# EFFECTS OF FORAGE CHOP LENGTH ON PHYSICALLY EFFECTIVE FIBRE, PRODUCTION, AND METABOLISM OF DAIRY COWS

BY

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A Thesis
Submitted to the Faculty of Graduate Studies
In Partial Fulfillment of the Requirements for the Degree of

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Department of Animal Science
University of Manitoba
Winnipeg, Manitoba

**THE UNIVERSITY OF MANITOBA**

**FACULTY OF GRADUATE STUDIES**

*****

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Effects of Forage Chop Length on Physically Effective Fibre, Production, and Metabolism of Dairy Cows

**BY**

Sanjiv Kumar Bhandari

**A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirement of the degree**

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**Master of Science**

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MASTER'S THESIS/PRACTICUM FINAL REPORT

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Effect of forage chop length on physically effective fibre, production, and metabolism of dairy cows

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Approved

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☒ **Thesis**

☐ **Practicum**

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# ABBREVIATIONS

**Diets**

Experiment1

| | |
|---|---|
| ALCL | diet containing long chop alfalfa silage and long chop corn silage |
| ALCS | diet containing long chop alfalfa silage and short chop corn silage |
| ASCL | diet containing short chop alfalfa silage and long chop corn silage |
| ASCS | diet containing short chop alfalfa silage and short chop corn silage |

Experiment 2

| | |
|---|---|
| ALOL | diet containing long chop alfalfa silage and long chop oat silage |
| ALOS | diet containing long chop alfalfa silage and short chop oat silage |
| ASOL | diet containing short chop alfalfa silage and long chop oat silage |
| ASOS | diet containing short chop alfalfa silage and short chop oat silage |

**Forages**

| | |
|---|---|
| AL | long chop alfalfa silage (19 mm), in experiment 1 |
| AS | short chop alfalfa silage (10 mm), in experiment 1 |
| CL | long chop corn silage (19 mm), in experiment 1 |
| CS | short chop corn silage (10 mm), in experiment 1 |
| AL | long chop alfalfa silage (19 mm), in experiment 2 |
| AS | short chop alfalfa silage (6 mm), in experiment 2 |
| OL | long chop oat silage (19 mm), in experiment 2 |
| OS | short chop oat silage (6 mm), in experiment 2 |

**Terms**

| | |
|---|---|
| ADF | acid detergent insoluble fibre |
| ADIP | acid detergent insoluble protein |
| ASAE | American Society of Agricultural Engineers |
| EDTA | ethylene diamine tetra acetic acid |
| eNDF | effective NDF |
| F:C | forage to concentrate ratio |
| HPLC | high performance liquid chromatography |
| Hz | hertz |
| MPL | mean particle length |
| NDF | neutral detergent fibre |
| nm | nanometre |
| NRC | National Research Council |
| NSC | non-structural carbohydrate |
| pef | physical effective factor |
| peNDF | physically effective fibre |
| $peNDF_M$ | physically effective NDF measured from tabular values of pef multiplied by dietary NDF |
| $peNDF_{NDF}$ | physically effective NDF measured as proportion of NDF retained by the 8 and 19 mm screens of Penn State Particle Size separator multiplied by the DM content |

| | |
|---|---|
| $peNDF_{PS}$ | physically effective NDF measured as proportion of DM retained by the 8 and 19 mm screens of Penn State Particle Size separator multiplied by the dietary NDF |
| $peNDF_{>1.18}$ | physically effective NDF measured as the proportion of DM retained by a 1.18 mm screen of multiplied by dietary NDF |
| PSPS | Penn State Particle Separator |
| SARA | Sub Acute Ruminal Acidosis |
| TLC | theoretical chop length |
| TMR | total mixed ration |
| VFA | volatile fatty acid |

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# ABSTRACT

Bhandari, Sanjiv Kumar. M.Sc., The University of Manitoba, February, 2006. Effects of forage chop length on physically effective fibre, production, and metabolism of dairy cows. Advisor: J. C. Plaizier.

Physically effective fibre is the fibre that stimulates saliva production, chewing activity and ruminal buffering. Feeding high concentrate diets that are low in physically effective fibre to dairy cows may lead to production losses due to sub acute ruminal acidosis (SARA). The symptoms of SARA include decreased feed intake, decreased fibre digestion, decreased milk fat, and laminitis. Conversely, coarse diets with excessively high physically effective fibre may limit the feed intake due to physical constraints resulting in production losses. Forage chop length is one of the main factors affecting the physically effective fibre level of forages and diets. The objectives of this study were to quantify: 1) the effects of forage chop length on physically effective fibre, dry matter intake, feeding behaviour, chewing activity, water intake, rumen fermentation, and milk production in dairy cows fed a barley grain based total mixed rations and 2) to compare the different measures of measurement of physically effective fibre, and to determine which of these measures is best measure of rumen buffering. These objectives were achieved in two separate experiments. In experiment 1, diets contained 21.7% DM (dry matter basis) each of either a short (10 mm) or long (19 mm) chop lengths of alfalfa and corn silage and 44.0% barley grain based supplement, 12.6% protein supplement. In experiment 2, diets contained 24% DM each of either a short (6 mm) or long (19 mm) chop lengths of alfalfa and oat silage and 42.0% barley grain based supplement, 10.0% protein supplement. Reducing alfalfa and corn silage chop length from 19 mm to 10 mm in experiment 1, reduced the proportion of DM retained by the 8 and 19 mm screens of

the Penn State Particle Separator from 55.0 to 46% DM, respectively. Reducing alfalfa and oat silage chop length from 19 mm to 6 mm in experiment 2, reduced the proportion of DM retained by the 8 and 19 mm screens of the Penn State Particle Separator from 71.4 to 64.6% DM, respectively. Reducing corn silage chop length from 19 mm to 10 mm in experiment 1 and reducing oat silage chop length from 19 mm to 6 mm in experiment 2, increased dry matter intake (DMI) from 22.3 to 23.2 kg/d ($P<0.003$) and from 19.4 to 21.1 kg/d ($P<0.001$), respectively. Chop length of alfalfa did not affect the DMI in either experiment. In Experiment 1, reducing the corn silage chop length did not alter rumen pH, rumen VFA, and milk production, however, reducing alfalfa chop increased total rumen VFA, acetate, and propionate without affecting the rumen pH, DMI, and milk production. In experiment 2, reducing the chop length of alfalfa and oat did not affect eating behaviour, water intake, rumen pH, rumen VFA, rumen fluid volume, rumen passage rate, blood metabolites, and milk production. Comparisons of the particle size distribution of the diets and their respective orts/weigh backs showed that cows selected against coarse feed particles in favour of finer feed particles. As measures of rumen buffering capacity did not vary among diets, it could not be assessed which measure of physically effective fibre is the most accurate indicator of rumen buffering capacity. Chopping corn at 10 mm, chopping alfalfa and oat at 6 mm using New Holland forage harvester (model 790) can increase feed intake without causing SARA compared to chopping these forages at theoretical chop length of 19 mm.

# FOREWORD

This thesis is written in manuscript style, with each manuscript having its own abstract, introduction, materials and methods, results and discussion sections. There is a general introduction and review of the literature prior to the manuscripts, which are followed by the general discussion and conclusions, and literature cited sections.

# 1.0 INTRODUCTION

Physically effective fibre is fibre that stimulates the saliva production, chewing activity, and contributes to the ruminal buffering (Mertens, 1997). High concentrate diets for the high producing cows are low in fibre, which can result in insufficient physically effective fibre and sub acute ruminal acidosis (SARA) (Beauchemin et al., 2003). SARA has been defined by rumen pH values between 5.2 and 5.6 (Cooper and Klopfenstein, 1996). This disease can result in decreased dry matter intake (DMI), fibre digestibility, milk yield, milk fat, and laminitis (Nocek, 1997; Beauchemin et al., 2003; Plaizier, 2004). The National Research Council (NRC) (2001) recommends a minimum inclusion of 25% neutral detergent fibre (NDF) in the ruminant diet and that 75% of this fibre should be from forages in order to prevent SARA. These NRC (2001) guidelines do not provide recommendations for the physically effective fibre in dairy diet due to absence of a standardized method for the determination of physically effective fibre of a diet and due to conflicting results reported in the literature.

Dietary particle size is the one of the most important factors affecting the physically effective fibre. It can also affect dry matter intake, chewing activities, and rumen fermentation (NRC, 2001; Mertens, 1997). Lammers et al. (1996) designed the Penn State Particle Separator (PSPS) as a quick, on-farm method for measuring the particle size distribution of the TMR and forages. The PSPS consists of 3 screens with 19 mm, 8 mm, and 1.18 mm diameter holes and a bottom pan. Heinrichs and Lammers (1997) recommended that between 50 to 60% and between 40 to 60% of particles of silages and TMR, respectively, should be retained by the 8- and 19 mm of the screens of the PSPS to provide sufficient rumen buffering. Plaizier et al. (2004) conducted a survey

on dairy farms across Manitoba and reported that only 35% and 30% of the alfalfa and corn silage samples, respectively, were within the recommended range provided by Heinrichs and Lammers (1997). These authors also reported that 40% of corn silage and 30% of alfalfa silage samples were too coarse, whereas, 30% of corn silage and 35% of the alfalfa silage were too fine compared to the recommendations of Heinrichs and Lammers (1997). Excessively coarse or fine diets deviating from the recommendations of Heinrichs and Lammers (1997) can affect the health and production of the dairy cows, as low physically effective fibre in finer diets can result in SARA (Mertens, 1997), whereas, the higher physically effective fibre in coarser diets can limit the intake (Allen, 2000).

Different methods for determining the physically effective fibre based on the particle size distribution of diets and forages have been used. These include, the product of amount of the DM retained by the 8 and 19 mm screens of the Penn State Particle Separator (PSPS) and dietary NDF ($peNDF_{PS}$, Yang et al., 2001a), the proportion of NDF retained on 8 and 19 mm screens of the PSPS multiplied by DM retained ($peNDF_{NDF}$, Calberry et al., 2003), the proportion of DM retained by 1.18mm screen multiplied by dietary NDF ($peNDF_{>1.18}$, Yang et al., 2001a), and dietary NDF content of feeds multiplied by a tabular physically effectiveness factor ($peNDF_{M}$, Mertens, 1997). Many studies have compared these measures and reported considerably different values among these measures (Yang et al., 2001a; Beauchemin et al., 2003; Plaizier, 2004). The $peNDF_{M}$ calculated based on the tabular values gives consistently higher values compared to other measures of physically effective fibre (Plaizier, 2004). Beauchemin et al. (2003) reported no correlation of the $peNDF_{PS}$, $peNDF_{M}$, and $peNDF_{>1.18}$ with the rumen pH, however, observed a higher correlation ($r= 0.55$, $P<0.05$) of $peNDF_{PS}$ with the time and

area below rumen pH 5.8 compared to other measures. There is no general agreement among studies regarding the best measure for the rumen buffering.

Forage chop length affects the dietary particle size and the dietary physically effective fibre (Mertens, 1997). During the past few years, several studies have been conducted on the effect of forage chop length on health and production of dairy cows (Krause et al., 2002a, 2002b; Soita et al., 2002, 2003; Kononoff and Heinrichs, 2003a, 2003b; Krause and Combs, 2003; Beauchemin and Yang, 2005). The results obtained from these studies were inconclusive, as it is difficult to compare studies due to differences in the techniques used to measure and by the expression of dietary particle size distribution and physically effective fibre, and due to interactions between levels of concentrate inclusion, concentrate source, and forage source among diets. Additionally, the current NRC (2001) recommendations for the NDF, physically effective fibre, and the dietary particle size have been developed for corn based diets. Plaizier et al. (2004) reported a high use of the barley grain based diets compared to corn grain in the province of Manitoba. Barley grain has higher NDF content (Beauchemin and Rode, 1997) and is more rapidly fermentable (McCarthy et al., 1989) than corn. The NDF requirements for barley based diets are higher (34% DM) than those for corn based diets (25% DM), in order to maintain a milk fat content of 3.5% (Beauchemin, 1991).

It is therefore important to develop and provide guidelines for the forage chop length and physically effective fibre levels for barley grain based diets that are commonly used in Manitoba. The objectives of these studies were to investigate the effect of the forage chop length of alfalfa, corn and oat silages on the dietary physically effective fibre, chewing activity, feeding behaviour, rumen conditions, production, and metabolism of the

dairy cows fed with barley grain based total mixed ration in order to provide general recommendations for the forage chop length and dietary particle size. Further, to compare the different methods of the physically effective fibre determination and to assess which of these methods is the best measure for the rumen buffering.

# 2.0 LITERATURE REVIEW

## 2.1 Introduction

Increased genetic potential of the dairy cows for milk production necessitates formulation of diets rich in concentrates in order to meet their higher energy demands. These energy rich diets are highly fermentable and can fall short in physically effective fibre (Mertens, 1997), i.e the fibre that stimulates chewing, saliva production, and provide rumen buffering. Inadequate physically effective fibre levels in the diets of these high yielding cows may lead to one or more metabolic disorders such as sub acute ruminal acidosis (SARA), reduced total DM digestibility, reduced milk fat percentage, displaced abomasums, increased incidence of laminitis, acidosis, and fat cow syndrome (Sudweeks et al., 1981; Nocek, 1997).

Plaizier et al. (2004) conducted a survey across the dairy farms in Manitoba and reported that a trend of increased usage of ensiled forages compared to dried hay. This survey revealed that alfalfa and mixed alfalfa-grass silages were the most commonly used (75% of diets), followed by corn silage (58% of diets), and alfalfa and mixed grass by (55% of diets) of farms. This data clearly show the considerable variation in feeding practices followed across the farms in the province. Plaizier et al. (2004) also reported that a wide range of forage chop lengths were used across the dairy farms. Heinrichs and Lammers (1997) recommended that the 8 and 19 mm screens of the Penn State Particle Separator (PSPS) should retain between 50 to 60% of forages and 40% to 60% of TMR particles to provide sufficient rumen buffering. In their survey, Plaizier et al. (2004) revealed that only 35% of alfalfa silage samples and 30% of the corn silage samples were

within the recommended range, whereas, 40% of corn silage samples and 30% of the alfalfa samples were too coarse and other 30% of corn silage and 35% of the alfalfa silage samples were too finer than the recommendations provided by the Heinrichs and Lammers (1997). Dietary particle size can affect dry matter intake, chewing activities and rumen fermentation and is linked to the ability of the ration to meet the animal's fibre requirement (NRC, 2001). Diets with low physically effective fibre (< 22% DM) can result in insufficient rumen buffering leading to ruminal acidosis (Mertens, 1997), conversely, diets with higher physically effective fibre levels can limit feed intake (Allen, 2000), affecting the production levels of high yielding animals. It is therefore imperative, to provide guidelines for dietary particle size and fibre content to ensure maximum production.

NRC (2001) only provides recommendations on minimum inclusion of neutral detergent fibre (NDF) of 25 % DM in dairy diets and 75 % of this fibre should be from the forage source. Beauchemin (1991) suggested that barley based diets should contain a minimum of 34% NDF in order to maintain a milk fat content of 3.5%. The NDF content of barley grain ranges (19-25%) compared to (7%) for corn grain. Additionally, barley has more rapid rumen fermentation rate compared to corn (Beauchemin and Rode, 1997). So, for barley based diets, NDF requirements are different than NRC (2001) recommendations. The NRC (2001) guidelines, in the absence of a validated technique for the physically effective fibre and particle size determination, do not provide recommendations for factors such as physical effectiveness of fibre, due to unknown interactions with non-fibrous carbohydrates, or animal attributes. Further, current recommendations for optimum dietary fibre level and forage particle size were primarily

developed for corn based diets. Therefore, there is a need to develop guidelines for particle size distribution and physically effective fibre levels for barley grain based diets that are commonly used in Manitoba.

## 2.2 Techniques for determining particle size

### 2.2.1 Wet versus dry sieving

Wet or as fed based techniques for determining the particle size for forages and TMR are preferred over the dry method. Drying a sample results in shattering of particles leads to further reduction in particle size during sieving (Finner et al., 1978). Wet sieving appears to be a better measure, as samples are of comparable consistency to what is being offered to the animals. However, adherence of the small particles due to high moisture content and loss of moisture during storage may affect the particle size distribution of forages. Kononoff et al. (2003b) reported only small differences in the particle size distribution of forages when the sample moisture loss was 40% of the original sample. However, these authors emphasized that the samples should be analyzed in the same physical form as that fed to the animal.

The American Society of Agricultural Engineers (ASAE) standard S424 device is the standard method for determining the particle size of forages. This ASAE, a laboratory scale separator consists of five screens with decreasing diameter of holes 19.0, 12.7, 6.3, 3.96, and 1.17 mm from top to bottom, respectively and a bottom pan to separate the particles into six unique fractions, on a wet basis (Lammer et al., 1996). The ASAE sieve stack should be driven with a frequency of 2.4 ± 0.08 $H_Z$ (144 ± cycles/min) (ASAE, 1992). The particle distribution of the forages is calculated using a lognormal

approach and data is presented as both geometric mean and standard deviation (Finner et al., 1978).

This ASAE method, a cumbersome laboratory procedure, is impracticable for farm use. There is a need for a rapid, inexpensive on farm method of particle size separation for regular on farm use (Lammers et al., 1996). As an alternative to the existing ASAE method, Yang et al. (2001a) measured particle size distribution of feeds and TMR by dry sieving using a vertical oscillating sieve shaker (Analysette 3). This shaker consists of six sieves arranged in descending mesh size of 9.5, 6.7, 3.35, 1.18, 0.6, and 0.15 mm, respectively and a bottom pan. The mean particle length was used to represent sample distribution and this was calculated as the particle length for which 50 percent of the cumulative percentage weight of the sample was retained (Yang et al., 2001a). Using mean particle length as a measure of particle length is limited in that it does not indicate variation around the average, or range of the sample being measured (Einarson, 2004). Lammers et al. (1996) developed the Penn State Particle Separator (PSPS) to mimic the complex ASAE method. Kononoff et al. (2003a) stated that the PSPS is a simple on-farm method for particle size determination and is designed to describe the particle size of feed offered to the animals. These authors emphasized that samples should not be altered chemically or physically before sieving, which agrees with Finner et al. (1978), who suggested that completely drying a sample results in shattering of particles and/or increased sample moisture increase the likelihood of particle adherence and may mispresent the true separation.

### 2.2.2 Penn State Particle Separator

The PSPS, a forage and TMR particle size separator, was designed as a quick, cost effective, and on-farm applicable device (Lammers et al., 1996). This separator consists of two sieves with apertures 19 and 8.0 mm with a thickness of 12.2 and 6.4 mm, respectively and a bottom pan, which was later modified by Kononoff et al. (2003b) by adding additional sieve with an aperture of 1.18 mm. Based on a survey, Heinrichs (1996) reported an average 57.5 percent of the forage and TMR passes through both sieves of the PSPS. Poppi and Norton, (1980) and Mertens (1997) suggested that 1.18 mm is the critical length governing the retention in the reticulo-rumen and these particles require mastication and chewing to reduce them in order to pass out of the rumen. Kononoff et al. (2003b) suggested a better characterization of these small particles required a new sieve beaded and these authors added a third sieve to the PSPS with an aperture of 1.18 mm.

Lammers et al. (1996) compared the results of PSPS and ASAE Standard S424 method and found no difference in ability to predict fractions of particle with maximum length of less than 8 and 19 mm. With its simple construction and size, the PSPS sieving method has been widely accepted and particle size measurement using the PSPS is now commonly reported in the scientific literature (Kononoff et al., 2003b). It is recommended that approximately 1.4 ± 0.5 L of wet sample be placed on the top screen of the PSPS (Lammers et al., 1996). The particle separation is performed by shaking the PSPS in one direction on a flat surface. This process is repeated for 5 times for a total of 8 sets or 40 shakes with the sieves rotated one-fourth after each set of 5 shakes, without any vertical motion during shaking (Heinrichs, 1996). Being a manually operated device, individual differences due to rate of shaking and shaking distance may exist. Kononoff et al. (2003b) recommended that the PSPS should be shaken at 1.1Hz (66 cycles/min) or greater with a

stoke of 17 cm.

## 2.3 Effective fibre

Dietary fibre is an essential component of diets for ruminants and is associated with rumen mat formation, chewing activity, salivation, rumen buffering, and rumen motility (Van Soest, 1994). Dietary effective fibre measured as an effective neutral detergent fibre (eNDF), is defined as the sum total ability of a feed to replace forage or roughage and effectively maintain milk fat percentage (Mertens, 1997). Effective NDF accounts for the chemical make up of the forages as its values were determined by conducting the animal response feeding studies. In these studies, eNDF value for the unknown nutrient in a test diet is determined against the standard forage (with index of 1.0) (Armentano and Pereira, 1997) and the response in milk fat measured. Other researchers have used different response variables such as rumen pH, VFA concentration (Pitt et al., 1996)

In general, eNDF may not be the most useful measure for defining fibre requirements, as it cannot be used to quantify acid production by fermentation of VFA, because distinct feed ingredients might have identical eNDF but different fermentabilities as observed with barley (Yang et al., 1997). This technique of determining eNDF has several flaws such as the "standard" forage varied among experiments and with the location, and other variables such as lactation stage and heat stress affect milk fat percentage, there is no standardized method of assessment of eNDF and this assignment of eNDF value to particular feeds is arbitrary, and there is no laboratory assessment of eNDF values of feed (Beauchemin and Yang, 2003).

## 2.4 Physically effective fibre

Mertens (1997) introduced the term physically effective fibre (peNDF), which is related to physical characteristics of fibre (primarily particle size) that influence chewing activity, salivary production, and the diurnal variation in ruminal pH. Dietary physically effective fibre levels are affected by many factor, such as forage particle size, type and amount of concentrates, forage type, forage to concentrate ratio, type of feeding, frequency of feeding (Beauchemin et al., 1997; Beauchemin et al., 2003; Mertens, 1997). Several researchers have devised several independent definitions as well as techniques for determining the dietary physically effective fibre levels. These multiple techniques make comparisons among studies difficult, and until now no standard validated method for determining the dietary physically effective fibre levels has been universally accepted. Lack of sufficient data on dietary physically effective fibre and discrepancies among studies on its effect on the animal health and production explain the absence of the recommendations on physically effective fibre levels in NRC (2001).

### 2.4.1 Physical effectiveness factor

Mertens (1997) described the importance of the physical characteristics of the dietary fibre and defined the term, physical effectiveness factor (pef), measured by assessing its ability to promote chewing and eating. This value vary from 0 to 1.0, 0 when NDF is completely ineffective in promoting chewing activity and 1.0 when NDF is fully effective against a reference feed (long grass hay containing 100% NDF) that is assigned a pef of 1.0 (Mertens, 1997). This author also provided a reference table for the pef values for various feed stuffs. However, these values are subjective, discontinuous, and cannot

be applied under different field conditions. To address this, Mertens (1997) developed a laboratory method to measure the pef based on the concept that long particles retained on sieves represent particles that require chewing. This author suggested that pef could be determined as the proportion of feed particles retained on a 1.18 mm screen (denoted $pef_M$) and the $peNDF_M$ is the product of the pef factor of the feed to the dietary NDF content.

Lammers et al. (1996) developed the PSPS and determined the pef based on the particle size distribution in the PSPS. The $peNDF_{PS}$ calculated based on the PSPS is the product of DM retained on 8 and 19 mm sieves and NDF of the diet (Yang et al., 2001a). Determination of particles >1.18 mm is important as these particles pass through rumen slower than the particles < 1.18 mm (Poppi et al., 1985). Taking this into account, many studies have calculated $peNDF_{>1.18}$ as the percentage of DM retained on a 1.18 mm screen after dry sieving multiplied by total dietary NDF content (Yang et al., 2001a). Further, Kononoff et al. (2003a) modified the PSPS by adding the third sieve with a mesh size of 1.18 mm. The usage of different techniques for peNDF measurement among studies makes comparison between studies difficult. Further, Calberry et al. (2003) emphasized that NDF levels vary across the different fractions of the PSPS and defined $peNDF_{NDF}$ as, the product of the DM retained on the top two sieves of PSPS with their respective NDF concentrations.

The dietary physically effective fibre level is affected by fibre concentration, particle size and is related to animal health, rumination stimulation, fibre retention in the rumen, determining the dynamics of ruminal fermentation and passage (Mertens, 1997). Beauchemin and Yang, (2005), Plaizier (2004), and Einarson et al. (2004) compared the

different measures of the physically effective fibre determination and reported that different measures gave different values. Einarson et al. (2004) reported that a reduction in barley silage chop length (from 19 to 10 mm) decreased the $peNDF_{PS}$ and $peNDF_{NDF}$. These authors also reported higher $peNDF_{NDF}$ values of 29.2 and 25.2% DM compared to $peNDF_{PS}$ values of 21.2 to 18.9% DM, for long chop (19 mm) and short chop (10 mm) at high concentrate levels, respectively.

Yang et al. (2001a), Beauchemin et al. (2003), and Beauchemin and Yang (2005) studied the Pearson correlation between various physically effective fibre measures ($peNDF_{PS}$, $peNDF_{M}$, $peNDF_{>1.18}$) and with parameters such as chewing activity and rumen pH. Beauchemin et al. (2003) reported that $peNDF_{M}$ and $peNDF_{>1.18}$ were significantly ($r \geq 0.52$, $P<0.05$) correlated with ruminating or total chewing time (min/d) but not with eating time. Only one measure ($peNDF_{PS}$) was significantly correlated to eating salivary output. Yang et al. (2001a) and Beauchemin et al. (2003) failed to find a significant correlation between the three measures of physically effective fibre and rumen pH. Beauchemin and Yang (2005) reported that dietary $peNDF_{PS}$ content was moderately correlated to number of chews during eating ($r = 0.41$, $P<0.05$) and the total chewing time ($r = 0.37$, $P<0.05$), however, they did not report significant correlations between dietary peNDF content and mean ruminal pH, area below pH 5.8 or 5.5, and time that pH was below 5.8 or 5.5. Based on the literature, it can be concluded that as different measures are yielding different values of physically effective fibre. So, in the absence of a standard validated technique for physically effective fibre determination, it is difficult to compare results from different studies and further, relate the physically effective fibre to chewing/rumination and rumen conditions.

2.4.2 Chop length and physically effective fibre

Forage particle size affects the physically effective fibre of the diet, besides other factors such as forage source, concentrate source, and forage to concentrate ratio. Mertens (1997) reported that as the forage particle size increases, it is believed that physically effective fibre content increases, resulting in an elevated total chewing time, salivary buffer secretions and ruminal pH. Increasing the forage particle size results in increased physically effective fibre levels (Einarson et al., 2004; Yang and Beauchemin, 2005), however, there was a positive curvilinear relationship (Mertens, 1997). Kononoff and Heinrichs (2003a) reported a numerical decrease in $peNDF_{PS}$ value with the decrease in chop length of alfalfa silage.

Various techniques to estimate the physical effective fibre yield considerably different values for physically effective fibre for a given feed and diet (Yang et al., 2001a; Einarson et al., 2004; Plaizier, 2004). Einarson et al. (2004) reported higher $peNDF_{NDF}$ values of the diet as compared to $peNDF_{PS}$ with the same chop length. Yang et al. (2001a) reported higher $pef_{M}$ and $pef_{>1.18}$ value than the $pef_{PS}$, but these authors reported a consistent decrease in physically effective fibre values from different measures with the reduction in the particle size. Plaizier (2004) compared the different measures for measuring the physically effective fibre values and reported that for high concentrate diet at two levels of forage chop length (19 and 10 mm) the values for $peNDF_{M}$ range (16.0 – 17.6% DM) was higher than $peNDF_{PS}$ (9.2 to 12.5% DM) and $peNDF_{NDF}$ (13.3 to 15.6% DM). This variation or dissimilarity between different measures for calculating the dietary physically effective fibre levels demonstrate the need to standardize and validate a method for physically effective fibre determination.

