Physically Effective NDF (peNDF) Requirements for Lactating Dairy Cows

A Thesis Submitted to

The Faculty of Graduate Studies

The University of Manitoba

By

Matthew Stephen Einarson

In Partial Fulfilment of Requirements for the Degree of

Master of Science

Department of Animal Science

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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ABSTRACT

Dairy cows require sufficient physically effective NDF (peNDF), i.e. fibre that stimulates rumen buffering, in order to prevent ruminal acidosis. Excess peNDF can limit intake. Variation in dietary peNDF can be the result of variation in the inclusion rates of forage and concentrate, as well as forage chop length. The objectives of this study were to examine the effects of: 1) replacing chopped alfalfa hay with alfalfa silage in barley grain based total mixed rations (TMR) and 2) chop length of barley silage when incorporated in a barley grain based TMR at two levels of concentrate inclusion, on dry matter intake (DMI), milk production and rumen fermentation. These objectives were achieved in three separate experiments. Experiment 1 diets contained 34.6% DM coarse barley silage, 51.8% DM concentrate, and 13.6% DM alfalfa, fed either as chopped hay, silage, or a 50:50 (DM basis) mix of hay and silage. Experiment 2 diets contained 30.5 % DM corn silage, 59.7% concentrate, and 9.8% DM alfalfa, fed either as chopped hay, silage, or a 50:50 (DM basis) mix of hay and silage. Experiment 3 TMR contained either 58.0 or 41.4% DM concentrate, with either short (10 mm) or long (19 mm) chopped barley silage. Replacing chopped alfalfa hay with alfalfa silage increased peNDF in experiment 1 and experiment 2, calculated as the proportion of the DM retained by the 8 and 19 mm screens of the Penn State Particle Separator multiplied by dietary NDF, from 16.2 to 21.9% and from 20.8 to 22.7% DM basis, respectively. Replacing chopped alfalfa hay with alfalfa silage reduced DMI tended to increase milk protein percentage and yield, but had no effect on milk yield, and rumen fermentation in experiment 1. Replacing chopped hay with alfalfa silage did not affect DMI, rumen fermentation and milk

production in experiment 2. Increasing chop length of barley silage increased peNDF from 24.6 to 29.5 % DM and from 18.8 to 21.5 % DM, respectively, at the lower and the higher concentrate inclusion, decreased DMI from 18.9 to 18.1 kg d^{-1} across concentrate levels, but did not affect milk production and rumen fermentation in experiment 3.

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I wish to express the most sincere gratitude to my supervisor, Dr. Kees Plaizier, for his guidance, advice, encouragement, and above all, friendship throughout my M.Sc. program. I am indebted to his support and abetment; for without which, I would have never succeeded. I would also like to extend my gratitude to my committee members, Dr. K. Wittenberg, Dr. A. Kennedy, and Dr. M. Entz for their helpful criticism of my experiments and thesis.

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I have to thank my family, above all, for whose love, support, and sacrifice have given me these great opportunities in life. I know I don't say it enough, but I thank and love you with all my heart.

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ABBREVIATIONS

Diets

H1	diet containing chopped alfalfa hay in experiment 1
HS1	diet containing both chopped alfalfa hay and alfalfa silage in experiment 1
S1	diet containing alfalfa silage in experiment 1
H2	diet containing chopped alfalfa hay in experiment 2
HS2	diet containing both chopped alfalfa hay and alfalfa silage in experiment 2
S2	diet containing alfalfa silage in experiment 2
HLC	higher concentrate, long chop diet in experiment 3
HSC	higher concentrate, short chop diet in experiment 3
LLC	lower concentrate, long chop diet in experiment 3
LSC	lower concentrate, short chop diet in experiment 3

Terms

Ac:Pr	acetate to propionate ratio
ADF	acid detergent fibre
ADIP	acid detergent insoluble protein
ASAE	American Society of Agricultural Engineers
BCS	body condition score
BW	body weight
Ca	calcium
cm	centimetre
CP	crude protein
d	day
DIM	days in milk
dL	decalitre
DM	dry matter
DMI	dry matter intake
eNDF	effective NDF
F:C	forage to concentrate ratio
g	gram
hr	hour
Hz	hertz
K	potassium
kg	kilogram
L	litre
LC	long chop barley silage

m	metre
MCP	microbial crude protein
mg	milligram
Mg	magnesium
min	minute
ml	millilitre
mm	millimetre
mM	millimol
MP	metabolizable protein
MPL	mean particle length
MY	milk yield
N	nitrogen
Na	sodium
NDF	neutral detergent fibre
nm	nannometre
NRC	National Research Council
NSC	non-structural carbohydrate
P	phosphorous
pef	physically effective factor
peNDF	physically effective NDF
$peNDF_{DM}$	proportion of DM retained by the 19 and 8 mm PSPS screens multiplied by dietary NDF
$peNDF_{NDF}$	proportion of dietary NDF retained by the 19 and 8 mm PSPS screens
PSPS	Penn State Particle Separator
RDP	rumen degradable protein
RUP	rumen undegradable protein
SARA	subacute ruminal acidosis
SC	short chop barley silage
SE	standard error
SP	soluble protein
TLC	theoretical length of chop
TMR	total mixed ration
USD	United States Dollar
VFA	volatile fatty acid
°C	degrees Celsius

INTRODUCTION

Energy nutrient demands of lactating dairy cows have dramatically increased in recent years as milk yields have increased (CDC, 2003). In 1990, dairy cows in Canada produced an average of 7, 412 kilograms of milk per cow (CDC, 2003). In 2000, dairy cows in Canada, produced an average of 9,152 kilograms of milk per cow (CDC, 2003). In order to meet their milk production potential, high producing dairy cows are typically fed high energy diets that are low in fibre, and high in starch, making them highly fermentable in the rumen. It is critical to balance high-energy diets with sufficient fibre, with an adequate particle length to stimulate chewing and salivary buffering of the rumen and prevent ruminal acidosis (Beauchemin and Rode, 1997).

A recent survey of dairy farms across the province of Manitoba (Plaizier et al., 2003) has indicated that ensiled forages are used more than other long hay in dairy cattle diets, and that the particle size of these ensiled forages is relatively short (50% of farms having >52.0% alfalfa and corn silage DM passing through both screens (19 and 8 mm) of the Penn State Particle Separator (PSPS)). This move to shorter particle lengths stems from a forage management point of view in that shorter particles ensile better than coarse particles (Khorasani, 1999). Feeding diets that are too coarse in length can also limit intake through physical fill of the rumen (Allen, 2000). Therefore, it is important to develop guidelines for dietary particle size and fibre content to insure that milk production potential is met, and rumen conditions are maintained. These guidelines need to be based on the chemical and physical nature of dietary fibre that influences chewing. One such measurement of these parameters is

physically effective NDF (peNDF), defined as fibre that stimulates chewing and rumen buffering (Mertens, 1997).

The term physically effective fibre was introduced to refine the concept of effective fibre (Mertens, 1997). Physically effective fiber relates solely to the physical characteristics of a feed (primarily particle size) and is an indication of the potential of a feed to stimulate chewing (Mertens, 1997). Thus, physically effective fibre differs from effective fibre in that effective fibre encompasses more factors, in its assessment of maintenance of milk fat percentage, than just chewing stimulation assessed by physically effective fibre.

Over the last six years of research on fibre and particle size, many researchers have devised several independent definitions of what constitutes physically effective fibre and physically effective neutral detergent fibre (peNDF), making comparisons between studies difficult. The NRC (2001) briefly touches on peNDF, but cites no recommendations due to the lack of a standard validated method for determining dietary peNDF.

There are no recommendations for dietary, and forage particle size distribution in the dairy NRC (2001) due to a lack of a standard validated technique, and due to the unknown interactions between forage and concentrate sources in a diet. The NRC (2001) recommends that 25% of DM constitute NDF, and of that, 75% should be NDF from forage to provide sufficient rumen buffering. Penn State University has developed guidelines for corn silage: 45 to 65% of silage material should remain on the middle (8 mm) sieve, 30 to 40% should be retained on the

bottom sieve (1.18 mm), and no more than 5% should pass through to the bottom pan of the PSPS (Heinrichs and Kononoff, 2002).

Current recommendations for optimum fibre levels and forage particle size available to producers in Western Canada were developed for diets based on corn grain rather than barley grain. There are several differences between barley and corn, such as a higher NDF content of barley (19 to 25%) than that of corn (7%), in addition to barley having a more rapid rumen fermentation rate (Beauchemin and Rode, 1997), leading one to deduce that fibre content and particle size recommendations would be different for barley grain based diets than for corn grain based diets. Beauchemin (1991) recommended that diets based on barley grain contain a minimum of 34% NDF in order to maintain a milk fat content of 3.5%. This recommendation exceeds the minimum amount of 25%, recommended by the NRC (2001) for corn grain based diets. Barley silage, like most small cereal silages, has a higher proportion of rumen fermentable carbohydrates such as starch, whereas grass or alfalfa silage provide proportionally more fermentable carbohydrate as NDF and, therefore, these two different sources are digested differently in the rumen (Charmley et al., 1996; Soita et al., 2003). Hence, guidelines for particle size distribution and fibre content developed for corn-based diets might not be applicable for barley-based diets that are commonly used in Manitoba.

The objectives of experiment 1 were to investigate rumen conditions and production of dairy cows fed coarsely chopped barley silage based TMR. Specifically, the effects of replacing alfalfa silage with chopped alfalfa hay on dietary particle size were considered. The objective of experiment 2 was to determine the

effects of increasing dietary peNDF content in corn silage based TMR fed to lactating dairy cows. Specifically, the effects of replacing alfalfa silage with chopped alfalfa hay on production and rumen conditions were considered. The objectives of experiment 3 were to study the effect of varying barley silage chop length on dietary peNDF, DMI, rumen fermentation and milk production when used with two barley grain inclusion rates.

LITERATURE REVIEW

Introduction

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Techniques for Determining Particle Size

Wet Versus Dry Sieving

Most particle size determination techniques for forages and TMRs are conducted by sieving on a wet, or as fed basis, but some methods are performed on a dry basis. Sieving on a wet basis would appear to be the more accurate measure of particle size distribution, because samples are of a comparable consistency to what the animal is actually fed. There have, however, been some adversaries to wet sieving because of the potential for sample moisture loss, which may occur during storage or transport, and for the potential of smaller particles to adhere to larger particles, misrepresenting true separation (Kononoff et al., 2003a).

The standard method for determining the particle size distribution of chopped forages is standard S424 of the American Society of Agricultural Engineers (ASAE) (Lammers et al., 1996). The ASAE device is a laboratory-scale separator of forage particle sizes, containing five screens of varying sizes and a bottom pan to separate

particles into six unique fractions, on a wet basis (Lammers et al., 1996). Currently, a generally accepted technique for assessing particle size distribution of TMRs is not available.

