	H	SE	Chopped	Not chopped	SE
Temperature over 70 days (°C)	27.1	28.5	27.3	0.4	27.9	27.3	0.4
Temperature in first 7 days (°C)	32.6	33.8	34.2	1.0	34.0	33.1	0.9
Maximum temperature (°C)	34.7 ^{b}	36.8 ^{a}	38.0 ^{a}	0.8	37.1	35.8	0.7
Days to reach maximum temperatur	6.3	3.4	1.0	2.4	4.7	2.4	2.0

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

T = time

lengths between the baler used in this trial (7 cm) and the mower macerator, as used by Charmley et al. (1999) (1.9 cm) may explain the differences reported in fibre content changes.

4.4.7.4 Water-soluble carbohydrates

Water-soluble carbohydrate losses were greatest in the low DM treatment ($P < 0.01$), but were not affected by processing technique ($P = 0.43$) (Table 3). The WSCHO levels in the low DM bales decreased by over 75% during the 70 days of storage. Water-soluble carbohydrates can be depleted by effluent loss, especially in low DM silages, through aerobic deterioration and during anaerobic fermentation (Van Soest, 1994). Effluent production ranged from less than one litre in the high DM bales to 5.4 litres in the low DM bales (Table 3). The loss of WSCHO in the low DM bales is supported by the increase in effluent production recorded in these bales (Table 3).

Silage WSCHO were significantly different between treatments, with the low DM bales containing 20.8 g kg^{-1} DM, compared to the medium and high DM bales which contained 37.7 g kg^{-1} DM and 51.8 g kg^{-1} DM, respectively (Table 4). Similarly, Nicholson et al. (1992) found that after 10 days of storage, WSCHO content was lower in wet (27% DM) bale silage (16.6 $\pm$ 0.8 g kg^{-1} DM) than in control (40% DM) alfalfa bale silage (19.7$\pm$0.8 g kg^{-1} DM). However, after 60 days of storage this difference in WSCHO levels between treatments was no longer evident. The observed DM*T interaction ($P = <0.05$) for WSCHO during 70 days of storage was expected as microbes use WSCHO as a source of energy. It is possible that the initially higher lactic acid

producing bacteria populations present in the low DM bales utilized WSCHO, leading to the observed lower pH vales.

The decrease in silage WSCHO levels in the low DM bales can also be an indication that secondary fermentation was occurring. Secondary fermentation occurs mainly due to the re-exposure of the silage to oxygen or if there is a lack of fermentable CHO to prevent a rapid drop in pH at the onset of the fermentation process (Van Soest 1994). Indicators of secondary fermentation are increased levels of butyric acid, rise in pH as yeast metabolizes lactic acid and increased levels of NH_3-N (Kung et al. 2000). Significantly higher ethanol concentrations during 70 days of storage in the low DM bales provide further evidence that secondary fermentation may have occurred. The low DM bales had high levels of butyric acid compared to the high DM bales during the 70 days of storage (Table 2) and > 100 g kg^{-1} total N at 70 days of storage (Table 4). This data suggests that secondary fermentation has occurred, but was not as a consequence of insufficient WSCHO levels. It is possible that the high butyric and NH_3-N may have occurred as oxygen was allowed to re-enter the bales during storage.

4.4.7.5 Relative feed value

Relative feed value has been used for years to compare the quality of legume and legume/grass hay and silages. At 70 days of storage RFV values ranged from 125 to 127 with no significant differences between treatments (Table 4).

4.4.7.6 Summary of silage quality

Although, initial WSCHO and lactic acid at the onset of storage were above the recommended levels, ideal fermentation did not occur. A possible explanation for this is some oxygen re-entering the anaerobic environment allowing microbial respiration to persist and causing ethanol production due to the presence of yeast. Although bales were flushed with CO_2 after each sampling, it is possible that oxygen entered during sampling or penetrated through the plastic wrapping. Penetration of oxygen through the plastic wrapping indicate that the number of layers of wrap were either inadequate or that the plastic had been punctured during the storage period. However, the bales were wrapped within the recommended range by MAFRI of five layers.

Processing technique did not affect forage quality or the fermentation process in big bale alfalfa-grass silage. The processing strategy used may not have resulted in sufficiently small enough particle size to lead to differences in packing density when compared to long fiber bales.

McDonald et al. (1991) have defined poor quality alfalfa silage as that with pH values of 7.0, WSCHO concentrations of 4 g kg^{-1} DM, trace amounts of lactic acid and, butyric acid concentrations of 8 g kg^{-1}. At 70 days of storage, it can be concluded that silage of mediocre quality was produced in the low, medium and high DM bales since WSCHO and lactic acid and levels exceeded those cited by McDonald et al. (1991). pH levels were also lower than 7.0 in all treatments. However, butyric acid levels were above 8.0 g kg^{-1} in both the medium and high DM bales indicating that unfavorable clostridial fermentation may be occurring. Also, CP of all bales at 70 days of storage were above the level reported in the medium quality alfalfa BBS in the Moshtaghi-Nia and Wittenberg (1997) study of 179.6 g kg^{-1}, but less than the high quality category of 239.2

g kg^{-1}. Similarly, RFV of all bales were greater than the medium quality BBS category of 112 in the Moshtaghi-Nia and Wittenberg (1997) study, but less than the high quality category of 155. Similarly, lactic acid levels in the low and medium DM bales were above the level reported by McDonald et al. (1991) in poor quality silage.

Although, based on the criteria mentioned above it appears that all DM levels produced silage of equal quality, the recommended DM level, based on this study, is in the medium DM range of 40.0% DM. This is according to the high levels of ethanol in the low DM bales it appears that good silage was not produced. Also, the low levels of lactic acid producing bacteria and lactic acid in the high DM bales indicate that proper lactic acid production did not occur at the onset of the fermentation process. According to the higher levels of lactic acid and slightly lower pH levels in the low DM bales, it appears that a moisture level of 26.4% DM is ideal. Conversely, increasing DM level resulted in favorable increases in WSCHO levels and decreases in ammonia N levels in the medium and high DM bales compared to the low DM bales. Although a DM level of approximately 26 % encourages a more favorable fermentation pattern, it may lead to problems during feed-out in the winter months if the forage freezes due to the high moisture content. This aspect of frozen bales and the effect of DM and processing on silage intake is examined further in Manuscript II. The higher level of effluent recorded in the low DM is another disadvantage of ensiling at 26% DM. As shown in this study effluent may lead to a loss of important nutrients such as CP. Therefore, overall, according to this study, a DM level of 40% is recommended to produce the highest quality alfalfa BBS.

4.5. CONCLUSION

This research demonstrates that processing did not have an effect on bale weight into storage, the fermentation processes, nor nutrient profile in BBS. Forage DM content, however, did impact fermentation processes. Although the low DM treatment was characterized by significantly higher lactic acid concentrations, higher ethanol concentrations were also observed. An examination of nutrient profiles indicates it is possible to produce high protein feed in BBS systems although CP may be lost through the effluent if bales are less than 30% DM.