2.4.3 Chop length and passage rate

The chemical and physical composition of ruminant rations affects the passage rate (Van Soest, 1994). In general, small particles have faster passage rate compared to large particles. The larger feed particles are filtered by the rumen mat and are disintegrated through rumination and digestion before being passed to omassum (Van Soest, 1994). Grinding of forages results in faster passage rate and decreased digestibility, however, chopping and grinding can also lead to reduced rumen pH (Staples et al., 1984), which may reduce the rates of fibre digestion in the rumen and account for a portion of this depression in digestibility (Soita et al., 2003). Feeding strategy also affects the passage rate as well as the rumen volume. Rumen liquid volume tends to increase with ad libitum feeding (Colucci, 1984). The effect of the particle size on the passage rate, rumen volume, and flow rate is variable among studies.

Many studies have reported increased passage rate, decreased digestibility, and lowered ruminal retention time with the reduction in the particle size of forages (Soita et al., 2003; Grant et al., 1990c; Yansari et al., 2004). Uden et al. (1982) reported an increase in microbial protein synthesis due to reduction in forage particle size because of an increased ruminal passage of solid digesta. Reducing forage particle size may increase the surface area available for microbial degradation, allowing a faster rate of degradation, and/or by increasing the passage rate of digesta through the digestive tract (Soita et al., 2003). Particle size and functional specific gravity are critical feed characteristics that influence the rate of passage from the rumen (Kaske and Engelhardt, 1990). Yansari et al. (2004) studied the alfalfa particle size and specific gravity and reported that reducing the forage particle size from 7.83 to 1.14 mm resulted in significant ($P<0.05$) increase in

forage specific gravity of alfalfa and total mixed ration. These authors also reported a significant increase in ruminal particulate passage rate ($P \leq 0.01$), decrease in rumen mean retention time (h) ($P \leq 0.01$) with the reduction in forage particle size. Krause et al. (2002b) reported an increased liquid outflow rate from the rumen with the coarse chop of corn silage and attributed this increase to a higher saliva production for cows fed with coarsely chopped corn silage. Maekawa et al. (2002) reported that total volume of saliva secretion per day was positively correlated ($r = 0.51$; $P<0.01$) to rumen liquid outflow rate. Similar findings were reported by Cassida and Stokes (1986) who concluded that saliva production is a major determinant of liquid outflow rate from the rumen.

Yang et al. (2001b) did not find an effect of chop length on the rate of rumen flow of either liquid or solid particles. These discrepancies in the results among studies may be due to differences in the methods for determination of the particle size and differences in the forage particle size (Soita et al., 2003). The absence of an effect of forage particle size on the ruminal liquid and solid flow rate by Yang et al. (2001b) compared to other studies (Krause et al., 2002b; Soita et al., 2003; Yansari et al., 2004) could be explained due to a narrow forage particle range, which ranged from 6.08 to 7.59 mm, as well as the finer diets used in their study.

### 2.5 Sub Acute Ruminal Acidosis (SARA)

The normal rumen contains bacterial and protozoal population living in an anaerobic environment with an average pH of 6.5 (Van Soest, 1994). There are several factors such as intake of fermentable organic matter, saliva production, water intake, VFA production, VFA absorption rate, physical form of diet and acidity of the diet affect the

diurnal rumen pH patterns (Van Soest, 1994). To a major extent, high buffering capacity of saliva and the removal of VFAs through absorption help in regulation of the rumen pH (Van Soest, 1994). Dairy cows with high milk production are fed with concentrate rich-low fibre diets to meet their energy demands. These diets can result in VFA accumulation in rumen leading to SARA (Nocek, 1997). Under farm conditions, the challenges and economic losses due to SARA are more severe (Stone, 1999) compared to those due to acute acidosis, which is defined by clear signs of off feed, pH below 5.0, a large increase in lactic acid, increase in VFA, a large decrease in total protozoa, sudden drop in milk yield and possibly death of the animal (Stone, 1999). Despite of that, SARA mostly goes undetected and untreated under farm conditions. Stone (1999) reported an economic impact of SARA as loss of 1.12 US dollar per cow per day.

SARA is characterized by repeated daily episodes of low rumen pH ranging from 5.2 to 5.6 (Cooper and Klopfenstein, 1996). Sub acute ruminal acidosis is a major problem during the transition phase (early lactation), as during this period cows are switched from a high forage diet to a high concentrate diet. During the transition phase, the rumen mucosa is not yet adapted to this dietary change, as during dry period the absorptive capacity of rumen is reduced by 50% (Dirksen et al., 1984). It takes several weeks to regain the absorptive capacity of the rumen, so this transition in feed to animal lead to accumulation of VFA resulting in SARA (Dirksen et al., 1984). The symptoms of the SARA are subtle and not considerably evident, so, this condition is often named as "sleepy disease" (Nocek, 1997). The symptoms include vague health problems, reduced and erratic variable feed intake, reduced protozoal population, reduced milk yield, milk fat depression, rough hair coat, laminitis, parakeratosis, and abscesses in the liver (Nocek,

1997; Nordlund, 2000; Oetzel, 2000). Sub acute ruminal acidosis is primarily a problem of the high producing cows due to the incorporation of the high concentrate-low fibre dietary regime. These high starch diets accompanied by low dietary physically effective fibre levels fail to provide the adequate ruminal buffering resulting in SARA and the associated production losses. Therefore, it is important to formulate diets for these high yielding animals keeping in mind the goal for meeting the energy demands as well as to provide sufficient effective fibre for rumen buffering activity to maintain rumen conditions.

## 2.6 Dietary particle size

### 2.6.1 Eating, rumination, and saliva production

Total chewing activity per unit of dry matter intake, includes eating time and rumination time, and is affected by the forage quality, forage type and physical form of the forage (Allen, 1997). Mertens (1997) defined physically effective fibre (peNDF) as fibre that stimulates chewing activity, which includes the eating and rumination. Chewing activity is an important event for ruminants to sustain themselves on fibrous diets (Van Soest, 1994) as well as to contribute to rumen buffering due to saliva production (Allen, 1997). Beauchemin (1991) reported that a healthy animal should spend at least 6 h/d eating and 8-10 h/d ruminating. Chewing time is an indicator of the physical effectiveness of the diet (Mertens, 1997). The physically effective fibre content of the diet and chewing time can be increased by increasing the fibre content and/or the particle size of the diet (Beauchemin, 1991). Increasing the alfalfa particle size from 3.7 to 13.6 mm resulted in an increased chewing activity by 4.3 h/d (Krause et al., 2002b). Poppi and Norton (1980)

suggested that 1.18 mm is the critical length governing the reticulo-rumen retention as particles >1.18 mm require more chewing in order to pass through omasum. High yielding animals are being fed with the high energy diets (high fermentable carbohydrates and low forage fibre), which usually leads to high VFA production. This rapid VFA production in conjunction with decreased VFA absorption and flow from the rumen results in low rumen pH leading to SARA. This highlights the importance of monitoring forage particle size in ruminant diets to enhance the chewing activity with longer forage particles to provide sufficient salivary buffering (Beauchemin and Yang, 2005). Allen (1997) reported that decreasing the forage particle size reduced the total chewing time, however, this is a curvilinear relationship. He suggested that the most dramatic reductions in chewing time occur when the particle size is reduced below 3 mm.

Many researchers have studied the relationship between the physical form of the diets and eating, rumination and saliva production. Forage particle size affects the chewing activity with higher chewing at longer forage chops than with shorter chop forages (Beauchemin et al., 1997). Chewing activity promotes the production of saliva, which contains bicarbonate and phosphate buffers that help in neutralizing the acidic effects of the $H^+$ ions (Beauchemin et al., 1991). The average bicarbonate and phosphate concentration in saliva is 126 and 26 meq/L, respectively (Bailey and Balch, 1961). Saliva composition is relatively constant and is not greatly affected by diet or feed intake (Erdman, 1988). Saliva secretion increases when cows chew during eating and ruminating, thus a diet that increases chewing time increases the buffering capacity within the rumen. Allen (1997) indicated that a lactating dairy cow eating 4.5 h/d and ruminating 6.7 h/d will produce a total salivary buffer flow of 273 L/d. This author calculated the

total salivary buffer flow based on flow rates of 0.151, 0.177, and 0.272 L/min for resting, eating, and ruminating activities (Allen, 1997). Kononoff and Heinrichs (2003a) reported increased eating (262 to 297 min/d), ruminating (460.2 to 479.1 min/d), and total chewing activity (723 to 777 min/d) per kilogram of DM with the increase in the theoretical length of chop of alfalfa silage from 4.8 to 22.3 mm.

Cassida and Stokes (1986) revealed that time spent chewing is a good indicator of rumen health because salivary secretions during chewing are higher than during resting. These authors reported that saliva secretion is about 1.5 to 2 times higher during chewing compared to during resting. Beauchemin et al. (2003) reported no effect of the forage particle size on the eating time but found a decrease in rumination time with the reduction in the forage particle size. A similar effect of the forage particle size on the ruminating activity was reported by Krause et al. (2002b), that increase in forage particle size results in increased time spent on rumination, number of rumination periods and also duration of each rumination period ($P = 0.07$). Colendrander et al. (1991) reported a decrease in rumination time and eating time by approximately 50% with the reduction in the particle size, with a larger effect (85%) of this decrease on chewing activity time due to the reduction in rumination time. Grant et al. (1990a) reported a decrease of more than 16% of the total chewing time with the reduction in mean particle length of chopped alfalfa hay from 2.3 to 0.9 mm. Grant et al. (1990b) reported a 21% reduction in chewing time when the mean particle size of alfalfa silage was decreased from 3.1 to 2.0 mm.

Based on several studies, De Brabander et al. (1999) concluded that the effect of forage particle size on chewing activity is ambiguous. These researchers reported increased chewing activity and increase in the dietary fibre content with the increase in

the particle size. The differences among the diets become negligible after correcting the chewing activity for the fibre content. De Bover et al. (1993) demonstrated a decrease in rumination time, when corn silage particle size reduced from 8 to 4 mm; however, increasing the forage chop length to more than 8 mm did not result in higher rumination time. Beauchemin and Yang (2005) studied the effect of the dietary physically effective fibre levels on the chewing activity by varying the corn silage chop length and reported an increased chewing activity due to the increase dietary physically effective fibre levels. Yansari et al. (2004) also reported an increase in the eating ($P<0.039$), ruminating ($P<0.0001$), and total chewing activity time ($P<0.0001$) with the increase in the alfalfa hay particle size from 1.14 to 7.83 mm.

In the absence of standard validated technique for the measurement of the particle size and determination of the physically effective fibre levels it is difficult to compare the results of different studies and quantify the relationship between forage particle size and physically effective fibre levels with chewing activity and saliva production. However, based on the literature we can conclude that in diets with low physically effective fibre levels, a positive relationship of the forage particle size and physically effective fibre levels with the chewing activity and saliva production exists but has less or no affect on cows fed diets with adequate physically effective fibre levels.

### 2.6.2 Rumen pH

Physically effective fibre in ruminant diets influences ruminal pH by increasing saliva flow via its effect on chewing activity and by diluting more fermentable feed components, which reduces fermentation acid production (Allen, 1997). Rumen pH is

predominately regulated by the bicarbonate and phosphate buffering system in saliva and VFA levels, however, rapid changes in diet upset this buffering mechanism (Allen, 1997) and also affect the rumen pH. This is apparent during the transition period (3 weeks prior to calving to 3 weeks post calving) in which an abrupt change in diet from high fibre to high concentrate diet may lead to rumen acidosis (Nocek, 1997). During dry period animals are fed high fibre-low concentrate diet, which reduces the ruminal absorptive capacity by 50% (Dirksen et al., 1984). It takes several weeks for rumen epithelium to adapt to the early lactation (high concentrate) diets and regain the absorptive capacity, which makes this transition from high forage diets to high concentrate diets difficult (Dirksen et al., 1984). After calving high concentrate diets are fed to the cows, which results in rapid VFA production and combined with reduced VFA absorptive capacity of rumen lead to drop in rumen pH (Nocek, 1997). This predisposes the animal to SARA, which is associated with reduced intake, rumen motility, microbial yield, and fibre digestion (Allen, 1997). Stone (1999) reported a significant ($1.12/cow/day (USD) economic impact of the low rumen pH and/or SARA in the dairy cows. Forage particle size and fibre content affect the chewing, ensalivation, and rumen pH (Mertens, 1997). So, the diets should be formulated with adequate particle size and fibre content to maintain rumen pH.

Mertens (1997) reported a curvilinear relationship between the physically effective fibre levels and the rumen pH based on 26 citations. This author reported a plateau at a dietary physically effective fibre level 22% and above which increasing the dietary physically effective fibre levels did not affect the rumen pH. Mertens (1997) also emphasized the importance of the forage particle size on the maintenance of optimal

rumen pH. The type of forage used in the diet also has an effect on rumen pH. The intrinsic buffering capacity of corn silage is lower compared to alfalfa silage (Mc Burney et al., 1983). Therefore, at low rumen pH, the risk for SARA induction is higher with corn silage based diets compared to alfalfa based diets.

Many researchers studied the effect of the forage chop length on the rumen pH and rumen conditions. Beauchemin et al. (2003) reported a significant effect of forage particle size on the rumen pH. These authors reported an increase in time (3.5 to 7.3 h/d) during which rumen pH was above 6.02 ($P<0.11$) and a decrease in time (13.5 to 7.5 h/d) for which rumen pH was less than 5.8, with an increase in the mean forage particle size from 2.22 to 5.67 mm. Beauchemin et al. (2003) also illustrated that changing the forage particle size had a greater affect on rumen pH than altering the ratio between silage to hay. Based on two studies Soita et al. (2002) reported that ruminally fistulated steers had lower($P<0.05$) rumen pH at 1000, 1200, 1800, 2000, and 2200 h when fed short barley chop (4.7 mm) compared to long chop (18.8 mm) barley. These authors also reported a significant reduction in the mean rumen pH from 6.46 to 6.27 with the reduction in the barley silage chop length. Krause et al. (2002b) reported a decrease in total ruminal VFA concentration and increase in mean rumen pH from 5.81 to 6.02 with an increase in forage particle size from 3.7 to 13.6 mm.

In a study by Beauchemin and Yang (2005), dietary $peNDF_{PS}$ levels were reduced from 11.5 to 8.9% DM by altering the corn silage chop length. These authors studied the diurnal ruminal pattern and reported that mean daily rumen pH, area under pH 5.8 or 5.5, and duration of pH <5.8 or pH <5.5 were not affected. The ruminal pH, however was lower after the 0800 and 1500 h feeding for the low physically effective

fibre diet ($P<0.15$) compared to high physically effective fibre level diet. Yansari et al. (2004) reported a significant ($P=0.0003$) decrease in rumen pH (6.52 to 6.12) with the reduction of the alfalfa hay particle size from 3.34 to 1.66 mm.

The inconsistent effect of the forage chop length and dietary physically effective fibre on the rumen pH reported in the literature suggests the possibility of other factors affecting the rumen pH, such as DMI, fermentability of the diet, feeding management, differences among different techniques for determination of dietary physically effective fibre and particle size (Beauchemin and Yang, 2005; Plaizier, 2004; Einarson et al., 2004). Besides these factors differences among different techniques used for measuring the rumen pH also have a confounding effect (Keefe and Ogilvie., 1997; Duffield et al., 2004). Rumen pH values are not consistent throughout the day but follow a diurnal pattern. Duffield et al. (2004) concluded that continuous rumen pH monitoring is the best representation of the diurnal pattern. Furthermore, this technique allows for additional calculations such as time below certain pH and area under curve. However, in large production trials this is not feasible and in these trials only one sample is taken per cow per day (Einarson et al., 2004). In such trials, it is necessary to perform rumen fluid sampling at a fixed time (4-6 hrs after feeding) (Keunen et al., 2002). From the literature, we can conclude that dietary particle size and physically effective fibre do affect the rumen pH in finer diets and in diets with low intrinsic buffering capacity compared to coarser diets and diets with high intrinsic buffering capacity.

### 2.6.3 Volatile fatty acids

The volatile fatty acid pool (VFA) in the rumen consists of end products of

organic matter fermentation of feedstuffs and VFA consumed in silage (Allen, 1997). Dairy cattle diets contain more than 65% carbohydrates (primarily non-structural) and the extent of fermentation of carbohydrates varies among feeds due to many factors such as particle size, fibre content, concentrate source, forage to concentrate ratio, and forage source (Van Soest, 1994; Allen, 1997). Further, the VFA concentration in the rumen at any time is regulated by the balance between production, absorption of VFA, and VFA flow to omasum, as well as rumen fluid pool size, flow rate and turnover (Van Soest, 1994). Readily fermentable dietary constituents such as concentrates/grains result in rapid increase in rumen VFA levels and result in an accumulation of VFA in rumen, which can lead to low rumen pH (Van Soest, 1994).

Maekawa et al. (2002), based on their study on eight ruminally cannulated cows, reported a 2.2 times higher saliva production during eating than during resting. Krause et al. (2002b) reported that as particle size decreases, there is greater VFA production by fermentation leading to lower rumen pH. Krause et al. (2002b) observed increased chewing activity from 9.8 to 12.4 h/d with coarser diets, resulting in an increased saliva flow and saliva has a diluting effect as well as increases liquid turnover rate in the rumen which might explain the lower VFA levels with coarser diets. Yang et al. (2001a) reported less saliva production, decreased liquid passage rate and volume of liquid digesta in the rumen with the reduction in the forage particle size from 7.59 to 6.08 mm, however, no effect was observed on individual VFA proportions or acetate to propionate ratio.

Beauchemin and Yang (2005) reported an increase in the total VFA concentration from 122 to 132 mM with the reduction in pef levels from 0.84 to 0.67

%DM. These authors also observed an increase in the molar proportion of propionate (28 to 32 mol/100mol) but reported an opposite effect on the butyrate molar proportion (12 to 10 mol/100mol) and acetate to propionate ratio, which was reduced from 2.04 to 1.69, with the reduction in the dietary physically effective fibre levels. Einarson et al. (2004) studied the effect of barley silage chop (19 and 10 mm) and reported a decrease in the acetate to propionate ratio from 3.1 to 2.7 but total VFA concentrations were not affected in this study. Yansari et al. (2004) studied the effect of alfalfa chop length on mid lactation dairy cows and reported increase in total VFA from 118.62 to 125.4 mM and propionate concentrations from 14.6 to 16.9 mol/ 100mol, but decrease in acetate concentration from 73.55 to 70.68 mol/100mol, and acetate to propionate ratio from 5.1 to 4.2 with the reduction in the particle size from 7.83 to 1.14 mm. A significant ($P<0.05$) increase in total VFA concentration and decrease ($P<0.05$) in acetate to propionate ratio with a reduction in the barley silage chop from 18.8 to 4.7 mm was reported by Soita et al. (2002). Beauchemin et al. (1991) reported a significant increase in the acetate to propionate ratio by increasing both the forage to concentrate ratio and forage NDF intake from 35:65 to 45:55 and 2.8 to 3.8 kg/d, respectively. A linear increase in acetate to propionate ratio with the increase in dietary fibre content was also reported by Beauchemin (1991).

Based on the literature it is difficult to draw a conclusive relationship between dietary particle size and the total rumen VFA content, as the concept of physically effective fibre does not account for differences in fermentability of feeds and does not predict differences in chewing and rumen pH due to grain fermentability (Beauchemin and Yang, 2003). Krause et al. (2003) reported a low rumen pH in cows fed with high

moisture corn (highly ruminal fermentable carbohydrate) compared to dry cracked shell corn (low rumen fermentable carbohydrate), even though diets fed were coarse. A similar effect was reported by Yang et al. (2000) and these authors suggested that diets with higher fermentable carbohydrate sources require a higher level of physically effective fibre to reduce the risk of acidosis. Chesson et al. (1995) reported that reducing the particle size increase the surface area for microbial action and may result in higher fermentation rate of carbohydrates compared to diets with longer particle size. It is therefore important to provide adequate particle size and forage content in high concentrate diets for high producing cows in order to reduce risk of SARA.

The dietary particle size and fibre content have a negative relationship with the ruminal propionate levels (Van Soest, 1994). Reducing the forage particle size and/or fibre content in high concentrate diets is related to decreased rumen pH (Beauchemin et al., 2003; Krause et al., 2002b; Soita et al., 2002). This decrease in rumen pH brings a change in rumen microflora, i.e there is reduction in fibrolytic bacteria and increase in amylolytic bacteria (Nocek, 1997). Berger (1988) reported increased propionic acid molar concentration and decreased acetic acid concentration, resulting in low acetate to propionate ratio by replacing concentrates with roughages in dairy cow diet. Therefore, the reduction in rumen pH associated with lower physically effective fibre levels could be related to increased ruminal propionate levels and lower acetate to propionate ratio.

### 2.6.4 Dry matter intake

Dry matter intake of ruminants is affected by the physical and chemical characteristics of the diet and also by the interactions between these factors (Allen, 1997).

The physical and chemical factors of the feed that control the DMI are also affected by the forage to concentrate ratio of the diets. DMI in high forage diets is mainly limited by distension of reticulo-rumen and in high concentrate diets DMI is mainly limited by the metabolic feed back (Allen, 1997). Beauchemin et al. (1994) conducted a study with two forage fibre levels (low forage, 35 %DM and adequate forage, 65 %DM) and at two chop length levels (short, 5 mm and long, 10 mm) and reported that decreased chop length of adequate forage diets resulted in increased DMI. Further, these authors reported no effect on DMI by reducing the chop length in low forage diets. Allen (1997) also suggested that if reticulo-rumen distension limits the DMI, then decreasing the forage particle size may lead to increased intake, as it decreases the filling effect of forage and increases the ruminal passage rate.

Einarson et al. (2004) reported increased DMI intake with the reduction in the barley chop length from 19 to 10 mm in low forage diets (42% DM). Beauchemin et al. (2003) observed a positive relationship between the dietary physically effective fibre levels and DMI. Beauchemin and Yang (2005) and Yang et al. (2001a) failed to find a relationship between dietary physically effective fibre level and DMI. Krause et al. (2002a) found no effect of reducing mean dietary particle size from 6.2 to 2.9 mm on dry matter intake. Beauchemin et al. (1994) reported an interaction ($P<0.01$) effect between alfalfa particle length (0.5 to 1 cm theoretical length of cut) and percentage of forage in the diet (35 or 65% DM). These authors reported that at long chop of alfalfa increasing the forage content from 35 to 65% reduced the intake by 3 kg/d, however, at short chop of alfalfa this reduction in intake was less than 0.5 kg/d.

Beauchemin et al. (1997) reported an increased DMI in lactating cows due to reduction in forage chop length in poor quality high forage diets. Leonardi et al. (2005) studied the effect of the different geometric particle length (6.68, 5.39, 5.19, 4.46, 4.35 mm) of oat silage on the DMI and reported a linear increase in the DMI with the increase in the dietary geometric mean particle size. These authors and as well as Leonardi and Armentano (2003) and Calberry et al. (2003) reported a linear increase in sorting against the longer feed particle with the increase in mean geometric particle length. Increased DMI due to reduction in the particle size could be due to increased passage rate and decreased sorting performed by the animals against the coarser feed particles. Contrary to that, Beauchemin and Yang (2005) reported sorting in favor of long feed particles and this effect was more pronounced in the finer diets. These discrepancies of sorting of feed particles among studies may be due to differences in the dietary physically effective fibre levels. The dietary $peNDF_{PS}$ levels were ranging between 20.1 to 23.3% (Calberry et al., 2003) as compared to the 5.7 to 9.4 % in study by Beauchemin and Yang (2005). Further, Beauchemin and Yang (2005) suggested that dairy cows may intentionally select in favor of long feed particles to meet their need for physically effective fibre in finer diets and when rumen pH is low. Soita et al. (2002; 2003) also reported an increase in the DMI in steers with the reduction in the theoretical chop length of the barley silage from 18.8 to 4.7 mm.

In the light of the current literature, we can conclude that the forage chop length has a variable effect on the DMI. Reduction in chop length could result in higher intake in high forage diets or when distension of the reticulo-rumen limits the intake (Allen, 1997). Contrary to that diets with low physically effective fibre can obscure the effect of particle

size on DMI by metabolic constraints. Further studies are required to study the effect of the chop length on DMI in high yielding animal taking into account the above mentioned factors and the interaction between them.

### 2.6.5 Milk yield

In the last few decades, research in the field of dairy production was primarily focused at improving the milk production. Today we have high producing dairy herds, but, getting maximum production from these animals is affected by several factors such as animal age, physiological status, nutrition, and climate (Illius and Jessop, 1996). The nutrient and energy requirements of high producing animals correspond to their production levels. The biggest hurdle to attain such high production levels is energy intake, which is determined by net energy content of the diet and DMI (Allen, 2000).

Reducing the chop length of forages did not affect the milk production in the cows in the studies from (Yang et al., 2001a; Krause et al., 2002a; Beauchemin et al., 2003; Calberry et al., 2003; Kononoff and Heinrichs, 2003a; Kononoff et al., 2003b; Einarson et al., 2004; Yang and Beauchemin, 2005) However, Krause et al. (2002b) reported an increase in milk yield with the increase in the forage particle size at high ruminally fermentable concentrates levels, but attributed this increase to the interaction effect of the forage particle size and increased starch content of the diet. Leonardi et al. (2005) studied the effect of the mean geometric particle length of oat silage on milk yield and reported a linear decrease in milk production with the increase in the forage geometric particle size. These authors reported a decrease of 0.91 kg milk per day for each 1 mm increase in the forage mean geometric particle size.

Beauchemin et al. (1994) reported a decrease in the milk yield due to higher dietary NDF content. These authors reported a drop in milk production by 3.2 kg per day because of low dietary energy, when the dietary NDF levels were increased from 32 to 40%. Yang and Beauchemin (2005) studied the effect of $peNDF_{PS}$ (11.5, 10.3, and 8.9 % DM) on milk yield and reported no significant improvement in the milk yield and milk composition. Fewer animals and short duration of the trials could be the possible reasons for the absence of effect of forage particle size on milk yield reported in literature. Increasing the duration might of studies might illustrate this effect, however, in doing so other confounding factors such as parity and stage of lactation might show their effect.

### 2.6.6 Milk fat

Milk fat is the most variable component in the ruminant milk and is predominately composed of triglycerides (Bauman & Griinari, 2001). In ruminants these milk fatty acids arise from de novo synthesis (50%) and the (50%) uptake of long chain fatty acids from the diets (Griinari et al., 1998). The milk fat levels are affected by many dietary and metabolic factors such as forage particle size, physically effective fibre, intrinsic rumen buffering capacity, intrinsic fragility of feed particles, inclusion of buffers, concentrate inclusion, rumen digestion rate, factors affecting rumen fermentation, rumen buffering, feed intake, and lactation stage. The most common high grain/low forage diets fall short in fibre effectiveness in maintaining the normal rumen function and de novo synthesis (Bauman and Griinari, 2001). These authors highlighted the importance of the dietary particle size and physically effective fibre levels with relation to the milk fat as suggested by Mertens (1997). The proposed theory for milk fat reduction by these high

grain/low forage diets is that these diets lead to decreased acetate levels in the rumen leading to low acetate to propionate ratio that corresponds to low rumen pH and results in low milk fat (Van Soest, 1994). Besides that at low rumen pH, increased insulin levels and trans-fatty acid production also have an inhibitory effect on the de novo milk fat synthesis. Increased insulin levels result in preferential channelling of the nutrients (acetate, β - hydroxybutyrate, and diet derived long chain fatty acids), resulting in a shortage of lipogenic precursors for the milk fat synthesis (Bauman and Griinari, 2001). Griinari et al. (1998) reported a negative effect of the increased total trans-fatty acids on the de novo fat synthesis associated with the high grain/low forage diets as well as diets rich in fat. Bauman and Griinari (2001) however, reported that the low milk fat production associated with these diets is due to an increase in trans-10 C18:1 rather than an increase in total trans-C18:1 content of the milk fat.