Although moisture content may affect sieving properties, Finner et al. (1978) stated that it is not practical to recommend analysis using the ASAE device at standard moisture content when field samples are taken. In association with this statement, Kononoff et al. (2003a) also stated that the Penn State Particle Separator (PSPS) is designed to describe the particle size of feed offered to the animal. Thus, it is recommended that samples should not be chemically or physically altered prior to sieving (Kononoff et al., 2003a). Kononoff et al. (2003a) found that small moisture losses of less that 40% of the original sample did affect particle size, but these differences, when observed, were small. Conversely, completely drying a sample resulted in large differences in particle size results (Kononoff et al., 2003a). These results are similar to Finner et al. (1978) who suggested that completely drying a sample results in shattering of particles and further size reduction during the sieving process. Therefore, sieving feeds on the same basis as that which is fed is more accurate.

American Society of Agricultural Engineers Standard for Particle Size Analysis

As previously mentioned, the ASAE laboratory-scale separator contains five sieves of varying sizes and a bottom pan, and was developed to assess the particle size distribution of chopped forages, although it is used for TMRs as well. The screens of the ASAE device have a width of 406 mm and a length of 565 mm (ASAE,

1992). The openings in the sieve screens are 19.0, 12.7, 6.3, 3.96, and 1.17 mm from the top to bottom screen respectively (ASAE, 1992). The particle distribution of the forage is calculated using a lognormal approach, and data is presented as both geometric mean and standard deviation (Finner et al., 1978). According to the ASAE standard (S424) (1992) the sieve stack should be driven with a frequency of 2.4 ± 0.08 Hz (144 ± 5 cycles/min).

Despite the fact that ASAE device is the standard for measuring particle size distribution of forages, it has met with some criticism (Lammers et al., 1996; Heinrichs and Kononoff, 2002; and Kononoff et al., 2003b). Nutritionists and farmers need a practical method that is rapid and inexpensive for regular use on the farm (Lammers et al., 1996). Because the ASAE is a cumbersome laboratory procedure, it is impractical for on farm use (Heinrichs and Kononoff, 2002).

Variations of the ASAE method exist. Yang et al. (2001a) used an Analysette 3 vertical oscillating sieve shaker to measure particle size distributions of feeds and TMRs on a dry basis. In this method, six sieves, with mesh sizes of 9.5, 6.7, 3.35, 1.18, 0.6 and 0.15 mm were used in addition to a bottom pan. Mean particle length (MPL) was used to represent sample distribution. Mean particle length was calculated as the particle length for which 50% of the cumulative percentage weight of the sample was retained (Yang et al., 2001a). Using MPL as a measure of particle length has its limitations in that it does not indicate variation around the average, or range of the sample being measured.

Penn State Particle Separator

The Penn State Particle Separator (PSPS) was designed to mimic the ASAE laboratory-scale separator, but on a quick and cost-effective basis (Lammers et al., 1996). The manually operated PSPS is constructed out of two sieves and a bottom pan (Heinrichs, 1996). Apertures of the two sieves measure 19.0 and 8.0 mm with a thickness of 12.2 and 6.4 mm (Heinrichs, 1996). Because of its simple construction and size, the PSPS sieving method may be implemented on farm, making it more versatile than the automated shaker methods.

It is recommended that approximately 1.4 ± 0.5 L of wet sample be placed on the top screen of the PSPS for proper analysis (Lammers et al., 1996). On a flat surface, the separator is shaken horizontally fives times in one direction, then rotated one-fourth turn, and again shaken five times, for a total of 8 turns (Heinrichs, 1996). Kononoff et al. (2003b) recommends that the PSPS be shaken at 1.1 Hz (66 cycles/min) or greater with a stroke of 17 cm. Because the PSPS is manually operated, occasional differences between devices and operators exists (Lammers et al., 1996).

In a recent survey of 831 TMR samples, Heinrichs et al. (1999) reported an average of 57.7% of the material passes through both sieves of the PSPS. Better characterization of these smaller feed particles required that an additional sieve be added (Kononoff et al., 2003b). As a result of this observation, a sieve with 1.18 mm apertures was incorporated in the PSPS device for a more detailed measurement (Kononoff et al., 2003b). It has been suggested that 1.18 mm is the critical length

governing retention in the reticulo-rumen, in that in order for particles greater than 1.18 mm to pass out of the rumen they would have to be reduced through mastication (Mertens, 1997).

Effective Fibre

Long forage particles in dairy cattle diets promote chewing and salivary buffering of the acids produced as a result of microbial feed fermentation (Beauchemin, 1991). Thus, particle size of forages can have a significant impact on rumen conditions, digestion, and animal production. The term effective fibre, measured as effective neutral detergent fibre (eNDF), attempts to quantify the role of fibre in ruminant diets.

Effective fibre is defined as the total ability of a feed to replace forage in a ration, so that milk fat percentage is maintained (Mertens, 1997). Therefore, dietary eNDF values are dependant upon particle size, intrinsic buffering capacity, fermentation rate, and other inherent characteristics (Beauchemin and Yang, 2003). Effective fibre values can be determined by conducting an animal feeding study in which standard forage is replaced by a test forage, and the response in milk fat measured (Armentano and Pereira, 1997). The feed is then assigned an eNDF value (expressed as a proportion of the NDF content of the feed) relative to the standard feed which has an index of 1.0 (Armentano and Pereira, 1997). Others have used variables such as rumen pH to assess eNDF (Pitt et al., 1996).

In common practice, when conducting a feeding study is impractical, eNDF values are drawn from tabular indexes based on previous feeding studies, to be used

in ration formulation (Beauchemin and Yang, 2003). This affects the accuracy of determining the effectiveness of forage fibre. In addition to this, there is no standardized method of assessment for the "standard" forage used to determine test forage fibre effectiveness, thus making the assignment of eNDF values to particular feeds arbitrary (Beauchemin and Yang, 2003). The use of rumen pH to assess eNDF is flawed because ruminal pH is not determined by the fibre content of the diet alone.

Physically Effective Fibre

Physical characteristics

The term physically effective fibre was introduced to refine the concept of effective fibre (Mertens, 1997). Physically effective fiber relates solely to the physical characteristics of a feed (primarily particle size) and is an indication of the potential of a feed to stimulate chewing (Mertens, 1997). Thus, physically effective fibre differs from effective fibre in that effective fibre encompasses more factors, in its assessment of maintenance of milk fat percentage, than just chewing stimulation assessed by physically effective fibre.

Over the last six years of research on fibre and particle size, many researchers have devised several independent definitions of what constitutes physically effective fibre and physically effective neutral detergent fibre (peNDF), making comparisons between studies difficult. The NRC (2001) briefly touches on peNDF, but cites no recommendations due to the lack of a standard validated method for determining dietary peNDF.

Physically effectiveness factor

Mertens (1997) developed a system to assess the physical effectiveness of feeds that is based on chewing activity. A forage or diet's physically effectiveness factor (pef) is measured by assessing its ability to promote chewing, and is expressed in an index from 0 to 1.0 (Mertens, 1997). By definition, pef varies from 0, when NDF is not effective, to 1.0 when NDF is fully effective in promoting chewing (Mertens, 1997).

This system is limited by the fact that using chewing time to indicate the physical effectiveness relies on book values. Individual feed samples may not exactly fit the tabular values, decreasing the accuracy of this measurement. To compensate for this fact, Mertens (1997) developed a laboratory method to measure physical effectiveness based on sieving. According to Mertens (1997) the sieving method is based on the concept that the long particles retained on the sieves represent particles that require chewing by the animal. Mertens (1997) suggested that pef be determined using a 1.18 mm screen, indicating that the particular aperature size is the critical length governing retention in the reticulo-rumen. Lammers et al. (1996) used the PSPS to determine pef values of feeds, where the percentage of dry matter retained on the top two sieves (>8 mm) was considered physically effective. However, values determined by Mertens (1997) and Lammers (1996) cannot be compared with one another due to differences in techniques in measuring peNDF.

Physically effective Neutral Detergent Fibre

In addition to manipulating dietary particle size to alter chewing and ruminating time, dietary fibre content can also be manipulated, with similar effect (Beauchemin et al., 1994). Generally, total chewing time (eating and rumination) increases approximately 2.5 to 3 h/d for every 1 kg/d increase in NDF intake (Beauchemin and Yang, 2003). Because of this inherent relationship, Mertens (1997) recommended multiplying the pef of the diet by its total NDF content to calculate peNDF. Due to the fact that this calculation of peNDF relies on pef values, it is limited by the inaccuracy of having to compare sample forages with tabular indexes.

Many studies have recently been conducted where peNDF has been calculated as the percentage of dry matter retained on the top two PSPS sieves (>8 mm), multiplied by total dietary NDF content (Yang et al., 2001a; Beauchemin et al., 2002; Yang et al., 2002; Beauchemin et al., 2003). This system is based on analysis of forages, and eliminates tabular comparison.

Mertens (1997) suggested that 1.18 mm is the critical particle length governing retention in the reticulo-rumen, indicating that in order for particles greater than 1.18 mm to pass out of the rumen they would have to be reduced through mastication. As a result of this comminution, these particles would stimulate more saliva secretion than those less than 1.18mm (Mertens, 1997). Taking this particular particle length into consideration, several studies have calculated peNDF as the percentage of dry matter retained on a 1.18 mm screen multiplied by total dietary NDF content (Kononoff et al., 2000; Kononoff and Heinrichs, 2003a; Kononoff and

Heinrichs, 2003b; Kononoff et al., 2003a). In order to facilitate this measurement, a 1.18 mm screen was added to the PSPS (Heinrichs and Kononoff, 2002).

More recently, Calberry et al. (2003) found that NDF content differed significantly among the various PSPS fractions. The top two sieves (>8mm) had much more NDF content than the bottom pan (Calberry et al., 2003). Previous calculations of peNDF assumed uniformity of NDF over PSPS fractions. Because this is not the case, calculations using total dietary NDF content discount the actual dietary peNDF content due to the reduced levels of NDF in the bottom fraction. Therefore, Calberry et al. (2003) made the recommendation that peNDF content be calculated as the proportion of NDF retained on the top two sieves (>8 mm) of the PSPS.

Comparison of various peNDF calculations

It can be assumed that because of the many variations in peNDF calculations, that different measures of peNDF will have varying effects on total chewing time, salivary output, and rumen pH. In an attempt to qualify these variations, research has been conducted on the correlation between various peNDF calculations and the previously mentioned parameters in the form of Pearson correlations (Yang et al., 2001a; Beauchemin et al., 2003). Yang et al. (2001a), and Beauchemin et al. (2003) both calculated peNDF three ways: 1) proportion of DM retained on both sieves of PSPS (>8 mm) multiplied by total NDF content = $peNDF_{PS}$ 2) pef, based on chewing determined from Mertens (1997), of steam-rolled barley (index of 0.7), multiplied by

total NDF content = $peNDF_M$ 3) proportion of DM retained on a 1.18mm screen multiplied by total NDF content = $peNDF_{1.18}$.

Beauchemin et al. (2003) found that both $peNDF_M$ and $peNDF_{1.18}$ were significantly correlated to total chewing time (min/d), and $peNDF_{PS}$ was not, with $peNDF_{1.18}$ having the highest correlation. For ruminating time (min/d) $peNDF_M$ was correlated the highest, and $peNDF_{PS}$ was the lowest. However, $peNDF_{PS}$ was significantly correlated to eating salivary output L/d, and the other two measurements were not. Both Yang et al. (2001a) and Beauchemin et al. (2003) found no significant correlations between the three peNDF calculations and rumen pH. Based on these two studies, it is impossible to determine which measure of peNDF is most related to chewing/ruminating, and rumen buffering. However, as $peNDF_{PS}$ was correlated with saliva output, it should provide an indication of rumen buffering capacity of a feed.