5.0 MANUSCRIPT II

Effects of processing and moisture content and intake

of alfalfa-grass ensiled as big bales

5.1. ABSTRACT

Advances in baling technology over the last decade have enabled producers to chop forage at the time of baling. Merit of chopping and forage moisture level at baling for an alfalfa –grass stand, cut at 10% bloom and ensiled as big bale silage (BBS), was evaluated with an animal intake trial. Alfalfa-grass forage was baled at low (55.5 %) and high (66.4 %) dry matter (DM) levels and fed to 24 crossbred steers, BW 260 SE $\pm$ 20.3 kg, for four 28-day periods. Both long-fibre and short-fibre bales were manufactured using a round baler equipped with knives spaced 7 cm apart. Bales were wrapped, within five hours of baling using five layers of plastic film. The six dietary treatments reflected two silage moisture levels and three methods of ensiled forage delivery, namely: forage chopped at baling (CC), long fibre chopped at feeding (LC) using a tub grinder, and long fibre silage that was not chopped at feeding (LL). Over the duration of the intake trial, core samples were taken from treatment bales and analyzed for DM, crude protein (CP), acid detergent fibre (ADF), ammonia nitrogen (NH_3 –N), water-soluble carbohydrate (WSCHO), pH, volatile fatty acid (VFA), lactic acid (LA), ethanol and 2,3-butanediol levels. At the time of feeding, grab samples were taken on a daily basis for the chopped diets and core samples were taken on a weekly basis from the bales in the round bale feeders for the long fibre diets. Silage samples taken at time of feeding were analyzed for DM, CP and NDF content and particle size for the chopped diets. Animal intake was determined by measuring feed offered and refused on a daily basis for the chopped diets and on a weekly basis for the long fibre diets. Processing had an effect on particle size distribution ($P < 0.05$). Silage that was chopped at baling had between 70 and 75% of the

particles greater than 19mm compared to only 27 to 27% of the particles greater than 19 mm in the bales that were chopped at the time of feeding.

Silage pH was not affected by moisture level or processing technique. Silage lactic, propionic, isobutyric, butyric acid concentrations as well as ethanol concentrations were greater in the low DM bales ($P< 0.05$) compared to the high DM bales. Silage WSCHO concentrations were 46.5 g kg^{-1} DM in high DM bales and 48.4 g kg^{-1} DM in the low DM bales and were not significantly different. Dry matter content had a significant impact on animal intake as animals receiving the high DM diets consumed 2.6% body weight (BW) and 3.6% $BW^{.75}$ compared to those receiving the low DM bales, which consumed 2.4% BW and 3.3% $BW^{.75}$. Differences in intake may be attributed to differences in fermentation end products, including LA, propionic, isobutyric, butyric and ethanol ($P<0.05$) levels that were all greater in the low DM bales. In conclusion, bales ensiled at 66.4% DM led to higher intake values than bales ensiled at 55.2% DM. Processing, however, did not have a significant effect on intake.

Abbreviations: ADF, acid detergent fibre; **BBS**, big bale silage; **BW**, body weight; **CC**, chopped at baling; **CP**, crude protein; **DM**, dry matter; **LA**, lactic acid: **LC**, chopped at feeding; **LL**, long fibre; **NDF**, neutral detergent fibre; **NH_3 –N**, ammonia nitrogen; **VFA**, volatile fatty acid; **WSCHO**, water soluble carbohydrate

Keywords: silage, bale, alfalfa, grass, intake, processing, moisture

5.2 INTRODUCTION

Alternative forage preservation systems are required as producers strive to generate high quality forage, especially in areas where weather conditions do not support hay production (Small and McCaughey 1999). Big bale silage (BBS) production is less dependent on the weather compared to hay production and provides a more flexible and lower cost (Holmes 1997) alternative to traditional chopped silage systems. Big bale silage may be particularly beneficial to western Canadian producers with backgrounding operations who make use of all-forage diets to add value to their animals before being shipped for finishing.

Silage DM intake may be influenced by a number of factors including dry matter (DM) level (Dulphy & van Os 1996), silage pH (Rook and Gill 1990), particle size (Charmley et al. 1999; Nicholson et al. 1991), method of feed delivery (Nicholson et al. 1991) and silage organic acid concentrations (Rook and Gill 1990). Although an acceptable range of DM values has been well established for traditional silage systems (McDonald et al. 1991), limited work has been conducted to explore the impact of DM on quality (Manuscript I) and subsequent intake of BBS. Current recommendations regarding production of BBS suggest that acceptable DM values range from 40 to 60 % DM (MAFRI 2008). It is not known how this range in DM affects BBS quality and subsequent dry matter intake (DMI).

Research at the University of Manitoba has demonstrated that calves, backgrounded on 100% forage rations ensiled as round bales may achieve substantial gains (0.89 – 1.65 kg DM d^{-1}) when fed high quality forage that was chopped prior to feeding (Moshtaghi-Nia and Wittenberg 1997). However, the effects of chopping high

quality forage at the time of baling for BBS are not clearly known. Typically, forage preserved as BBS has little, if any, particle reduction during the harvest period. As such, it has been reported that BBS, which has long forage particles, has reduced fermentation and higher pH levels compared to shorter forage particle lengths stored in silos (Nicholson et al. 1991). Advances in baling technology have now made it possible to alter fibre length by chopping the forage at the time of baling through the use of precutter devices. Shinners (2003) reported that these devices offer the following advantages: 1) bale density is increased up to 15% which is believed to lead to improved baler productivity and silage quality; 2) bales are more easily handled by total mixed ration (TMR) mixer-feeders and 3) forage is easier to take off the bale by the animal.

The advantages and disadvantages of chopping high quality forage at the time of baling compared to that which is chopped at the time of feeding or fed as long fibre have not been fully explored. The objectives of this research were to 1) determine the value of processing forage at baling as assessed by silage quality at feed-out 2) assess potential benefits of big bale processing at feed-out, and 3) determine the effect of silage DM content on intake of BBS.

5.3 MATERIALS AND METHODS

5.3.1 Dietary treatments

Three alfalfa-grass stands were used as replicates when harvesting forage as BBS. Botanical composition of the stand biomass consisted of 72% legumes, 21% grass and 7% weeds, DM basis. Targeted bale DM levels were 50% for the low DM treatment and

65% for the high DM treatment. The low DM bales for this trial were produced at the same time as the medium DM bales in Manuscript I. The high DM bales were produced at the same time as the high DM bales in Manuscript I. Big bale silage was produced using a Claas Variant 180 Roto-cut baler. One third of the bales produced were chopped at the time of baling with the knives (spaced 7 cm apart) engaged, whereas all remaining silage was produced with the knives disengaged. Bales were wrapped within 5 h of baling with five layers of plastic film (25.4 um thickness and 750 mm width, Max tech Superior Cling Silage Film ^{TM} Tyco Plastics). The six dietary treatments, included two DM levels and three BBS delivery systems: 1) forage chopped at the time of baling and fed using a feed bunk (CC), 2) silage chopped at the time of feeding using a 1994 Case 8610 tub grinder with a theoretical chop length of 7 cm and fed using a feed bunk (LC) and 3) silage that was not chopped and fed using a round bale feeder (LL). Bales were stored uncovered for approximately five to eight months until feeding.

5.3.2 Animal management

Twenty-four crossbred steers with a body weight (BW) of 260 (SE $\pm$ 20.3) kg were housed in 24 individual pens. At the onset of the trial, steers were vaccinated with CattleMaster ® 4 (Pfizer Animal Health), One Shot ® (Pfizer Animal Health), Covexin ® 8 (Schering-Plough Animal Health) and implanted with Ralgro ® implants (Schering-Plough Animal Health). Animals also received an injection of Vitamin AD-500 (Citadel Animal Health) and were treated with Dectomax ® Pour-On (Pfizer Animal Health). Trace mineralized salt blocks and fresh water were provided on a free-choice basis.