Based on the 36 citations, Mertens (1997) concluded a positive curvilinear relationship between the dietary physically effective fibre levels and milk fat. Mertens (1997) also suggested that diets which have lower $peNDF_M$ levels than minimum recommended level of 20% DM will have more reduction in milk fat compared to diets having higher physically effective fibre levels. Kononoff et al. (2000) studied the effect of the barley silage chop length on the milk composition and reported that reducing the barley chop length from 9.0 to 4.8 mm did not affect the milk fat percent and fat yield. These authors suggested that diets included in this trial met the minimum fibre requirement recommended by (NRC, 2001) to maintain the milk fat above 3.5%. Other researchers (Krause et al., 2002a; Beauchemin et al., 2003; Kononoff and Heinrichs, 2003a; Calberry et al., 2003) reported no effect of the forage particle size on the milk fat

percentage. Plaizier (2004) also concluded that the negative effects of forage particle size on milk fat percentage are likely to be observed when the dietary NDF levels below the minimum requirements recommended by the NRC (2001).

Beauchemin et al. (1997) reported a significant decrease in milk fat percentage when animals were fed with short chop (5 mm) of alfalfa compared to long chop (10 mm). Clark and Armentano (1999) reported an increase in milk fat yield with reduction in the chop length of corn silage. Beauchemin and Yang (2005) reported no effect of the dietary physically effective fibre on the milk yield and milk components. Yang et al. (2001a) studied the effect of the chop length on the milk fat and reported a decrease in the milk fat levels with the reduction in the chop length of forages from 7.59 to 6.08 mm. Therefore, variable effects of forage particle size on milk fat levels are reported in literature. However, we can conclude that the chance of observing an effect of dietary particle size on milk fat level are higher in diets with low NDF content than the recommendation of NRC (2001) and/ or with physically effective fibre lower than the recommendations of Mertens (1997).

### 2.6.7 Milk protein

Milk protein levels are positively related to dietary energy levels and supply of metabolizable protein (Ensminger, 1993). Clark and Armentano (1999) reported an increase in the milk protein levels with an increase in the starch digestibility. Clark et al. (1992) also emphasized the importance of amino acid profile of the diet and suggested that diets deficient in amino acids (rumen degradable) decrease the milk protein level.

Yang et al. (2001a) reported a decrease in the milk protein levels with the reduction in the chop length of forages from 7.59 to 6.08 mm. Kononoff and Heinrichs (2003a) reported a quadratic effect of the dietary particle size on the milk protein percentage. However, Kononoff and Heinrichs (2003b) reported an increase in the milk protein levels by 0.03 kg/d with the reduction in the forage particle size from 22.3 to 4.8 mm and attributed this increase in milk protein to the improved starch digestion resulting in more energy for milk production. Other researchers (Calberry et al., 2003; Beauchemin and Yang, 2005; Kononoff et al., 2000) could not find an effect of the forage chop length on the milk protein percentage.

Krause et al. (2002a) studied the effect of forage chop length at two levels of the ruminally fermentable carbohydrates (RFC) and reported an interaction between forage particle size and RFC levels for milk protein percentage. These authors reported an increase in protein percentage, when the forage particle size was increased in high moisture corn and decreased in the dry corn. They attributed this interaction effect to an increase in the microbial protein synthesis in high moist corn diet at long chop length leading to higher milk protein levels.

Low rumen pH has a negative effect on the microbial protein yield by increasing the maintenance requirement of microbes and by decreasing the yield efficiency of fibrolytic bacteria (Van Soest, 1994). Yang et al. (2002) reported a decrease in microbial N synthesis by 48 g/d at low physically effective fibre diet achieved by reducing the forage particle size. Krause and Comb (2003) failed to find an effect of particle size on rumen pH as well as on microbial nitrogen supply. Further research is needed to study the effect of forage particle size on milk protein content including the interaction of factors

such as particle size, physically effective fibre level, rumen pH, energy status, microbial protein synthesis.

## 2.7 Summary

The literature citied revealed the importance of the adequate particle size and physically effective fibre levels in the concentrate rich/low forage diets for high yielding dairy cows, in order to prevent the production and economic losses suffered due to decreased intake, SARA, and a range of other metabolic disorders. A substantial amount of research has been done to determine the relationships of dietary particle size and physically effective fibre levels with the feeding behaviour, chewing/ensalivation, dry matter intake, rumen conditions, and milk production of dairy cows. The current recommendations for the NDF, physically effective fibre, and the dietary particle size have been developed for the corn grain based diets. A recent survey across the dairy farms in Manitoba province revealed a higher usage of barley grain compared to corn grain in dairy diets. These changed nutritional practices utilized across the dairy farms of Manitoba, suggest it is necessary to develop guidelines for the dietary physically effective fibre and particle size to ensure the optimal health of dairy animals along with a reduction in economic and production losses.

Different techniques are used for measuring the dietary physically effective fibre level as well the particle size, which makes the comparison among studies difficult. So studies are warranted to compare and standardize the method for determining the dietary particle size and physically effective fibre levels and further to provide the prairie specific

guidelines for physically effective fibre levels for the barley based diets used in Manitoba, in relation to their impact on the production and metabolism of the dairy cows.

## 2.8 HYPOTHESES AND OBJECTIVES

### Hypotheses

1) Reducing the forage chop length reduces the physically effective fibre level in diets of high yielding dairy cows.
2) The reduction in forage chop length and dietary physically effective fibre in barley grain based diets, decreases the chewing activity, rumination, and saliva production, leading to less ruminal buffering and SARA. This reduced forage chop length and dietary physically effective fibre affects the feeding behaviour, dry matter intake, milk production, rumen fluid composition, and blood composition of high yielding dairy cows fed with barley grain based high concentrate diets.

### Objectives

The general objectives of the thesis research were:

1) to investigate the effect of forage chop length on the dietary physically effective fibre levels, chewing activity, feeding behavior, rumen conditions, production, and metabolism of the dairy cows fed with barley grain based total mixed rations.
2) to determine and provide guidelines for the dietary particle size and physically effective fibre levels to be included in the barley grain based diets.
3) to compare the different measures for physically effective fibre determination and to determine which of these is best measure for rumen buffering.

# 3.0 Effect of chop lengths of alfalfa and corn silage on productivity, rumen fermentation, and blood parameters of dairy cows fed total mixed rations

## 3.1 Abstract

Effects of chop length (short: 10 mm or long: 19 mm) of alfalfa silage and corn silage on feed intake, rumen conditions, and milk production were investigated in 16 mid-lactation Holstein cows using 4 by 4 Latin squares with experimental periods consisting of two adaptation weeks and one sampling week. Cows were fed total mixed rations (TMR) containing (DM basis) 44.0% barley grain based supplement, 12.6% protein supplement, and 21.7% DM long chop or short chop alfalfa silage and 21.7% DM long chop or short chop corn silage. Rumen fluid samples were collected twice during the sampling week using an oral probe 4h after feeding. The $peNDF_{PS}$ calculated as the proportion of DM retained by the 8 and 19 mm screens of the Penn State Particle Separator (PSPS) multiplied by dietary NDF ranged from 16.4 to 19.1% DM among diets. The $peNDF_{NDF}$ calculated as the NDF retained by the PSPS screens ranged from 19.6% to 23.5% DM among diets. The peNDF>1.18 calculated as the proportion of DM retained by a 1.18 mm screen multiplied by dietary NDF did not differ among diets and averaged 28.9% DM across diets. Different methods for the estimation of dietary physically effective fibre resulted in different values and affected differences in this measure among diets. Forage chop lengths did not affect chemical composition of diets. Reducing corn silage chop length increased dry matter intake (DMI) from 22.3 to 23.2 kg/d ($P<0.003$), but did not alter rumen pH, rumen VFA, and milk production. Reducing alfalfa chop

increased total rumen VFA, acetate, and propionate, but did not affect rumen pH, DMI, and milk production. Chop length of alfalfa and corn silage did not affect the blood parameters. Comparisons of the particle size distribution of the diets and their respective orts/weigh backs showed that cows selected against coarse feed particles in favour of finer feed particles.

(**Key words:** forage chop length, dairy cows, physically effective NDF, particle size, rumen pH, feed intake, volatile fatty acids, milk production)

**Abbreviation key: AL** = long chop alfalfa silage (19 mm); **AS** = short chop alfalfa silage (10 mm); **CL** = long chop corn silage (19 mm); **CS** = short chop corn silage (10 mm); **ALCL** = diet containing long chop alfalfa silage and long chop corn silage; **ALCS** = diet containing long chop alfalfa silage and short chop corn silage; **ASCL** = diet containing short chop alfalfa silage and long chop corn silage; **ASCS** = diet containing short chop alfalfa silage and short chop corn silage; **peNDF$_{PS}$** = physically effective NDF measured as proportion of DM retained by the 8 and 19 mm screens of Penn State Particle Size separator multiplied by the dietary NDF; **peNDF$_{M}$** = physically effective NDF measured from tabular values multiplied by dietary NDF (Mertens, 1997); **peNDF$_{NDF}$** = physically effective NDF measured as proportion of NDF retained by the 8 and 19 mm screens of Penn State Particle Size separator multiplied by the DM; **peNDF$_{>1.18}$** = physically effective NDF measured as the proportion of DM retained by a 1.18 mm screen of multiplied by dietary NDF; **PSPS** = Penn State Particle Separator; **SARA** = sub acute ruminal acidosis.

## 3.2 Introduction

High yielding dairy cows are fed high concentrate diets to meet their high energy demands. These diets can result in compromised rumen function due to accumulation of VFA and insufficient rumen buffering, which may result in metabolic sub acute ruminal acidosis (SARA), milk fat depression, and laminitis (Nocek, 1997; Beauchemin et al., 2003). Conversely, coarser diets may limit feed intake due to physical fill constraints and, as a result, reduce milk production (Allen, 1997). The component of diets and feeds that stimulates chewing activity, saliva production and rumen buffering is referred to as physically effective fibre, which is affected by forage chop length, forage source, concentrate source, and forage to concentrate ratio (Mertens, 1997). Forage chop length can also affect the silage quality, as long chop lengths can result in poor silage fermentation, due to difficulty in packing and maintaining anaerobic conditions (Johnson et al., 2003).

The National Research Council (NRC, 2001) recommends that diets for dairy cows should contain a minimum of 25% NDF, with 75% of the total dietary NDF supplied by forages, in order to provide sufficient rumen buffering. Beauchemin (1991) suggested a minimum of 34% NDF for barley based diets. However, this recommendation does not account for the differences in rumen buffering capacity among forages and differences in rumen degradability among grains. Currently, NRC (2001) guidelines do not provide recommendations for physically effective fibre, due to the lack of a standard validated technique to quantify the physical effectiveness of feeds or entire diets. The Penn State Particle Separator (PSPS) is one tool available for rapid, on-farm analysis of particle size distribution of forage and TMR (Lammers et al., 1996). The PSPS consists of

3 screens with 19 mm, 8 mm, and 1.18 mm diameter holes and a bottom pan. Heinrichs and Lammers (1997) recommend that the 8 and 19 mm screens of the PSPS should retain between 50 to 60% of forages and 40% to 60% of TMR particles. Plaizier et al. (2004) observed that only 35% of alfalfa silage samples and 30% of the corn silage samples obtained from Manitoba dairy farms were within this recommended range. Based on the PSPS guidelines (Heinrichs and Lammers, 1997), 30% of alfalfa silage samples were too coarse, 35% of alfalfa silage samples were too fine, 40% of corn silage samples were too coarse, and 30% of corn silage samples were too fine (Plaizier et al., 2004).

Poppi and Norton (1980) suggested that 1.18 mm is the critical length governing retention of particles in the reticulorumen. It is important to measure the particle mass <1.18 mm while interpreting the results of experiments designed to evaluate the effects of diets with different physical forms (Kononoff et al., 2003a), as particles greater than 1.18 mm require reduction through mastication and microbial digestion to pass out of rumen. NRC (2001) suggests that alfalfa silage based diets with a mean particle length below 3 mm result in depressed milk fat and decreased rumen pH as a consequence of decreased digestibility, and increased passage rate which decreases buffering capacity in the rumen.

Various methods are used to estimate the physical effective fibre content of feeds and diets. These include, the product of amount of the DM retained by the 8 and 19 mm screens of the Penn State Particle Separator (PSPS) and dietary NDF ($peNDF_{PS}$, Yang et al., 2001a), the proportion of NDF retained on 8 and 19 mm screens of the PSPS ($peNDF_{NDF}$, Calberry et al., 2003), the proportion of DM retained by 1.18mm screen multiplied by dietary NDF ($peNDF_{>1.18}$, Yang et al., 2001a), and dietary NDF content of feeds multiplied by tabular physically effectiveness factor ($peNDF_{M}$, Mertens, 1997).

These techniques yield considerably different values for physically effective fibre for a given diet (Beauchemin et al., 2003; Yang et al., 2001a). Beauchemin et al. (2003) reported a positive correlation ($r = 0.44$, $P < 0.10$) between $peNDF_{PS}$ levels and ruminating time, but no correlation between $peNDF_{M}$, $peNDF_{>1.18}$ and $peNDF_{PS}$ and mean ruminal pH. They also observed a strong inverse relationship ($r = 0.55$, $P < 0.05$) of $peNDF_{PS}$ with time and area below rumen pH 5.8. Higher dietary $peNDF_{PS}$ may be associated with the reduction in the incidence of SARA (Beauchemin et al., 2003). Mertens (1997) suggested minimum $peNDF_{M}$ requirements of 22% DM and 20% DM to maintain an average rumen pH of 6.0 and milk fat percentage of 3.4 in the mid lactation cows, respectively.

Many studies have been conducted to explore the effect of particle size on feed intake, rumen fermentation, milk production and nutrient digestibility in cows (Kononoff et al., 2000; Yang et al., 2001ab; Krause et al., 2002ab; Soita et al., 2002; Beauchemin et al., 2003; Kononoff & Heinrichs, 2003ab; Soita et al., 2003). Results obtained are inconclusive as it is difficult to compare among studies due to differences in the techniques used to measure dietary particle size and physically effective fibre, and variation in the dietary concentrate to forage ratio, concentrate source and forage source among diets.

Plaizier et al. (2004) conducted a survey of Manitoba farms and indicated that alfalfa silage and corn silage were the most commonly used forages in lactation TMRs diets fed in the province. Further, these authors reported that in over 25% of TMR-fed herds included in this survey, the proportion of forages and TMR retained by the 8 and 19 mm screens of the PSPS was less than the minimum recommended level of 50% and

40%, respectively (Heinrichs and Lammers, 1997). The potentially negative impacts of these excessively fine TMR and forages on milk yield and milk compositions were not obvious (Plaizier et al., 2004). It is therefore, important to establish guidelines for physically effective fibre requirements and chop lengths of forages in TMR based diets and to determine the effects on productivity and metabolism of the cow. The objective of this study was to determine the effects of chop length of alfalfa and corn silage on dietary physically effective fibre, dry matter intake, milk production, rumen fermentation, blood parameters, and silage fermentation and to compare various measures for dietary physically effective NDF.

## 3.3 Materials and methods

### 3.3.1 Experimental procedures

Sixteen multiparious lactating Holstein cows, housed in a tie-stall barn at the Glenlea Research Station, University of Manitoba, were used in a 4 x 4 Latin square design with four 3-week experimental periods. Each experimental period consisted of a 14-d adaptation period, followed by a 7-d collection period. Animals were cared for in accordance with the Canadian Council for Animal Care guidelines (CCAC, 1984). Upon commencement of the experiment, cows averaged 78 ± 37 days in milk (DIM) (mean ± SD), had an average body condition score (BCS) of 3.55 ± 0.49 on a 5 point scale (Edmonson et al., 1989) and had an average body weight of 756 ± 37 kg.

Second cut alfalfa (Pickseed, custom forage mix) was harvested in the late bud stage. Corn (Pioneer, 39T71) silage was harvested at second maturity (2/3 milk line). Corn and alfalfa were chopped at 10 mm (short chop) or 19 mm (long chop) using a New

**Table 3.1** Ingredients and nutrient composition of experimental diets containing a short (10 mm) or long (19 mm) chop lengths of alfalfa and corn silages. [1]

| | Diet [2] | | | | | Effect[3] | | |
|---|---|---|---|---|---|---|---|---|
| Diet ingredients, % of DM | ALCL | ALCS | ASCL | ASCS | SE | AC | CC | AC x CC |
| Short chop alfalfa silage | | | 21.7 | 21.7 | | | | |
| Long chop alfalfa silage | 21.7 | 21.7 | | | | | | |
| Short chop corn silage | | 21.7 | | 21.7 | | | | |
| Long chop corn silage | 21.7 | | 21.7 | | | | | |
| Energy supplement | 44.0 | 44.0 | 44.0 | 44.0 | | | | |
| Protein supplement | 12.6 | 12.6 | 12.6 | 12.6 | | | | |
| Nutrient Composition[4] | | | | | | | | |
| Dry matter, % | 54.7 | 55.3 | 53.8 | 53.2 | 1.37 | 0.16 | 0.99 | 0.55 |
| Crude protein, % DM | 18.6 | 18.6 | 18.7 | 18.6 | 0.72 | 0.98 | 0.87 | 0.98 |
| SP, % Crude protein | 6.7 | 7.5 | 7.7 | 6.7 | 0.71 | 0.80 | 0.84 | 0.10 |
| ADIP, % DM | 1.7 | 1.6 | 2.6 | 1.6 | 0.63 | 0.38 | 0.23 | 0.38 |
| NDF, % DM | 34.8 | 35.3 | 36.7 | 35.7 | 1.47 | 0.28 | 0.80 | 0.48 |
| ADF, % DM | 20.1 | 20.6 | 20.7 | 19.3 | 1.08 | 0.66 | 0.57 | 0.24 |
| Ether extract, % DM | 4.5 | 4.5 | 4.9 | 4.8 | 0.39 | 0.27 | 0.86 | 0.86 |
| Ash, % DM | 9.9 | 10.1 | 9.9 | 9.9 | 0.55 | 0.92 | 0.85 | 0.84 |
| NFC, % DM | 28.6 | 28.2 | 26.7 | 28.1 | 2.06 | 0.51 | 0.74 | 0.54 |
| Calcium, % DM | 1.34 | 1.35 | 1.44 | 1.40 | 0.12 | 0.45 | 0.72 | 0.66 |
| Phosphorus, % DM | 0.52 | 0.52 | 0.55 | 0.50 | 0.03 | 0.18 | 0.95 | 0.95 |
| Potassium, % DM | 1.92 | 2.04 | 1.94 | 1.92 | 0.11 | 0.53 | 0.54 | 0.32 |
| Magnesium, % DM | 0.36 | 0.36 | 0.35 | 0.37 | 0.02 | 0.77 | 0.38 | 0.62 |
| Sodium, % DM | 0.43 | 0.47 | 0.49 | 0.48 | 0.05 | 0.34 | 0.74 | 0.41 |
| Zinc, ppm | 87.6 | 91.3 | 89.3 | 91.7 | 14.9 | 0.92 | 0.78 | 0.96 |
| Manganese, ppm | 74.5 | 82.1 | 78.7 | 81.7 | 8.6 | 0.83 | 0.38 | 0.79 |
| Copper, ppm | 21.4 | 21.3 | 19.6 | 23.8 | 4.19 | 0.90 | 0.50 | 0.48 |
| Iron, ppm | 382.3 | 328.5 | 305.7 | 401.5 | 63.5 | 0.97 | 0.65 | 0.12 |
| Molibedinium, ppm | 1.55 | 1.43 | 1.50 | 1.70 | 0.10 | 0.19 | 0.73 | 0.06 |

[1](n = 4) for each diet.

[2]ALCL = alfalfa long chop and corn long chop; ALCS = alfalfa long chop and corn short chop; ASCL = alfalfa short chop and corn long chop; ASCS = alfalfa short chop and corn short chop.

[3]AC = alfalfa chop effect; CC = corn chop effect; AC x CC = interaction effect of alfalfa and corn chop lengths.

[4]Nutrient compositions of experimental diets were measured for each experimental period

Holland Forage Harvester, model 790 (New Holland Inc., New Holland, PA), from the same field on the same day. These silages were ensiled and stored in plastic covered piles of approximately 30 tons without additives or inoculants for 3 months prior to the beginning of the experiment. Cows were assigned to one of four total mixed rations (TMR) (ALCL, ALCS, ASCL, and ASCS) during each experimental period (Table 3.1). Each diet contained (DM basis) 44.0% barley grain based energy supplement (containing 42% pellets) and 12.6% protein supplement (containing 58% pellets) (Table 3.1 and appendix 1), 21.7% of either long or short chop of alfalfa and 21.7% of either long or short chop of corn silage. The forage to concentrate ratio was 43.4:56.6.

The diets were mixed using a Data Ranger mixer (American Calan, Northwood, NH) with a Weigh Tronix weigh head (Model 1000, American Calan, Northwood, NH). The TMR were fed once daily for *ad libitum* consumption allowing 5-10% orts. Cows had unlimited access to water.

### 3.3.2 Dry matter intake and feed analyses

During the collection periods, the amount of TMR offered and refused was recorded daily for each cow. Diet samples were collected daily and pooled for each collection period. Individual ort samples from each cow were obtained daily during each collection period and pooled by weight. Forages were sampled once per collection period and pooled across collection periods. The DM content of pooled diets, forages and ort samples was determined by drying at 60°C for 48 hr. Dried feed samples were ground using a Wiley mill through a 1 mm screen (Thomas-Wiley, Philadelphia, PA) and stored at -20 °C until analysis. All feed samples were analyzed for CP using the $CuSO_4/TiO_2$

Mixed Catalyst Kjeldahl procedure (AOAC 988.05, 1990), NDF (Van Soest et al., 1991) using α amylase (Sigma no. A3306: Sigma Chemical Co., St. Louis, MO), sodium sulfite and corrected for ash concentration adapted for Ankom 200 Fibre Analyzer (Ankom Technology, Fairport, NY), ADF (AOAC 973.18, 1990), ether extract (AOAC 920.39, 1990), and Ash (AOAC 942.05, 1990). Soluble protein was determined according to Licitra et al. (1996). Calcium, P, K, Mg, and Na were measured by inductively coupled plasma emission spectroscopy (AOAC 968.08, 1990) using an Atom Scan 25 plasma spectrometer (Thermo Jarrell Ash Corp, Grand Junction, CO) after acid digestion.

Particle size distribution was determined for all TMR, pooled refusals, and forage samples using the Penn State Particle Separator (PSPS) (Kononoff et al., 2003a). Approximately 150 g of wet sample was placed on the top screen of the PSPS. The PSPS was shaken 40 times (5 times in each direction, twice) (Heinrichs and Lammers, 1997; Kononoff et al., 2003a). The contents of each fraction were weighed and analyzed for DM and NDF as described earlier. Physically effective fibre was determined as the proportion of the dietary NDF retained by the PSPS sieves ($peNDF_{NDF}$) and the proportion of dietary DM retained by the PSPS sieves multiplied by the dietary NDF content ($peNDF_{PS}$). The $peNDF_{M}$ was measured using tabular values for the physical effectiveness of feeds as recommended by Mertens (1997).

Particle size distribution of TMR was also measured by dry sieving using a vertical oscillating test sieve shaker (EFL 1 KII, Endecotts Ltd., London, UK) equipped with a stack of 6 brass sieves and a bottom pan with a 200 mm diameter (ASTM E11, Endecotts Ltd., London, UK) arranged in descending mesh size. Sieve mesh sizes were 19, 9.5, 6.3, 4.0, 1.18, and 0.6 mm. Approximately 200 g was placed on the top screen,

and the stack of sieves was shaken until the distribution of materials did not change (approx. 15 min). The $peNDF_{>1.18}$ was determined as the proportion of DM retained on and above the 1.18 mm screen multiplied by dietary NDF.

### 3.3.3 Milk yield and composition analysis

Cows were milked twice daily in their stalls and milk production was measured using Tru Test regulation meters (Westfalia Surge, Mississauga, ON). Milk samples were collected from four consecutive milkings in 50 ml vials in each collection period and preserved with 2-bromo-2-nitropropane–1,3 diol. Milk samples were stored at 4 °C until analyzed for fat and protein at the laboratory of the Dairy Farmers of Manitoba (Winnipeg, MB) by near infrared analysis using the Milk-O-Scan 303AB (Foss Electric, Hillerød, Denmark).

### 3.3.4 Rumen pH measurement and blood sample collection

Rumen fluid and peripheral blood were sampled on day 1 and 3 during each 7 d collection period at 4 to 5 hr post-feeding. Rumen fluid was aspirated using a Geishauser oral probe (Geishauser, 1993). The first 200 mL of collected rumen fluid was discarded and the subsequent 50 mL of rumen fluid were kept for subsequent analysis and processing. Rumen fluid pH was measured using an Accumet Basic 15 pH meter and an Accumet gel-filled, polymer- body combination pH electrode (Fischer Scientific, Fairlawn, NJ), calibrated with pH 4.0 and pH 7.0 buffer solutions (Fisher Scientific, Fairlawn, NJ). Rumen fluid samples were centrifuged at 1900 x *g* for 10 min and the supernatant stored at -20 °C until further analysis. Blood samples were collected by

coccygeal venipuncture in heparinized 10 mL vacutainers, centrifuged at 1900 x *g* for 10 min. Subsequently, the plasma was aspirated and stored at -20 °C until further analysis.

3.3.5 VFA, ammonia, and blood plasma analysis

Frozen rumen fluid samples were thawed at room temperature and 1 ml of 25% meta-phosphoric acid solution was added to 5 ml of rumen fluid. The tubes were vortexed and placed in a -20 °C freezer for 17 hr. Thawed samples were centrifuged for 10 min at 1900 x *g*. Approximately 2 ml of supernatant were decanted into a clean dry vial. The samples were capped and placed into the autosampler device (Model 8100, Varian, Walnut Creek, CA) for analysis. Concentrations of VFA were determined by gas chromatography (Model 3400 Star, Varian, Walnut Creek, CA) using a 1.83 m glass column (Model 2-1721, Supelco, Oakville, ON) (Erwin et al., 1961). The injector and detector temperatures were set at 170 °C and 195 °C, respectively, with initial and final column temperatures set at 120 °C and 165 °C, respectively. The runtime was 4 min followed by a 2 min thermal stabilization period.

Ammonia nitrogen concentration of rumen fluid samples was determined using the method described by Novozamsky et al. (1974). Absorbance was read at 630 nm on a Pharmacia Biotech Ultraspec 2000 UV/visible spectrophotometer (Biochrom, Cambridge, UK).