Subacute Ruminal Acidosis

Most rumen disorders involve some disruption of the balance and control of the internal rumen environment as a result of the sudden introduction of feed to which the rumen microflora and epithelium are unaccustomed (Van Soest, 1994). As a result, ruminal pH is substantially reduced by excessive VFA production and accumulation, which can overwhelm the ruminal and the host system (Nocek, 1997). Acute acidosis, characterized by a dramatic reduction in ruminal pH (<5.0) and a large increase in lactic acid production, results in very sick animals (Nocek, 1997). Acute acidosis presents specific signs and symptoms, and is a recognized disease in dairy herds. These symptoms include: off-feed, sudden drop in milk, malodorous

diarrhoea, and, possibly, sudden death (Brand et al., 2003). However, symptoms of subacute ruminal acidosis (SARA) are much less overt, and many times go untreated (Garrett et al., 1999). Stone (1999) projected that the economic impact of SARA in a dairy herd is as high as $1.12/cow/day (USD). Because SARA goes virtually undetected and untreated, its presence in a dairy herd has a major impact on producers (Stone, 1999).

Subacute ruminal acidosis is defined as daily episodes of pH <5.5 for given periods of time that impair microbial function (Nocek, 1997; Garrett et al., 1999) and is often associated with feeding high grain diets, with insufficient peNDF, in transition cows, as well as cows at peak production. During the dry period, a mainly forage based diet is fed, reducing the absorptive capacity of the rumen by 50% (Dirksen et al., 1984). It takes several weeks for the absorptive capacity of the rumen to return to normal (Dirksen et al, 1984). Because of the reduced absorptive capacity, the rumen tends to accumulate VFA, dropping the pH. The major clinical expression of SARA is reduced or inconsistent feed intake (Nocek, 1997). Other associated indications include decreased efficiency of milk production, milk fat depression, poor body condition despite adequate energy intake, liver abscesses, and unexplained diarrhoea (Underwood, 1992; Nocek, 1997). Long-term effects of several severe acidotic episodes can ultimately lead to laminitis (Nocek, 1997). The symptoms of SARA occur typically as a result of feeding diets with a high starch content that are insufficient in peNDF. In contrast, high forage diets that promote maximum gut fill and that provide adequate physically effective fibre to maximize the buffering activity by stimulating rumination and salivation, reduce the prevalence of SARA, but often

limit the cow's energy intake capabilities resulting in decreased milk production (Brand et al., 2003). Therefore, it is important that a balance be met between energy in concentrate form, and buffering activity in the form of forage, to maintain rumen conditions.

Dietary Particle Size and Fibre

Chewing and Saliva Production

Ruminants require a minimum amount of large forage particles in their diets to maximize production and to maintain their health by sustaining a stable environment in the rumen (Allen, 1997). The ability of forages to stimulate chewing has been investigated extensively because of the relationship between chewing and salivary buffering of fermentation acids in the rumen (Allen, 1997). The physical form of the diet affects the amount of time an animal spends chewing during eating and ruminating (Beauchemin and Buchanan-Smith, 1989). Mertens (1997) suggested that 1.18 mm is the critical particle length governing retention in the reticulo-rumen. Therefore, particles longer than this length require more chewing to pass on to the omasum. High yielding dairy cattle diets often consist of large amounts of non-structural carbohydrates that are readily fermentable and cause a rapid decline in ruminal fluid pH. This rapid decline in rumen pH is caused by an accumulation of VFA due faster production rates, than absorption. It is, therefore, important that adequate dietary particle length be maintained to ensure sufficient salivary buffering of the rumen through chewing longer forage particles (Beauchemin and Buchanan-Smith, 1989).

Saliva is by far the most important mechanism for removal of hydrogen ions from rumen fluid (Allen, 1997). Saliva contains bicarbonate and hydrogen phosphate ions that remove hydrogen ions from solution by a combination of alkalization and buffering. Saliva composition has been reported to be relatively constant and not greatly affected by diet or feed intake (Erdman, 1988). Saliva flow rates increase quadratically with increasing chewing activity, in that it increases until a point, when the effect of chewing on saliva production is diminishing. Cassida and Stokes (1986) measured resting and eating saliva flow with multiparous Holstein cows in early lactation. Mean saliva flow rate (ml/min) during eating were 15% higher than during resting, and 100% higher during ruminating (Cassida and Stokes, 1986). Therefore, the longer cows chew, the more saliva is produced.

Salivary secretion per minute of mastication is relatively constant, and is not usually affected by diet (Cassida and Stokes, 1986). Therefore, its important to increase time spent chewing to improve salivary secretion. Beauchemin et al. (2003) found that increasing dietary particle size, by increasing the proportion of alfalfa hay relative to alfalfa silage, in the diet of cows required more time for eating. Maekawa et al. (2002) also found that chewing time, as well as ensalivation rate, linearly increased with increasing proportion of forage in the TMR. Krause et al. (2002b) also found that time spent eating and ruminating increased with increasing forage particle size.

The NRC (2001) recommends a minimum particle length of 3 mm for alfalfa hay to maintain chewing activity and rumen pH. Diets using alfalfa forage source that had a mean particle length less than 3 mm resulted in reduced time spent chewing

(Beauchemin et al, 1994b). According to Allen (1997), there is a clear break point at approximately 3 mm in alfalfa particle length at which point no further increase in particle length affected total chewing time.

The NRC (2001) recommends a minimum of 25 to 28% NDF for cows in early lactation and higher concentrations in later lactation with 75% of the dietary NDF supplied from forages. Beauchemin (1991) found that total time spent chewing each day, and the number of chews increased linearly as dietary NDF concentration increased from 31 to 37%, mainly attributed to an increase in total time spent eating. As the proportion of NDF in a diet increases, each unit of dry matter is chewed for a longer period of time (Beauchemin, 1991). Beauchemin et al. (1994b) also found that as the amount of fibre in the diet increased, so did the time spent chewing and ruminating, as well as the number of chews during eating.

Though it is difficult to compare studies due to differences in particle size determination and variation in feed ingredients, it can be concluded that dietary particle size and fibre content are positively related to chewing and saliva production. By increasing the proportion of peNDF in high-energy rations fed to lactating dairy cows, chewing and saliva production are increased, and the natural buffers contained within the saliva help to moderate rumen pH, which might otherwise be low and adverse.

Rumen pH

Physically effective fibre stimulates chewing activity, which in turn stimulates saliva secretion (Allen, 1997). Bicarbonate and phosphate buffers in saliva neutralize

acids produced by the fermentation of organic matter in the rumen. The balance between production of fermentation acids and buffer secretion is a major determinant of ruminal pH (Allen, 1997). Finding a proper balance is particularly important in transition cows. During the dry period, a mainly forage based diet is fed which reduces the absorptive capacity of the rumen by 50% (Dirksen et al., 1984). It takes several weeks to adapt to high concentrate diets, making the transition from high forage diets to high concentrate diets difficult (Dirksen et al, 1984). Because of the reduced absorptive capacity during the dry period, cows are at risk of SARA because the rumen tends to accumulate VFA, dropping the pH when high concentrate diets are introduced after freshening. Low rumen pH can impair feed intake and milk production; therefore, diets should be formulated with adequate particle size and fibre content to maintain adequate rumen pH. Generally speaking, as dietary particle length and fibre content increase, risk of SARA is reduced.

The type of dietary forage source chosen has an effect on rumen pH. McBurney et al. (1983) found that corn silage has a lower intrinsic buffer capacity than alfalfa silage or alfalfa hay. Therefore, lower rumen pH and an increased risk of SARA is more likely to be expected in corn silage based diets compared with alfalfa-based diets.

Mertens (1997) indicated the presence of a plateau, or a threshold, above which increasing dietary peNDF content would not affect rumen pH. Evidently, due to the many variations in peNDF determination and factors affecting rumen pH, the level at which this occurs is difficult to define and will depend on dietary factors such as forage source, concentrate source, and inclusion of buffers. According to

calculations by Mertens (1997), the effect of peNDF on rumen pH is diminished when dietary peNDF is above 22%, as rumen pH reaches its plateau.

Allen (1997) found that forage particle length was more influential on the range of in ruminal pH than dietary NDF contents. In a study by Krause et al. (2002b), mean rumen pH decreased from 6.02 to 5.81 when mean forage particle size was reduced from 3.7 to 3.0 mm. Beauchemin et al. (2003) also found that when dietary particle size was reduced by substituting ground hay for chopped hay, mean rumen pH significantly decreased, in addition to significantly increasing the time spent below a pH of 5.8. Calberry et al. (2003) found a significant decrease in rumen pH from 6.47 to 6.27 when the amount of DM passing through the top two screens (19 and 8 mm) of the PSPS was increased from 55.2 to 61.9%. A study was conducted on six ruminally fistulated steers, by Soita et al. (2002), where rumen pH was significantly decreased from 6.46 to 6.27 when barley silage theoretical chop length was reduced from 18.8 to 4.7 mm.

Maekawa et al. (2002) found that mean rumen pH was elevated when forage NDF was increased from 28.3 to 32.2%. The amount of time under pH of 5.8 (h/d) was significantly reduced from 11.2 to 8.4 in diets with a forage to concentrate ratio of 40:60 and 60:40, respectively (Maekawa et al., 2002). This is consistent with Beauchemin et al. (1994b) who found that rumen pH was significantly lower in diets with a 32% NDF compared to diets with 40% NDF. Beauchemin (1991) also found that as dietary NDF content increased from 31 to 37%, mean rumen pH increased from 5.63 to 6.08, when early bloom stage alfalfa hay was fed and from 5.57 to 5.92 when midbloom stage alfalfa hay was fed.

It is fairly difficult to draw conclusions regarding the effect of peNDF on rumen pH, not only due to differences in methods of peNDF determination, particle size determination, and forage source, but also due to differences in rumen fluid collection techniques (Keefe and Ogilvie, 1997; Duffield et al., 2000). Rumen pH fluctuates substantially throughout the day. It is then important in large production trials when only one sample is taken per cow, per day, that it is sampled at a fixed time, after feeding. Continuous rumen pH monitoring is recommended, but might not be suitable for production trials with large animal numbers (Keunen et al., 2002). With continuous data collection, other calculations including area under the curve, and time below a certain pH, can be performed. One conclusion can, however, be drawn: the effect of peNDF is greater in fine diets compared to coarse diets, and greater in diets with low intrinsic buffering capacity compared to diets with high intrinsic buffering capacity.

Volatile Fatty Acids

Volatile fatty acids are short-chain fatty acids that are produced in the rumen, derived from feedstuffs as end products of organic matter fermentation (Allen, 1997). Carbohydrates normally constitute the major proportion of the DM of dairy cattle diets, and the extent of carbohydrate fermentation is variable amongst particle lengths and fibre contents (Allen, 1997). Variation in ruminal fermentation within feeds tends to be higher for fine, nonforage fibre sources, compared to coarse forage fibre sources because of differences in ruminal retention time, which are probably affected by ruminal consistency (Grant, 1997). In addition to this, feeding high concentrate diets

tends to cause more diurnal variation in rumen pH (Plaizier et al., 1999). High forage diets tend to produce less total VFA because the total fermentation of non-structural carbohydrates is higher and occurs faster than structural carbohydrates, allowing for less accumulation of VFA (Van Soest, 1994).