Steers were fed one of six diets for four, 28-day periods. Each period consisted of 14 d adaptation to diets and 14 d of intake measurements. Chopped diets were fed in a feed bunk, with a target weighback of 10-15%, while the diets that were not chopped were fed as a bale in a round bale feeder. The silage for the chopped diets that were chopped at the time of baling was removed by hand from the bale and weighed in plastic containers before delivery to the steers individually. The silage that was chopped at the time of feeding was chopped with the tub grinder as needed. This feed was also weighed into plastic containers before being delivered to the steers individually. Feed offered and refused for the chopped diets were recorded daily. Weekly feed offered and refused for the long fiber diets was determined by weighing the bale at the beginning and end of each 7-day period using a calibrated platform scale. Visible wasted feed that had been pulled from the round bale feeder or feed bunk was collected once a day and placed back into the feeders.

Animals were weighed at the beginning and end of each of the two 7-day intake periods for a total of three times per period or 12 times over the course of the entire trial (four periods). Canadian Council on Animal Care Guidelines were followed in the care and management of the steers (CCAC 1984).

5.3.3 Sample collection and chemical analysis

As an indication of forage quality at feed-out, grab samples of feed delivered were collected on an individual basis for both the chopped diets (daily) and long fibre diets (weekly) and immediately frozen. For DM analysis, samples were thawed at room temperature and dried as explained above. Subsequently, feed samples for each animal

were composited for each 7-day period and analysed for CP, as described above, and neutral detergent fiber (Komarek et al. 1994) using the ANKOM Fiber Analyzer #F200 (Fairport, NY).

When the bales from the previous trial (Manuscript I) were unwrapped at 70 days of storage, it was predicted that quality changes may continue to occur between day 70 and feed-out if the bales did not remain in a stable state. Therefore, to characterize any changes that may have occurred between day 70 of storage and feed-out, core samples from six bales per replicate were collected throughout the duration of the intake trial and frozen (-20°C) until analysed. After thawing at room temperature, approximately half of each of the original core samples was dried at 60°C for 48 h in a forced-air oven. Dried samples were ground through a 1-mm screen (Tecator Cylotec 1093) and analyzed for DM, acid detergent fibre (ADF) and water-soluble carbohydrates (WSCHO). Acid detergent fibre (Komarek et al. 1994) was determined using the ANKOM Fiber Analyzer #F200 (Fairport, NY). Water-soluble carbohydrate content of forages was determined using the procedure described by Slominski et al. (1993).

The remaining half of each core sample was analyzed for pH, VFA, lactic acid, ethanol, 2,3 butanediol and ammonia nitrogen. Forage pH was determined by immersing a 12.5 gram sample in 50 mL of distilled water and soaking for 2 h. An Accumet pH meter, model 810 (Fisher Scientific) with a gel-filled combination electrode (Orion model 91-05) was used to determine pH of the resulting solution. Volatile fatty acids, lactic acid, ethanol, 2,3 butanediol and ammonia nitrogen were extracted by placing a 10-g sample into a 125 mL Erlenmeyer flask and adding 50 mL of 0.1 M HCl. The contents were shaken at 180 rpm at room temperature overnight using a Controlled Environment

Incubator Shaker (New Brunswick Scientific Co. Inc., Eidson, NJ). Volatile fatty acid, lactic acid, ethanol and 2,3-butanediol analyses were performed by placing 1 mL of 25 % metaphosphoric acid and 5 mL of sample solution into a 10 mL centrifuge tube (Erwin et al. 1961), vortexing and freezing until analyzed. Before analysis, the solution was thawed at room temperature, 0.4 mL 25% NaOH was added and the solution was vortexed. Then 0.64 mL of 0.3 M oxalic acid was added and the solution was again vortexed and then centrifuged at 3000 rpm for 20 minutes (Erwin et al., 1961). To determine volatile fatty acid (VFA) and 2,3 butanediol concentration, one to two millilitres of the supernatant were drawn off using a Pasteur pipette as described by Di Corcia and Samperi (1974). 1-uL sample was injected onto a 2 m x 2 mm ID glass column packed with 80/120 Carbopack^{TM} B-DA/4% CARBOWAX$^{®}$ 20M (Supelco1-1889, 15g) installed in a Varian gas chromatograph, model 3400, with flame ionization detector and model 8100 auto sampler. Injector and detector temperatures were 200°C. The column temperature was held at 175°C for 20 min and then increased to 215°C for 2 min. The flow rate of the helium carrier gas was 24 mL min^{-1}. Lactic acid and ethanol analyses were completed by diluting the original sample concentration by one half and the column temperature was decreased to 165°C. Forage ammonia nitrogen analysis was conducted as described by Novozamsky et al. (1974).

5.3.4 Particle size determination

Samples representing six bales of each of the four chopped diets from the three replicates were collected throughout the duration of the feeding trial to examine particle size distribution using the Penn State Particle Separator (PSPS) (Heinrichs 1997). The

PSPS consists of three screens and a bottom pan. The diameters of the screen holes were 19, 8 and 1.18 mm for the top, 2nd and 3rd screen, respectively. Approximately 150 g of wet forage was placed on the top screen of PSPS. The PSPS was shaken a total of 40 times, five times in each direction, twice, as described by Heinrichs 1997. The contents of each fraction were weighed and analyzed for DM. The percent retained for each screen was determined by subtracting the total weight of the sample from the weight retained on the screen and dividing the difference by the total weight of the sample and multiplying by 100.

5.3.5 Statistical analysis

The trial was designed as an incomplete Latin square and individual animals were designated as the experimental unit. Effect of forage moisture level at baling, forage fibre length at baling and their interaction on forage DM, crude protein (CP), neutral detergent fibre (NDF) content and particle size were analyzed using the following model:

$$Y_{ijkm} = \mu + P_i + D_j + PD_{ij} + T_m + e_{ijkm}$$

where, Y_{ijkm} = an observation resulting from the application of the i^{th} processing technique (i = chopped at baling, chopped at feeding, not chopped), the j^{th} moisture level (j = low or high) and the k^{th} animal (k =1 to 24) during the m^{th} period (m = 1 to 4), u = population mean, P_i = the deviation from u that results from the i^{th} processing technique, D_j = the deviation from μ that results from the j^{th} moisture level, PD_{ij} = the deviation from μ that results from the interaction between P and D, T_m = the effect of the m^{th} period, e_{ijkm} = the error deviation.

Effect of forage moisture level at baling, forage fibre length at baling and their interaction effect on initial silage DM, acid detergent fibre (ADF), water-soluble carbohydrate (WSCHO), pH, VFA, lactic acid (LA), ethanol, 2,3 butanediol and ammonia nitrogen (NH_3-N) were assessed using the following model:

$$Y_{ijk} = u + P_i + D_j + PD_{ij} + e_{ijk}$$

where, Y_{ijk} = an observation resulting from the application of the i^{th} processing technique (i = chopped at baling, chopped at feeding, not chopped), the j^{th} DM level (j = low or high) and the k^{th} bale (k =1 to 6), u = population mean, P_i = the deviation from u that results from the i^{th} processing technique, D_j = the deviation from u that results from the j^{th} DM level, PD_{ij} = the deviation from u that results from the interaction between P and D, e_{ijkl} = the error deviation.