Blood plasma was analyzed for glucose, urea, and lactate using a Nova Stat profile M blood gas and electrolyte analyzer (Nova Biomedical Corporation, Waltham, MA).

### 3.3.6 Silage fermentation profile

Alfalfa silage and corn silage were analyzed for VFA, lactate, and ethanol using High Performance Liquid Chromatography (HPLC) with the method described by Siegfried et al. (1984). This method uses 0.015N $H_2SO_4$ and 0.25 mM EDTA (free acids) as the mobile phase. The temperature of the column (Bio Rad Aminex Ion exclusion HPX- 87H [300 x 7.8 mm]) was kept at 42°C and the flow was maintained at 0.6 ml/min.

Forage pH was measured using an ORION 710 pH/ISE meter calibrated with pH 4.0, 7.0, and pH 10.0 buffer solutions. A representative raw, undried, unground silage sample was mixed with distilled water in a plastic vial and kept for 10 minutes before measurement (Buchanan-Smith & Yao, 1981). Ammonia nitrogen concentration of silage samples was determined according to AOAC. 920.03 (1990).

### 3.3.7 Statistical analysis

Analysis of variance for weekly averages of rumen fluid, blood plasma variables, milk, and intakes was conducted using the SAS MIXED procedure (SAS, 1990). The effect of alfalfa chop length and corn chop length were considered fixed. Cow and period effects were considered random.

Analysis of variance for physical composition of diets was conducted using the same model, with the exception that the cow effect was excluded. Statistical significance was set at a $P \leq 0.05$. Differences among treatment means were tested for significance using Tukey's multiple range test (SAS, 1990).

## 3.4 Results and discussion

### 3.4.1 Chemical and physical compositions of forage ingredients and experimental diets

Ingredient and nutrient compositions of experimental diets are given in Table 3.1 and appendix 1. The nutritional composition of the forages included in the experimental diets is given in Table 3.2. Diets did not vary in chemical composition. Chop length did not affect chemical composition of the silages, with the exception that DM was higher for the long chop alfalfa silage (43.4%) than for the short chop alfalfa silage (38.1%). As chemical composition did not vary among diets, observed differences in DMI, rumen fermentation, blood parameters, and milk production must have been due to physical or non measured chemical differences among diets.

Silage fermentation profile was not indicative of most desirable fermentation characteristics (Kung & Shaver, 2001) (Table 3.3). pH was higher for all the forages included in the diets than the optimal range (3.7 to 4.2 and 4.0 to 4.3) for corn silage and alfalfa silage, respectively (Kung and Shaver, 2001). Long chop alfalfa silage (AL) had considerably fewer particles passing through the 8 and 19 mm PSPS screens than the short chop alfalfa silage (AS) (14.6% vs. 25.0%, Table 3.4). As a result, diets that contained AL silage had less DM passing through the 8 mm screen of the PSPS and more DM retained by the 19 mm screen (Table 3.5) than diets incorporating the AS silage. Long chop corn silage (CL) also had considerably fewer particles passing through the 8 and 19 mm PSPS screens than the short chop corn silage (CS) (11.0% vs. 24.1%, Table 3.4). As a result, diets that contained the CL silage had less DM passing through the 8 mm screen of the PSPS and more DM retained by the 19 mm screen than diets containing the CS silage (Table 3.5). In diets containing CL, reduction in alfalfa chop length reduced

$peNDF_{PS}$ and $peNDF_{NDF}$ from 19.1 to 18.2 % DM and from 23.5 to 22.5 % DM, respectively. In diets containing CS, reduction in alfalfa chop length reduced $peNDF_{PS}$ and $peNDF_{NDF}$ from 18.2 to 16.4% DM and from 21.0 to 19.6% DM, respectively. In diets containing AL, reduction in corn silage chop length reduced $peNDF_{PS}$ and $peNDF_{NDF}$ from 19.1 to 18.2 % DM and from 23.5 to 21.0 % DM, respectively. In diets containing AS, reduction in corn silage chop length reduced $peNDF_{PS}$ and $peNDF_{NDF}$ from 18.2 to 16.4% DM and from 22.5 to 19.6% DM, respectively. Different techniques used for measuring dietary physically effective fibre levels resulted in different values. This disparity is due to differences among techniques in the criteria for measuring the particle size and ability to measure effective fibre. The need for a standardized method for the particle size determination is apparent. Determination of the $peNDF_{PS}$ assumes that NDF across all the fractions of PSPS is same. This is an erroneous assumption as the NDF levels varied between different PSPS fractions (Table 3.5). Contents of the top screen of the PSPS were higher in NDF levels than in the bottom pan fraction of the PSPS for all diets (Table 3.5). As a consequence, diets had lower values for $peNDF_{PS}$ than for $peNDF_{NDF}$. Theoretically, $peNDF_{NDF}$ seems to be the best indicator of physically effective fibre levels of a diet.

The diets used in this experiment exceeded the NRC (2001) minimum NDF recommendation of 25% dietary DM, with 75% of NDF from forage and also exceeded the current recommendations for minimum NDF content (34%) in barley based diets (Beauchemin, 1991). Heinrichs and Lammers (1997) recommended that the 19 mm sieve, the 8 mm sieve, and the bottom pan of the PSPS should retain 6 to 10%, 30 to 50%, and

**Table 3. 2** Nutrient composition of the forages included in the experimental diets. (SD within brackets)

| | Forage[1] | | | |
|---|---|---|---|---|
| | AL | AS | CL | CS |
| Nutrient Composition[2] | | | | |
| Dry matter, % | 43.4 (1.5) | 38.1 (1.9) | 36.2 (0.4) | 35.3 (1.8) |
| Crude protein, % DM | 21.2 (0.5) | 20.9 (0.8) | 6.9 (0.2) | 6.2 (0.4) |
| SP, % Crude protein | 13.5 (0.4) | 13.6 (0.3) | 3.2 (0.4) | 2.7 (0.4) |
| ADIP, % DM | 1.2 (0.2) | 2.6 (3.1) | 0.6 (0.1) | 0.5 (0.1) |
| NDF, % DM | 36.8 (3.2) | 37.1 (2.0) | 51.7 (2.5) | 49.4 (3.0) |
| ADF, % DM | 27.5 (1.9) | 27.5 (1.9) | 28.1 (1.5) | 27.4 (2.0) |
| Ether extract, % DM | 2.6 (0.3) | 2.9 (2.9) | 2.1 (0.1) | 2.2 (0.1) |
| Ash, % DM | 11.2 (0.2) | 11.7 (0.3) | 6.9 (0.5) | 7.1 (0.6) |
| NFC, % DM | 24.7 (2.5) | 23.9 (1.0) | 29.4 (2.2) | 32.3 (3.7) |
| Calcium, % DM | 1.5 (0.1) | 1.5 (0.1) | 0.3 (0.0) | 0.3 (0.1) |
| Phosphorus, % DM | 0.3 (0.0) | 0.3 (0.0) | 0.2 (0.0) | 0.2 (0.0) |
| Potassium, % DM | 3.4 (0.1) | 3.4 (0.1) | 1.6 (0.0) | 1.5 (0.1) |
| Magnesium, % DM | 0.3 (0.0) | 0.3 (0.0) | 0.3 (0.3) | 0.3 (0.0) |
| Sodium, % DM | 0.03 (0.0) | 0.03 (0.0) | 0.01 (0.0) | 0.01 (0.0) |
| Zinc, ppm | 22.8 (0.5) | 23.1 (0.2) | 25 (22.1) | 22.6 (0.7) |
| Manganese, ppm | 22.1 (2.7) | 24.8 (1.1) | 18.9 (3.9) | 24.2 (9.6) |
| Copper, ppm | 9.2 (9.2) | 9.1 (0.4) | 3.9 (0.3) | 3.6 (0.5) |
| Iron, ppm | 194.2 (100.5) | 298.0 (149.8) | 255.5 (81.4) | 529.3 (284.5) |
| Molybdenum, ppm | 2.1 (0.0) | 2.5 (0.1) | 0.80 (0.1) | 0.83 (0.2) |

[1]AL = alfalfa long chop (19 mm); AS = alfalfa short chop (10 mm); CL = corn long chop (19 mm); CS = corn short chop (10 mm).
[2]Nutrient composition of forages were determined for each experimental period (n = 4)

**Table 3.3** Silage fermentation profile of the forages included in the experimental diets. (SD within brackets)

| | Forage[1] | | | |
|---|---|---|---|---|
| | AL | AS | CL | CS |
| pH | 5.08 (0.33) | 5.23 (1.0) | 4.85 (0.30) | 5.03 (1.05) |
| VFA (% DM) | 0.46 (0.47) | 1.53 (0.32) | 0.20 (0.18) | 0.00 (0) |
| Lactic Acid (% DM) | 2.64 (0.35) | 4.72 (0.63) | 1.51 (0.53) | 1.31 (0.50) |
| Total VFA (% DM) | 3.10 (0.75) | 6.25 (0.90) | 1.70 (0.71) | 1.31 (0.50) |
| Acetic Acid (% DM) | 0.46 (0.47) | 1.53 (0.32) | 0.15 (0.10) | 0.01 (0) |
| Ethanol (% DM) | 0.01 (0) | 0.01 (0) | 0.01 (0) | 0.01 (0) |
| Ammonia (% CP) | 16.67 (0.88) | 17.26 (1.68) | 14.73 (3.10) | 9.21 (3.77) |

[1]AL = alfalfa long chop (19 mm); AS = alfalfa short chop (10 mm); CL = corn long chop (19 mm); CS = corn short chop (10 mm).

[2]Fermentation profile of the forages were determined for each experimental period (n = 4)

**Table 3.4** Penn State particle size analysis of short (10 mm) or long (19 mm) chop lengths of alfalfa and corn silages[1]. (SD within brackets)

| | Forages[1] | | | |
|---|---|---|---|---|
| PSPS distribution[2] | AL | AS | CL | CS |
| | % retained, as fed basis | | | |
| Top screen (19 mm) | 40.7 (5.1) | 22.7 (3.3) | 31.9 (1.0) | 14.7 (2.2) |
| Second screen (8 mm) | 44.7 (3.8) | 52.3 (3.6) | 57.1 (1.3) | 61.2 (4.1) |
| Third screen (1.18 mm) | 12.7 (1.5) | 24.1 (4.3) | 10.4 (0.5) | 23.1 (3.0) |
| Bottom pan | 1.9 (0.3) | 0.9 (0.3) | 0.6 (0.3) | 1.0 (0.3) |
| | % retained, DM basis | | | |
| Top screen (19 mm) | 40.2 (4.7) | 19.7 (5.9) | 29.5 (0.9) | 14.4 (2.4) |
| Second screen (8 mm) | 44.1 (3.7) | 54.7 (7.4) | 57.0 (1.4) | 60.3 (4.0) |
| Third screen (1.18 mm) | 12.9 (1.4) | 24.4 (4.1) | 12.4 (0.4) | 23.6 (3.4) |
| Bottom pan | 2.8 (0.5) | 1.2 (0.3) | 1.1 (0.5) | 1.7 (0.5) |

[1]AL = alfalfa long chop (19 mm); AS = alfalfa short chop (10 mm); CL = corn long chop (19 mm); CS = corn short chop (10 mm).

[2]Penn State Particle Separator distributions of experimental silages were calculated for each period (n = 4) for each treatment and analyzed by analysis of variance.

**Table 3.5** Penn State Particle Separator analysis of experimental diets containing a short (10 mm) or long (19 mm) chop lengths of alfalfa and corn silages.

| | Diet[1] | | | | | Effect[6] | | |
|---|---|---|---|---|---|---|---|---|
| PSPS distribution[2] | ALCL | ALCS | ASCL | ASCS | SE | AC | CC | AC x CC |
| | % retained, as fed basis | | | | | | | |
| Top screen (19 mm) | 20.8 | 15.4 | 13.3 | 8.7 | 1.12 | <0.01 | <0.01 | 0.62 |
| Second screen (8 mm) | 40.8 | 42.2 | 41.5 | 42.3 | 1.38 | 0.82 | 0.53 | 0.85 |
| Third screen (1.18 mm) | 31.9 | 36.3 | 39.0 | 42.3 | 0.89 | <0.01 | 0.04 | 0.78 |
| Bottom pan | 6.5 | 6.1 | 6.2 | 6.7 | 0.31 | 0.82 | 0.89 | 0.53 |
| | % retained, DM basis | | | | | | | |
| Top screen (19 mm) | 17.4 | 12.9 | 10.7 | 6.9 | 0.36 | <0.01 | <0.01 | 0.48 |
| Second screen (8 mm) | 37.6 | 38.8 | 38.9 | 39.1 | 0.74 | 0.69 | 0.72 | 0.80 |
| Third screen (1.18 mm) | 37.7 | 41.5 | 43.5 | 46.4 | 0.49 | 0.01 | 0.09 | 0.83 |
| Bottom pan | 7.3 | 6.8 | 7.0 | 7.6 | 0.20 | 0.75 | 0.97 | 0.46 |
| | NDF retained in fraction % DM | | | | | | | |
| Top screen (19 mm) | 49.7 | 50.6 | 47.3 | 46.6 | 1.47 | 0.46 | 0.16 | 0.90 |
| Second screen (8 mm) | 36.7 | 30.5 | 32.8 | 33.6 | 1.47 | 0.80 | 0.10 | 0.03 |
| Third screen (1.18 mm) | 21.9 | 24.3 | 21.1 | 23.6 | 1.58 | 0.65 | 0.14 | 0.97 |
| Bottom pan | 30.4 | 30.0 | 30.4 | 31.8 | 0.68 | 0.20 | 0.45 | 0.22 |
| $peNDF_{PS}$[3], % DM | 19.1 | 18.2 | 18.2 | 16.4 | 0.96 | 0.18 | 0.19 | 0.68 |
| $peNDF_{NDF}$[4], % DM | 23.5 | 21.0 | 22.5 | 19.6 | 1.02 | 0.28 | 0.02 | 0.82 |
| $peNDF_{M}$[5], % DM | 23.7 | 22.8 | 23.4 | 22.4 | 0.44 | | | |

[1]ALCL = alfalfa long chop and corn long chop; ALCS = alfalfa long chop and corn short chop; ASCL = alfalfa short chop and corn long chop; ASCS = alfalfa short chop and corn short chop.

[2]Penn State Particle Separator distributions of experimental diets were calculated for each period (n = 4) for each treatment and analyzed by analysis of variance.

[3]$peNDF_{PS}$ = proportion of DM retained by the 19 and 8 mm PSPS screens multiplied by dietary NDF content.

[4]$peNDF_{NDF}$ = proportion of NDF retained by the 19 and 8 mm PSPS screens.

[5]$peNDF_{M}$ = dietary NDF content of feeds multiplied by tabular physically effectiveness factor.

[6]AC = alfalfa chop effect; CC = corn chop effect; AC x CC = interaction effect of alfalfa and corn chop lengths.

**Table 3.6** Particle size distribution of experimental diets as determined by dry sieving and $peNDF_{>1.18.}$ [1]Diets contained a short (10 mm) or long (19 mm) chop lengths of alfalfa and corn silages.

| | Diet[1] | | | | | Effect[2] | | |
|---|---|---|---|---|---|---|---|---|
| Retained on[3] Sieve | ALCL | ALCS | ASCL | ASCS | SE | AC | CC | AC x CC |
| | % retained, DM basis | | | | | | | |
| $S_1$ (19mm) | 1.1 | 0.6 | 0.7 | 0.4 | 0.22 | 0.26 | 0.11 | 0.59 |
| $S_2$ (9.5mm) | 2.9 | 1.9 | 5.1 | 2.2 | 0.55 | 0.08 | 0.01 | 0.13 |
| $S_3$ (6.3mm) | 10.7 | 8.3 | 11.5 | 8.0 | 2.54 | 0.94 | 0.28 | 0.83 |
| $S_4$ (4.0mm) | 25.3 | 31.2 | 28 | 34.8 | 2.43 | 0.22 | 0.03 | 0.86 |
| $S_5$ (1.18mm) | 40.9 | 39.0 | 37.2 | 36.4 | 4.02 | 0.46 | 0.74 | 0.89 |
| $S_6$ (0.6mm) | 10.4 | 10.3 | 8.9 | 10.4 | 0.55 | 0.26 | 0.16 | 0.13 |
| $S_7$ (pan) | 8.7 | 8.7 | 8.8 | 7.7 | 0.60 | 0.43 | 0.35 | 0.37 |
| <1.18mm | 19.1 | 19.0 | 17.5 | 18.1 | 0.89 | 0.23 | 0.78 | 0.70 |
| $peNDF_{>1.18}$[4] mm | 28.4 | 28.6 | 29.3 | 29.2 | 0.85 | 0.41 | 0.94 | 0.89 |

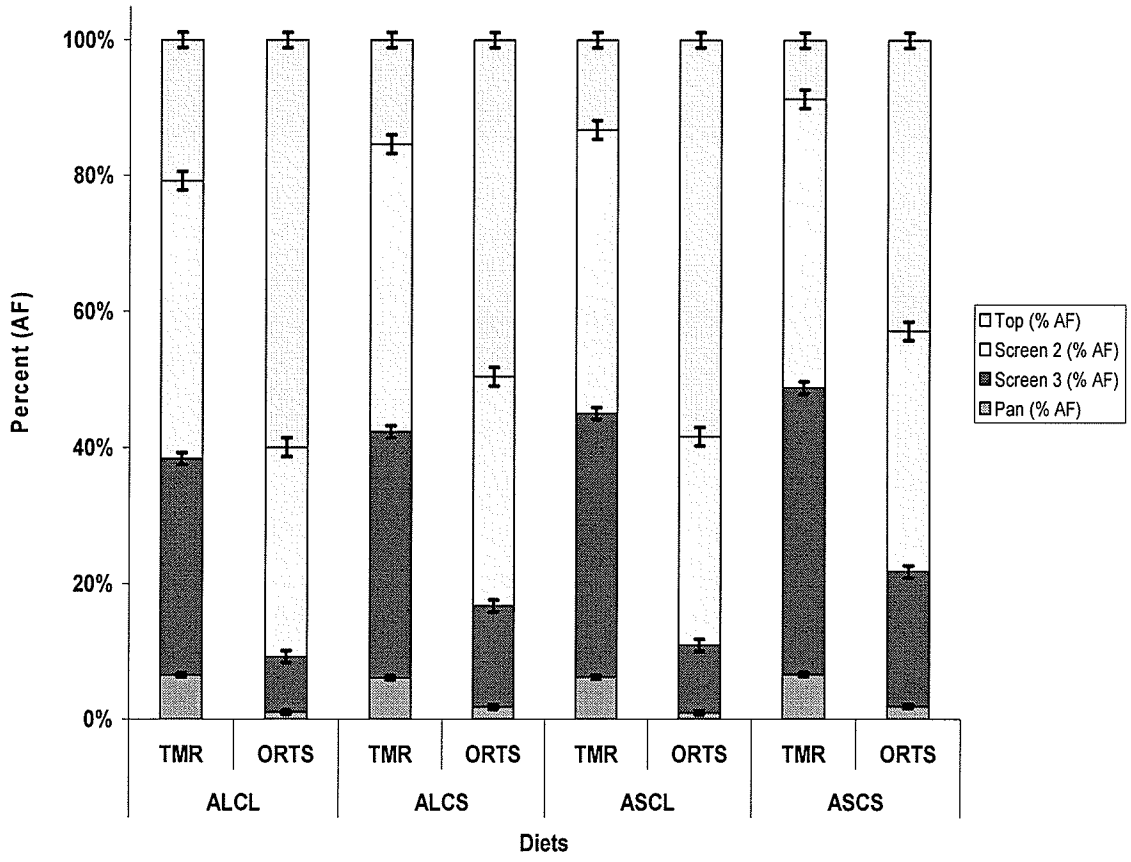
[1]ALCL = alfalfa long chop and corn long chop; ALCS = alfalfa long chop and corn short chop; ASCL = alfalfa short chop and corn long chop; ASCS = alfalfa short chop and corn short chop.

[2]AC = alfalfa chop effect; CC = corn chop effect; AC x CC = interaction effect of alfalfa and corn chop length.

[3]Dry sieve distributions of experimental diets were calculated for each period (n = 4) for each treatment and analyzed by analysis of variance.

[4]$peNDF_{>1.18mm}$ = proportion of NDF retained by ≥ 1.18mm screens.

**Figure 3.1** Penn State Particle Size Distribution of Diets[1] and Orts (as fed Basis) containing a short (10 mm) or long (19 mm) chop lengths of alfalfa and corn silages.




[1]ALCL = alfalfa long chop and corn long chop; ALCS = alfalfa long chop and corn short chop; ASCL = alfalfa short chop and corn long chop; ASCS = alfalfa short chop and corn short chop.

40 to 60%, respectively, of TMR on a wet basis. In the current experiment, all diets were coarser than these guidelines. Comparing particle size distribution of TMR and their orts show that orts had a larger percentage of DM retained by the 19 mm screen of the PSPS than TMR (Figure 1). This observation was also reported by Calberry et al. (2003), and demonstrates that cows selected against large feed particles in favour of small feed particles.

### 3.4.2 Dry matter intake

Alfalfa chop length did not affect DMI, however, decreasing the chop length of corn silage increased DMI (21.9 to 23.4 kg/d) of diets containing AL and increased DMI (22.6 to 23.0 kg/d) in diets containing AS ($P = 0.003$) (Table 3.7). Increases in DMI associated with reduction in the particle size of forages have been reported (Soita et al., 2002; Kononoff & Heinrichs, 2003a). Some researchers have reported decreased DMI as a consequence of reduced particle size (Kononoff et al., 2000; Krause & Combs, 2003), whereas others did not notice any effect of chop length on DMI (Krause et al., 2002a; Beauchemin et al., 2003; Calberry et al., 2003; Kononoff & Heinrichs, 2003b). This discrepancy in results may be due to variation in the range of chop length of forages, dissimilarity in measurement of particle size and physically effective fibre. Feeding long, coarse particles in the diet may cause reticulorumen distension, slow passage rate (Gherardi et al., 1992; Allen, 2000) and increase sorting (Calberry et al., 2003), thereby resulting in decreased feed intake. Increased sorting with long chop of alfalfa and corn silage was also observed in this study (Figure 1). Chopping at 19 mm failed to fully chop up the corn cobs, leaving unpalatable chunks. These chunks may not be ingested and

result in higher refusals for the ALCL & ASCL diets as compared to ALCS & ASCS diets, lead to higher DMI with short corn chop in this study (Table 3.7). Kononoff et al. (2003b) also reported an inverse relationship between DMI and sorting with long chopped corn silage.

The lack of response of alfalfa chop length on DMI may be attributed to a low forage inclusion in diets (Beauchemin et al., 1994). The extent of response of chop length on the voluntary dry matter intake is more pronounced in the high forage diets (Calberry et al., 2003) than low forage diets, because in high concentrate diets metabolic constraints rather than physical constraints, limit short term voluntary feed intake (Allen, 2000). Silage quality and fermentation products affect the DM intake (Dulphy & Van, 1996). The typical range of pH for corn silage and alfalfa silage is 3.7 to 4.2 and 4.0 to 4.3, respectively (Kung & Shaver, 2001). Typically, the concentrations of fermentation products of a silage on DM basis ranges from 4 to 7% for lactic acid, 1 to 3% for acetic acid, <0.1% for propionic acid, 0% for butyric acid and 5 to 7% for $NH_3$ N (% of CP). Kung & Shaver (2001) also indicated that lactic acid should be 65 to 70% of total acids. Silage pH of all the forages used in this study was higher than the range considered typically. Shaver et al. (1985) and Erdman (1988) reported increased DM intake when silage pH increased, however, other researchers reported a negative effect of high silage ammonia concentration on DM intake (Wilkins et al., 1971; Brown & Radcliffe, 1972). The higher silage ammonia concentration might have restricted the DM intake of AL, AS, and CL forages compared to CS in present study, which might explain the higher DM intake with the CS. (Table 3.3).

**Table 3.7** Dry matter intake and milk production of experimental diets containing a short (10 mm) or long (19 mm) chop lengths of alfalfa and corn silages.

| | Diet[1] | | | | | Effect[3] | | |
|---|---|---|---|---|---|---|---|---|
| Item[2] | ALCL | ALCS | ASCL | ASCS | SE | AC | CC | AC x CC |
| DMI, kg/d | 21.9 | 23.4 | 22.6 | 23.0 | 0.31 | 0.68 | 0.003 | 0.12 |
| Orts, % of feed provided | 9.9 | 8.5 | 9.0 | 8.6 | 0.32 | 0.23 | 0.01 | 0.11 |
| Milk yield, kg/d | 37.7 | 37.8 | 38.2 | 38.9 | 0.54 | 0.14 | 0.40 | 0.52 |
| Milk Components | | | | | | | | |
| Fat, % | 2.72 | 2.63 | 2.57 | 2.56 | 0.10 | 0.22 | 0.58 | 0.65 |
| Fat yield, kg/d | 1.00 | 0.99 | 0.95 | 0.99 | 0.04 | 0.43 | 0.88 | 0.43 |
| Protein, % | 3.30 | 3.28 | 3.29 | 3.29 | 0.03 | 0.83 | 0.69 | 0.68 |
| Protein yield, kg/d | 1.22 | 1.22 | 1.24 | 1.25 | 0.02 | 0.41 | 0.83 | 0.82 |

[1]ALCL = alfalfa long chop and corn long chop; ALCS = alfalfa long chop and corn short chop; ASCL = alfalfa short chop and corn long chop; ASCS = alfalfa short chop and corn short chop.

[2]Feed intake and milk production variables were averaged for each animal during each period (n = 16) for each treatment and analyzed by analysis of variance.

[3]AC = alfalfa chop effect; CC = corn chop effect; AC x CC = interaction effect of alfalfa and corn chop lengths.

3.4.3 Rumen fermentation

The chop length of alfalfa silage (P = 0.48) did not affect rumen pH, whereas reducing the chop length of corn silage significantly (P>0.05) increased the rumen pH (Table 3.8). No effect of the alfalfa chop on rumen pH was found in this study. Similar results were reported by Kononoff and Heinrichs (2003b) and Einarson et al. (2004). Contrary to that, Krause and Combs, (2003) and Beauchemin et al. (2003) reported a decrease in ruminal pH with decreased chop length of forages.

The observed increase in the pH associated with a reduction in the corn particle size is contrary to the finding of Soita et al. (2002), Beauchemin et al. (2003), Krause & Combs (2003), and Plaizier (2004), who reported a decrease in rumen pH with the reduction in forage chop length. Yang et al. (2002) reported decreased rumen pH with a reduction in the particle size of corn silage even though the chop length range was coarser than the studies by Krause et al. (2002b) and Beauchemin et al. (2003). This discrepancy in results may be due to the fact that many factors including particle size, physically effective fibre, intrinsic buffering capacity, water intake, forage pH, forage organic acid concentrations effect rumen pH. Reduction in rumen pH with the reduction in particle size reported in studies from Beauchemin et al. (2003) and Krause and Combs (2003) could be explained as a result of inclusion of finer diets in these studies compared to our study and other studies by Kononoff and Heinrichs (2003b) and Einarson et al. (2004), who failed to find such an effect. A wider chop length range (18.8 to 4.7 mm) used by Soita et al. (2002) might explain the reduction in rumen pH reported with the reduction in particle size.