It has been suggested that diets that increase chewing time and saliva flow may lower the concentration of VFA because saliva flow has a dilution effect and increases the turnover rate of rumen liquid (Krause et al., 2002b). In support of this, Krause et al. (2002b) found that total ruminal VFA concentration decreased with increasing particle size. Ruminal proprionate concentration decreased with increasing forage particle size, causing an increase in acetate to propionate ratio (Krause et al., 2002b). In a study conducted on ruminally fistulated steers, Soita et al. (2002) found that the TLC of barley silage was reduced from 18.8 to 4.7 mm, total VFA concentration increased significantly, and acetate to propionate ratio significantly decreased. Total concentration of VFA was increased with a reduction in particle size of both alfalfa silage (Kononoff and Heinrichs, 2003a) and corn silage (Kononoff et al., 2003a). Similar to the previously mentioned studies, Kononoff and Heinrichs (2003a) found that acetate to propionate ratio also significantly decreased with a reduction in forage particle size. The study by Kononoff et al. (2003a) differs from the previously mentioned studies in that the highest molar proportion of acetate and the lowest molar proportion of propionate were observed on diets of intermediate particle length, possibly as a result of two competing effects, namely starch digestibility and fibre intake. Kononoff et al. (2003a) speculated that a lower proportion of acetate and a higher proportion of propionate on diets of shortest

particle size may have been due to increased starch digestibility, which may have been associated with reducing corn silage particle size (Johnson et al., 2003).

In contrast to these previously mentioned studies, Yang et al., (2001a) found that when the amount of DM passing through the top two sieves (19 and 8 mm) of the PSPS was increased from 60.9 to 67.5%, total VFA concentration decreased from 117.6 to 110.8 m*M* /L. No effect was observed on individual VFA proportions, or acetate to propionate ratio.

Beauchemin (1991) found that total VFA concentration increased linearly as dietary NDF concentration increased from 31 to 37%. Individual molar proportions of acetate, butyrate, isobutyrate, and isovalerate increased; proportions of propionate and valerate decreased as NDF concentration of the diet increased (Beauchemin, 1991). Consequently, the acetate to propionate ratio increased linearly as fibre content of the diet increased. Beauchemin et al. (1991) also found that as forage to concentrate ratio increased from 35:65 to 45:55 and forage NDF intake increased from 2.8 to 3.8 kg/d, acetate to propionate ratio significantly increased. Yang et al. (2001a) found that acetate to propionate ratio increased significantly from 2.99 to 3.62 when dietary NDF content increased from 31.7 to 37.8%, despite the lack of an effect on total VFA concentration.

The effect of varying dietary particle size on total ruminal VFA content appears to be variable. Increasing the ratio of non-structural to structural carbohydrate inevitably decreases peNDF, and allows for more complete ruminal carbohydrate digestion, thus increasing the total VFA concentration (Soita et al., 2003). Non-structural carbohydrates are broken down faster in the rumen than structural

carbohydrates (Tamminga et al., 1990). Increasing the level of non-structural carbohydrates will only improve the completeness of digestion to a point, at which over accumulation of VFA occurs resulting in SARA and a reduction in ruminal digestion (Brand et al, 2003). Reducing particle size increases the surface area available for ruminal fermentation of carbohydrates, allowing for more complete digestion and total VFA production (Soita et al., 2003). A reduction in dietary particle size will only improve the completeness of digestion to a point, at which the lack of salivary buffering will depress rumen pH, reducing ruminal digestion (Brand et al., 2003).

There is a negative effect on propionate content when dietary particle size and fibre content are increased, which is consistent in most studies in the literature. When peNDF content is decreased by an increase in concentrate inclusion rate, there is a change in rumen microflora (Van Soest, 1994). There is a reduction in a fibre digesting fibrolytic bacteria, and due to an increase in available substrate, an increase in starch digesting, amylolytic bacteria (Van Soest, 1994). This shift in the rumen population results in more propionate-producing bacteria. Acetate producing fibrolytic bacteria are negatively affected by low rumen pH (Van Soest, 1994). Therefore, a reduction in peNDF resulting a reduction in rumen pH would cause a decrease in acetate to propionate ratio.

Dry Matter Intake

Dry matter intake is limited by physical fill in high forage diets, and by metabolic factors in high concentrate diets (Allen, 2000). Feed intake is regulated by

distension and hypertonicity in the reticulo-rumen, and effects of metabolic fuel stimulation of rumen epithelial receptors (Allen, 2000). Feeds with a rapid rate of fermentation are expected to result in shorter meal length and size (Illius and Jessop, 1996). The reticulo-rumen is generally regarded as the site at which distension most often regulates DMI of ruminants, by both volume and weight of the digesta (Allen, 1996).

According to Allen (2000) when distension in the reticulo-rumen limits DMI, decreasing forage particle size could result in increased DMI if the density of swallowed particles or the time available for rumination increases. Beauchemin et al. (1994b) reported that DMI was reduced nearly 3 kg/d when forage content was increased from 35 to 65% with diets containing long chop (10 mm TLC) alfalfa silage, but less than 0.5 kg/d with diets containing the short chopped (5 mm TLC) forage. Kononoff et al. (2003a) found that DMI was increased when corn silage particle size was reduced from a geometric mean length of 12.9 to 9.2 mm. Krause et al. (2002a) found no effect of mean dietary particle size from 6.2 mm to 2.9 mm on DMI. Beauchemin et al. (2003) and Soita et al. (2000) found no effect of altering forage particle size on DMI. However, Beauchemin et al. (2003) did see a positive relationship between dietary peNDF and DMI. This could be due to the fact that increasing the proportion of alfalfa hay relative to alfalfa silage decreased dietary content peNDF. Beauchemin et al. (1997) found that DMI was higher for cows fed alfalfa hay than for cows fed alfalfa silage. Belyea et al. (1985) reported that decreased forage particle size increased intake of cows fed only forage, but forage particle size had no effect on intake of cows fed forage plus concentrate because

ruminal fill was not a limiting factor for DMI. This fact is further evidenced by Soita et al. (2002), who found that DMI increased when forage particle size was reduced from 18.8 to 4.7 mm, in strictly forage diets, fed to steers.

The fibre component of feedstuffs is generally retained in the reticulo-rumen longer than other feed components and variation in NDF digestion kinetics can influence the filling effect of feeds over time (Allen, 2000). Changing the NDF content of a diet by substituting grain for forage should result in a cubic response in DMI; DMI increases until it is no longer limited by fill and decreases when limited by an excess of metabolic fuels (Allen, 2000). Beauchemin et al. (1994b) found that DMI significantly decreased by 1.6 kg/d when dietary NDF content was increased from 27.3 to 33.2%.

According to Allen (2000) the extent to which DMI of lactating dairy cows is regulated by distension in the reticulo-rumen depends upon the animal's energy requirement and the filling effect of the diet offered. Reductions in DMI with added inert fill of approximately 25% were observed only when cows were in a negative or slightly positive energy balance (Allen, 1996).

A negative relationship tends to exist between dietary fibre content and DMI, which is heavily supported by the literature. The effect of dietary particle size on DMI is variable in the literature. When chop length is reduced in high forage diets, DMI is increased. When forage and concentrate are both fed to lactating dairy cows, metabolic factors limit intake and there is minimal effect of altering dietary particle size. The effect of peNDF on DMI can be obscured by the fact that excessive dietary peNDF can reduce DMI by physical fill limitations, and insufficient peNDF can

reduce DMI by metabolic restraint. Further study is required to investigate the interaction between particle size and fibre content on DMI, as well as determining the range at which physical and metabolic limits are not restraining DMI.

Milk Yield

The maximal productive capacity of an animal will depend on its genetic potential and will vary over the animal's lifetime according to its age, physiological status, and climate (Illius and Jessop, 1996). Energy intake is a primary limitation on milk yield for high producing dairy cows and is determined by net energy content of the diet and DMI (Allen, 2000). The amount of lactose in milk is constant (Ensminger, 1993). Lactose in milk is largely responsible for the osmotic pressure exerted by milk, or the regulation of milk volume (Ensminger, 1993). Since particle size has no real bearing on lactose content, it can be assumed that particle size does not affect milk yield (Krause et al., 2002a; Krause and Combs, 2003). Altering dietary particle length, without altering level of concentrate, has no effect on milk yield (Soita et al., 2000; Yang et al., 2001a; Krause et al., 2002a; Beauchemin et al., 2003; Kononoff et al., 2003a; Kononoff and Heinrichs, 2003a).

The negative effect of dietary NDF content on milk yield is fairly well documented (Beauchemin, 1991; Beauchemin and Buchannan-Smith, 1989; and Beauchemin et al., 1994a). An increase in dietary NDF content is generally associated with a decrease in dietary energy, reducing the milk yield (Beauchemin et al., 1994a). Beauchemin et al. (1994a) found that when dietary NDF content was increased from 32 to 40%, milk yield dropped by 3.2 kg/d because of a reduction in dietary energy.

Beauchemin et al. (2003) found no effect of varying NDF content on milk yield, and attributed this to a lack of effect on DMI.

Milk Fat

Milk fat content is affected by many dietary and metabolic factors (Griinari et al., 1998). A reduction in the availability of acetic acid, an important milk fat precursor, will cause a reduction in milk fat content (Griinari et al., 1998). As mentioned previously, peNDF content has an effect on ruminal acetate to propionate ratio. An increase in circulating insulin levels causes preferential channelling of lipid precursors (namely acetate) to adipose tissue, away from the mammary gland, thereby reducing milk fat synthesis (Sutton, 1989; Griinari et al., 1998). According to Griinari et al. (1998) circulating insulin levels are increased when high concentrate diets are fed. Approximately fifty percent of milk fat comes from de novo synthesis (Ensminger, 1993). Trans-fatty acids have a negative effect on de novo milk fat synthesis (Griinari et al., 1998). When rumen pH decreases, possibly due to a reduction in peNDF content, the production of trans-fatty acids increases, and milk fat content decreases (Griinari et al., 1998). In addition to this, diets that are high in fat content inevitably contain high amounts of unsaturated fatty acids, that are converted to trans-fatty acids when rumen pH is low, and which will have a negative impact on milk fat production (Grinnari et al., 1998).

Sutton (1989) clearly identified the importance of roughage in maintaining milk fat concentration, which has been reiterated throughout the literature (Mertens, 1997). Sutton (1989) particularly emphasized the significance of physical structure as

opposed to the chemically determined "fibre". With the importance of the relationship between forage and milk fat content in mind, the term effective NDF (eNDF) was invoked to relate the sum total ability of a feed to replace forage or roughage in a ration so that the percentage of fat in milk produced by cows eating the ration is effectively maintained (Mertens, 1997).