The data was statistically analyzed using the Proc Mixed procedure of the Statistical Analysis System Institute, Inc. (1999). Contrast statements were used to elicit differences when treatment differences were observed ($P < 0.05$). The main effects for DM and P were reported if DM x P interactions were not significant.

Data from period 1 was removed from statistical analysis for forage quality parameters at feed-out and for intake values. The average weighback during this period was 14.3%. However, due to fluctuating weather conditions, intake was variable leading to days in which weighback was less than 1%. As such, the assumption of ad libitum intake could not be validated.

5.4 RESULTS AND DISCUSSION

The mean daily temperatures (+/- SE) for periods 1, 2, 3 and 4 of the intake trial were: - 4.0 (± 3.9), - 15.7 (± 8.0), - 20.9 (± 6.2) and - 14.6 (± 10.2) °C, respectively (Environment Canada, Winnipeg International Airport 2002). Initial bale weights for the low and high DM bales were 407 ± 22 and 321 ± 18 kg wet basis, respectively. Initial bale DM weights into storage ranged from 162 to 200 kg and were significantly different across treatments (Table 3).

According to the daily (chopped) and weekly (not chopped) grab samples collected at feed-out, the mean DM levels of the ensiled forage were 55.2 and 66.4 % DM for the low and high DM bales, respectively, as indicated in Table 6. Although the bales designated as low and high DM in this trial were baled at the same time as the medium and high DM bales, respectively, in Manuscript I, there were differences in DM levels. Manuscript I bales had DM levels of 40.0% and 62.4% at baling (Appendix 1) and 44.4 and 63.7 at 70 days of storage (Table 4). The increase in DM level from day 70 to feed-out may be attributed to sampling strategy at feed-out. All of the feed-out samples were taken from unwrapped bales over the feed-out period. During this time drying may have occurred from the surface of the exposed bales.

To eliminate this drying effect and to determine if changes in silage quality had occurred between day 70 of storage and feed-out, six samples per treatment per block were collected from wrapped bales for nutrient and fermentation end product analysis. The results of these samples are included in Table 7 and show that the mean DM levels of the ensiled forage were 41.8 and 62.9% DM. These DM levels are very similar to the initial DM levels of the medium and high DM bales from Manuscript I which were baled at the same time. Therefore, it is thought that the mean DM level of the low DM bales at

feed-out was between 41.8 and 55.2% DM and that the mean DM level of the high DM bales was between 62.9 and 66.4 %DM.

For the grab samples taken at feed-out, a significant DM x P interaction ($P < 0.01$) occurred for DM content suggested that water loss from bales was dependent on processing (Table 6). This interaction may be attributed to differences in surface area in the processed bales leading to differences in rate of drying at the time of feed-out. Also, as indicated above, a greater proportion of the low DM bales, particularly those chopped at the time of baling, were frozen at feed-out. Frozen material was avoided at sampling and as such may have influenced the DM of the sample.

The mean silage dry matter intake values were 7.5, 7.7 and 8.3 kg day $^{-1}$ for the CC, LC, and LL treatments, respectively (Table 7). These mean intake values are similar to that reported by Charmley et al. (1999) who found DMI values of steers fed legume/grass to be 8.21 to 8.35 kg day $^{-1}$. Based on body weight (BW), the mean silage dry matter intake values in this study were 2.4, 2.5 and 2.6 % BW for the CC, LC and LL treatments, respectively. These values are also similar to those reported by Han et al. (2004) who found voluntary intake values of alfalfa BBS to range from 2.0 to 2.1 % BW. Period (T_m) did have an effect on intake values with period two having lower intake values compared to periods three and four. This is as expected since the animals were larger in size during periods three and four. Period did not have an effect ($P = 0.40$) when intake was expressed as a percent of body weight.

TABLE 6. Forage quality parameters at feed-out and intake of alfalfa-grass ensiled as big bales at 55.2 (L) and 66.4 (H) % DM levels using three processing techniques (chopped at baling (CC), chopped at feeding (LC) or not chopped (LL)) (without Period 1)

	Dry matter level (DM)			Processing technique (P)				P-value		
	L	H	SE	CC	LC	LL	SE	DM	P	DM x P
DM (%)	55.2	66.4	0.6	61.7	60.4	60.2	0.7	<0.01	0.22	<0.01
Nutrients										
CP (g kg^{-1}DM)	181.5	181.4	0.3	181.7	179.5	183.2	0.3	0.98	0.22	0.83
NDF (g kg^{-1}DM)	462.0	457.2	0.3	461.4	463.3	454.2	0.4	0.33	0.30	0.38
Intake										
kg DM day^{-1}	7.5	8.1	0.3	7.5	7.7	8.3	0.3	0.08	0.15	0.45
% BW day^{-1}	2.4 [a]	2.6 [b]	0.1	2.4	2.5	2.6	0.1	0.04	0.15	0.20
% $BW^{.75}$ day^{-1}	3.3 [a]	3.6 [b]	0.2	3.3	3.4	3.6	0.2	0.04	0.24	0.14

a-b Means in the same row followed by a different letter differ (P<0.05)

TABLE 7. Forage quality parameters of alfalfa-grass ensiled as big bales at 41.8 (L) and 62.9 (H) % DM using two processing techniques (chopped or not chopped) (n=6)*

	Dry matter level (DM)			Processing technique (P)			P-value		
	L	H	SE	Choppped	Not chopped	SE	DM	P	DM x P
DM (%)	41.8 [a]	62.9 [b]	1.6	52.4	52.3	1.6	0.01	0.97	0.35
Nutrients									
Ammonia N (g kg^{-1}total N)	61.0	23.3	7.1	45.7	38.6	6.0	0.06	0.38	0.72
ADF (g kg^{-1}DM)	332.9	334.3	0.6	332.7	334.6	0.5	0.76	0.88	0.10
WSCHO (g kg^{-1}DM)	48.8	46.5	1.8	50.4 [a]	44.4 [b]	1.4	0.53	0.05	0.06
Fermentation end products									
Lactic (g kg^{-1}DM)	65.5 [a]	18.4 [b]	7.5	40.0	43.9	7.5	0.05	0.75	0.87
Acetic (g kg^{-1}DM)	10.0	3.6	1.4	7.0	6.6	1.5	0.06	0.85	0.59
Lactic:Acetic	6.8	6.1	0.9	6.8	6.1	0.9	0.61	0.67	0.22
Propionic (g kg^{-1}DM)	0.9 [a]	0.4 [b]	0.1	0.7	0.6	0.1	0.02	0.89	0.26
Isovaleric (g kg^{-1}DM)	0.3	0.1	0.1	0.2	0.1	0.1	0.20	0.35	0.56
Valeric (g kg^{-1}DM)	0.0	0.0	0.0	0.1	0.0	0.0	0.68	0.26	0.80
Isobutyric (g kg^{-1}DM)	5.7 [a]	1.1 [b]	0.6	3.0	3.8	0.6	0.02	0.40	0.35
Butyric (g kg^{-1}DM)	2.3 [a]	0.5 [b]	0.3	1.4	1.3	0.4	0.02	0.81	0.12
2, 3 butanediol (g kg^{-1}DM)	0.2	0.0	0.1	0.2	0.1	0.1	0.29	0.50	0.79
Ethanol (g kg^{-1}DM)	20.4 [a]	8.3 [b]	1.5	12.4	16.3	1.5	0.02	0.14	0.78
pH	6.2	6.0	0.2	6.3	5.9	0.2	0.59	0.27	0.20

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

* Analysis completed on core samples from 6 bales per treatment per block

Table 8. Penn State Particle Separator analysis of alfalfa-grass ensiled as big bales at 55.2 (L) and 66.4 (H) % DM levels using two processing techniques (chopped at baling (CC) or chopped at feeding (LC)) (n = 6).