The increased pH due to reduced corn chop length may be attributed to the higher silage pH found in short corn chop than long corn chop silage (Table 3.3). Thomas and Wilkinson (1975) reported a small but definite effect of silage pH on the rumen pH and this effect increased as the quality of the silage decreased. Secondly, this discrepancy of increased rumen pH with short chop of corn silage found in the present study could be explained by the variation due to time of sample in relation to diurnal variation in ruminal pH. As in this study, rumen fluid was collected 4 h after feeding using a Geishauser oral probe (Geishauser, 1993), as Keunen et al. (2002) reported that rumen pH reaches to its lowest point at this time. Duffield et al. (2004) reported that the pH of rumen fluid sampled using an oral probe was on average 0.13 units higher because of salivary contamination than the pH of rumen fluid sample obtained from the caudal rumen via a rumen canula of lactating dairy cows. These authors also explained that these single spot rumen samples are like a snap shot. Rumen pH remained above 6.10 across diets throughout the study, suggesting that none of the diets resulted in SARA even after adjusting pH using a correction factor of 0.13 units for saliva contamination, as suggested by Duffield et al. (2004).

Reducing the chop length of alfalfa and corn silage reduced the $peNDF_{PS}$ and $peNDF_{NDF}$ but did not affected $peNDF_{M}$ and $peNDF_{>1.18}$ (Table 3.5 and 3.6). Furthermore, no relationships between different measures of physically effective fibre and rumen pH were found in present study, which is similar to the findings of Krause & Combs (2003). However, several researchers (Krause et al., 2002b; Beauchemin et al., 2003; Calberry et al., 2003) demonstrated a positive correlation between the $peNDF_{NDF}$, $peNDF_{PS}$ with the rumen pH. This discrepancy may be attributed to the coarseness of the diets and higher

**Table 3.8** Rumen fluid composition for experimental diets containing a short (10 mm) or long (19 mm) chop lengths of alfalfa and corn silages.

| | Diet[1] | | | | | Effect[3] | | |
|---|---|---|---|---|---|---|---|---|
| Item[2] | ALCL | ALCS | ASCL | ASCS | SE | AC | CC | AC x CC |
| pH | 6.12 | 6.17 | 6.12 | 6.23 | 0.04 | 0.48 | 0.05 | 0.38 |
| VFA, mM/L | | | | | | | | |
| Total | 109.1 | 107.7 | 118.1 | 120.9 | 3.79 | <0.01 | 0.80 | 0.45 |
| Acetate (A) | 60.5 | 59.5 | 67.2 | 70.1 | 3.06 | <0.01 | 0.66 | 0.37 |
| Propionate (P) | 28.1 | 27.2 | 29.5 | 29.4 | 1.03 | 0.02 | 0.51 | 0.60 |
| Butyrate | 15.0 | 15.1 | 16.2 | 15.4 | 0.71 | 0.13 | 0.54 | 0.37 |
| A:P | 2.2 | 2.2 | 2.3 | 2.4 | 0.12 | 0.14 | 0.55 | 0.68 |
| Ammonia (mg/dL) | 10.6 | 11.3 | 10.5 | 11.0 | 1.14 | 0.88 | 0.58 | 0.90 |

[1]ALCL = alfalfa long chop and corn long chop; ALCS = alfalfa long chop and corn short chop; ASCL = alfalfa short chop and corn long chop; ASCS = alfalfa short chop and corn short chop.

[2]Rumen fluid composition variables were averaged for each animal during each period (n= 16) for each treatment and analyzed by analysis of variance.

[3]AC = alfalfa chop effect; CC = corn chop effect; AC x CC = Interaction effect of alfalfa and corn chop lengths.

physically effective fibre levels of the diets used in present study compared to other studies by Krause et al. (2002b), Beauchemin et al. (2003), and Calberry et al. (2003). The narrower range in forage particle length (2.7-5.8 mm) used by Krause and Combs (2003) might explain the absence of correlation between the physically effective fibre and rumen pH in their study, even though the diets included in their study were finer than those used in present study.

Results show increasing the chop length of alfalfa silage increased total VFA, acetate, and propionate. The chop length of corn silage did not affect any of these parameters (Table 3.8). Increase in the total VFA with reduced particle size has been reported in studies by Krause et al. (2002b), Calberry et al. (2003), Kononoff & Heinrichs (2003a), and Kononoff et al. (2003a), while Yang et al. (2001a) reported a decrease in VFA levels. Other researchers (Kononoff & Heinrichs, 2003b; Beauchemin et al., 2003; Krause & Combs, 2003; Soita et al., 2003) reported no effect on total VFA concentrations due to differences in particle size. These discrepancies in the results explain the complexity of the factors affecting the rumen VFA levels in rumen such as VFA production, absorption, rumen pool size, rumen turn over, rumen degradation (Soita et al., 2002 & 2003), water consumption, saliva production, liquid passage rate, and volume of liquid digesta in rumen (Yang et al., 2001b; Krause et al., 2002a; Yang et al., 2002; Plaizier, 2004).

Reducing the dietary particle size of forages might increase the microbial digestion due to increased surface area for microbial attachment, but may not affect the ruminal digestion and VFA production, as there is increased particulate passage rate (Soita et al., 2002; Soita et al., 2003). Further several researchers have reported a

decreased liquid passage rate and volume of liquid digestion in the rumen due to less saliva production with reduced dietary particle size (Yang et al., 2001ab; Krause et al., 2002a; Yang et al., 2002). Therefore, an increase in total VFA, propionate, and acetic acid concentrations with AL and no response with corn silage can be due to differences between forages in their ability to stimulate saliva production, ruminal degradability, as well as differences among animals (Plaizier, 2004).

Reducing particle size across diets did not affect rumen acetate:propionate ratio across diets. This finding is similar to others studies by Yang et al. (2001b), Beauchemin et al. (2003), and Plaizier, (2004). However, an increased acetate:propionate ratio associated with increased chop length has been reported by Soita et al. (2002), Krause et al. (2002b), Krause and Combs, (2003), and Soita et al. (2003). Reducing chop length of alfalfa and corn silage did not affect the rumen ammonia concentration (Table 3.8). No effect of the particle size on the rumen ammonia concentration have been reported by Beauchemin et al. (2003), Calberry et al. (2003), Kononoff and Heinrichs, (2003ab), and Kononoff et al. (2003b). However, Soita et al. (2003), and Plaizier, (2004) reported increased ammonia concentration with increases dietary particle size and Einarson et al. (2004) reported a decrease in rumen ammonia concentration with decrease in forage chop length. These discrepancies could be explained as rumen ammonia concentration are more affected by differences in dietary protein content rather than differences in particle size (Einarson et al., 2004).

### 3.4.4 Blood variables

The plasma concentrations of glucose, urea, and lactate did not differ among

**Table 3.9** Concentrations of metabolites in blood plasma for experimental diets containing a short (10 mm) or long (19 mm) chop lengths of alfalfa and corn silages.

| | Diet[1] | | | | | Effect[3] | | |
|---|---|---|---|---|---|---|---|---|
| Item[2] | ALCL | ALCS | ASCL | ASCS | SE | AC | CC | AC x CC |
| Glucose (mM/L) | 4.14 | 4.19 | 4.12 | 4.20 | 0.07 | 0.99 | 0.38 | 0.86 |
| Lactate (mM/L) | 1.02 | 1.04 | 1.03 | 1.33 | 0.10 | 0.14 | 0.11 | 0.16 |
| BUN (mg/dL) | 17.0 | 18.4 | 18.2 | 19.5 | 0.84 | 0.18 | 0.12 | 0.94 |

[1]ALCL = alfalfa long chop and corn long chop; ALCS = alfalfa long chop and corn short chop; ASCL = alfalfa short chop and corn long chop; ASCS = alfalfa short chop and corn short chop.

[2]Blood plasma variables were averaged for each animal during each period (n=16) for each treatment and analyzed by analysis of variance.

[3]AC = alfalfa chop effect; CC = corn chop effect; AC x CC = interaction effect of alfalfa and corn chop lengths.

diets (Table 3.9). Although diets in the study did not affect blood parameters, reducing the chop length of corn silage resulted in numeric increased BUN (P=0.12) and lactate (P=0.11). This numeric increase in the BUN levels might be explained due to decreased starch availability in the rumen because of the increased passage rate of the digesta with the short chop of the corn silage (Soita et al., 2002; Soita et al., 2003). Increased CS passage rate from rumen may result in low lactate utilization for VFA production and more of lactate passes to small intestine resulting in higher blood lactate levels (Godfrey et al., 1992; Owens et al., 1998). However, these numeric increases in BUN and lactate may not only be due to particle size, but may be due to other factors like digestibility, saliva production, as well as site of starch digestion (Yang et al., 2001a; Yang et al., 2002).

### 3.4.5 Milk production and composition

Reducing the chop length of alfalfa silage and corn silage did not affect milk yield and milk composition (Table 3.7). The absence of an effect of forage chop length on milk yield, milk fat percent, milk protein percent, protein yield, and milk fat yield across diets agrees with Beauchemin et al. (2003), Krause et al. (2002a), and Calberry et al. (2003). The low milk fat percentages of all diets found in this study were suggestive of SARA, however, these low fat levels may be attributed to several other factors. Firstly, the herd from which the cows for this trial originated has practiced selection for low milk fat content for the past few decades. Secondly, it may be due to interaction between forage particle sizes and forage source on milk fat as described by Krause and Combs (2003). Krause and Combs (2003) observed a decrease in fat percent when both alfalfa

and corn silages were included in the diet but not when only alfalfa silage was included. These authors explained that the interaction between forage particle size and forage source on milk fat could be due to differences in digestibility of the alfalfa silage and corn silage. That is, higher starch content and low intrinsic buffering capacity of corn silage as compared to alfalfa silage requires greater rumen buffering compared to alfalfa silage alone (McBurney et al., 1983). This is not a plausible explanation for the results observed hare as diets in this study did not vary in the starch content (Table 3.1).

There are several theories to account for the reduced fat percentage due to small dietary particle size. Mertens (1997) based on compilation of 36 citations reported a curvilinear relationship between $peNDF_M$ and milk fat percent, with a greater reduction in milk fat percent at low $peNDF_M$ levels than at higher $peNDF_M$ levels. Low $peNDF_M$ resulted in decreased rumen pH, leading to increased synthesis of trans-fatty acids in the rumen (Griinari et al., 1998). As trans-fatty acids have a negative effect on de novo milk fat synthesis (Griinari et al., 1998), this would reduce milk fat content. However, in this study there was no direct relationship found between the rumen pH and particle size. Another theory states that reduction in acetate and an increase in propionate concentrations in the rumen leads to milk fat depression (Griinari et al., 1998). Reduction in acetate, a precursor for de novo fat synthesis, could reduce fat percentage. However, this study, as well as in others (Beauchemin et al., 2003; Krause et al., 2002a; Plaizier, 2004), failed to find an inverse relationship between forage particle size and milk fat content. Krause and Combs (2003) reported a significant decrease in milk fat percentage with reduced chop length, where other researchers (Yang et al., 2001a; Calberry et al., 2003; Kononoff and Heinrichs, 2003b) reported a numerical decrease in the milk fat

percentage with reduction in particle size, as observed in this study. This discrepancy in results observed in our study and those of Krause and Combs (2003) may be due to short dietary particle sizes (<6mm) used in their study compared to other studies (Beauchemin et al., 2003; Kononoff et al., 2003b; Yang et al., 2001a; Calberry et al., 2003) with no such effect.

The lack of agreement regarding the effect of particle size on milk fat percent may be attributed to the fact that milk fat may be affected by many factors including forage particle size, physically effective fibre, intrinsic rumen buffering capacity, intrinsic fragility of feed particles, inclusion of buffers, concentrate inclusion, rumen digestion rate, factors affecting rumen fermentation, rumen buffering, feed intake, and lactation stage (Beauchemin et al., 1994; Mertens, 1997; Allen, 2000; Plaizier, 2004). In addition to the complexity of these factors, absence of a standard method for physically effective fibre determination, as well as variation of particle size among studies complicates comparisons as well as determination of a relationship between particle size and milk fat percentage (Plaizier, 2004). A lack of effect of the particle size of alfalfa and corn silages on the protein yield could be explained as CP content of the diets did not differ (Table 3.1). Similar results were reported in studies by Krause et al. (2002a), Beauchemin et al. (2003), and Calberry et al. (2003).

### 3.5 Conclusions

Reducing the chop length of alfalfa and corn silage from 19 mm to 10 mm did not affect the nutrient composition of the diets, but reduced the proportion of TMR retained by the 19- and 8-mm screen of the PSPS from 55% for coarser diet (ALCL) to

46% for finer diet (ASCS). Reducing alfalfa chop length did not affect production parameters, rumen pH, rumen ammonia concentration, but increased the total and individual VFA concentrations. However, reducing whole corn chop from 19 to 10 mm increased DMI and rumen pH but did not affect VFA, ammonia concentration and production parameters. Animals selected against the coarser diets and in favor of the finer diets. Different techniques for estimating physically effective fibre yield different values indicating that there is need for standardization and determination of the best indicator for rumen buffering. Further study is warranted to investigate the effect of chop length of forages on optimal physically effective fibre content in dairy cows diets. However, the results of this study demonstrate that chopping the forages (alfalfa and corn) using New Holland forage harvester (model 790) as low as 10 mm does not lead to SARA, as long as the proportion of concentrates in the diet does not exceed 55.6%.

# 4.0 Effect of chop lengths of alfalfa and oat silage on eating behaviour, chewing activity, productivity, rumen fermentation, and blood parameters of dairy cows fed total mixed rations

## 4.1 Abstract

Effects of chop length (short: 6 mm or long: 19 mm) of alfalfa silage and oat silage on, feed intake, water intake, rumen conditions, and milk production were investigated in 16 mid-lactation Holstein cows using 4 by 4 Latin squares with experimental periods consisting of two adaptation weeks and one sampling week. Feeding behaviour and chewing activity were investigated using the Growsafe equipment (Growsafe system Ltd., Airdire, AB) and video taping using four low light level black and white cameras (WV-BP 134 Panasonic) in 8 cows. Four cows out of 16 were rumen fistulated for the continuous pH monitoring and measuring the rumen liquid volume and flow rate. Cows were fed total mixed rations (TMR) containing (DM basis) 42.0% barley grain based energy supplement, 10% protein supplement, and 24% DM long chop or short chop alfalfa silage and 24% DM long chop or short chop oat silage. Reducing the chop length affected the particle size distribution across the diets, with higher proportion of the particles (71.4 % DM) were retained for the coarser diet compared to the finer diets (64.6% DM) by the 19 and 8 mm screens of Penn State Particle Separator (PSPS). Different measures of physically effective fibre yielded different values, the $peNDF_{>1.18}$ and $peNDF_{M}$ gave consistently higher values than $peNDF_{PS}$ and $peNDF_{NDF}$. The $peNDF_{PS}$ calculated as the proportion of DM retained by the 8 and 19 mm screens of the PSPS multiplied by dietary NDF ranged from 21.6 to 24.5% DM among diets. The $peNDF_{NDF}$

calculated as the NDF retained by the PSPS screens ranged from 24.3% to 28.1% DM among diets. Reducing oat silage chop length increased dry matter intake from 19.4 to 21.1 kg/d ($P<0.001$), but did not alter rumen pH, rumen VFA, rumen fluid volume, liquid flow rate, and milk production. Reducing alfalfa chop did not affect dry matter intake, rumen pH, rumen VFA, rumen fluid volume, liquid flow rate, and milk production. Chop length of alfalfa and oat silage did not affect the eating, ruminating, drinking activities, eating rate, number of meals and blood parameters. Comparisons of the particle size distribution of the diets and their respective orts/weigh backs showed that cows selected against coarse feed particles in favour of finer feed particles. It can be concluded that reducing the alfalfa and oat forage length to 6 mm with 66% particles retained on and above 8 mm screen of PSPS, improves the silage quality, increases dry matter intake without inducing SARA.

(**Key words:** forage chop length, chewing activity, feeding behaviour, dairy cows, physically effective NDF, particle size, rumen pH, feed intake, volatile fatty acids, milk production)

**Abbreviation key:** **AL** = long chop alfalfa silage (19 mm); **AS** = short chop alfalfa silage (6 mm); **OL** = long chop oat silage (19 mm); **OS** = short chop oat silage (6 mm); **ALOL** = diet containing long chop alfalfa silage and long chop oat silage; **ALOS** = diet containing long chop alfalfa silage and short chop oat silage; **ASOL** = diet containing short chop alfalfa silage and long chop oat silage; **ASOS** = diet containing short chop alfalfa silage and short chop oat silage; **$peNDF_{PS}$** = physically effective NDF measured as proportion of DM retained by the 8 and 19 mm screens of Penn State Particle Size separator multiplied by the dietary NDF; **$peNDF_{M}$** = physically effective NDF measured

from tabular values of pef multiplied by dietary NDF (Mertens, 1997); **peNDF$_{NDF}$** = physically effective NDF measured as proportion of NDF retained by the 8 and 19 mm screens of Penn State Particle Size separator multiplied by the DM; **peNDF$_{>1.18}$** = physically effective NDF measured as the proportion of DM retained by a 1.18 mm screen of multiplied by dietary NDF; **PSPS** = Penn State Particle Separator; **SARA** = sub acute ruminal acidosis.

## 4.2 Introduction

Proper nutritional management of the high producing dairy cows is of utmost importance in order to achieve the production levels up to their genetic potential. High energy diets need to be fed to these cows. These energy rich diets are highly fermentable and low in forage fibre, and may lead to number to metabolic disorders, including sub acute ruminal acidosis (SARA), decreased fibre digestion, milk fat depression, laminitis, and fat cow syndrome (Beauchemin et al., 1997; Beauchemin et al., 2003; Nocek, 1997). To account the importance of the fibre in dairy diets for the prevention of SARA, NRC (2001) recommends that diets should contain a minimum of 25% neutral detergent fibre (NDF), with 75% of the total dietary NDF supplied by forages. However, NDF is based on chemical characteristics and does not account for the physical characteristics of fibre such as particle size and density (Mertens, 1997). Mertens (1997) defined physically effective fibre (peNDF) as the fibre that stimulates the chewing activity, saliva production, and contributes to ruminal buffering. However, the current NRC (2001) does not provide guidelines for the physically effective fibre, due to the lack of a standard validated technique to quantify the physical effectiveness of feeds or entire diets and due to conflicting results in literature.

Forage chop length affects the physically effective fibre level of forages and diets. Fine high concentrate diets may result in SARA leading to production losses due to such as decreased intake, fibre digestion, milk fat depression, and laminitis (Nocek, 1997). Conversely, coarser diets may limit the feed intake because of limits to physical fill in the rumen and result in production losses (Allen, 2000). Currently, many techniques are used to measure the particle size distribution of diets and forages. These include; the product of amount of the DM retained by the 8 and 19 mm screens of the Penn State Particle Separator (PSPS) and dietary NDF ($peNDF_{PS}$, Yang et al., 2001a), the proportion of NDF retained on 8 and 19 mm screens of the PSPS with their respective DM retained ($peNDF_{NDF}$, Calberry et al., 2003), the proportion of DM retained by 1.18 mm screen multiplied by dietary NDF in dry sieving ($peNDF_{>1.18}$, Yang et al., 2001a), and dietary NDF content of feeds multiplied by tabular physically effectiveness factor ($peNDF_{M}$, Mertens, 1997). Many studies have compared these measures and reported considerably different values among these measures (Yang et al., 2001a; Beauchemin et al., 2003; Plaizier, 2004). The $peNDF_{M}$ calculated based on the tabular values gives consistently higher values compared to other measures of physically effective fibre (Plaizier, 2004). In order to validate these measures there is a need to determine which of these methods is most closely related to saliva production and to rumen buffering. Such a determination is difficult, as saliva production is very difficult to measure and rumen buffering is affected by many factors other than physical effective fibre. Determining a correlation between different measures of physical effective fibre, chewing activity, and rumen pH is required for the validation of these measures.

The PSPS is a quick on-farm method for measuring the particle size distribution of forages and diets (Lammers et al., 1996). The PSPS consists of 3 screens with 19 mm, 8 mm, and 1.18 mm diameter holes and a bottom pan. Heinrichs and Lammers (1997) recommend that the 8 and 19 mm screens of the PSPS should retain between 50 to 60% of forages and 40% to 60% of TMR particles. Based on their survey on Manitoba dairy farms, Plaizier et al. (2004) reported that a wide range of forage particle lengths are used in the industry and they observed that only 35% of alfalfa silage samples and 30% of the corn silage samples were within this recommended range.

Many researchers have studied the effect of forage particle size on chewing activity, dry matter intake, rumen fermentation, milk production and nutrient digestibility in cows (Soita et al., 2002; Beauchemin and Yang, 2005; Kononoff & Heinrichs, 2003ab; Soita et al., 2003). Results obtained were inconclusive, as it is difficult to compare among studies due to differences in the techniques used to measure dietary particle size and physically effective fibre, and differences in the dietary forage to concentrate ratio, concentrate source and forage source among studies. It is important to establish guidelines for the chop lengths of the forages and the dietary physically effective fibre in TMR based diets by determining their effects on productivity and metabolism of the cow. The objective of this study was to determine the effects of chop length of alfalfa silage and oat silage on dietary physically effective fibre levels, dry matter intake, chewing activity, milk production, rumen fermentation, blood parameters, and silage fermentation and to compare various measures for dietary physically effective fibre.

## 4.3 Materials and methods

4.3.1 Experimental procedures

Sixteen multiparious lactating Holstein cows, housed in a tie-stall barn at the Glenlea Research Station, University of Manitoba, were used in a 4 x 4 Latin square design with four 3-week experimental periods. Four animals were fistulated in order to monitor rumen pH continuously, and to measure rumen liquid flow rate. Eight cows, including the four fistulated cows, were housed in the metabolism unit. The other eight cows were housed in the main barn and used for the production data. Each experimental period consisted of a 14-d adaptation period, followed by a 7-d collection period. Animals were cared for in accordance with the Canadian Council for Animal Care guidelines (CCAC, 1984). Upon commencement of the experiment, cows averaged 64.9 ± 25.9 days in milk (DIM) (mean ± SD), had an average body condition score (BCS) of 3.09 ± 0.25 on a 5 point scale (Edmonson et al., 1989) and had an average body weight of 611 ± 78 kg. The experimental design used was balanced for the residual effects.

Second cut alfalfa (Pickseed, custom forage mix) was harvested in the late bud stage. Oats (Ronald) was harvested in the milk stage. Oat and alfalfa were chopped at 6 mm (short chop) or 19 mm (long chop) using a New Holland Forage Harvester, model 790 (New Holland Inc., New Holland, PA), from the same field on the same day. Alfalfa silage was ensiled and stored in plastic covered piles of approximately 30 tons without additives or inoculants for 3 months prior to the beginning of the experiment. The oat silage was ensiled and stored in plastic covered piles of approximately 30 tons without additives or inoculants for 4 wks prior to the beginning of the experiment. Cows were assigned to one of four total mixed rations (TMR) (alfalfa long oat long, ALOL; alfalfa long oat short, ALOS; alfalfa short oat long, ASOL, and alfalfa short oat short, ASOS)

during each experimental period (Table 4.1). Each diet contained (DM basis) 42.0% barley grain based energy supplement (containing 42% pellets) and 10% protein supplement (containing 58% pellets) (Table 4.1 and Appendix 1), 24% of either long or short chop of alfalfa and 24% of either long or short chop of oat silage. The forage to concentrate ratio was 48:52.

The diets were mixed using a Data Ranger mixer (American Calan, Northwood, NH) with a Weigh Tronix weigh head (Model 1000, American Calan, Northwood, NH). The TMR were fed once daily for *ad libitum* consumption allowing 5-10% orts. Cows had unlimited access to fresh water.

4.3.2 Dry matter intake and feed analyses

During the collection periods, the amount of TMR offered and refused was recorded daily for each cow. Diet samples were collected daily and pooled for each collection period. Individual ort samples from each cow were obtained daily during each collection period and pooled by weight. Forages were sampled once per collection period and pooled across collection periods. The DM content of pooled diets, forages and ort samples was determined by drying at 60°C for 48 hr. Dried feed samples were ground using a Wiley mill through a 1 mm screen (Thomas-Wiley, Philadelphia, PA) and stored at -20 $^{o}$C until analysis.

All feed samples were analyzed for CP using the $CuSO_4/TiO_2$ Mixed Catalyst Kjeldahl procedure (AOAC 988.05, 1990), NDF (Van Soest et al., 1991) using $\alpha$ amylase (Sigma no. A3306: Sigma Chemical Co., St. Louis, MO), sodium sulfite and corrected for ash concentration adapted for Ankom 200 Fibre Analyzer (Ankom Technology, Fairport,

**Table 4.1** Ingredients and nutrient composition of experimental diets containing a short (6 mm) or long (19 mm) chop lengths of alfalfa and oat silages. [1]

| | Diet [2] | | | | | Effect[3] | | |
|---|---|---|---|---|---|---|---|---|
| Diet ingredients, % of DM | ALOL | ALOS | ASOL | ASOS | SE | AC | OC | AC x OC |
| Short chop alfalfa silage | | | 24 | 24 | | | | |
| Long chop alfalfa silage | 24 | 24 | | | | | | |
| Short chop oat silage | | 24 | | 24 | | | | |
| Long chop oat silage | 24 | | 24 | | | | | |
| Energy supplement | 42 | 42 | 42 | 42 | | | | |
| Protein supplement | 10 | 10 | 10 | 10 | | | | |
| Nutrient Composition[4] | | | | | | | | |
| Dry matter, % | 46.3 | 48.1 | 46.9 | 49.8 | 0.46 | 0.09 | <0.01 | 0.44 |
| Crude protein, % DM | 15.8 | 15.4 | 15.5 | 16.7 | 0.17 | 0.06 | 0.09 | <0.01 |
| NDF, % DM | 34.3 | 33.9 | 34.1 | 33.5 | 1.21 | 0.71 | 0.57 | 0.90 |
| ADF, % DM | 27.2 | 30.3 | 28.1 | 26.1 | 0.72 | 0.13 | 0.61 | 0.03 |
| Calcium, % DM | 1.20 | 1.11 | 1.05 | 1.08 | 0.04 | 0.09 | 0.54 | 0.26 |
| Phosphorus, % DM | 0.49 | 0.43 | 0.49 | 0.53 | 0.02 | 0.09 | 0.05 | 0.04 |
| Potassium, % DM | 2.22 | 2.33 | 2.19 | 2.15 | 0.09 | 0.38 | 0.76 | 0.55 |
| Magnesium, % DM | 0.39 | 0.38 | 0.36 | 0.37 | 0.01 | 0.26 | 0.85 | 0.35 |
| Sodium, % DM | 0.49 | 0.43 | 0.46 | 0.50 | 0.02 | 0.56 | 0.71 | 0.14 |

[1](n = 4) for each diet.

[2]ALOL = alfalfa long chop and oat long chop; ALOS = alfalfa long chop and oat short chop; ASOL = alfalfa short chop and oat long chop; ASOS = alfalfa short chop and oat short chop.

[3]AC = alfalfa chop effect; OC = oat chop effect; AC x OC = interaction effect of alfalfa and oat chop lengths.

[4]Nutrient compositions of experimental diets were measured for each experimental period

NY), ADF (AOAC 973.18, 1990), ether extract (AOAC 920.39, 1990), and ash (AOAC 942.05, 1990). Soluble protein was determined according to Licitra et al. (1996). Calcium, P, K, Mg, and Na were measured by inductively coupled plasma emission spectroscopy (AOAC 968.08, 1990) using an Atom Scan 25 plasma spectrometer (Thermo Jarrell Ash Corp, Grand Junction, CO) after acid digestion.