The effect of forage particle size on milk fat content reported in the literature is variable. Beauchemin et al. (1994b) and Beauchemin et al. (1997) concluded that the effects of particle size on milk fat content were likely to be observed when NDF levels were below minimum requirements recommended by the NRC (2001). Yang et al (2001a) found that even though dietary NDF levels were adequate, milk fat content was significantly higher when mean particle length was increased from 5.6 to 7.0 mm. Beauchemin et al. (1997) found that cows fed short alfalfa silage (TLC of 5 mm) produced milk with lower fat percentages than cows fed long alfalfa silage (TLC of 10 mm). Krause et al. (2002a) found that even though alfalfa silage particle size was reduced from 13.6 to 3.7 mm, no reduction in milk fat content was observed. Considering that diets containing the shorter chopped alfalfa silage did have numerically lower milk fat percentages than diets containing the longer chopped alfalfa silage, a possible explanation may have been that the number of animals used in the trial may not have been adequate enough to detect differences in milk components caused by dietary treatments. Kononoff and Heinrichs (2003a) also did not find an effect of decreasing alfalfa haylage particle size on milk fat content. They suggest that this is most likely due to the fact that the diets used in the experiment met NRC (2001) requirements for NDF content.

The effect of dietary NDF content on milk fat content is fairly well documented in the literature. In a study using varying amounts of alfalfa hay and silage, Beauchemin et al. (1994b) found that milk fat content was substantially higher for cows fed diets containing 65% forage, rather than 35% forage. Beauchemin (1991) found that milk fat content increased as NDF concentration in the diet increased. Beauchemin et al. (1991) also found that when dietary NDF content increased, through feeding more fibrous feedstuffs, that milk fat content was increased.

The response in milk fat percentage to reducing forage particle size varies and depends on the actual particle size reduction due to chopping or grinding (Soita et al., 2000); therefore, an interpretation of studies using different methods to determine particle size distribution is difficult. When NDF content is lower than the recommended NRC (2001) levels, the likelihood of observing an effect of forage particle size on milk fat content is increased.

Milk Protein

The amount of protein in milk is affected by numerous dietary factors (Ensminger, 1993). Supply of metabolizable protein (MP) in the diet is positively related to milk protein yield (Ensminger, 1993). If the amino acid profile (the supply of individual amino acids) of the MP is not ideal for milk production, the amount of milk protein will be diminished (Clark et al., 1992). The effect of particle size on milk protein can be confounded, in that a reduction in dietary particle by an increase

concentrate level will also increase dietary crude protein content, possibly resulting in improved milk production (NRC, 2001; Kononoff and Heinrichs, 2003b).

The effect of forage particle size on milk protein was found to be variable. Many studies have observed no effect of altering forage particle size without changing concentrate levels, on milk protein content (Kononoff et al., 2000; Krause et al., 2002a; Kononoff and Heinrichs, 2003a; Kononoff et al., 2003a; Beauchemin et al., 2003; and Calberry et al., 2003). However, Kononoff and Heinrichs (2003b) saw an increase in milk protein by 0.03 kg/d when TLC was reduced from 22.3 to 4.8 mm. Additionally, Soita et al. (2003) that when TLC was reduced from 18.75 to 4.68 mm, milk protein increased by 0.04 kg/d. Kononoff and Heinrichs (2003b) speculated that the increase in milk protein as a result of reducing particle length was due to improved starch digestion, providing more energy for milk production.

A positive relationship exists between rumen pH and microbial growth, in that when pH drops, so does the population of most rumen microbes (Van Soest, 1994). Since reducing particle length can cause a reduction in rumen pH, a reduction in particle size could have a negative impact on microbial growth. According to Soita et al. (2003), a reduction in particle size causes a decrease in rumen digestion of carbohydrates, or substrate for microbial protein synthesis. Therefore, the total effect of particle size and rumen pH on MP supply depends on a balance between these two factors. Krause and Combs (2003) found that particle size had no effect on microbial nitrogen supply. However, no effect was observed of particle size on pH in this trial. There is a need for more research on the interactions between particle size, rumen pH, and microbial protein synthesis.

Considering altering NDF content is generally associated with a change in concentrate level, increasing dietary NDF content has a negative effect on milk protein synthesis (Beauchemin et al., 1994b). Beauchemin et al. (1994a) found that increasing dietary NDF content from 32 to 40% decreased milk protein percentage linearly. Beauchemin et al. (1994b) found that increasing forage inclusion from 35 to 65% caused a reduction in milk protein from 3.48 to 3.30%. Both studies attributed the reduction in milk protein to a reduction in dietary energy content. Milk protein production is increased as a result of an increase in metabolizable protein supply, due to increased MCP synthesis (NRC, 2001). Microbial crude protein synthesis is increased when there is an increase in fermentable organic matter for rumen microbes (NRC, 2001).

Summary

The importance of maintaining adequate dietary particle size distribution, and ensuring minimum fibre content, to prevent rumen acidosis by providing sufficient buffering, can not be emphasized enough given the increase in energy nutrient demands as genetic milk production potential of lactating dairy cows increases. The groundwork has been laid in determining trends in relationships between particle size and fibre, on chewing/salivation, DMI, rumen conditions, and milk production. Guidelines for peNDF need to be developed for dairy cow diets containing feeds commonly used in Manitoba, to ensure optimal rumen conditions, milk composition, and feed intake, as well animal health.

Insufficient dietary peNDF will reduce rumen pH, DMI, and milk fat content once peNDF drops below a critical level. Excess peNDF will reduce DMI once its increased beyond another critical level. Many dietary factors, such as forage source, concentrate source, inorganic buffer inclusion, will affect these levels.

Comparison of studies on the effect of particle size distribution on various aspects of dairy nutrition is quite difficult. The variety of techniques for assessing particle size distribution, as well as variation in forage source, disallows for comparison. A standard validated technique for measuring particle size distribution is required to facilitate generation of recommendations. Measuring peNDF content as the amount of NDF retained on the top two sieves of the PSPS appears to such a technique.

HYPOTHESES AND OBJECTIVES

Hypotheses

Altering particle size distribution of a diet, within a range indicative of production in Manitoba, by varying forage chop length, forage to concentrate ratio, and various inclusion rates of forages of variable chop lengths, affects rumen conditions, dry matter intake, and milk production of lactating dairy cows. Requirements of coarse feed particles for rumen buffering in barley grain based diets are affected by forage source.

Objectives

The general objectives of the thesis research were 1) to investigate rumen conditions and production of dairy cows fed coarsely chopped barley silage based TMR where specifically, the effects of replacing alfalfa silage with chopped alfalfa hay on dietary particle size, while maintaining consistent NDF content, were considered, 2) to determine the effects of increasing dietary peNDF content in corn silage based TMR fed to lactating dairy cows where specifically, the effects of replacing alfalfa silage with chopped alfalfa hay, while maintaining consistent NDF content, on production and rumen conditions were considered, and 3) to study the effect of varying barley silage chop length on milk production and rumen conditions at two concentrate inclusion rates.

MANUSCRIPT I

Effects of replacing chopped alfalfa hay with alfalfa silage in a barley grain based total mixed ration on production and rumen conditions of lactating dairy cows

ABSTRACT

The effects of replacing chopped alfalfa hay with alfalfa silage in two barley grain based total mixed rations (TMR) that contained either coarse barley silage (Experiment 1) or corn silage (Experiment 2) were evaluated. The TMR used in experiment 1 contained (DM basis) 34.6% coarse barley silage, 3.5% sunflower seeds, 33.6% commercial energy supplement and 14.7% commercial protein supplement. In experiment 2, the TMR contained (DM basis) 38.5% barley grain based energy supplement, 30.5% corn silage, 17.0% protein supplement, and 4.2% sunflower seeds. Diets varied in inclusion rates of chopped alfalfa hay and alfalfa silage. In experiment 1, diets either contained (DM basis) 13.6% alfalfa hay (H1), 6.8% alfalfa hay and 6.8% alfalfa silage (HS1), or 13.6% alfalfa silage (S1). In experiment 2, diets either contained (DM basis) 9.8% alfalfa hay (H2), 4.9% alfalfa hay and 4.9% alfalfa silage (HS2), or 9.8% alfalfa silage (S2). Crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and mineral content did not differ significantly among diets within each experiment. Replacing chopped alfalfa hay with alfalfa silage increased physical effective NDF (peNDF) in both experiments, as the proportion of the DM passing through the 8 and 19 mm screens of the Penn State Particle Separator (PSPS) was reduced ($P < 0.05$) from 60.7 to 47.4% (Experiment 1) and from 52.4 to 47.9% (Experiment 2). Increasing peNDF reduced ($P < 0.05$) dry matter intake (DMI), tended ($P < 0.1$) to increase milk protein percentage and yield, but had no effect on milk yield (MY), rumen pH, and rumen ammonia in experiment 1. Milk fat content was the highest in diet HS1. In the second

experiment, increasing peNDF, did not affect DMI, MY, milk composition, rumen pH and rumen ammonia.

Keywords: physically effective NDF, rumen pH, particle size, dairy cows

Abbreviation key: **H1** = diet containing chopped alfalfa hay in experiment 1; **HS1** = diet containing both chopped alfalfa hay and alfalfa silage in experiment 1; **S1** = diet containing alfalfa silage in experiment 1; **H2** = diet containing chopped alfalfa hay in experiment 2; **HS2** = diet containing both chopped alfalfa hay and alfalfa silage in experiment 2; **S2** = diet containing alfalfa silage in experiment 2; **MCP** = microbial crude protein; **MPL** = mean particle length; **peNDF** = physically effective NDF; **peNDF$_{DM}$** = proportion of DM retained by the 19 and 8 mm PSPS screens multiplied by dietary NDF content; **peNDF$_{NDF}$** = proportion of dietary NDF retained by the 19 and 8 mm PSPS screens; **PSPS** = Penn State Particle Separator; **SARA** = sub-acute ruminal acidosis.

INTRODUCTION

High concentrate, low fiber diets are fed to meet the energy nutrient demands of lactating dairy cows. Energy content is maximized by increasing the non-structural carbohydrate (NSC) content of a diet (NRC, 2001). Increasing dietary energy by increasing the ratio of NSC to structural carbohydrates has a limitation, as a minimum amount of fiber is required for salivary rumen buffering (Beauchemin and Rode, 1997). Insufficient rumen buffering capacity will have a detrimental effect on rumen conditions leading to a variety of metabolic disorders, including ruminal acidosis (NRC, 2001). Excess fiber in a ration results a lower energy density with intake limited by rumen fill, thus compromising production (Allen, 2000). This has brought forth an increase in research on measurements of rumen buffering capacity and minimum dietary fiber requirements.

Fiber quantity, as well as its physical characteristics, including particle size, affects rumen buffering capacity, utilization of the diet, and animal performance (Mertens, 1997). As a result, forage fiber stimulates rumen buffering much more that non-forage fiber (NRC, 2001). It is, therefore, imperative that a validated unit, or measure, for buffering capacity provided by a diet be established (Mertens, 1997). Physically effective NDF (peNDF) has been used to assess rumen-buffering capacity of a diet (Mertens, 1997). This measure reflects the ability of physical characteristics of fiber, mainly particle size, to stimulate chewing and saliva buffering in the rumen. The amount of peNDF in a diet is affected by forage chop length, concentrate:forage ratio, and dietary NDF content (Mertens, 1997). Mertens (1997) suggested that the

requirement for peNDF of dairy cows should be 22% of ration DM, in order to maintain an average rumen pH of 6.0, and 20% of ration DM to maintain a milk fat percentage of 3.4 in midlactation Holstein cows. The Nutrient Requirements of Cattle (NRC, 2001) recommends 25% of DM as NDF, of which 75% must be from forage sources. This ignores differences in rumen buffering capacity between forages due to differences in particle size and intrinsic rumen buffering capacity. The NRC (2001) provides no recommendations for peNDF inclusion, due to the lack of a standard, validated technique to quantify the physically effective properties of fiber in a diet. This suggests a need a validated technique.