	Diet					
	CC			LC		
	L	H	SE	L	H	SE
PSPS distribution	% retained, as is					
Top screen (> 19mm)	71.2 [a]	74.2 [a]	3.4	27.4 [c]	37.2 [b]	3.4
2nd screen (> 8 mm)	15.5 [c]	12.5 [c]	1.8	40.8 [a]	24.6 [b]	1.8
3rd screen (> 1.18 mm)	8.9 [b]	9.2 [b]	1.9	27.3 [a]	30.5 [a]	1.9
Bottom pan	1.8 [b]	1.7 [b]	0.5	1.7 [b]	4.9 [a]	0.5

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

McDonald et al. (1991) reported that silage with a DM content of less than 30% resulted in decreased voluntary intake. The DM level of the low DM bales in this experiment was approximately 55 %. However, there was a trend (P = 0.08) for intake levels to be affected by DM level with intake values of 7.53 and 8.11 kg DM day $^{-1}$ for the low and high DM bales, respectively (Table 7). Furthermore, when intake was examined according to % BW, the high DM bales had significantly higher intake levels. A general observation made consistently throughout the trial was that dry matter level had an effect on bale freezing with the low DM bales freezing to a much greater extent compared to the high DM bales. Dry matter level and processing technique had no significant effect on CP or NDF levels.

The mean NH_3-N levels were 61.0 and 23.3 g kg^{-1} total N for the low and high DM bales, respectively. Generally, NH_3-N less than 100 g kg^{-1} of total N have no effect on voluntary intake (Mc Donald et al. 1991). Therefore, ammonia nitrogen levels were not in the range to have an adverse effect on intake levels.

The chopped bales (50.5 g kg^{-1} DM) had significantly higher WSCHO levels compared to the unchopped bales (44.4 g kg^{-1} DM). Processing did not have an effect on WSCHO in Manuscript I.

Overall, fermentation end product analysis indicated that DM had an effect, while processing had no effect (Table 7). Lactic acid levels were greater in the low DM bales indicating that the fermentation process of these bales was more efficient in producing high levels of lactic acid. Higher levels of lactic acid also indicate greater storage stability in the low DM bales. Dulphy and van Os (1996) reported that with

concentrations up to 100 g kg^{-1} DM, lactic acid tended to be positively related to intake, likely because it reflected better preservation. Although the LA concentrations in this study were higher in the low DM bales (65.5 g kg^{-1} DM) compared to the high DM bales (18.4 g kg^{-1} DM), intake of the high DM bales was higher. Furthermore, propionic, isobutyric, butyric and ethanol levels were all greater in the low DM bales. Generally, fermentation end products were equal or greater for low as compared to high DM bales. Although the low DM bales did have higher lactic acid concentrations, the final pH for both silages was above 6.0, which was high. High levels of butyric acid (BA) are considered unfavourable in silage since high levels are an indication that lactic acid is being converted into BA, indicating the presence of clostridia (McDonald et al.1991). Although butyric acid levels have been negatively correlated with intake by beef cattle, it is difficult to define a precise range at which butyric acid will affect intake because it is speculated that intake is dependent on a combination of a number of factors, including moisture, VFAs, NH_3, amine and BA levels. McDonald et al. (1991) reported that typical butyric acid levels of conventional ryegrass and maize silages were 1 g kg^{-1} DM. These same authors also reported butyric acid levels range from 8 to 36 g kg^{-1} DM in badly preserved silage, in which either enterobacteria or clostridia or both have dominated the fermentation. The BA levels were higher in the low DM bales compared to the high DM bales, but were below 2.5 g kg^{-1} DM so is not expected that butyric acid levels were in the range to categorize this silage as badly preserved.

Although the low DM bales had a higher level of lactic acid, pH levels were not affected by DM level ($P = 0.59$) or processing technique ($P = 0.27$) and ranged from 5.9

to 6.3 (Table 7). This is a higher pH range compared to that reported by Han et al. (2004) of 4.7 – 5.1 for alfalfa BBS.

Processing had an effect on the particle size distribution when measured using the Penn State Particle Separator with the particle size of the bales chopped at baling being of greater length than the forage chopped at feeding (Table 8). Silage that was chopped at the time of baling had 71.2% - 74.2 % of DM retained in the top screen (>19mm) compared to 27.4 % – 37.2 % when chopped at the time of feeding. Dry matter content had a significant effect on particle length when the tub grinder was used at the time of feed-out. The smallest average particle size (8.79 mm) was observed for low DM bales, which approached 50% of the length observed for the high DM bales (15.21 mm). The average particle size for forage chopped at the time of baling was 15.0 mm. The ideal particle size to maximize intake of alfalfa silage has been reported to be between 6 and 13 mm (Seglar 1997). As such, the particle size in this trial was on average approximately 2 mm greater than the optimal particle size to maximize intake.

This study did not indicate that chopping at the time of baling results in increased forage intake. However, the economic cost of chopping at baling compared to chopping at feeding may be lower since it has been reported that the cutting mechanism on the baler requires only slightly more average power than a baler without the cutting mechanism (Holmes, 1997). Also, less labor would be required if the silage is chopped at the time of baling since baling and chopping would be done in one step instead of two. There may be a difference in diet selection for the two systems as the bales which are chopped at the time of baling are fed in a round baler whereas the bales chopped at the time of feeding are often fed in bunks.

5.5. CONCLUSION

In this study, processing did not have a benefit on fermentation and did not lead to an increase in DMI. This may be due to the fact that chop length of forage generated via a forage cutting device was too course to affect the fermentation process and also greater than the optimal particle size to maximize intake.

Dry matter level did have an effect on intake values with the high DM bales having a higher intake compared to the low DM bales. Therefore, a DM level of 63 to 66% was more favorable than a DM content of 42 to 55% for alfalfa-grass ensiled as BBS for optimizing DM intake.

6.0. GENERAL DISCUSSION

6.1 Forage quality

The rising cost of grains and an interest in forage-finishing beef has created renewed interest for strategies, such as BBS, to produce high quality forage. When considering RFV, the alfalfa BBS produced in this trial, with a RFV of approximately 125, could be classified as medium quality silage (Moshtaghi-Nia and Wittenberg 1997). However, it is important to emphasize that RFV, which is mainly influenced by plant maturity, should not be the only parameter to consider when assessing forage quality. This is especially the case when assessing silage quality. Indicators of the fermentation process, such as pH, organic acid content, WSCHO and ammonia levels, should be included in silage evaluations.

However, the link between fermentation parameters and intake are not clearly defined for BBS (Charmley 2001). It is very difficult to define high quality silage based solely on fermentation characteristics. It is not clearly understood which fermentation parameters influence intake and at what levels these parameters become significant in traditional silage systems. Since BBS is not as common as other traditional hay and silage systems, even less information is available. Therefore, it is important to link BBS DM and particle size with fermentation qualities and animal intake. Table 9 indicates minimum and maximum levels that have been reported in the literature for alfalfa-grass silage. When available, values were reported for BBS, otherwise conventional silage ranges are included.