Particle size distribution was determined for all TMR, pooled refusals, and forage samples using the Penn State Particle Separator (PSPS) (Kononoff et al., 2003a). Approximately 150 g of wet sample was placed on the top screen of the PSPS. The PSPS was shaken 40 times (5 times in each direction, twice) (Heinrichs and Lammers, 1997; Kononoff et al., 2003a). The contents of each fraction was weighed and analyzed for DM and NDF as described earlier. Physically effective fibre was determined as the proportion of the dietary NDF retained by the PSPS sieves ($peNDF_{NDF}$) and the proportion of dietary DM retained by the PSPS sieves multiplied by the dietary NDF content ($peNDF_{PS}$). The $peNDF_{M}$ was measured using tabular values for the physical effectiveness of feeds as recommended by Mertens (1997).

Particle size distribution of TMR was also measured by dry sieving using a vertical oscillating test sieve shaker (EFL 1 KII, Endecotts Ltd., London, UK) equipped with a stack of 6 brass sieves and a bottom pan with a 200 mm diameter (ASTM E11, Endecotts Ltd., London, UK) arranged in descending mesh size. Sieve mesh sizes were 19, 9.5, 6.3, 4.0, 1.18, and 0.6 mm. Approximately 200 g was placed on the top screen, and the stack of sieves was shaken until the distribution of materials did not change (approx. 15 min). The $peNDF_{>1.18}$ was determined as the proportion of DM retained on and above the 1.18 mm screen multiplied by dietary NDF.

4.3.3 Feeding behaviour

Feeding behaviour was monitored continuously over a 24 h period using a weigh cell system (Growsafe systems Ltd, Airdire, AB) with in the manger. Each manger was connected to weight acquisition system (Model 4000/E) consisting of 800 lbs load cell bars connected with A/D converter. The output signals were recorded in a computer using appropriate software (Model 4000 Data acquisition software ver 1.2 MOD). Feed bunk was cleaned daily before the next feed allocation. A meal was defined as eating activity in which at least 0.3 kg of fresh feed was consumed, and the activity had to occur after at least 20 min without eating activity (Beauchemin et al., 2002).

4.3.4 Eating, ruminating, and standing/lying behaviour

Animals in the metabolism unit were video taped for 3 consecutive days using four low light level black and white video cameras (WV-BP 134 Panasonic), a multiplexer (WJ-FS 216 Digital Panasonic) and a time-lapse video recorder (Panasonic AG 6720A). The four cameras were placed about 5 m above the floor to allow complete visualization of the two pens. Cameras were linked to a central monitor and video recorder. Barn lights were turned off between 10.00pm and 3.30 am and to help video recording at night seven red light (bulb, 40 watt) were used with total light intensity of less than 3 lux. In order to study the eating, and ruminating activities instantaneous scan sampling was performed at 5-min intervals. All other activities of the animals excluding the eating and ruminating activities were assigned as idle. Similar scan sampling was used to measure standing and lying activities of the animals over the period of 72hrs. These activities were recorded for 1 minute after every five minutes and were assumed that each

activity persists for the entire 5 minutes interval (Maekawa et al., 2002). Total time spent on chewing was calculated as the total of eating and ruminating.

4.3.5 Milk yield and composition analysis

Cows were milked twice daily in their stalls and milk production was measured using Tru Test regulation meters (Westfalia Surge, Mississauga, ON). Milk samples were collected from four consecutive milkings in 50 ml vials in each collection period and preserved with 2-bromo-2-nitropropane–1,3 diol. Milk samples were stored at 4 $^{o}$C until analyzed for fat and protein at the laboratory of the Dairy Farmers of Manitoba (Winnipeg, MB) by near infrared analysis using the Milk-O-Scan 303AB (Foss Electric, Hillerød, Denmark).

4.3.6 Rumen pH and rumen liquid flow rate measurement

Rumen fluid from non-rumen fistulated cows was sampled on day 1 and 3 during each 7 d collection period at 4 to 5 hr post-feeding. Rumen fluid was aspirated using a Geishauser oral probe from the 12 non fistulated cows (Geishauser, 1993). The first 200 mL of collected rumen fluid was discarded and the subsequent 50 mL of rumen fluid were kept for subsequent analysis and processing. Rumen fluid sampling from the fistulated cows was done from the cranial ventral region. Rumen fluid pH was measured using an Accumet Basic 15 pH meter and an Accumet gel-filled, polymer-body combination pH electrode (Fischer Scientific, Fairlawn, NJ), calibrated with pH 4.0 and pH 7.0 buffer solutions (Fisher Scientific, Fairlawn, NJ). Rumen fluid samples were

centrifuged at 1900 x *g* for 10 min and the supernatant stored at -20 °C until further analysis.

Rumen pH was measured continuously from day 1 to day 5 in the sampling week using the indwelling pH probes in the four fistulated cows. Indwelling pH probes were placed in the ventral sac of the rumen of each cow as described by Cumby et al. (2001). Measurements were taken every second and averaged over 60 s. Rumen fluid pH data were summarized as average pH, time below pH 6.0, and time below pH 5.6, area (time x pH) below pH 6.0, and area (time x pH) below pH 5.6 for each 24-h period.

The rumen volume, flow rate and retention time was measured using Cr-EDTA. The method used to prepare the Cr-EDTA was that of Uden et al. (1980). A solution of Cr-EDTA in distilled water was introduced into several sites in the rumen through the ruminal canula at 0900 h. Ruminal fluid samples were at 0, 2, 4, 6, 8, 12, 16, 24, 48, 72 h post dosing from the ventral sac of the rumen.

4.3.7 Blood sampling and analysis

Blood samples were collected by coccygeal venipuncture in heparinized 10 mL vacutainers, centrifuged at 1900 x *g* for 10 min. Subsequently, the plasma was aspirated and stored at -20 °C until further analysis. Blood plasma was analyzed for glucose, urea, and lactate using a Nova Stat profile critical care express (CЄ) blood gas and electrolyte analyzer (Nova Biomedical Corporation, Waltham, MA).

4.3.8 VFA, ammonia and rumen fluid marker analysis

Frozen rumen fluid samples were thawed at room temperature and 1 ml of 25% meta-phosphoric acid solution was added to 5 ml of rumen fluid. The tubes were vortexed

and placed in a -20 °C freezer for 17 hr. Thawed samples were centrifuged for 10 min at 1900 x *g*. Approximately 2 ml of supernatant were decanted into a clean dry vial. The samples were capped and placed into the autosampler device (Model 8100, Varian, Walnut Creek, CA) for analysis. Concentrations of VFA were determined by gas chromatography (Model 3400 Star, Varian, Walnut Creek, CA) using a 1.83 m glass column (Model 2-1721, Supelco, Oakville, ON) (Erwin et al., 1961). The injector and detector temperatures were set at 170 °C and 195 °C, respectively, with initial and final column temperatures set at 120 °C and 165 °C, respectively. The runtime was 4 min followed by a 2 min thermal stabilization period.

Ammonia nitrogen concentration of rumen fluid samples was determined using the method described by Novozamsky et al. (1974). Absorbance was read at 630 nm on a Pharmacia Biotech Ultraspec 2000 UV/visible spectrophotometer (Biochrom, Cambridge, UK).

Rumen fluid samples were analyzed for the Cr concentration using the Inductive Coupled Plasma - Optical Emission (ICP-OES) (Vista - MPX, Varian, Walnut Creek, CA) and the liquid rate of passage from the rumen was estimated as the slope of the regression of the natural logarithm of the concentration with time post dosing.

### 4.3.9 Silage fermentation profile

Alfalfa silage and corn silage were analyzed for VFA, lactate, and ethanol using High Performance Liquid Chromatography (HPLC) with the method described by Siegfried et al. (1984). This method uses 0.015N $H_2SO_4$ and 0.25 mM EDTA (free acids)

as the mobile phase. The temperature of the column (Bio Rad Aminex Ion exclusion HPX- 87H [300 x 7.8 mm]) was kept at 42°C and the flow was maintained at 0.6 ml/min.

Forage pH was measured using an ORION 710 pH/ISE meter calibrated with pH 4.0, 7.0, and pH 10.0 buffer solutions. A representative raw, undried, unground silage sample was mixed with distilled water in a plastic vial and kept for 10 minutes before measurement (Buchanan-Smith & Yao, 1981). Ammonia nitrogen concentration of silage samples was determined according to AOAC. 920.03 (1990).

### 4.3.10 Statistical analysis

Analysis of variance for weekly averages of rumen fluid (spot sampling), blood plasma variables, milk, and intakes were conducted using the SAS MIXED procedure (SAS, 1990). The effect of alfalfa chop length and oat chop length were considered fixed. Cow and period effects were considered random. Analysis of variance for physical composition of diets was conducted using the same model, with the exception that the cow effect was excluded. For the continuous rumen pH, the average values were calculated per day with in a period and then were analyzed with SAS MIXED procedure (SAS, 1990) with day affect taken as replicate and cow and period as random factors.

Duration of meals and amounts consumed per meal for each cow per experimental period with Growsafe equipment data were summarized as in standard deviation, minimums, $25^{th}$ percentile, $50^{th}$ percentile, $75^{th}$ percentiles, and the maximums of the measured variables using the mean procedure of the SAS institute, Inc. (1990). Data for the video taping and grow safe for eating, ruminating activities and feeding behaviour was analyzed with SAS MIXED procedure (SAS, 1990) with day affect taken

as replicate and cow and period as random factor. Rumen fluid volume, liquid passage rate was measured using the marker Cr-EDTA and was estimated using the formula:

$$C(t) = C_0 e^{-kt}$$

where:

C(t) = marker concentration in rumen at time t,

$C_0$ = marker concentration in rumen at time of infusion (t=0)

k = rumen flow rate (outflow/volume) per time unit.

Statistical significance was set at a $P \leq 0.05$. Differences among treatment means were tested for significance using Tukey's multiple range test (SAS, 1990).

**4.4 Results and discussion**

4.4.1 Chemical and physical compositions of forage ingredients and experimental diets

The chemical composition of the TMR, and the energy and protein supplements used in the TMR are given in Table 4.1 and Appendix 1. The nutritional composition and the fermentation profile of the forages is given in Tables 4.2 and 4.3, respectively. TMR containing short chop oat silage had higher dry matter (DM) content than the TMR containing long chop oat silage. The crude protein, phosphorus, and acid detergent fibre had interaction effect across the diets. However, the differences in DM, crude protein, phosphorus, and acid detergent fibre concentrations among the diets were not large enough to have an affect on intake, rumen conditions and the production of the cows. Ipharraguerre et al., (2005) reported that reducing the dietary crude protein from 16 to 14% did not affect the dry matter intake, rumen fermentation and the milk production. Lopez et al. (2004) reported no affect on the milk production and cow health when cows were fed diets varied in phosphorus concentrations either 0.37% or 0.57% DM.

**Table 4.2** Nutrient composition of the forages included in the experimental diets [1]. (SD within brackets)

| | Forage[1] | | | |
|---|---|---|---|---|
| | AL | AS | OL | OS |
| Nutrient Composition[2] | | | | |
| Dry matter, % | 42.1 (1.41) | 40.3 (2.68) | 29.6 (0.71) | 30.0 (1.88) |
| Crude protein, % DM | 16.9 (0.93) | 17.3 (0.17) | 10.5 (0.53) | 11.1 (0.83) |
| NDF, % DM | 48.4 (2.16) | 48.0 (0.79) | 59.4 (1.86) | 59.9 (4.22) |
| ADF, % DM | 34.8 (2.16) | 33.8 (1.68) | 36.8 (1.52) | 36.0 (1.18) |
| Calcium, % DM | 1.30 (0.06) | 1.37 (0.16) | 0.41 (0.22) | 0.41 (0.19) |
| Phosphorus, % DM | 0.27 (0.02) | 0.29 (0.02) | 0.33 (0.04) | 0.36 (0.02) |
| Potassium, % DM | 3.00 (0.23) | 3.13 (0.28) | 2.05 (0.27) | 2.41 (0.27) |
| Magnesium, % DM | 0.39 (0.03) | 0.41 (0.04) | 0.26 (0.08) | 0.26 (0.03) |
| Sodium, % DM | 0.05 (0.01) | 0.04 (0.00) | 0.29 (0.07) | 0.32 (0.03) |

[1]AL = alfalfa long chop (19 mm); AS = alfalfa short chop (6 mm); OL = oat long chop (19 mm); OS = oat short chop (6 mm).
[2]Nutrient composition of forages were determined for each experimental period (n = 4)

**Table 4.3** Silage fermentation profile of the forages included in the experimental diets. (SD within brackets)

| | Forage[1] | | | |
|---|---|---|---|---|
| | AL | AS | OL | OS |
| pH | 6.0 (1.5) | 4.9 (0.4) | 5.4 (1.3) | 4.6 (0.2) |
| VFA (% DM) | 4.7 (1.8) | 5.3 (0.7) | 3.1 (1.9) | 4.4 (0.3) |
| Lactic Acid (% DM) | 2.3 (1.9) | 3.9 (0.8) | 0.8 (0.5) | 1.3 (1.0) |
| Ammonia (% DM) | 18.2 (6.9) | 14.2 (6.2) | 13.2 (2.5) | 17.3 (1.2) |

[1]AL = alfalfa long chop (19 mm); AS = alfalfa short chop (6 mm); OL = oat long chop (19 mm); OS = oat short chop (6 mm)

**Table 4.4** Penn State particle size analysis of short (6 mm) or long (19 mm) chop lengths of alfalfa and oat silages[1].

| | Forages[1] | | | |
|---|---|---|---|---|
| PSPS distribution[2] | AL | AS | OL | OS |
| | % retained, as fed basis | | | |
| Top screen (19 mm) | 39.3 (7.1) | 20.6 (2.7) | 29.4 (5.6) | 13.3 (2.5) |
| Second screen (8 mm) | 45.1 (3.9) | 55.5 (2.7) | 56.1 (3.1) | 63.0 (4.0) |
| Third screen (1.18 mm) | 14.2 (3.9) | 22.5 (4.1) | 13.9 (5.1) | 23.1 (5.7) |
| Bottom pan | 1.4 (0.3) | 1.4 (0.7) | 0.6 (0.8) | 0.6 (0.7) |
| | % retained, DM basis | | | |
| Top screen (19 mm) | 39.2 (7.0) | 20.6 (2.7) | 25.7 (5.6) | 12.2 (2.9) |
| Second screen (8 mm) | 44.3 (3.2) | 54.7 (2.7) | 56.1 (3.1) | 62.6 (4.1) |
| Third screen (1.18 mm) | 14.4 (4.0) | 23.0 (4.0) | 17.0 (5.8) | 24.2 (5.8) |
| Bottom pan | 2.2 (0.4) | 1.8 (1.0) | 1.8 (1.4) | 1.1 (1.2) |

[1]AL = alfalfa long chop (19 mm); AS = alfalfa short chop (6 mm); OL = oat long chop (19 mm); OS = oat short chop (6 mm).

[2]Penn State Particle Separator distributions of experimental silages were calculated for each period (n = 4) for each treatment.

**Table 4.5** Penn State Particle Separator analysis of experimental diets containing a short (6 mm) or long (19 mm) chop lengths of alfalfa and oat silages.

| | Diet[1] | | | | | Effect[2] | | |
|---|---|---|---|---|---|---|---|---|
| PSPS distribution[3] | ALOL | ALOS | ASOL | ASOS | SE | AC | OC | AC x OC |
| | % retained, as fed basis | | | | | | | |
| Top screen (19 mm) | 23.1 | 17.3 | 16.4 | 10.0 | 3.0 | 0.01 | 0.02 | 0.90 |
| Second screen (8 mm) | 52.5 | 52.7 | 56.2 | 56.4 | 1.7 | 0.01 | 0.82 | 0.98 |
| Third screen (1.18 mm) | 22.2 | 27.5 | 25.0 | 30.6 | 3.3 | 0.22 | 0.04 | 0.95 |
| Bottom pan | 2.2 | 2.5 | 2.4 | 3.0 | 0.4 | 0.13 | 0.30 | 0.59 |
| | % retained, DM basis | | | | | | | |
| Top screen (19 mm) | 18.5 | 14.5 | 12.8 | 7.9 | 2.6 | 0.01 | 0.03 | 0.82 |
| Second screen (8 mm) | 52.9 | 54.4 | 56.5 | 56.7 | 1.9 | 0.05 | 0.56 | 0.63 |
| Third screen (1.18 mm) | 25.7 | 28.2 | 27.6 | 31.8 | 3.5 | 0.29 | 0.21 | 0.75 |
| Bottom pan | 2.9 | 3.0 | 3.2 | 3.8 | 0.5 | 0.20 | 0.37 | 0.48 |
| | NDF retained in fraction % DM | | | | | | | |
| Top screen (19 mm) | 53.9 | 51.5 | 54.7 | 56.3 | 1.2 | <0.01 | 0.62 | 0.03 |
| Second screen (8 mm) | 34.4 | 36.9 | 34.2 | 35.1 | 1.4 | 0.31 | 0.09 | 0.43 |
| Third screen (1.18 mm) | 29.3 | 32.2 | 30.0 | 33.6 | 0.9 | 0.15 | <0.01 | 0.60 |
| Bottom pan | 34.6 | 36.0 | 35.7 | 35.5 | 0.9 | 0.62 | 0.33 | 0.25 |
| | Comparison of TMR & orts (% retained, as fed basis) | | | | | | | |
| D1[4] | -24.9 | -31.2 | -20.0 | -22.5 | 6.33 | 0.16 | 0.34 | 0.69 |
| D2[5] | 14.6 | 16.1 | 12.2 | 12.9 | 4.05 | 0.35 | 0.70 | 0.89 |
| D12[6] | -10.3 | -15.0 | -7.8 | -9.6 | 3.27 | 0.77 | 0.18 | 0.55 |
| $peNDF_{PS}$[7], % DM | 24.5 | 23.4 | 23.7 | 21.6 | 1.64 | 0.28 | 0.19 | 0.67 |
| $peNDF_{NDF}$[8], % DM | 28.1 | 27.5 | 26.3 | 24.3 | 2.10 | 0.11 | 0.41 | 0.65 |
| $peNDF_{M}$[9], % DM | 38.9 | 37.9 | 37.7 | 34.4 | 1.50 | | | |

[1]ALOL = alfalfa long chop and oat long chop; ALOS = alfalfa long chop and oat short chop; ASOL = alfalfa short chop and oat long chop; ASOS = alfalfa short chop and oat short chop.

[2]AC = alfalfa chop effect; OC = oat chop effect; AC x OC = interaction effect of alfalfa and oat chop lengths.

[3]Penn State Particle Separator distributions of experimental diets were calculated for each period (n = 4) for each treatment and analyzed by analysis of variance.

[4]D1 = is the proportion of particles retained by 19mm PSPS screen for TMR minus orts.

[5]D2 = is the proportion of particles retained by 8mm PSPS screen for TMR minus orts.

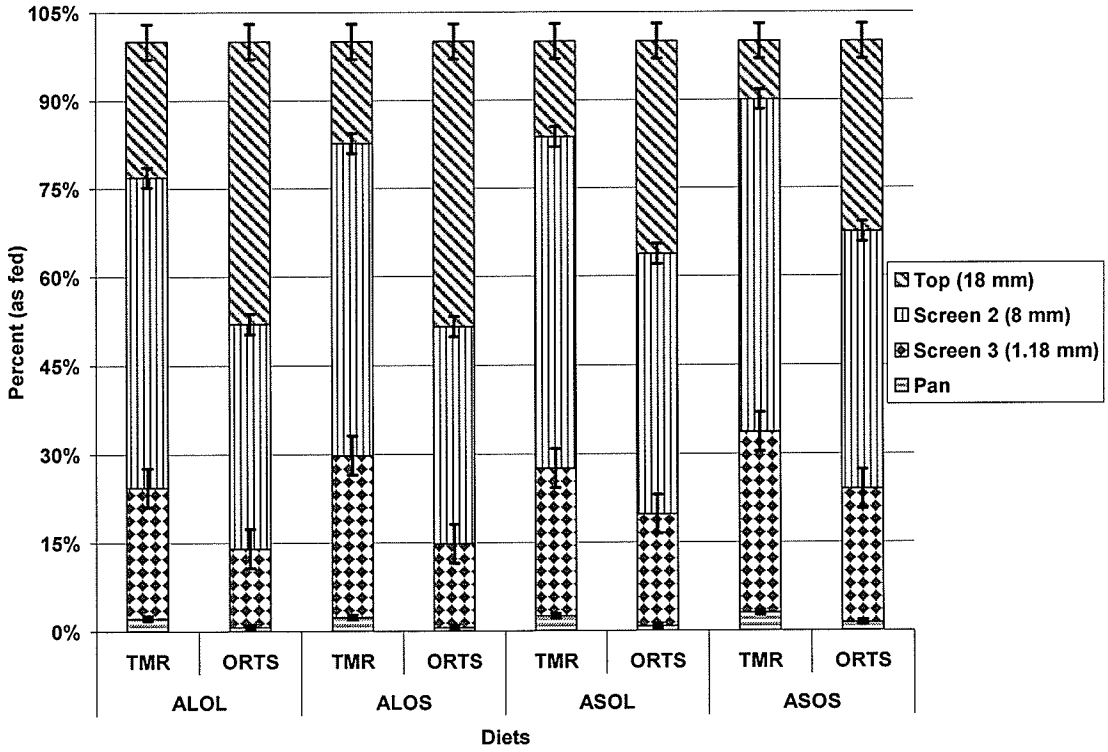
[6]D12 = is the sum of proportion of particles retained by 19mm and 8mm PSPS screens for TMR minus orts.

[7]$peNDF_{PS}$ = proportion of DM retained by the 19 and 8 mm PSPS screens multiplied by dietary NDF content.

[8]$peNDF_{NDF}$ = proportion of NDF retained by the 19 and 8 mm PSPS screens.

[9]$peNDF_{M}$ = dietary NDF content of feeds multiplied by tabular physically effectiveness factor.

**Figure 4.1** Penn State Particle Size Distribution of Diets[1] and Orts (as fed Basis) containing a short (6 mm) or long (19 mm) chop lengths of alfalfa and oat silages.




[1]ALOL = alfalfa long chop and oat long chop; ALOS = alfalfa long chop and oat short chop; ASOL = alfalfa short chop and oat long chop; ASOS = alfalfa short chop and oat short chop.

**Table 4.6** Particle size distribution of experimental diets as determined by dry sieving and $peNDF_{>1.18}$. [1]Diets contained a short (6 mm) or long (19 mm) chop lengths of alfalfa and oat silages

| | Diet[1] | | | | | Effect[2] | | |
|---|---|---|---|---|---|---|---|---|
| Retained on[3] Sieve | ALOL | ALOS | ASOL | ASOS | SE | AC | OC | AC x OC |
| | % retained, DM basis | | | | | | | |
| $S_1$ (19mm) | 3.2 | 2.5 | 2.3 | 0.8 | 0.3 | <0.01 | <0.01 | 0.11 |
| $S_2$ (9.5mm) | 0.9 | 1.0 | 1.1 | 1.0 | 0.5 | 0.73 | 1.00 | 0.78 |
| $S_3$ (6.3mm) | 3.5 | 2.4 | 4.0 | 2.4 | 1.2 | 0.77 | 0.14 | 0.77 |
| $S_4$ (4.0mm) | 28.0 | 27.5 | 25.8 | 31.0 | 2.1 | 0.70 | 0.14 | 0.08 |
| $S_5$ (1.18mm) | 52.4 | 54.6 | 55.4 | 48.4 | 3.1 | 0.48 | 0.30 | 0.06 |
| $S_6$ (0.6mm) | 6.2 | 7.2 | 5.8 | 10.4 | 1.6 | 0.24 | 0.03 | 0.13 |
| $S_7$ (pan) | 5.7 | 4.9 | 5.8 | 6.2 | 1.2 | 0.44 | 0.82 | 0.47 |
| $peNDF_{>1.18}$[4] mm | 40.8 | 41.8 | 40.6 | 36.6 | 0.8 | <0.01 | 0.03 | <0.01 |

[1]ALOL = alfalfa long chop and oat long chop; ALOS = alfalfa long chop and oat short chop; ASOL = alfalfa short chop and oat long chop; ASOS = alfalfa short chop and oat short chop.

[2] AC = alfalfa chop effect; OC = oat chop effect; AC x OC = Interaction effect of alfalfa and oat chop lengths.

[3]Dry sieve distributions of experimental diets were calculated for each period (n = 4) for each treatment and analyzed by analysis of variance.

[4]$peNDF_{>1.18mm}$ = proportion of DM retained by 1.18 mm screen multiplied by dietary NDF in dry sieving.

The silage fermentation profile shows that reducing alfalfa and oat chop length from 19 mm to 6 mm reduced silage pH from 6.0 to 4.9 and from 5.3 to 4.6, respectively, and resulted in better silage quality (Kung and Shaver, 2001). Reducing the chop length affected the particles size distribution of forages and TMR. Reducing the chop length of alfalfa silage from 19 mm (AL) to 6 mm (AS) resulted in more particles passing through the 8 and 19 mm PSPS screens (15.6% vs. 23.9%, as fed basis, Table 4.4). As a result, diets that contained AL silage had less DM passing through the 8 mm screen of the PSPS and more DM retained by the 19 mm screen (Table 4.5) than diets incorporating the AS silage.

Reducing the chop length of oat silage from 19mm (OL) to 6mm (OS) also had increased particles passing through the 8 and 19 mm PSPS screens (14.5% vs. 23.7%, as fed basis, Table 4.4). As a result, diets that contained the OL silage had less DM passing through the 8 mm screen of the PSPS and more DM retained by the 19 mm screen than diets containing the OS silage (Table 4.5). In diets containing OL, reduction in alfalfa chop length reduced $peNDF_{PS}$ and $peNDF_{NDF}$ from 33.0 to 31.8 % DM and from 28.1 to 26.3 % DM, respectively. In diets containing OS, reduction in alfalfa chop length reduced $peNDF_{PS}$ and $peNDF_{NDF}$ from 32.7 to 28.3% DM and from 27.5 to 24.3% DM, respectively. In diets containing AL, reduction in oat silage chop length reduced $peNDF_{PS}$ and $peNDF_{NDF}$ from 33.0 to 32.7 % DM and from 28.1 to 27.5 % DM, respectively. In diets containing AS, reduction in oat silage chop length reduced $peNDF_{PS}$ and $peNDF_{NDF}$ from 31.8 to 28.3% DM and from 26.3 to 24.3% DM, respectively.

Different methods used for measuring dietary physically effective fibre levels resulted in different values (Table 4.5 and 4.6). The $peNDF_{>1.18}$ and $peNDF_{M}$ gave

consistently higher values than the $peNDF_{PS}$ and $peNDF_{NDF}$. The $peNDF_{PS}$ yielded higher values than $peNDF_{NDF}$ and this variation could be explained due to differences among techniques in the criteria for measuring the particle size and ability to measure effective fibre. Determination of the $peNDF_{PS}$ assumes that NDF across all the fractions of PSPS is same. This is an erroneous assumption, as the NDF levels varied between different PSPS fractions and the top fraction of the PSPS had a higher NDF compared to the other fractions in all the diets (Table 4.5). This resulted in higher values for the $peNDF_{NDF}$ compared to $peNDF_{PS}$. The $peNDF_{M}$ yielded considerably higher values and is an arbitrary measure because it is calculated based on the tabular values, which are subjective and discontinuous. The $peNDF_{>1.18}$ is calculated based on the dry sieving, which results in shattering of the particles and is not a true representation of the sample what is being offered to the animals. The need for a standardized method for the particle size determination is apparent.