The peNDF value of a feedstuff has been calculated by Mertens (1997) as the product of the NDF content of the feed, and its physical effectiveness factor. This factor is a tabular value, between 0 and 1.00, where long grass hay is equal to 1.00, and is fully effective in stimulating chewing activity, and where the NDF of a feedstuff with a value of 0 will be ineffective in promoting chewing (Mertens, 1997). There is a potential limitation to this system in that it assumes a constant physical effectiveness factor for individual feedstuffs grown and harvested in a wide range of management and environmental conditions.

Yang et al. (2002), measured peNDF as the proportion of DM retained by the top two Penn State Particle Separator (PSPS) sieves, multiplied by total dietary NDF content. This method is limited by the assumption that NDF is uniformly distributed over all particle sizes, and that chewing activity is equal for all particles retained by the PSPS sieves (Mertens, 1997).

The peNDF value of a feed has also been determined as the amount of NDF retained on the top two sieves of the PSPS (Lammers et al., 1996), multiplied by the respective DM% of the individual sieves. This method takes into account the variation in distribution of NDF throughout the PSPS.

Most recommendations for optimum fiber levels and physical effectiveness have been developed using corn grain based, and not barley grain based diets (Beauchemin et al., 1991; Beauchemin and Rode, 1997; Soita et al., 2002). There are several differences between barley and corn, such as a higher NDF content of barley (19 to 25%) than that of corn (7%) (Beauchemin and Rode, 1997). Also, barley starch is more rapidly fermentable than corn (McCarthy et al., 1989). As a result, Beauchemin (1991) recommended that diets based on barley grain should contain 34% NDF in order to maintain a milk fat content of 3.5%, which exceeds the amount recommended for corn grain diets (Mertens, 1997). This suggests that minimum peNDF requirements should be higher for barley grain diets versus corn grain diets. At this time there is insufficient information available to establish recommendations for peNDF (Yang et al., 2001a).

Due to differences observed between cereal grain silages and alfalfa silage in reticular motility, time spent ruminating, and number of chews per kg DM and kg NDF consumed (Okine et al. 1994), it is likely that saliva production and rumen buffering vary. Therefore, forage source needs to be considered in the determination of minimal physically effective NDF requirements.

The objectives of experiment 1 were to investigate rumen conditions and production of dairy cows fed coarsely chopped barley silage based TMR.

Specifically, the effects of replacing alfalfa silage with chopped alfalfa hay on dietary particle size were considered. The objective of experiment 2 was to determine the effects of increasing dietary peNDF content in corn silage based TMR fed to lactating dairy cows. Specifically, the effects of replacing alfalfa silage with chopped alfalfa hay on production and rumen conditions were considered.

MATERIALS AND METHODS

Experimental Procedures for Experiments 1 & 2

Twelve multiparious lactating Holstein cows, housed in a tie-stall barn at the Glenlea Research Station, University of Manitoba, were used in a 3 x 3 Latin square design with three 3-week experimental periods, for both experiments. Each experimental period consisted of 14-d of adaptation to the experimental diet and 7-d of data collection. Animals were cared for in accordance with the Canadian Council for Animal Care (CCAC) guidelines. Upon commencement of experiment 1, cows averaged 142 ± 62.3 days in milk (DIM), had an average body condition score (BCS) of 3.0 ± 0.40, and had an average body weight (BW) of 625 ± 76.7kg. At the start of experiment 2, cows averaged 165 ± 102.2 DIM, had an average BCS of 3.5 ± 0.34, and had an average BW of 700 ± 56.1 kg.

Cows were assigned one of three total mixed rations (TMR) (S1, HS1, and H1) during each experimental period for experiment 1 (Table 1). Each diet contained (DM basis) 34.6% coarse barley silage, 33.6% barley grain based commercial energy supplement, 14.7% commercial protein supplement, 3.5% sunflower seeds, and 13.6% alfalfa forage. Source of alfalfa forage was alfalfa silage and no chopped alfalfa hay in S1; 50:50 ratio of alfalfa silage to chopped alfalfa hay in HS1, and chopped alfalfa hay and no alfalfa silage in H1.

Table 1. Ingredients and nutrient composition of experimental with fixed inclusion rates of barley silage, energy supplement and protein supplement and varying inclusion rates of chopped alfalfa hay and alfalfa silage. Experiment 1.[1]

	Diet, % DM basis				
Diet ingredients	H1	HS1	S1	SE	*P* value
Barley silage	34.6	34.6	34.6	—	—
Energy supplement	33.6	33.6	33.6	—	—
Protein supplement	14.7	14.7	14.7	—	—
Chopped alfalfa hay	13.6	6.8	0.0	—	—
Sunflower seeds	3.5	3.5	3.5	—	—
Alfalfa silage	0.0	6.8	13.6	—	—
Nutrient composition[2]					
DM, %	54.9[a]	51.1[ab]	45.2[b]	0.75	<0.01
CP, % of DM	15.8	16.0	15.6	0.33	0.69
Soluble protein, % of CP	28.2[b]	30.0[ab]	35.9[a]	1.71	0.04
NDF, % of DM	41.2	40.7	41.7	1.19	0.96
ADF, % of DM	27.4	27.0	30.1	1.58	0.39
Ca, % of DM	1.07	1.18	1.01	0.11	0.56
P, % of DM	0.57	0.55	0.51	0.03	0.34
K, % of DM	2.20	2.08	2.19	0.05	0.22
Mg, % of DM	0.37	0.36	0.36	0.01	0.75
Na, % of DM	0.32	0.30	0.29	0.03	0.76

[1]Means for nutrient composition in same row followed by a different superscript letters differ significantly ($P < 0.05$).

[2]Actual nutrient composition of experimental diets were measured for each period

Cows were assigned one of three TMR diets (H2, HS2, and S2) during each experimental period (Table 2) for experiment 2. Each diet contained (DM basis) 31.7% barley grain based energy supplement, 40.5% corn silage, 13.9% protein supplement, 4.2% sunflower seeds, and 9.7% alfalfa forage source. Source of alfalfa forage was alfalfa silage and no chopped alfalfa hay in S2; 50:50 ratio of alfalfa silage to chopped alfalfa hay in HS2, and chopped alfalfa hay and no alfalfa silage in H2.

In both experiments, the hay was chopped with an 8610 Tub Grinder (JI Case International, Rancine, WI). 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). TMR were fed once daily for *ad libitum* consumption allowing for between 5% and 10% orts. Cows had unlimited access to water.

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 cow ort samples were obtained daily during the collection period and pooled by weight and period. Forages were sampled once per collection period and pooled across collection periods. Dry matter content of pooled diets, forages and ort samples were determined by drying at 60°C for 48 hr (AOAC,

1990). 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.

Table 2. Ingredients and nutrient composition of experimental diets with fixed inclusion rates of barley silage, energy supplement and protein supplement and varying inclusion rates of chopped alfalfa hay and alfalfa silage. Experiment 2.[1]

	Diet, % DM basis				
Diet ingredients	H2	HS2	S2	SE	*P* value
Corn silage	40.4	40.4	40.4	—	—
Energy supplement	31.7	31.7	31.7	—	—
Protein supplement	13.9	13.9	13.9	—	—
Chopped alfalfa hay	9.8	4.9	0.0	—	—
Sunflower seeds	4.2	4.2	4.2	—	—
Alfalfa silage	0.0	4.9	9.8	—	—
Nutrient composition[2]					
DM, %	48.9[a]	46.7[b]	45.0[b]	0.47	0.003
CP, % of DM	14.7	15.1	14.1	0.57	0.53
SP, % of CP	33.3	31.1	30.5	1.72	0.52
RUP, % of CP	38.0	40.5	42.1	1.52	0.04
NDF, % of DM	43.8	43.7	45.5	0.81	0.57
ADF, % of DM	23.1	24.6	25.3	1.31	0.54
Ether extract, % of DM	6.1	5.9	6.3	0.35	0.85
Ca, % of DM	0.87	0.84	0.87	0.06	0.95
Starch, %, of DM	16.1	17.4	17.5	1.08	0.64
P, % of DM	0.54	0.55	0.54	0.01	0.36
K, % of DM	1.53	1.52	1.51	0.07	0.97
Mg,% of DM	0.27	0.27	0.27	0.01	0.88
Na, % of DM	0.39	0.39	0.40	0.02	0.84
pH	4.78	4.81	4.77	0.05	0.85

[1]Means for nutrient composition in same row followed by a different superscript letters differ significantly ($P < 0.05$).
[2]Actual nutrient composition of experimental diets were measured for each period.

All feed samples were analyzed for CP using the $CuSO_4/TiO_2$ Mixed Catalyst Kjeldahl procedure (AOAC, 1990); NDF (National Forage Testing Association, 1993) using alpha-amylase, ADF (AOAC, 1990); and for rumen undegradable protein (RUP, Licitera et al., 1999); ether extract (AOAC, 1990); and starch (McRae and Armstrong, 1968). Calcium, P, K, Mg, and Na were measured by inductively coupled plasma emission spectroscopy (AOAC, 1990) using an Atom Scan 25 plasma spectrometer (Thermo Jarrell Ash Corp, Grand Junction, CO) after acid digestion. Acid detergent lignin was determined in forage samples according to AOAC (1990). The pH of the TMR was determined according to Buchanan-Smith and Yao (1981), 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).

Particle size distributions were determined for all TMR, pooled refusals, and forage samples using the Penn State Particle Separator (PSPS) (Heinrichs 1996; Lammers et al., 1996). The PSPS has two screens and a bottom pan. The diameters of holes of the screens were 19 and 8 mm for the top and middle screen, respectively. Approximately 150 g wet sample was placed on the top screen of the PSPS. The PSPS was shaken for a total of 40 times (5 times in each direction, twice) (Heinrichs 1996). The contents of each fraction were weighed and analyzed for DM and NDF as described earlier. The $peNDF_{DM}$ was determined as the proportion of DM retained by the 19 and 8 mm PSPS screens multiplied by dietary NDF content. The $peNDF_{NDF}$ was determined as the proportion of NDF retained by the 19 and 8 mm PSPS screens.

Milk yield and composition analysis

Cows were milked twice daily and milk production was determined using Tru Test regulation meters (Westfalia Surge, Mississauga, ON). Milk samples were collected from four subsequent 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 Manitoba Milk Producers (Winnipeg, MB) by near infrared analysis using the Milk-O-Scan 303AB (Foss Electric, Hillerød, Denmark).