TABLE 9. Typical ranges of forage quality parameters of well preserved alfalfa-grass silage

	Typical range			Manuscript I		Manuscript II	
	Min.	Max.	Source	Min.	Max.	Min.	Max.
DM (%)	35.0	65.0	MAFRI 2006; Hunhke et al. 1997	26.0	64.0	42.0	63.0
Bacteria populations (at day 0)							
Lactic acid bacteria (Log 10 CFU g^{-1}wet forage)	7.0	9.0	Nicholson et al. 1991	5.4	7.5	N/A	N/A
Total bacteria (Log 10 CFU g^{-1}wet forage)	8.1	8.1	McDonald et al. 1991	8.1	8.2	N/A	N/A
Nutrients							
CP (g kg^{-1}DM)	77.0	160.0	Steen et al. 1998	170.0	187.0	180.0	183.0
Ammonia N (g kg^{-1}total N)	N/A	100.0	Nicholson et al. 1992	28.0	100.0	23.0	61.0
ADF (g kg^{-1}DM)	268.0	452.0	Steen et al. 1998	328.0	350.0	333.0	335.0
NDF (g kg^{-1}DM)	413.0	662.0	Steen et al. 1998	452.0	475.0	454.0	463.0
WSCHO (g kg^{-1}DM)	17.0	N/A	Haigh 1990	20.0	52.0	44.0	48.0
Fermentation end products							
Lactic (g kg^{-1}DM)	20.0	70.0	Cherney and Cherney 2003; Nicholson et al. 1992	2.0	32.0	18.0	66.0
Acetic (g kg^{-1}DM)	2.0	12.0	Nicholson et al. 1992; Muller et al. 2007	27.0	85.0	3.6	10.0
Propionic (g kg^{-1}DM)	0.5	1.0	Charmley et al. 1999	1.0	8.0	0.4	0.9
Isobutyric (g kg^{-1}DM)	0.2	0.5	Cushnahan and Mayne 1995	6.0	28.0	0.6	5.7
Butyric (g kg^{-1}DM)	N/A	5.0	McPherson and Violante 1966	2.0	24.0	0.5	2.3
2, 3 butanediol (g kg^{-1}DM)	N/A	21.0	Muller et al. 2007	0.7	4.4	0.0	0.2
Ethanol (g kg^{-1}DM)	3.0	6.0	McDonald et al. 1991 Charmley et al. 1999	2.0	11.0	8.0	20.0
pH	N/A	5.0	Borreani and Tobacco, 2006	5.7	6.3	5.9	6.3
Particle size (mm)	6.0	13.0	Beauchemin et al. 2003	N/A	N/A	9.0	15.0

N/A = not applicable

The suggested optimal DM levels reported in previous studies range from 35 to 65% DM. A minimum DM level of 35% has been recommended to prevent frozen bales during feed-out that may be caused if the bale moisture level is high (MAFRI 2006). The dry matter level of the low DM bales in Manuscript I (26.3% DM) was below this minimum and there were a greater proportion of frozen bales in the low treatment at feed-out, which made it difficult to remove the feed from the bale prior to delivery to the steers. In comparison, the long fibre bales that were fed in round bale feeders were not frozen to the same extent, which made it much easier and quicker to weigh and distribute the feed. In a commercial setting, more energy and time would be needed for these animals to consume the feed if it was frozen and forage was difficult to remove from the bale. This could also lead to increased labor by the producer if the silage had to be removed manually from the bale.

One of the most important parameters at the beginning of the ensiling process is the level of lactic acid producing bacteria. Initial lactic acid producing bacteria levels were below the minimum suggested value of 7.0 Log 10 CFU g^{-1} wet forage in the high DM bales, however there was not a significant difference between treatments so the optimum DM and processing technique can not be clearly defined by referring to initial lactic acid producing bacteria levels.

Initial WSCHO levels are also important for sufficient fermentation to occur. At 70 days of storage (Manuscript I) and at feed-out (Manuscript II) levels of WSCHO were above the minimum suggested level of 17.0 g kg^{-1} DM with the high DM (62.4% DM) having significantly higher levels of WSCHO throughout the 70 days of storage. This

would suggest that the optimum DM level would be approximately 62% DM to have high levels of WSCHO available for livestock at feed-out.

Lactic acid levels can be used as an indicator of silage quality since high levels lead to lower pH values which stabilize silage. The lactic acid levels at 2.30 g kg^{-1} DM were below the recommended minimum value of 20 g kg^{-1} DM in the high DM bales at 70 days of storage (Manuscript I) and at feed-out (Manuscript II). The high DM bales (62.9% DM) had significantly (P=0.05) lower levels of lactic acid compared to the low DM (41.8% DM) bales at feed out. This suggests that a DM level of approximately 40% is more favorably than a DM level of approximately 60 to 65%.

Ethanol was significantly higher (P=0.02) in the low DM bales in both Manuscript I (10.9 g kg^{-1} DM) and Manuscript II (20.4 g kg^{-1} DM) compared to the medium and high DM bales and were well above the typical maximum level of 6.0 g kg^{-1} DM. This suggests that a DM level above 40% leads to lower ethanol levels.

Ammonia nitrogen is often associated with reduced silage intake (Wright et al. 2000). The typical maximum NH_3-N levels are 100 g kg^{-1} DM (Table 9). Therefore, although NH_3-N levels were significantly higher in the low (26.4% DM) bales in Manuscript I at 100.3 g kg^{-1} total N, it is not expected that this would have a significant effect on intake since it is well below the maximum suggested level.

Butyric acid was been reported to reduce intake (Harris and Raymond 1963). The maximum suggested level is 7.0 g kg^{-1} DM. Therefore, although there was significantly higher (P=0.02) butyric acid levels reported in Manuscript II in the 42% DM bales (Table 8) it is not expected that this low level (2.3 g kg^{-1} DM) would affect intake.

In Manuscript II, it was reported that moisture loss during baling or ensiling was greatest in the low DM bales that were chopped at the time of baling. This agrees with results from Manuscript I where effluent production was higher in the low DM bales. Effluent loss could be a concern since nutrients, such as WSCHO and protein, could be lost, resulting in lower quality forage for the animal. Measuring the nutrient content of the effluent that was collected in this trial may have helped to explain the WSCHO and CP losses that were reported in the low DM bales. Low DM bales that were chopped at the time of baling also lost more moisture than the high DM bales and the low DM bales that were not chopped at baling, making it more difficult to achieve optimal moisture content required for fermentation to occur.

There was some mold that was observed primarily in the low DM bales when they were unwrapped and weighed at 70 days of storage. The mold was generally isolated to the area surrounding the sampling location. Mold was not as evident at feed-out since the majority of the low DM bales were frozen. However, some of the bales were very dusty at feed-out, which is an indicator of mold and poorer quality silage. Mold on the bales that were opened at 70 days of storage could have been caused from oxygen entering the bales during sampling, even though flushing with carbon dioxide was done. Therefore, in normal production systems where bales would not be sampled, this would not be a problem. Mold could also have been caused by puncture in the wrap between baling and feed-out.