Heinrichs and Lammers (1997) recommended that after wet sieving the 19 mm sieve, the 8 mm sieve, and the bottom pan of the PSPS should retain 6 to 10%, 30 to 50%, and 40 to 60%, respectively, of TMR on a wet basis. In the current experiment, all diets were coarser than these guidelines. Comparing particle size distribution of TMR and their orts, show that orts had a larger percentage of DM retained by the 19 mm screen of the PSPS than TMR (Figure 1). This observation is similar to the results of our previous experiment as well as other studies by (Calberry et al., 2003; Leonardi and Armentano, 2003; Leonardi et al., 2005) and demonstrates that cows selected against large feed particles in favor of small feed particles.

4.4.2 Dry matter intake

Reducing the chop length of alfalfa did not affect the dry matter intake (DMI), however, reducing the oat chop length from 19 mm to 6 mm resulted in increased DMI ($P<0.001$) (Table 4.7). Leonardi et al. (2005), Kononoff and Heinrichs, (2003a), Soita et al. (2002) reported an increase in the DMI with the reduction in the forage chop length, however, another study (Krause and Combs, 2003) reported a negative effect on DMI with the reduction in the forage chop length. Other researchers (Beauchemin and Yang, 2005; Beauchemin et al., 2003; Calberry et al., 2003) reported no effect of the forage chop length on the DMI. A wider forage chop length range compared to this study might explain the increased DMI with the reduction in the forage chop length in studies from Soita et al. (2002) and Kononoff and Heinrichs (2003a). The absence of an effect of the forage chop length on the DMI reported in studies from Beauchemin et al. (2003), Calberry et al. (2003), and Beauchemin and Yang (2005) could be explained due to coarser diets and narrower range of the forage chop length used in these studies compared to the studies by (Kononoff and Heinrichs, 2003a; Soita et al., 2002; Leonardi et al., 2005).

Krause et al. (2003) and Krause and Combs, (2003) reported a negative effect on DMI with the reduction in dietary particle size, which could be explained by reduction in rumen pH and occurrence of SARA reported in these studies. SARA is often associated with decreased intake, reduced ruminal protozoal populations, and vague health problems (Nocek, 1997). A reduction in chop length can increase feed intake when intake is limited by physical fill constraints of the rumen. In such a situation, a reduction in feed particles size will increase digesta passage rate through the rumen. The absence of the effect of

**Table 4.7** Dry matter intake, water intake, and milk production of experimental diets containing a short (6 mm) or long (19 mm) chop lengths of alfalfa and oat silages.

| | Diet[1] | | | | | Effect[2] | | |
|---|---|---|---|---|---|---|---|---|
| Item[3] | ALOL | ALOS | ASOL | ASOS | SE | AC | OC | AC x OC |
| DMI, kg/d | 19.0 | 21.3 | 19.7 | 21.0 | 0.45 | 0.61 | <0.001 | 0.29 |
| Orts, % of feed provided | 11.7 | 7.1 | 6.4 | 6.5 | 2.67 | 0.19 | 0.26 | 0.37 |
| Water intake (L/d) | 77.8 | 82.3 | 82.4 | 81.6 | 2.80 | 0.33 | 0.36 | 0.20 |
| Milk yield, kg/d | 35.0 | 36.3 | 36.7 | 36.2 | 0.81 | 0.65 | 0.33 | 0.27 |
| Milk Components | | | | | | | | |
| Fat, % | 2.95 | 2.99 | 3.06 | 3.03 | 0.10 | 0.97 | 0.43 | 0.75 |
| Fat yield, kg/d | 1.02 | 1.08 | 1.11 | 1.10 | 0.03 | 0.58 | 0.08 | 0.29 |
| Protein, % | 3.18 | 3.15 | 3.14 | 3.18 | 0.03 | 0.72 | 0.74 | 0.30 |
| Protein yield, kg/d | 1.08 | 1.13 | 1.15 | 1.15 | 0.02 | 0.47 | 0.10 | 0.51 |

[1]ALOL = alfalfa long chop and oat long chop; ALOS = alfalfa long chop and oat short chop; ASOL = alfalfa short chop and oat long chop; ASOS = alfalfa short chop and oat short chop.

[2]AC = alfalfa chop effect; OC = oat chop effect; AC x OC = interaction effect of alfalfa and oat chop lengths.

[3]Feed intake and milk production variables were averaged for each animal during each period (n = 16) for each treatment and analyzed by analysis of variance.

alfalfa chop found in this study could be explained as alfalfa silage cut at 19 mm did not appear to limit feed intake.

The comparison of the particle size distribution of the TMR with their respective orts revealed that animal sorted against the coarser feed particles in favour of finer (Figure 1). This finding is similar to the results of other studies by Calberry et al. (2003), Leonardi and Armentano (2003), and Leonardi et al. (2005). This means that the ingested dietary particle size distribution was less coarse than what was offered to the animals. However, Beauchemin and Yang (2005) reported sorting in favour of longer feed particles and Plaizier (2004) reported no differences between the TMR and orts particle size distribution. This discrepancy in results among studies could be explained by the finer diets and lower physically effective fibre levels in studies from Beauchemin and Yang (2005) and Plaizier (2004) compared to our study and the studies from (Leonardi and Armentano, 2003; Calberry et al., 2003; Leonardi et al., 2005). Beauchemin and Yang (2005) suggested that when finer diets are fed and when rumen pH is low, cows may intentionally select long feed particles to meet their need for physically effective fibre.

### 4.4.3 Feeding behaviour

The overall time spent on eating ranged from 5.9 to 6.4 h/d, and the time spent ruminating ranged from 9.4 to 9.9 h/d (Table 4.8). The time spent on chewing and ruminating in this study was more than the minimum requirements for chewing and ruminating activities suggested by Beauchemin et al. (1994) under tie stall conditions. These authors suggested that a healthy high producing animal should spend ≥10 h/d on

**Table 4.8** Feeding behaviour and chewing activity of animals recorded by video monitoring (with scan sampling at 5 minutes interval) fed experimental diets containing a short (6 mm) or long (19 mm) chop lengths of alfalfa and oat silages.

| | Diet[1] | | | | | Effect[2] | | |
|---|---|---|---|---|---|---|---|---|
| Item[3] | ALOL | ALOS | ASOL | ASOS | SE | AC | OC | AC x OC |
| Eating (h/d) | 6.0 | 6.4 | 5.9 | 5.9 | 0.23 | 0.09 | 0.28 | 0.26 |
| Ruminating (h/d) | 9.5 | 9.4 | 9.4 | 9.9 | 0.29 | 0.37 | 0.28 | 0.18 |
| Idle (h/d) | 8.5 | 8.2 | 8.7 | 8.2 | 0.37 | 0.68 | 0.13 | 0.73 |
| Standing (h/d) | 12.0 | 12.5 | 11.7 | 11.9 | 0.71 | 0.39 | 0.49 | 0.79 |
| Lying (h/d) | 12.0 | 11.5 | 12.3 | 12.1 | 0.71 | 0.39 | 0.49 | 0.79 |

[1]ALOL = alfalfa long chop and oat long chop; ALOS = alfalfa long chop and oat short chop; ASOL = alfalfa short chop and oat long chop; ASOS = alfalfa short chop and oat short chop.

[2]AC = alfalfa chop effect; OC = oat chop effect; AC x OC = Interaction effect of alfalfa and oat chop lengths.

[3]Feeding behaviour parameters were averaged for each animal during each period (n=8) for each treatment and analyzed by analysis of variance.

**Table 4.9** Comparison of the Growsafe equipment (GS) and Video recording (with scan sampling at 5 minutes interval) data for eating activity and comparison of Growsafe equipment and manual recording data for intake in animals fed experimental diets containing a short (6 mm) or long (19 mm) chop lengths of alfalfa and oat silages.

| | Diet[1] | | | | | Effect[2] | | |
|---|---|---|---|---|---|---|---|---|
| Item[3] | ALOL | ALOS | ASOL | ASOS | SE | AC | OC | AC x OC |
| Eating - GS(h/d) | 4.8 | 4.8 | 4.9 | 4.4 | 0.25 | 0.49 | 0.20 | 0.18 |
| Eating - Video (h/d) | 6.0 | 6.4 | 5.9 | 5.9 | 0.23 | 0.09 | 0.28 | 0.26 |
| Intake - GS (kg/d)[4] | 36.4 | 35.5 | 37.0 | 35.5 | 1.71 | 0.79 | 0.32 | 0.81 |
| Intake - Manual (kg/d)[5] | 39.2 | 40.4 | 40.5 | 38.9 | 0.88 | 0.89 | 0.67 | 0.03 |
| Intake - GS (Start - final)[6] | 40.9 | 39.0 | 40.7 | 40.3 | 1.38 | 0.57 | 0.27 | 0.44 |
| Eating rate (g/min) | 126.9 | 121.8 | 129.6 | 137.4 | 5.11 | 0.01 | 0.71 | 0.08 |

[1]ALOL = alfalfa long chop and oat long chop; ALOS = alfalfa long chop and oat short chop; ASOL = alfalfa short chop and oat long chop; ASOS = alfalfa short chop and oat short chop.
[2]AC = alfalfa chop effect; OC = oat chop effect; AC x OC = Interaction effect of alfalfa and oat chop lengths.
[3]Feeding behaviour parameters were averaged for each animal during each period (n=8) for each treatment and analyzed by analysis of variance.
[4]Intake calculated from Growsafe equipment = sum of all meals.
[5]Intake calculated by manual recording = feed offered minus weigh back.
[6]Intake calculated from Growsafe equipment = TMR provided minus weigh back.

chewing and ≥6 h/d on rumination. Reducing the chop length of alfalfa and oat silage did not affect the time spent on eating and ruminating activity. These results were similar to the other reports (Kononoff et al., 2003a; Schwab et al., 2002). Other researchers reported higher eating, ruminating and total chewing activity with the long chop forages compared to short chop forages (Beauchemin et al., 2003; Krause and Combs, 2003; Yansari et al., 2004; Beauchemin and Yang, 2005; Leonardi et al., 2005).

The absence of an effect of forage chop length on the eating, ruminating, and chewing activity in the present study as well as in studies by (Schwab et al., 2002; Kononoff et al., 2003a) could be explained by the coarseness of diets included in these studies compared to studies by Beauchemin et al. (2003), Yansari et al. (2004), and Beauchemin and Yang (2005) who reported an increased chewing activity with the increase in forage chop length. A smaller forage chop length ranging 2.8 to 4.2 mm and from 4.4 to 6.7 mm used in the studies by Krause and Combs (2003) and Leonardi et al. (2005), respectively, might explain the increased eating, ruminating, and chewing activities with the increase in forage chop length, as, Allen (1997) reported a curvilinear relationship between the forage chop length and total chewing time and the most dramatic reduction in chewing time occur when particle size is reduced below 3 mm. The lower dietary $peNDF_{PS}$ levels ranging 7.5 to 15% DM and 8.9 to 11.5% DM in studies by Beauchemin et al. (2003) and Beauchemin and Yang (2005), respectively, compared to the present study and other studies (Schwab et al., 2002; Kononoff et al., 2003a) might explain the effect of forage particle size on eating and ruminating activity in these studies.

The highest eating activity was recorded in the first three hours after the feeding (Figure 4.2). Similar results were reported by the other studies (Beauchemin et al., 2002;

Tolkamp et al., 2000). There was a large difference in the meal sizes throughout the day. The largest meal was recorded after the morning feeding and thereafter the meal size decreased as the day progressed (Figure 4.2). The minimum intake and the minimum time per meal did not vary among the diets, however, the maximum intake per meal and the maximum time spent per meal was higher for the coarser diet (ALOL) compared to fine diet (ASOS). There was a considerable difference between the meal sizes for all the diets throughout the day as illustrated by the large standard deviation values (Table 4.10).

The overall eating time did not differ across the diets as recorded by Growsafe equipment and video monitoring (Table 4.8 and 4.9). Comparing the techniques, i.e the Growsafe equipment and the video taping for time spent eating, revealed that Growsafe equipment consistently gave lower values for time spent eating. This discrepancy in results among the two techniques could be explained based on the selection of the time frequency while scanning the video recording data and the selection of meal criteria while reading the data in Growsafe equipment. In our study, the video monitored data was scan sampled for 1 minute after every five minutes for the eating and ruminating activities and was assumed that each activity persists for the entire 5 minutes interval. Maekawa et al. (2002) and Deschanps et al. (1989) have reported different activities in dairy animals using similar scanning criteria for video monitored data. In our study, a meal was defined as eating activity in which at least 0.3 kg of fresh feed was consumed, and the activity had to occur after at least 20 min without eating activity (Beauchemin et al., 2002). All those eating activities which occurred after 20 min but with intake less than 0.3 kg, were excluded from the total time as well as from the total intake, which also explains the lower time recorded with Growsafe equipment compared to the video monitoring. This

**Table 4.10** Summarization of feeding behaviour measured with Growsafe equipment in animals fed experimental diets containing a short (6 mm) or long (19 mm) chop lengths of alfalfa and oat silages.

| | Diet [2] | | | | | Effect[3] | | |
|---|---|---|---|---|---|---|---|---|
| Item[3] | ALOL | ALOS | ASOL | ASOS | SE | AC | OC | AC x OC |
| Minimum intake (kg/meal) | 0.73 | 1.18 | 0.72 | 0.67 | 0.26 | 0.17 | 0.28 | 0.18 |
| Maximum intake (kg/meal) | 18.95 | 16.81 | 17.29 | 14.39 | 1.29 | 0.04 | 0.01 | 0.68 |
| SD[4] of intake (kg/meal) | 6.43 | 6.01 | 5.60 | 4.74 | 0.49 | 0.01 | 0.08 | 0.54 |
| 25$^{th}$ percentile of intake (kg/meal) | 1.61 | 1.93 | 1.47 | 1.93 | 0.27 | 0.72 | 0.06 | 0.73 |
| 50$^{th}$ percentile of intake (kg/meal) | 2.55 | 3.02 | 2.38 | 3.560 | 0.38 | 0.50 | 0.01 | 0.20 |
| 75$^{th}$ percentile of intake (kg/meal) | 5.05 | 6.65 | 4.68 | 5.170 | 0.90 | 0.16 | 0.12 | 0.40 |
| Minimum time(min) | 5.03 | 10.0 | 4.2 | 4.3 | 2.5 | 0.09 | 0.17 | 0.19 |
| Maximum time (min) | 137.3 | 128.8 | 136.7 | 102.7 | 11.3 | 0.11 | 0.02 | 0.13 |
| SD of time/meal | 46.5 | 47.2 | 45.0 | 34.6 | 4.3 | 0.03 | 0.12 | 0.08 |
| 25$^{th}$ percentile of time/meal | 13.6 | 17.2 | 12.8 | 12.2 | 3.4 | 0.24 | 0.54 | 0.38 |
| 50$^{th}$ percentile of time/meal | 24.2 | 28.8 | 21.8 | 26.4 | 3.9 | 0.38 | 0.11 | 0.99 |
| 75$^{th}$ percentile of time/meal | 43.1 | 58.8 | 40.8 | 43.6 | 6.9 | 0.09 | 0.07 | 0.20 |
| Number of meals/d | 7.8 | 7.4 | 8.8 | 8.0 | 0.7 | 0.15 | 0.26 | 0.72 |

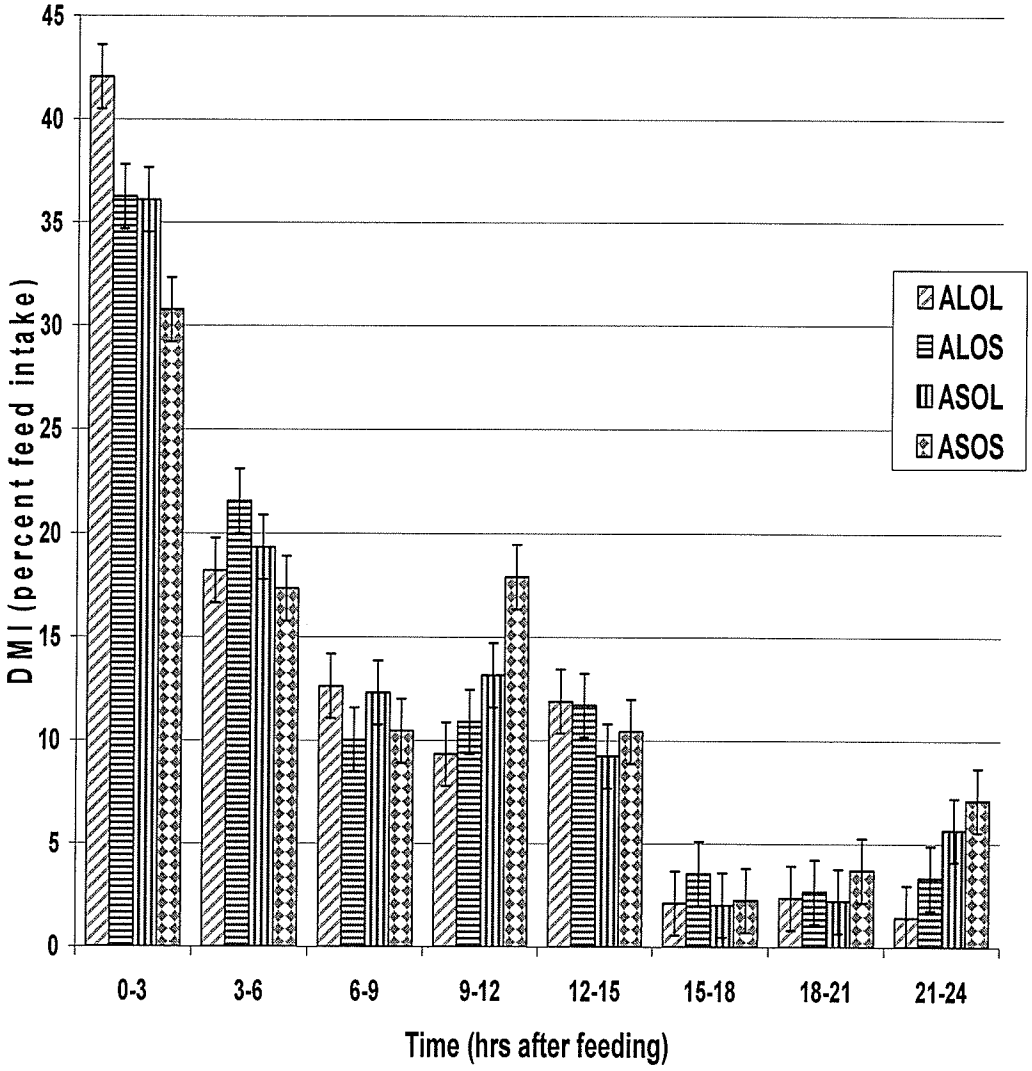
[1]ALOL = alfalfa long chop and oat long chop; ALOS = alfalfa long chop and oat short chop; ASOL = alfalfa short chop and oat long chop; ASOS = alfalfa short chop and oat short chop.

[2]AC = alfalfa chop effect; OC = oat chop effect; AC x OC = Interaction effect of alfalfa and oat chop lengths.

[3]Feeding behaviour parameters were averaged for each animal during each period (n=8) for each treatment and analyzed by analysis of variance.

[4]SD = standard deviation.

**Figure 4.2** Diurnal variation in the feed intake of high yielding dairy cows fed with diets containing a short (6 mm) or long (19 mm) chop lengths of alfalfa and oat silages.




[1]ALOL = alfalfa long chop and oat long chop; ALOS = alfalfa long chop and oat short chop; ASOL = alfalfa short chop and oat long chop; ASOS = alfalfa short chop and oat short chop.

also explains the lower intake (kg/day, as fed basis) calculated by the Growsafe equipment compared to the manual recording of intake (Table 4.9). This difference in measurement of intake per day decreased when we calculated the intake per day using the Growsafe equipment as the difference between TMR offered and weigh backs. These results illustrate the importance and subjectivity of the meal criteria used in order to calculate the intake.

Number of meals per day did not vary across the diets and ranged from 7.4 to 8.0 among diets (Table 4.10). Dado and Allen (1993) and Beauchemin et al. (2003) reported higher number of meals per day (10.9 and 10, respectively), where as, Cassida and Stokes (1986) reported a range of 9 to 14 meals per day for dairy cows fed with TMR diets. This variation in the results could be explained due to difference in the meal criteria among the studies. Cassida and Stokes (1986) used the used electronic weigh cell and sampling for saliva and bolus collections for determining the eating behaviour patterns. Forage chop length did not affect the total water intake per day (Table 4.8), and these values for water intake found in our study for high producing dairy cows conforms with the findings by Dado and Allen (1993).

### 4.4.4 Rumen fermentation

The average rumen pH obtained using a stomach tube ranged from 6.13 to 6.26 for coarsest (ALOL) to finest (ASOS) diets, respectively (Table 4.11). There was an effect of oat chop on the rumen pH ($P = 0.06$), as reduction in oat chop resulted in increased mean rumen pH. The mean rumen pH obtained from the continuous pH measurement using indwelling probes ranged from 6.21 to 6.27 for coarsest (ALOL) and

finest (ASOS) diets, respectively (Table 4.12) and was not affected by the forage chop length of alfalfa silage and oat silage. There was a numerical (0.01 to 0.08 units) difference in the mean rumen pH among the two techniques and higher values were obtained with the continuous pH monitoring using the indwelling probes than the spot sampling. These results are opposite to findings of Duffield et al. (2004), who reported non-significant (0.02 units) difference for mean rumen pH among these two techniques, with higher values obtained with oro-ruminal probe method. Duffield et al. (2004) reported that the continuous monitoring of rumen pH by cranial ventral rumen approach using indwelling probes is a more sensitive and accurate method than the oro-ruminal probe method. The higher mean rumen pH found with the reduction of the oat chop might be explained by variation due to time of spot sampling in relation to the diurnal variation in the rumen pH. In this study, rumen fluid was collected 4 h after feeding using a Geishauser oral probe (Geishauser, 1993), as Keunen et al. (2002) reported that rumen pH reaches to its lowest point at this time. However, the rumen pH obtained from a single sample may not be the true representation of effect of the factors interested, as Duffield et al. (2004) reported that the point samples are indeed just a snap short and may result in confounding affects. This might also explain the higher mean rumen pH values obtained in our study with the spot sampling compared to the continuous pH monitoring.

The absence of effect of the alfalfa chop and oat chop on rumen pH found in our study conforms the findings of Kononoff and Heinrichs (2003b) and Einarson et al. (2004). Other researchers reported a decrease in rumen pH with reduction in chop length of the forages (Krause and Combs, 2003; Beauchemin et al., 2003; Soita et al., 2002, 2003). Krause and Combs (2003) and Beauchemin et al. (2003) reported decreased rumen

**Table 4.11** Rumen fluid composition based on spot sampling using stomach tube for experimental diets containing a short (6 mm) or long (19 mm) chop lengths of alfalfa and oat silages.

| | Diet[1] | | | | | Effect[2] | | |
|---|---|---|---|---|---|---|---|---|
| Item[3] | ALOL | ALOS | ASOL | ASOS | SE | AC | OC | AC x OC |
| pH | 6.13 | 6.19 | 6.24 | 6.26 | 0.05 | 0.35 | 0.06 | 0.73 |
| VFA, mM/L | | | | | | | | |
| Total | 121.0 | 125.8 | 116.6 | 127.2 | 7.56 | 0.77 | 0.16 | 0.58 |
| Acetate (A) | 77.5 | 82.9 | 74.0 | 83.3 | 6.36 | 0.74 | 0.11 | 0.66 |
| Propionate (P) | 25.6 | 23.3 | 24.2 | 24.6 | 1.74 | 0.97 | 0.42 | 0.27 |
| Other | 3.9 | 4.8 | 4.1 | 4.4 | 0.37 | 0.72 | 0.02 | 0.22 |
| A:P | 3.4 | 3.7 | 3.4 | 3.5 | 0.37 | 0.71 | 0.41 | 0.67 |
| Ammonia (mg/dL) | 10.9 | 11.6 | 12.1 | 11.1 | 0.65 | 0.73 | 0.56 | 0.15 |

[1]ALOL = alfalfa long chop and oat long chop; ALOS = alfalfa long chop and oat short chop; ASOL = alfalfa short chop and oat long chop; ASOS = alfalfa short chop and oat short chop.

[2]AC = alfalfa chop effect; OC = oat chop effect; AC x OC = Interaction effect of alfalfa and oat chop lengths.

[3]Rumen fluid composition variables were averaged for each animal during each period (n= 16) for each treatment and analyzed by analysis of variance.

**Table 4.12** Rumen parameters based on continuous rumen pH monitoring using indwelling probes for experimental diets containing a short (6 mm) or long (19 mm) chop lengths of alfalfa and oat silages.

| | Diet[1] | | | | | Effect[2] | | |
|---|---|---|---|---|---|---|---|---|
| Item[3] | ALOL | ALOS | ASOL | ASOS | SE | AC | OC | AC x OC |
| pH | 6.21 | 6.22 | 6.27 | 6.27 | 0.07 | 0.30 | 0.94 | 0.94 |
| SD[4] | 0.43 | 0.40 | 0.41 | 0.40 | 0.02 | 0.63 | 0.39 | 0.54 |
| T <6 (min/d)[5] | 490.3 | 482.1 | 394.1 | 434.8 | 94.9 | 0.33 | 0.82 | 0.73 |
| T < 5.6 (min/d)[6] | 197.2 | 128.1 | 120.6 | 129.4 | 59.6 | 0.41 | 0.50 | 0.39 |
| A < 6 (min x pH/d)[7] | 172.6 | 123.9 | 122.0 | 129.4 | 45.6 | 0.51 | 0.55 | 0.42 |
| A < 5.6 (min x pH/d)[8] | 37.0 | 14.8 | 21.7 | 19.5 | 12.2 | 0.56 | 0.21 | 0.29 |
| K (%/h ) | 11.3 | 11.8 | 12.1 | 11.5 | 0.75 | 0.66 | 0.89 | 0.35 |
| Rumen volume (L) | 89.7 | 93.5 | 85.1 | 104.7 | 9.51 | 0.64 | 0.13 | 0.29 |
| Flow rate (L/h) | 9.9 | 10.9 | 10.3 | 12.0 | 0.80 | 0.26 | 0.05 | 0.55 |
| Retention time (h) | 9.1 | 8.5 | 8.3 | 8.8 | 0.69 | 0.59 | 0.96 | 0.31 |

[1]ALOL = alfalfa long chop and oat long chop; ALOS = alfalfa long chop and oat short chop; ASOL = alfalfa short chop and oat long chop; ASOS = alfalfa short chop and oat short chop.

[2]AC = alfalfa chop effect; OC = oat chop effect; AC x OC = Interaction effect of alfalfa and oat chop lengths.

[3]Rumen parameters were averaged for each animal during each period (n=4) for each treatment and analyzed by analysis of variance.

[5] T <6 (min/d) = time below rumen pH 6.0 per day

[6] T < 5.6 (min/d) = time below rumen pH 5.6 per day

[7] A < 6 (min x pH/d) = area below rumen pH 6.0 per day

[8] A < 5.6 (min x pH/d) = area below rumen pH 6.0 per day

pH with the decrease in forage chop length. These findings can be explained by inclusion of finer diets in studies of (Krause and Combs, 2003; Beauchemin et al., 2003) compared to present study and the studies of (Kononoff and Heinrichs, 2003; Einarson et al., 2004). The reduction in rumen pH associated with the reduction in forage chop length reported in studies by Soita et al. (2002) and (2003) could be explained due to wider (18.8-4.7 mm) forage chop length range used in these studies compared to our study and the studies of (Kononoff and Heinrichs, 2003; Einarson et al., 2004) which failed to find such an effect.