Rumen pH measurement

Rumen fluid was sampled twice during each collection period (Tuesday and Thursday afternoons) at 4 to 5 hr post-feeding. Approximately 50 ml of fluid was aspirated using a Geishauser oral probe (Geishauser, 1993; Green et al., 1999). 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.

VFA and ammonia analysis

Frozen rumen fluid supernatant was 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, with initial and final column temperatures were 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).

Statistical analysis

Analysis of variance for weekly averages of rumen fluid, milk, and intakes was conducted using the SAS Mixed Procedure (SAS, 1990). The effect of diets was considered fixed. The effects of cow and period were considered random. Analysis of variance for physical and chemical 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 value of equal to or less than 0.05. Differences between treatment means were tested for significance using Tukey's multiple range test (SAS, 1990). Reported SE are those used for the comparison of treatment means.

RESULTS

Experiment 1

Substituting alfalfa silage with chopped alfalfa hay increased ($P < 0.05$) dietary DM content from 45.2 to 54.9% (Table 1). Dietary CP, NDF, ADF, and minerals were the same across diets (Table 1). Alfalfa silage had considerably less DM passing through the 8 mm screen of the PSPS to the bottom pan than did chopped alfalfa hay (Table 4). As a result, substituting alfalfa silage with chopped alfalfa hay increased ($P < 0.05$) the amount of DM passing through an 8 mm screen from 47.4 to 60.7% (Table 5). Replacing alfalfa silage with chopped alfalfa hay increased $peNDF_{DM}$ from 16.2 to 21.9% DM (Table 5). Analysis of particle size distribution of the TMR and orts is shown in Figure 1. The DM distribution averaged across all three diets was 19.7, 27.7, and 52.5% in the 19 mm screen, 8 mm screen, and bottom pan fraction of the PSPS, respectively. The orts had a greater ($P < 0.05$) proportion of particles that were retained by the 19 mm and 8 mm PSPS screens compared with the TMR (Figure 1). Dietary treatment had no effect on particle size distribution between diet and orts (Figure 1).

Increasing $peNDF_{DM}$ content by replacing alfalfa silage with chopped alfalfa hay in the experimental TMR had no effect ($P = 0.57$) on rumen pH and ammonia concentration (Table 6).

Substituting alfalfa silage with chopped alfalfa hay significantly ($P < 0.05$) increased DMI from 19.1 to 21.2 kg/d^{-1} (Table 7). Feeding the HS1 diet resulted in a higher fat content than the H1 and S1 diets (Table 7). No effects were seen on fat yield. As alfalfa silage was substituted for chopped alfalfa hay, there was a trend

towards increased protein content in milk, as well as an increase in overall protein yield (Table 7).

Experiment 2

Replacing alfalfa silage with chopped alfalfa hay significantly ($P < 0.05$) increased dietary DM content from 45.0 to 48.9%, and significantly ($P < 0.05$) decreased dietary RUP content from 42.1 to 38.0% of CP (Table 2). Dietary CP, NDF, ADF, starch, ether extract, mineral contents, and pH remained unaffected (Table 2). Chopped alfalfa hay had more feed particles pass through the 8 mm screen of the PSPS to the bottom pan than did alfalfa silage (Table 9). As a result, replacing alfalfa silage with chopped alfalfa hay significantly ($P < 0.05$) increased dietary DM from the TMR passing the 8 mm screen from 47.9 to 52.4%, and reduced the dietary $peNDF_{DM}$ content from 22.7 to 20.8% DM ($P < 0.05$), and $peNDF_{NDF}$ content from 29.1 to 25.4% DM ($P < 0.05$) (Table 10).

The NDF content of the three PSPS fractions did not differ significantly between diets (Table 9). NDF contents averaged across diets were 63.2, 50.4, and 34.3% DM, in the 19 mm screen, 8 mm screen, and bottom pan fraction of the PSPS, respectively. Dietary NDF content of the three experimental TMR differed significantly ($P < 0.05$) among PSPS fractions. Analysis of the particle size distribution is shown in Figure 2.

The particle size distribution averaged across all three diets were 17.7, 34.6, and 47.6% DM in the 19 mm screen, 8 mm screen, and bottom pan fraction of the PSPS, respectively. The orts had a greater ($P < 0.05$) proportion of particles that were retained by the 19 mm and 8 mm PSPS screens compared with the TMR (Figure 2).

Table 3. Nutrient composition of forages. Experiment 1.

Chemical component	Alfalfa silage	Alfalfa hay	Barley silage
DM, %	53.9	87.3	38.6
Nutrient profile, % DM basis		% of DM	
CP	19.5	19.2	9.7
NDF	43.9	47.4	57.7
ADF	33.6	34.3	38.1
Ca	1.30	1.27	0.60
P	0.33	0.26	0.23
K	2.89	2.89	1.94
Mg	0.33	0.25	0.19

Table 4. Penn State Particle Size analysis of forages included in the experimental diets. Experiment 1.[1]

	Alfalfa silage	Alfalfa hay	Barley silage
PSPS sieving	% retained, as is		
Top screen (> 19 mm)	36.4	19.1	42.0
Middle screen (> 8 mm)	40.7	22.9	32.7
Bottom pan	22.9	58.1	25.3
	% retained, DM basis		
Top screen(> 19 mm)	36.8	13.2	39.9
Middle screen (> 8 mm)	37.5	21.7	32.0
Bottom pan	25.7	56.6	28.2

[1]Penn State Particle Separator distributions of forages included in experimental diets were calculated for each period (n= 3)

Table 5. Penn State Particle Separator analysis of experimental diets with fixed inclusion rates of barley silage, energy supplement and protein supplement and varying inclusion rates of chopped alfalfa hay and alfalfa silage. Experiment 1.[1]

	Diet				
	H1	HS1	S1	SE	*P* value
Particle distribution[2]	% retained, as is				
Top screen (> 19 mm)	12.9	15.2	22.9	4.71	0.17
Middle screen (> 8 mm)	27.3	26.2	30.9	1.91	0.64
Bottom pan	59.8[a]	58.7[ab]	46.2[b]	3.72	0.04
Particle distribution[2]	% retained, DM basis				
Top screen (> 19 mm)	11.6	16.8	20.1	3.43	0.07
Middle screen (> 8 mm)	27.7	26.7	32.6	2.38	0.30
Bottom pan	60.7[a]	56.5[ab]	47.4[b]	3.21	0.03
$peNDF_{DM}$[3], % DM	16.2[b]	17.7[ab]	21.9[a]	1.00	0.03

[1] Diets contained 13.6% DM chopped alfalfa hay (H1), 6.8% DM chopped alfalfa hay and 6.8% DM alfalfa silage (HS1), or 13.6% DM alfalfa silage (S1).

[2]Penn State Particle Separator distributions of experimental diets were calculated for each period (n= 3) for each treatment and analyzed by analysis of variance. Means for percentage of particles in a fraction in same row followed by a different superscript letters differ significantly ($P < 0.05$).

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

Table 6. Rumen fluid composition for cows fed experimental diets with fixed inclusion rates of corn silage, energy supplement and protein supplement and varying inclusion rates of chopped alfalfa hay and alfalfa silage. Experiment 1.[1]

	Diet				
Item[2]	H1	HS1	S1	SE	*P* value
pH	6.60	6.53	6.52	0.06	0.50
Ruminal ammonia, mg dL^{-1}	13.2	14.9	14.9	1.13	0.48

[1]Diets contained 13.6% DM chopped alfalfa hay (H), 6.8% DM chopped alfalfa hay and 6.8% DM alfalfa silage (HS), or 13.6 %DM alfalfa silage (S).

Table 7. Feed intake and milk production of cows fed experimental diets, with fixed inclusion rates of corn silage, energy supplement and protein supplement and varying inclusion rates of chopped alfalfa hay and alfalfa silage. Experiment 1.[1]

	Diet				
Item[2]	H1	HS1	S1	SE	*P* value
DMI, kg d^{-1}	21.2[a]	20.4[ab]	19.1[b]	0.61	0.009
Orts, % of feed provided	10.6	11.0	7.5		
Milk yield, kg d^{-1}	32.4	32.1	33.4	0.39	0.96
Milk components					
Fat, %	3.08[b]	3.31[a]	3.02[b]	0.09	0.02
Fat yield, kg d^{-1}	0.98	1.04	1.00	0.05	0.36
Protein, %	2.89	2.91	3.04	0.07	0.08
Protein yield, kg d^{-1}	0.93	0.92	1.01	0.05	0.08

[1] Diets contained 13.6% DM chopped alfalfa hay (H), 6.8% DM chopped alfalfa hay and 6.8% DM alfalfa silage (HS), or 13.6 %DM alfalfa silage (S).

[2]Feed intake and milk production variables were averaged for each animal during each period (n= 12) for each treatment and analyzed by analysis of variance. Means for variables in same row followed by a different superscript letters differ significantly ($P < 0.05$).

Figure 1. Penn State particle size distribution of diets and orts (DM basis). Diets contained 13.6% DM chopped alfalfa hay (H1), 6.8% DM chopped alfalfa hay and 6.8% DM alfalfa silage (HS1), or 13.6% DM alfalfa silage (S1). Experiment 1.

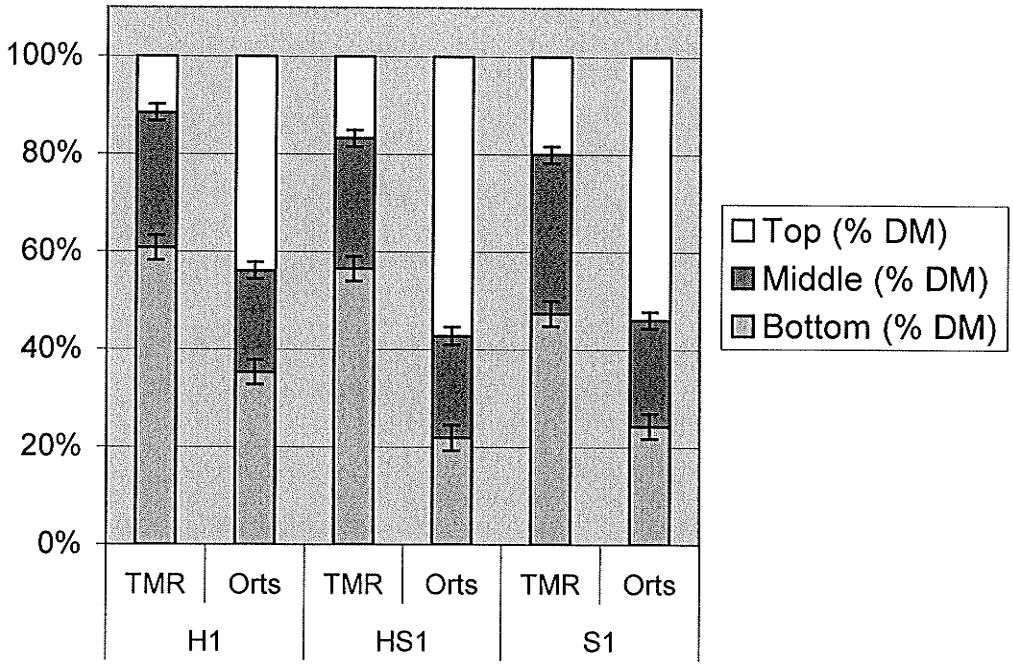


Table 8. Nutrient composition of the forages included in the experimental diets. Experiment 2.