6.2 Chopping techniques and particle size

Processing forage at baling and at the time of feeding did not significantly impact the fermentation process or forage intake in steers. This may be because the difference in particle size between treatments was not of sufficient magnitude to alter either fermentation process or forage intake. The bale DM density values reported in this trial ranged from 96 kg DM m^{-3} in the low (26.4%) DM bales to 148 kg DM m^{-3} in the high (62.4%) DM bales. These values are also all below the DM density values found by Borreani and Tabacco (2006) for alfalfa bales ensiled as round bales, which were between 182 and 204 kg DM m^{-3} for bales ranging from 35% to 49% DM. Therefore, processing may not have had an effect in this study because the density was not high enough to impact the amount of more oxygen present in the bales. Pre-trial work may have been beneficial to compare the range of particle sizes that were possible with both the baler and the tub grinder. It may have been possible to alter the baler and chopper settings to see a greater range in particle size between the two processed treatments.

Although the fermentation pattern and intake levels were not affected by processing, there may be other advantages associated with chopping silage at the time of baling as opposed to chopping at the time of feeding. For example, the chopping and baling processes can be combined in one step resulting in a lower labour requirement and tractor power. Also, chopping at baling may be more effective when ensiling grass versus alfalfa due to the higher buffering capacity of alfalfa.

6.3 Recommendations

It is difficult to establish a clear DM recommendation for alfalfa-grass ensiled as BBS from the results of this trial because it is thought that successful fermentation did

not occur in any of the treatments. The main indicator of unsuccessful fermentation is the high pH level during the 70 days of storage (Table 2) and at 70 days of storage (Table 4). The reasons that successful fermentation did not occur include the high pH values and the low DM densities that were seen in all treatments.

The main factors that are important when producing high quality silage are initial lactic acid producing bacteria levels, WSCHO levels and the rate of oxygen removal. At day one and day two post baling lactic acid producing bacteria made up 100% of the total bacteria compared to only 12 to 37% in the medium DM bales and less than 0.5% in the high DM bales. The high proportion of lactic acid producing bacteria in the low DM bales corresponded to a lower ($P<0.05$) pH in the low DM bales during 70 days of storage. Since there was sufficient WSCHO (Haigh 1990) and initial lactic acid producing bacteria (Nicholson et al. 1992), it is thought that initial conditions were appropriate for proper fermentation to occur.

Furthermore, the total pH drop in the medium and high DM bales was less than 0.25 units (Appendix 3). The pH drop of 0.64 (Appendix 3) was greatest in the low DM bales, which corresponded with the high proportion of lactic acid producing bacteria (Table 1) and the lowest pH during 70 days of storage (Table 2). At 70 days of storage the pH values were 5.7, 6.3 and 6.0 in the low, medium and high DM bales, respectively. This is almost one unit lower than the pH range reported by Nicholson et al. (1992) of 4.48 to 4.87 in alfalfa BBS. The small decrease in pH observed in this trial is thought to have occurred because of the buffering capacity of alfalfa and because the forage was not chopped enough to increase density and remove oxygen rapidly.

A third aspect for proper fermentation to occur is the rapid removal of oxygen from the silo or bale. The bale densities in this study were 96, 120 and 148 kg DM m^{-3} in the low, medium and high DM bales, respectively (Table 1). These values were below values reported by Borreani and Tabacco (2006) for alfalfa bales ensiled at a similar DM range which were between 182 and 204 kg DM m^{-3}. The DM density values in this trial were also below the average for BBS reported by Huhnke et al. (1997) of 159 kg DM m^{-3}. Furthermore, the density values reported in this trial were well below the densities seen in typical bunker silage systems for hay and corn silage of 237 and 232 kg DM m^{-3} respectively (Muck and Holmes, 2000). The low density values indicate that more oxygen would be available in these bales produced in this trial compared to silage in traditional systems or well packed BBS. The higher amount of oxygen would mean that it would take longer to reach anaerobic conditions which may have been another factor contributing to the small drop in pH.

Results from Manuscript II indicated that the bales ensiled at 66% DM led to higher ($P < 0.05$) intake levels compared to bales ensiled at 55% DM when intake was expressed as % BW and % $BW^{.75}$. This suggests that a DM level greater than 55% is preferred. Furthermore, the low DM bales led to a higher proportion of frozen bales and may have influenced intake. Although it appears that proper fermentation did not occur, the intake values in this trial which ranged from 3.3 to 3.6 % $BW^{.75}$ (Table 7) were similar to those reported in the literature (Charmley et al. 1999; Han et al. 2004). Factors that may decrease intake such as butyric acid, 2-3 butanediol and ethanol were below the threshold and as a result did not affect intake. Also, it has not been clearly defined which silage parameters affect intake. One of the major factors that may have limited intake in

this trial was gut fill. The steers were on high fibre, 100% forage diets, which would have been limited by gut fill to a greater extent than concentrated grain diets. Secondly, although mold did not visually appear to be a concern at 70 days of storage some bales were dusty at feedout, which can indicate mold contamination and reduce intake levels.

Therefore, based on the large proportion of frozen bales and effluent produced in the low DM bales, the higher intake values of the 66 %DM bales compared to the 55.2 %DM bales and the lower pH and higher lactic acid producing bacteria levels as DM decrease, a DM range of 55% to 60% is recommended to achieve the highest quality of alfalfa-BBS to optimize intake.

Although forage LAB and WSCHO were conducive to good fermentation, these conditions did not persist over the storage period. This was most evident for low DM bales. Lactic acid producing bacteria concentrations increased over the first and second day suggesting that anaerobic conditions were achieved. However, the fermentation resulted in low LA concentrations and bale stability became an issue over the storage period. Processing did not affect bale density, O_2 exclusion, LA or other fermentation end products. These findings are consistent with those reported by Borreani and Tabacco (2006). In the current trial, bale density was lower than observed by Borreani and Tabacco (2006). Although their study was conducted using 100% legume bales while the current study included bales consisting of approximately 20% grass, bale density in the current study may not have been high enough to encourage rapid O_2 elimination and thus resulted in less than optimum fermentation.

6.4 Future research work

This study demonstrated that there are a number of factors that may influence both silage quality and animal intake. There is little work examining the relationship between these two factors. A number of studies report optimum fermentation levels but the link to animal intake is often not clearly defined. In this study, it was evident that a DM level below 40% is unfavorable in western Canada where temperatures drop well below freezing in the winter months. However, the upper limit on DM level was not evident from the results of this trial. Also, the effect of processing should be further examined to test whether an increase in density can be achieved by chopping at the time of baling. As such, a positive effect on fermentation and intake may also be possible.

Since voluntary feed intake can be affected by a multitude of factors it is difficult to define the optimum forage quality parameters to optimize intake. The use of silage additives may be a tool to improve silage quality and requires further study in BBS systems.

7.0 CONCLUSION

Dry matter had an effect on silage quality and intake of alfalfa-grass ensiled as BBS. Since successful fermentation did not occur in any of the treatments in this trial the optimum DM range is unclear. However, based on a combination of fermentation parameters, labor requirement and intake values, a targeted DM level between 55 and 60% DM is suggested. Processing at baling or feeding did not have an effect on silage quality or intake.