Average time and area with ruminal pH below 6.0 or 5.6 did not differ among the diets (Table 4.12). Krause et al. (2002a) and Beauchemin et al. (2003) reported an effect of the forage chop length on the diurnal ruminal pH pattern. Allen (1997) suggested a positive curvilinear relationship between the dietary physically effective fibre levels and the rumen pH. This discrepancy in the results could be explained as diets used in our study were coarser compared to diets used in the studies by Krause et al. (2002a) and Beauchemin et al. (2003). The dietary $peNDF_{PS}$ levels ranged between 7.2 to 15% DM in the study by Beauchemin et al. (2003) compared to 28.3 to 33.0% DM in our study.

In our study, animals spent around 2 h per day with rumen pH less than 5.6 and surprisingly the time spent below rumen pH 5.6 was highest (197.2 min/d) for the coarsest diet (ALOL). However, the time and area spent below rumen pH 5.6 did not differ across diets and the rumen pH data obtained from both using Geishauser oral probe and the indwelling probes suggested that none of diets resulted SARA. Gozho et al. (2005) reported induction of SARA in steers with a time spent below pH 5.6 of 187 and 174 min/d. Defining SARA based on rumen fluid pH is a controversial issue and different

studies have set different upper threshold limit values such as 5.5 (Hibbard et al., 1995), 5.6 (Cooper and Klopfenstein, 1996), 5.8 (Beauchemin et al., 2003), and 6.0 (Kriehbiel et al., 1995), in order to define SARA (Gozho et al., 2005). Additionally, the duration for which the rumen pH must remain below this threshold has not been properly defined across studies.

Reducing the forage chop length reduced the $peNDF_{PS}$, $peNDF_{>1.18}$, $peNDF_{M}$ and $peNDF_{NDF}$, but diets did not affect the rumen pH. Hence, we could not find a correlation between different measures of physically effective fibre and rumen pH. This is similar to the findings of Krause & Combs (2003). Other researchers (Beauchemin et al., 2003; Calberry et al., 2003) found a positive correlation between the $peNDF_{NDF}$, $peNDF_{PS}$ with rumen pH. This discrepancy may be attributed to the coarseness of the diets and higher physically effective fibre levels of the diets used in our study compared to those used in studies by Beauchemin et al. (2003) and Calberry et al. (2003). The narrow forage particle size range (5.8-2.7 mm) used by Krause and Combs (2003) might explain the absence of correlation between the physically effective fibre and rumen pH in their study, even though the diets included in their study were finer than those used in our study. Calberry et al. (2003) reported a decrease in rumen pH with reduction in dietary particle size by adding chopped alfalfa hay to a coarse corn silage based TMR. This could be explained by factors other than particle size, such as intrinsic buffering capacity, which affects rumen buffering. Corn silage has a lower intrinsic buffering capacity than alfalfa silage (Mc Burney et al., 1983), facilitating a lower rumen pH, which might explain the effect reported by Calberry et al. (2003), despite the coarser diets in other studies.

Forage chop length did not affect the total rumen VFA as well as the individual VFA concentrations (Table 4.11). Kononoff & Heinrichs (2003b), Beauchemin et al. (2003), Plaizier (2004), and Soita et al. (2003) reported similar results. Other researchers (Calberry et al., 2003; Kononoff & Heinrichs, 2003a; Kononoff et al., 2003a) reported an increase in the total VFA concentration with reduction in forage particle size, whereas, Yang et al. (2001a) and Leonardi et al. (2005) reported a decrease in VFA levels due to reduction in forage chop length. Reduction in particle size increases the surface area of the particles for the microbial attachment which can increase the ruminal digestion and VFA production. However, reduction in particle size may not result in increased ruminal digestion and VFA production because of increased particulate passage rate and decreased digestibility (Soita et al., 2003). Other researchers (Yang et al., 2001a, 2001b; Krause et al., 2002a) reported a decrease in liquid passage rate and volume of liquid digesta in the rumen due to decreased saliva production with the reduction in particle size. This may result in increased VFA concentration provided the production and absorption of VFA are not affected.

Rumen fluid volume and passage rate of liquid were not affected by the forage particle size in this study (Table 4.12). Values for these measures were similar to that reported in other studies (Krause et al., 2002a; Leonardi et al., 2005). Increasing the dietary particle size increases the dietary physically effective fibre levels (Calberry et al., 2003; Beauchemin et al., 2003; Beauchemin and Yang, 2005) and affects the ruminal pH by increasing saliva flow. This increased saliva flow can dilute VFA thereby reducing VFA concentration (Allen, 1997). Nocek and Russell (1988) reported a wide range (29 to 90%) of variation in ruminal degradation of DM across feeds and further, (Allen, 1997)

reported that within feed type variation in ruminal degradation of DM is much less than the variation across feeds. So, the variation of feed stuffs among different studies also explains the differences in VFA production in rumen. Increased DMI due to reduction in forage particle size was reported by Allen (2000), Kononoff et al. (2003), and Beauchemin and Yang (2005). Such an increase in DMI can also increase rumen digestion and the production of VFA. When reduction in particle size decreases rumen pH and induces SARA (Krause et al., 2002a, 2002b; Beauchemin et al., 2003; Calberry et al., 2003; Krause et al., 2003) results in decreased ruminal digestion of DM and NDF, which could also decrease VFA production (Allen, 1997; Plaizier et al., 2001). This shows that dietary particle size affects the ruminal VFA levels in many different ways. Hence, the discrepancies among studies were to be expected.

Reducing particle size did not affect the acetate to propionate ratio across diets (Table 4.11). This finding confirms results from studies by Leonardi et al. (2005), and Plaizier, (2004). However, decreased acetate to propionate ratio associated with decreased dietary particle size was reported in studies by Soita et al. (2002), Krause et al. (2002b), Krause and Combs, (2003), and Soita et al. (2003). Comparison of the above studies reveals that a greater increase of the acetate to propionate ratio was observed in studies, where decrease in the forage particle size resulted in reduction of rumen pH. This is expected as the decrease in rumen pH is associated with decreased cellulolytic bacteria correspond to low acetate production and result in low acetate to propionate ratio in the ruminal fluid. In our study and other studies by Plaizier (2004) and Leonardi et al. (2005), diets did not affect rumen pH, which might explain the absence of a dietary effect on the acetate to propionate ratio.

Reducing chop length of alfalfa and oat silage did not affect the rumen ammonia concentration (Table 4.11). Other researchers (Leonardi et al., 2005; Beauchemin et al., 2003) did not observe such an effect. Other researchers (Soita et al., 2003) reported increased ammonia concentration with increased dietary particle size and Einarson et al. (2004) reported a decrease in rumen ammonia concentration with decrease in forage chop length. The discrepancies in rumen ammonia concentrations among studies could be explained due to the differences in the dietary crude protein content among studies. A lower dietary crude protein content with short barley (10 mm) chop compared to long barley chop (19 mm) in study from Einarson et al. (2004) resulted in reduced rumen ammonia levels. Einarson et al. (2004) suggested that the variation in the dietary protein content has more effect on the rumen ammonia concentrations than differences in particle size. The absence of effect of dietary particle size on rumen ammonia levels found in current study and other studies (Beauchemin et al., 2003; Leonardi et al., 2005), could be explained by no difference in dietary crude protein levels across diets compared to study by Soita et al. (2003), who increased rumen ammonia levels with the increase in forage chop length.

### 4.4.5 Blood variables

Blood glucose, urea, and lactate concentrations were not affected across the diets (Table 4.13). The values for the blood glucose and blood urea were within the normal range of 40-80 mg/dl and 2.5-7.5 mmol/L, respectively (Dukes et al., 1993). However, the values for the blood lactate found in the all the diets were towards the lower limits of the normal range (1.25-5 mmol/L) (Dukes et al., 1993). The absence of effect of forage chop

**Table 4.13** Concentrations of metabolites in blood plasma for experimental diets containing a short (6 mm) or long (19 mm) chop lengths of alfalfa and oat silages.

| | Diet[1] | | | | | Effect[2] | | |
|---|---|---|---|---|---|---|---|---|
| Item[3] | ALOL | ALOS | ASOL | ASOS | SE | AC | OC | AC x OC |
| Glucose (mg/dL) | 68.2 | 65.0 | 67.6 | 68.1 | 1.14 | 0.13 | 0.11 | 0.03 |
| Lactate (mM/L) | 1.05 | 0.89 | 0.89 | 0.87 | 0.08 | 0.11 | 0.11 | 0.25 |
| Urea (mM/L) | 6.0 | 6.0 | 5.9 | 5.8 | 0.16 | 0.15 | 0.76 | 0.78 |

[1]ALOL = alfalfa long chop and oat long chop; ALOS = alfalfa long chop and oat short chop; ASOL = alfalfa short chop and oat long chop; ASOS = alfalfa short chop and oat short chop.
[2]AC = alfalfa chop effect; OC = oat chop effect; AC x OC = Interaction effect of alfalfa and oat chop lengths.
[3]Blood plasma variables were averaged for each animal during each period (n=16) for each treatment and analyzed by analysis of variance.

length on the blood urea could be explained by the absence of a dietary effect on the rumen ammonia concentrations. Acute acidosis is often associated with the decreased blood pH and increased concentration of lactate. Huntington et al. (1981) fed the lambs with the increasing amount of grains in a gradual fashion and reported a less dramatic response as well as comparatively lower values of the plasma lactate levels than normal. In the present study, the animals might have adapted to the high concentrate diet and resulting in lower plasma lactate levels compared to the normal.

### 4.4.6 Milk production and composition

Alfalfa and oat chop did not affect milk yield and milk composition (Table 4.7). These results agree with studies from Yang and Beauchemin (2005), Beauchemin et al. (2003), and Calberry et al. (2003). These authors reported no effect of forage chop length on milk yield and composition despite an increase in DMI. This could be due to the short duration of the experimental periods. However, expanding these periods would also have confounded the effect of forage particle size on milk yield and milk components with factors such as stage of lactation, pregnancy, and prohibited the use of a Latin square design. In this study, the average milk fat percent for all the diets were lower than the normal range (3.5-3.6) for the Holstein cows (WCDHIS, 2003). This low milk fat level could suggests the occurrence of the SARA but the mean rumen pH and the continuous rumen pH data found in this study do not show this. These low fat values may be attributed to the fact that the herd from which the cows for this trial originated has practiced selection for low milk fat content from past few decades.

In the literature, several theories account for the reduced fat percentage due to

small dietary particle size. A positive curvilinear relationship between the $peNDF_M$ and milk fat percent have been reported by Mertens (1997), with a greater reduction in milk fat percent at low $peNDF_M$ levels than at higher $peNDF_M$ levels. However, our study could not find a relationship between various measures of physically effective fibre and milk fat percent. This may be attributed to the fact that the physically effective fibre levels of all the diets were higher than the threshold below which further reduction of physically effective fibre affect the milk fat (Mertens, 1997). A reduction in rumen pH due to decreased low dietary physically effective fibre levels, can lead to increased synthesis of trans-10 C18:1 in the rumen (Griinari et al., 1998). These trans-fatty acids can reduce the de novo milk fat synthesis, leading a reduction in milk fat levels. However, in our study diet did not affect the rumen pH, which explains the absence of a diet effect on milk fat.

Another theory for reduction in milk fat levels is based on the reduction of acetate to propionate ratio. Acetate is a precursor for the de novo fat synthesis of the milk fatty acids. The low ruminal acetate levels can result in low milk fat levels due to decreased de novo fat synthesis in the deficiency of its precursor (Bauman and Griinari, 2001). This study failed to find an effect of the forage particle size on the acetate to propionate ratio and on total VFA, which might have contributed to the absence of effect of diets on the milk fat levels. Similar findings were reported in other studies (Beauchemin et al., 2003; Plaizier, 2004; Beauchemin and Yang, 2005), however, Krause and Combs (2003) reported a significant decrease in milk fat percentage with reduced forage chop length. Other researchers (Yang et al., 2001a; Calberry et al., 2003; Kononoff and Heinrichs, 2003b) reported numerical decreases in the milk fat percentage with

reduction in particle size. The low milk fat levels found by Krause and Combs (2003) may be attributed to short dietary particle sizes (<6 mm) and low rumen pH reported in their study compared to other studies (Beauchemin et al., 2003; Plaizier, 2004) that did not show such an effect. Furthermore, Krause and Combs (2003) explained the reduction in milk fat based on the diet composition in their study. Krause and Combs (2003) observed a greater reduction in milk fat with reduction in particle size for diets containing a mixture of alfalfa and corn silage, compared to diets that contain only alfalfa silage. This could be explained by the higher starch content and lower intrinsic rumen buffering capacity of the corn silage compared to alfalfa (Mc Burney et al., 1983), which results in greater need for rumen buffering for corn silage based diets compared to the alfalfa based diets. This might also explain the absence of any significant effect on milk fat percentages with reduction in dietary particle size reported in studies involving the affect of alfalfa silage chop length (Yang et al., 2001a; Beauchemin et al., 2003; Plaizier, 2004).

The lack of agreement regarding the effect of particle size on milk fat percent may be attributed to the fact that milk fat may be affected by many factors other than forage particle size, physically effective fibre such as intrinsic buffering capacity, intrinsic fragility of feed particles, inclusion of buffers, concentrate inclusion, rumen digestion rate, factors affecting rumen fermentation, rumen buffering, feed intake, and lactation stage (Beauchemin et al., 1994; Mertens, 1997; Allen, 2000; Plaizier, 2004). In addition to the complexity of these factors, the absence of a standard method for physically effective fibre determination, as well as variation in the range of particle size among studies complicates comparisons and complicates determining the relationship between particle size and milk fat percentage (Plaizier, 2004).

The particle size of alfalfa and oat silages did not affect the milk protein percent and protein yield (Table 4.7), although, the diets differed in the CP levels and both alfalfa ($P>0.06$) and oat (0.09) chops had significant effect on dietary crude protein levels. Ipharraguerre et al. (2005) varied the dietary CP levels from 18% DM to 14% DM and reported no affect on the milk protein, and total solids. Frank and Swensson (2002) reported a decrease in the amount of milk protein percentage but no influence on the production of protein or the percentage and yield of fat with the reduction in dietary CP levels from 17% DM to 13.5% DM. In present study the differences in the dietary CP levels were not large enough (Frank and Swensson, 2002; Ipharraguerre et al., 2005) to have an effect on the milk production and composition. Other researchers reported the similar findings (Beauchemin et al., 2003; Calberry et al., 2003; Beauchemin and Yang, 2005).

**4.5 Conclusions**

Reducing the chop length of alfalfa and oat silage from 19 mm to 6 mm decreased the proportion of TMR retained by the 19 and 8 mm screen of the PSPS from 75.6% for coarsest diet (ALOL) to 66.4% for finest diet (ASOS). Reducing alfalfa chop length did not affect DMI, chewing behaviour, milk production, rumen pH, rumen ammonia concentrations, total VFA as well as individual VFA concentrations. However, reducing chop length of oat silage from 19 to 6 mm increased DMI and but did not affect chewing activity, rumen pH, VFA, ammonia concentration and production parameters. Animals selected against the long feed particles and in favor of the finer feed particles. Different techniques for estimating physically effective fibre yielded different values

indicating that there is need for standardization and determination of the best indicator for rumen buffering. The diets included in this study were coarse and the dietary physically effective fibre was higher than the minimum recommendations, so, this study did not show a relationship between different measures of dietary physically effective fibre and the rumen pH. It is difficult to provide general recommendations for a theoretical length of cut. The effective forage particle size is also affected by other factors such as sharpness of the knives, dryness of the feed, fragility of the feed and the type of the harvester used, besides setting the theoretical chop length. So, it is recommended to determine the particle size distribution with PSPS immediately after chopping the first batch of forage in the field and compared it to the guidelines and if required chop length should be adjusted. It can be concluded that reducing alfalfa and oat forage chop length to 6 mm with 10% and 56% particles retained on 19 and 8 mm screens of PSPS, respectively, improves the silage quality and increases dry matter intake without inducing SARA in diets containing 52% of concentrate.

# 5.0 GENERAL DISCUSSION

Two experiments were conducted to determine the effect of the forage chop length on the dietary physically effective fibre, chewing activity, feeding behavior, rumen conditions, milk production, and metabolism of the dairy cows fed barley grain based total mixed rations. Different methods for determining the physically effective fibre available in the literature were compared to assess which of these is the best measure for the rumen buffering. In the first experiment, the effect of long (19 mm) and short (10 mm) chop lengths of alfalfa and corn silages were studied and in second study, a wider range of the forage chop lengths (long, 19 mm and short 6 mm) of alfalfa and oat silage were used. In first study, the effects of alfalfa and corn chop lengths were studied on physically effective fibre, intake, rumen fermentation, blood metabolites, and milk production. In second study more parameters were studied, including feeding behaviour, eating, and ruminating activities and water intake. In the second experiment continuous rumen pH monitoring was conducted along with the spot sampling once daily as performed in the first study.

In Experiment 1, the chop lengths of alfalfa silage and corn silage did not affect the chemical composition of the TMR and the silages. The pH of all silages was higher than 4.5, which was higher than recommended for well preserved silage (Kung and Shaver, 2001). In experiment 2, the diets differed in the dry matter, crude protein (CP), acid detergent fibre (ADF), and phosphorus concentrations. However, these differences were not large enough to affect the intake, rumen conditions and production of the cows (Allen, 2000). In experiment 2, reducing chop lengths of alfalfa and oats reduced silage

pH from 6.0 to 4.9 and from 5.4 to 4.6, respectively. The pH of the short chop of alfalfa and oat silages was higher that the recommended 4.5 (Kung and Shaver, 2001), but lower than the pH of the short chops of silages in experiment 1. This shows that reducing forage chop length to 6 mm increases the silage quality. This could be due to less compaction achieved with the coarser diets resulting in oxygen entrapment which negatively affects the silage fermentation.

In both the studies, the different measures of the physically effective fibre resulted in the different values. Across diets, the values for $peNDF_{M}$ and $peNDF_{>1.18}$ were similar but were consistently higher than $peNDF_{PS}$ and $peNDF_{NDF}$. The $peNDF_{M}$ is an arbitrary measure, as it is calculated based on the tabular values, which are subjective and discontinuous. The $peNDF_{>1.18}$ is calculated based on dry sieving of the TMR sample which results in shattering of feed particles and is not the true representation of the sample what is being offered to the animals. The comparison between the $peNDF_{PS}$ and $peNDF_{NDF}$ revealed that the $peNDF_{NDF}$ yielded consistently higher values than $peNDF_{PS}$. This difference between the two techniques is due to the different NDF contents in the different PSPS fractions. The top fraction of the PSPS had a higher NDF (average $\geq$ 50 % DM) compared to the other fractions (average $\leq$ 37% DM) in all the diets, which explains the higher values for the $peNDF_{NDF}$ compared to $peNDF_{PS}$ found in both studies. The values for the $peNDF_{NDF}$ and $peNDF_{PS}$ varied across the diets in both experiments and were significantly ($P<0.05$) higher for coarser diets compared to the finer diets. The $peNDF_{NDF}$ can be the best measure for determining the physically effective fibre under laboratory conditions and the $peNDF_{PS}$ can be an on-farm based technique for the

determination of the physically effective fibre, as NDF measurement of all PSPS fractions is not practical.

The chop length of alfalfa silage did not affect feed intake in experiment 1 and experiment 2. Reducing the chop length of corn silage from 19 mm to 10 mm increased dry matter intake in experiment 1 and reducing the chop length of oat silage from 19 mm to 6 mm increased dry matter intake in experiment 2. A reduction in forage chop length can increase feed intake when this intake is limited by physical fill of the rumen, as in such a situation a reduction in feed particles size will increase digesta passage rate through the rumen. The results indicate that chopping whole crop corn and oat silage at 19 mm creates excessively coarse silage that limited intake in cows, which might explain the higher intake with short chop of corn and oat in experiment 1 and experiment 2, respectively. Alfalfa silage cut at 19 mm did not appear to limit feed intake. This might be due to differences other than dietary particle size between alfalfa silages and grain crop silages including rumen digestibility. In both experiments, animal selected against the coarser feed particles in favour of finer feed particles as reflected from the particle size distribution of the TMR to their respective orts. Hence, dietary particle size distribution of ingested feed was different from that of the TMR offered to the cows.

Forage chop length did not affect the eating and rumination behaviour in experiment 2, so, this study could not demonstrate a correlation between different measures of physically effective fibre and chewing behavior. In the literature inconsistent results of forage particle size on the eating and ruminating activities have been reported. Many factors such as coarseness of the diets, dietary particle range, forage source, and concentrate source affect the chewing behavior of the animal. Diets in our experiment

were coarser than the diets used in the studies of Yansari et al. (2004), Beauchemin and Yang (2005), and Leonardi et al. (2005). This might explain why no effect of forage chop length on chewing behavior was found in our study, whereas the earlier studies reported such an affect. Allen (1997) reported that decreasing the forage particle size reduces the total chewing time curvilinearily and that the most dramatic reduction in chewing time occurs when particle size is reduced below 3 mm. However in our studies, the forages included were coarser than 3 mm, which might explain the absence of effect of forage particle size on chewing activity.

The feeding behaviour of the animals in our study revealed a considerable variation in meal sizes within a day and differences in eating behaviour among cows. Comparison of the eating time calculated from the Growsafe equipment and video taping, and feed intake (as fed basis) from Growsafe equipment and manual recording, revealed that Growsafe equipment yielded lower values for both the time spend eating and the amount compared to video taping and manual recording, respectively. This disparity could be explained by the subjectivity of the meal criteria used for analyzing the Growsafe data, as many small meals which fail to meet the set meal criteria fail to add to the total amount eaten and time taken resulting in the differences.

Alfalfa chop length did not affect the rumen pH in both the studies. However, we found increased rumen pH with the reduction in the chop lengths of corn silage in the first study and of oat silage in second study. This increased rumen pH found with the reduction of the short chop might be explained by differences in diurnal variation in rumen pH resulting in the time of lowest rumen pH among diets. With the continuous pH monitoring in the second trial no such affect was seen. Duffield et al. (2004) also reported

that 24 h continuous pH monitoring using the indwelling pH probes is better and provides a better representation of the rumen pH compared to the single oral probe sampling of rumen fluid.

The average rumen pH for all diets at 4 h after feeding was never less than 6.12 and the diets did not affect the rumen pH in both studies. Hence, we could not find a correlation between different measures of physically effective fibre and rumen pH. In the second experiment, continuous rumen pH monitoring revealed that animals did spend around 2 h/d with rumen pH less than 5.6. However, none of the diets resulted in SARA, defined as rumen pH values between 5.2 and 5.6 for more than 3 h/d (Cooper and Klopfenstein, 1996; Gozho et al., 2005). In the literature, conflicting result of forage particle size on the rumen pH have been reported. This disparity in results of forage chop length on the rumen pH among studies can be explained by the inclusion of finer diets in studies by Krause and Combs (2003) and Beauchemin et al. (2003) and a wide forage chop length range used by Soita et al. (2002) and Soita et al. (2003) compared to our study and other studies (Kononoff and Heinrichs, 2003; Einarson et al., 2004) which failed to find an effect of forage chop length on rumen pH. Mertens (1997) suggested a positive curvilinear relationship between the physically effective fibre and the rumen pH and reported a threshold (22% $peNDF_M$) below which reduction in physically effective fibre and dietary particle size result in rumen pH suppression. This explains why reduction in forage particle size in fine diets affects the rumen pH, but not in the coarser diets such as the diets included in our studies.

In both experiments the chop lengths of the forages did not affect milk production, despite increase in feed intake due to reduction in chop lengths of corn silage

and oat silage in experiment 1 and 2, respectively. This could be due to the short duration of the experimental periods. Increasing the length of these periods might have accentuated the effect of increasing feed intake on milk production. However, extending these periods would also have prohibited the use of a Latin square design. The Latin square balanced for residual effects was the design of choice for these studies, as four times the number of cows would have been required to achieve the same statistical power if randomized complete block design had been used.

Forage chop length did not affect the milk fat percent and yield in both experiments. Krause and Combs (2003) reported a reduction in milk fat with the reduction in forage particle size which was associated with SARA. In the present study, rumen pH did not drop to very low levels to affect the de novo milk fat synthesis which explains the absence of effect of rumen pH on milk fat. In both studies the average milk fat percent for all the diets were lower than the normal range (3.5-3.6) for Holstein cows (WCDHIS, 2003), which might suggest the existence of SARA in these animals. However, the mean rumen pH and the continuous rumen pH data found in these studies do not suggest the presence of SARA. These low milk fat values may be attributed to the fact that the herd from which the cows for this trial originated has practiced selection for low milk fat content from past few decades.

No effect of the forage chop length was found on milk protein percent and protein yield in both experiments. Einarson et al. (2004) suggested that differences in dietary protein contents among diets, rather than differences in dietary particle size, are responsible for differences in rumen ammonia. In the second experiment, diets differ in their crude protein content, but forage chop length did not affect the milk protein content.

This could be explained as the differences in dietary crude protein content among diets were not large enough to affect the intake and milk parameters. Allen (2000) reported that decreasing the crude protein content of diet of lactating animals has a minor effect on intake and milk parameters unless it is reduced less than 12% DM.

Reducing forage chop length of alfalfa, corn and oats affected the particle size distribution and reduced the physically effective fibre across diets. Chopping alfalfa and oats at 6 mm and corn silage at 10 mm using New Holland forage harvester model 790 with concentrate levels ranging 52-56.6 % (DM basis) in diets of high yielding dairy cows resulted in higher dry matter intake but did not affect the eating behavior, chewing activity, rumen parameters, and milk production. Reducing the alfalfa and oat chop length to 6 mm resulted in better ensiling and diets did not induce SARA or negatively impact the production and health of the high yielding dairy cows.

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# APPENDIX

**Appendix 1** Ingredient composition of energy supplement and protein supplement (%).

| Ingredient | Energy supplement | Protein supplement |
|---|---|---|
| Rolled barley | 55.8 | - |
| Luprosil salt (Calcium propionate) | 0.2 | - |
| Dairy supplement[1] | 40.0 | - |
| Vegetable oil | 4.0 | |
| Dried distillers grain | - | 42.0 |
| Fish meal | - | 7.0 |
| Canola meal | - | 22.7 |
| Soybean meal | - | 20.0 |
| Beet molasses | - | 3.0 |
| Niacin | - | 0.3 |
| Sodium bicarbonate | - | 5.0 |

[1]Protein pellets contain 46.1% soybean meal, 2.6% wheat shorts, 40.0% canola meal, 5.0% oat hulls, 0.3% pellet binder, 1.0% cane molasses, and 5.0% corn gluten meal.
[2]Dairy supplement contains 0.13% vitamin ADE premix (Vit A, 16800 IU/kg; Vit D, 2215 IU/kg; Vit E, 75 IU/kg, DM basis), 0.13% trace mineral premix, 2.6% soybean meal, 0.06% selenium, 39.1% wheat shorts, 5.0% distillers grain, 17.5% canola meal, 15.0% ground wheat, 1.7% dicalcium phosphate, 1.6% salt, 2.0% dynamite, 0.3% pellet binder, 1.0% cane molasses, 3.7% calcium carbonate, and 10.0% corn gluten meal.