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9.0 APPENDIX

Appendix 1. Nutrient profile of alfalfa-grass ensiled as big bales at 26.4 (L), 40.0 (M) and 62.4 (H) %DM (n=8) using two processing techniques (chopped or not chopped) (n=12)

Day	Dry matter level (DM)				Processing technique (P)		
	L	M	H	SE	Chopped	Not chopped	SE
				Dry Matter (%)			
0	26.4	40.2	62.4	1.7	44.2	41.8	1.4
7	25.8	41.0	60.5	1.7	43.8	41.1	1.4
42	25.6	39.7	62.8	1.7	42.9	42.6	1.4
70	26.3	44.4	63.7	1.7	44.9	44.7	1.4
				Crude protein (g kg^{-1} DM)			
0	187.5	187.5	177.2	9.2	180.2	187.9	8.4
7	178.0	184.8	181.5	9.2	178.9	183.9	8.4
42	174.8	188.7	182.9	9.2	183.3	180.9	8.4
70	170.9	184.7	186.3	9.2	181.9	179.3	8.4
Mean	177.8	186.4	182.0	8.6	181.1	183.0	8.0
				ADF (g kg^{-1} DM)			
0	301.4	315.1	314.5	9.3	317.6	303.1	8.3
7	338.5	337.5	330.2	9.3	339.7	331.0	8.3
42	347.5	345.9	329.8	9.3	341.3	340.8	8.3
70	349.8	344.7	328.2	9.3	337.4	344.4	8.3
Mean	334.3	335.8	325.7	7.8	334.0	329.8	7.2
				NDF (g kg^{-1} DM)			
0	458.5	434.8	435.0	28.0	450.7	434.8	25.8
7	449.0	446.2	447.4	28.0	460.1	435.0	25.8
42	481.7	430.3	459.6	28.0	465.4	449.0	25.8
70	474.6	452.6	468.2	28.0	465.0	465.3	25.8
Mean	466.0	441.0	452.5	26.2	460.3	446.0	24.5
				WSCHO (g kg^{-1} DM)			
0	81.5	83.1	80.8	3.5	84.8	78.8	2.8
7	30.2	51.2	61.4	3.5	48.8	46.4	2.8
42	24.5	39.9	48.9	3.5	35.0	40.5	2.8
70	20.8	37.7	51.8	3.5	36.7	36.8	2.8
Mean	39.2	53.0	60.7	2.3	51.3	50.6	1.9

Appendix 2. Fermentation end products in alfalfa-grass ensiled as big bales at 26.4 (L), 40.0 (M) (n=8) and 62.4 (H) %DM (n=8) using two processing techniques (chopped or not chopped) (n=12)

Day	Dry matter level (DM)				Processing technique (P)		
	L	M	H	SE	Chopped	Not chopped	SE
				Lactic acid (g kg^{-1} DM)			
0	0.4	0.3	0.4	2.9	0.2	0.6	2.4
2	25.1	8.0	5.9	2.9	11.2	14.8	2.4
21	39.8	19.0	1.8	2.9	20.9	19.5	2.4
70	31.9	16.0	2.3	2.9	16.9	16.6	2.4
				Acetic acid (g kg^{-1} DM)			
0	3.2	12.6	16.0	6.2	12.1	9.1	5.6
2	14.6	10.7	7.2	6.2	10.5	11.1	5.6
21	17.0	36.2	24.1	6.2	28.0	23.6	5.6
70	85.4	47.7	26.5	6.2	56.9	49.5	5.6
				Lactic:Acetic			
0	0.2	0.0	0.1	0.3	0.1	0.1	0.3
2	2.1	1.0	0.9	0.3	1.6	1.0	0.3
21	2.6	0.9	0.1	0.3	1.2	1.2	0.3
70	0.4	0.4	0.1	0.3	0.3	0.3	0.3
				Propionic (g kg^{-1} DM)			
0	0.1	1.2	12.3	1.9	3.2	5.8	1.5
2	0.4	0.5	0.5	1.9	0.4	0.5	1.5
21	0.9	2.1	4.6	1.9	1.5	3.6	1.5
42	3.8	4.5	1.7	1.9	3.2	3.4	1.5
70	7.8	2.6	1.1	1.9	4.5	3.2	1.5
				Isovaleric (g kg^{-1} DM)			
0	0.0	0.2	0.1	0.5	0.2	0.0	0.4
2	0.1	0.1	0.1	2.9	0.1	0.1	0.4
21	0.2	0.6	0.5	2.9	0.4	0.5	0.4
42	0.7	0.7	0.2	2.9	0.8	0.3	0.4
70	3.7	1.7	1.0	2.9	1.4	2.9	0.4
				Valeric (g kg^{-1} DM)			
0	0.0	0.1	0.0	0.1	0.1	0.0	0.1
2	0.2	0.3	0.0	0.1	0.2	0.1	0.1
21	0.0	0.0	0.0	0.1	0.0	0.0	0.1
42	0.1	0.0	0.0	0.1	0.1	0.0	0.1
70	0.5	0.2	0.0	0.1	0.5	0.0	0.1
				Isobutyric (g kg^{-1} DM)			
0	0.1	0.3	0.3	5.1	0.2	0.2	4.4
2	4.6	5.3	2.1	5.1	4.1	3.9	4.4
21	5.6	23.6	20.2	5.1	12.7	20.3	4.4
42	16.4	24.1	5.3	5.1	15.0	15.6	4.4
70	17.9	28.4	5.9	5.1	17.5	17.3	4.4
				Butyric acid (g kg^{-1} DM)			
0	0.0	0.0	0.1	2.8	0.0	0.1	2.3
2	0.0	0.0	0.0	2.8	0.0	0.0	2.3
21	1.8	3.0	0.5	2.8	1.8	1.7	2.3
42	15.5	10.6	2.3	2.8	11.2	7.7	2.3
70	24.4	11.0	2.3	2.8	16.8	8.4	2.3

Appendix 3. Fermentation end products, pH and ammonia nitrogen levels in alfalfa-grass ensiled as big bales at 26.4 (L), 40.0 (M) (n=8) and 62.4 (H) %DM (n=8) using two processing techniques (chopped or not chopped) (n=12)

Day	Dry matter level (DM)				Processing technique (P)		
	L	M	H	SE	Chopped	Not chopped	SE
				2,3 Butanediol (g kg^{-1} DM)			
0	0.0	0.0	0.1	0.6	0.0	0.0	0.5
2	0.0	0.0	0.1	0.6	0.1	0.0	0.5
21	0.0	0.0	0.4	0.6	0.2	0.1	0.5
42	0.5	0.5	0.1	0.6	0.7	0.1	0.5
70	0.7	4.4	2.4	0.6	2.8	2.2	0.5
				Ethanol (g kg^{-1} DM)			
0	17.5	1.3	5.9	3.3	11.0	5.5	2.7
2	13.4	8.8	4.1	3.3	9.6	8.0	2.7
21	5.0	9.0	3.8	3.3	6.0	5.9	2.7
70	10.9	6.6	1.8	3.3	5.5	7.3	2.7
				pH			
0	6.4	6.3	6.2	0.2	6.3	6.3	0.1
1	6.2	6.2	6.1	0.2	6.2	6.2	0.1
2	5.6	6.2	6.1	0.2	6.0	5.9	0.1
7	5.5	6.0	6.1	0.2	5.9	5.8	0.1
21	5.4	5.8	5.9	0.2	5.7	5.7	0.1
42	5.5	6.3	6.3	0.2	6.2	5.8	0.1
70	5.7	6.3	6.0	0.2	5.9	6.1	0.1
				Ammonia N (g kg^{-1} total N)			
0	2.9	3.2	3.2	5.6	3.0	3.2	4.6
7	48.8	32.5	12.6	5.6	29.9	32.7	4.6
42	86.2	65.9	21.1	5.6	59.7	55.8	4.6
70	100.3	56.9	28.7	5.6	58.0	65.9	4.6