Chemical component	Corn silage	Alfalfa silage	Alfalfa hay
DM%	29.0	47.2	87.3
Nutrient profile, % DM basis		% of DM	
CP	7.7	17.3	16.9
NDF	58.9	56.5	57.4
ADF	34.3	47.1	46.4
ADL	3.0	8.6	6.9
Starch	19.4	12.3	11.8
Ca	0.28	1.12	1.13
P	0.27	0.30	0.26
K	1.30	3.01	2.45
Mg	0.19	0.29	0.18

Table 9. Penn State Particle Size analysis of forages included in the experimental diets. Experiment 2.[1]

	Corn silage	Alfalfa silage	Alfalfa hay
PSPS sieving	% retained, as is		
Top screen (> 19 mm)	11.9	26.6	31.4
Middle screen (> 8 mm)	56.4	44.6	24.6
Bottom pan	31.7	28.8	44.1
	% retained, DM basis		
Top screen(> 19 mm)	12.6	30.0	31.6
Middle screen (> 8 mm)	54.1	41.1	24.6
Bottom pan	33.3	28.9	43.8
$peNDF_{DM}$[2], % DM	39.3	40.2	32.3

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

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

Table 10. Penn State Particle Separator analysis of experimental diets.[1] Diets contained 9.8% DM chopped alfalfa hay (H2), 4.9% DM chopped alfalfa hay and 4.9% DM alfalfa silage (HS2), or 9.8% DM alfalfa silage (S2). Experiment 2.

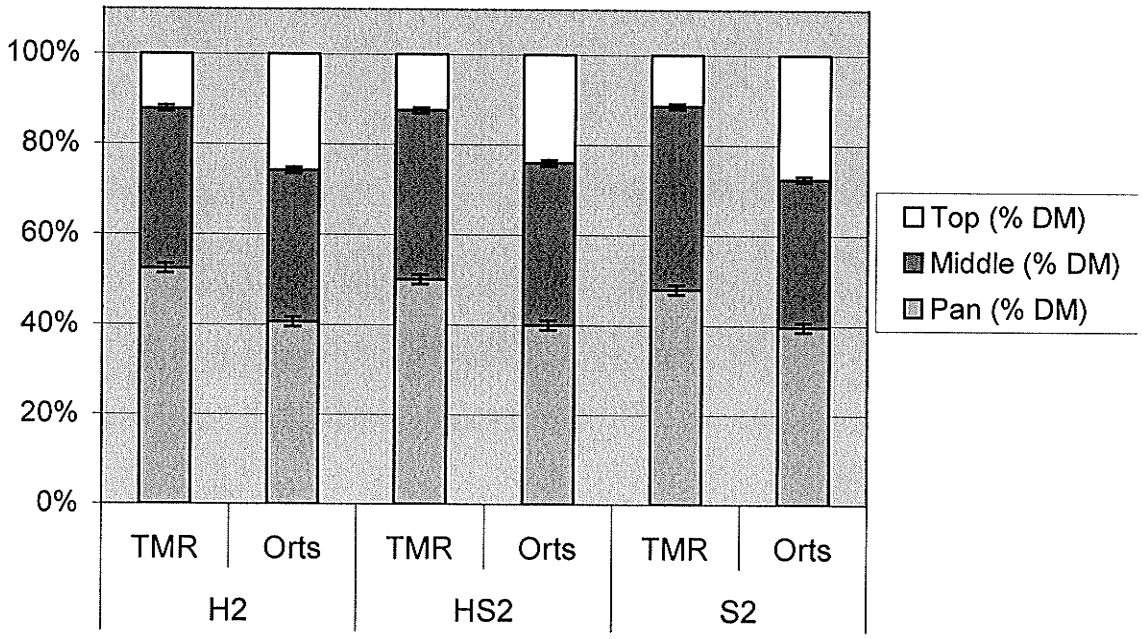
	Diet				
	H2	HS2	S2	SE	*P* value
PSPS distribution	% retained, as is				
Top screen (> 19 mm)	14.5	13.6	14.0	0.80	0.73
Middle screen (> 8 mm)	38.2 ^{b}	41.2 ^{ab}	42.0 ^{a}	0.56	0.004
Bottom pan	47.3 ^{a}	45.1 ^{ab}	43.9 ^{b}	0.45	0.003
PSPS distribution	% retained, DM basis				
Top screen (> 19 mm)	12.1	12.4	11.3	0.75	0.40
Middle screen (> 8 mm)	35.4^{b}	37.5^{ab}	40.7^{a}	1.06	0.03
Bottom pan	52.4^{a}	50.1^{ab}	47.9^{b}	1.18	0.02
PSPS distribution	NDF in retained fraction, % of DM				
Top screen (> 19 mm)	63.7	64.5	61.5	1.33	0.32
Middle screen (> 8 mm)	52.1	50.5	48.7	1.42	0.30
Bottom pan	35.2	34.1	33.8	1.03	0.63
$peNDF_{DM}$[2], % DM	20.8^{b}	21.6 ^{ab}	22.7 ^{a}	0.52	0.02
$peNDF_{NDF}$[3], % DM	25.4^{b}	26.9^{ab}	29.1^{a}	0.51	0.04

[1]Penn State Particle Separator distributions of experimental diets were calculated for each period (n= 3) for each treatment and analyzed by analysis of variance. Means for percentage of particles in a fraction in same row followed by a different superscript letters differ significantly ($P < 0.05$).

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

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

Figure 2. Penn State particle size distribution of diets and orts (DM Basis). Diets contained 9.8% DM chopped alfalfa hay (H2), 4.9% DM chopped alfalfa hay and 4.9% DM alfalfa silage (HS2), or 9.8% DM alfalfa silage (S2). Experiment 2.



Diet type had no effect on difference in particle size distribution between diet and orts (Figure 2).

Replacing alfalfa silage with chopped alfalfa hay in the experimental diets had no effect on rumen pH, rumen ammonia levels, total and individual VFA concentrations, as well as acetate to propionate ratio (Table 11). Replacing alfalfa silage with chopped alfalfa hay did not affect DMI, milk yield, and milk composition (Table 12).

Table 11. Effects of replacing chopped alfalfa hay with alfalfa silage in a total mixed ration on rumen fluid composition[1]. Diets contained 9.8% DM chopped alfalfa hay (H2), 4.9% DM chopped alfalfa hay and 4.9% DM alfalfa silage (HS2), or 9.8% DM alfalfa silage (S2). Experiment 2.

	Diet				
	H2	HS2	S2	SE	*P* value
pH	6.29	6.27	6.32	0.07	0.72
VFA, mM L^{-1}					
Total	91.1	91.6	89.6	3.30	0.83
Acetate	56.3	56.7	54.7	2.20	0.65
Propionate	22.0	22.6	22.6	1.01	0.76
Butyrate	10.0	9.8	9.7	0.50	0.83
Other	2.8	2.5	2.5	0.09	0.08
[2]Ac:Pr	2.6	2.6	2.4	0.06	0.14
Ruminal ammonia, mg dL^{-1}	6.97	5.82	6.17	0.65	0.22

[1]Rumen fluid composition variables were averaged for each animal during each period (n= 12) for each treatment and analyzed by analysis of variance. differ significantly ($P < 0.05$).

[2]acetate:propionate ratio

Table 12. Effects of replacing chopped alfalfa hay with alfalfa silage in a total mixed ration on milk production and feed intake[1]. Diets contained 9.8% DM chopped alfalfa hay (H2), 4.9% DM chopped alfalfa hay and 4.9% DM alfalfa silage (HS2), or 9.8% DM alfalfa silage (S2). Experiment 2.

	Diet				
	H2	HS2	S2	SE	*P* value
DMI, kg d^{-1}	22.2	22.3	22.0	0.67	0.83
Orts, % of feed provided	8.52	7.43	5.87		
Milk yield, kg d^{-1}	37.0	36.8	36.2	1.17	0.75
Milk Components					
Fat, %	2.90	2.99	2.75	0.16	0.54
Fat yield, kg d^{-1}	1.04	1.08	1.00	0.07	0.51
Protein, %	3.03	3.03	3.14	0.06	0.11
Protein yield, kg d^{-1}	1.11	1.13	1.12	0.04	0.85

[1]Feed intake and milk production variables were averaged for each animal during each period (n= 12) for each treatment and analyzed by analysis of variance.

DISCUSSION

Chemical and physical compositions of forage ingredients and experimental diets

Chopped alfalfa hay was substituted for alfalfa silage to decrease dietary $peNDF_{DM}$ content in experiment 1. In doing so, the proportion of particles passing through the 19 and 8 mm screens of the PSPS was increased (Table 5). This substitution increased dietary DM content, but did not affect dietary CP, NDF, ADF, and mineral contents (Table 1). It can, therefore, be inferred that differences in intake and production can be mainly attributed to differences in the physical, rather than chemical, characteristics of the diets.

The barley silage used in experiment 1 was chopped coarse. A recent survey of 42 randomly selected dairy farms across the province of Manitoba showed that on average 42.5% DM passed through the 19 mm and 8 mm screens of the PSPS, and 49.9% DM was retained on the 8 mm screen of the PSPS, of the farms that included barley silage in their rations (Plaizier et al., unpublished data). In the current study, only 28.2% DM passed through both PSPS screens, and only 32.0% DM was retained by the 8 mm screen (Table 4), confirming that in fact the barley silage used was of a coarser nature that what is commonly seen across the province.

Alfalfa silage was substituted for chopped alfalfa hay to increase dietary peNDF content in experiment 2. This replacement decreased the proportion of particles passing through the 19 and 8 mm screens of the PSPS (Table 10); decreased dietary DM content; increased dietary RUP content; but had no affect on diet pH, or CP, NDF, ADF, ether extract, and mineral contents (Table 2). Therefore, differences in

intake and production can be attributed to differences in the physical, rather than chemical construct of the diets.

The PSPS top sieve values obtained in experiment 2 were marginally coarser than those recommended by Mertens (1997). The remainder of the particle distribution was on par with these recommendations. The dietary $peNDF_{DM}$ values in this experiment ranged from 20.8 to 22.7% DM, and met Mertens (1997) recommendations.

Minimal recommendations regarding particle size distribution of diets are found in the NRC (2001). Some generally preferred recommendations for particle size distribution are 6 to 10% of the particles in the top sieve of the PSPS, 30 to 50% in the middle sieve, and 40 to 60% in the bottom pan in rations for high producing dairy cows (Heinrichs, 1996). In the current experiment, all diets were coarser than these guidelines, with the exception of diet H1, which was on par with these recommendations.

The NRC (2001) provides minimal recommendations for amount, as well as physical quality of dietary fiber. The diets used in this experiment exceeded the NRC (2001) minimum NDF recommendation of 25% dietary DM, with 75% of NDF from forage. The diets used in this experiment exceeded the current recommendation for minimum NDF content, 34% DM, in barley silage based diets (Beauchemin, 1991). Mertens (1997) recommends that physically effective fibre content, calculated using tabular physical effectiveness factors multiplied by total NDF, should be 22% of ration DM, in order to maintain an average rumen pH of 6.0, and 20% of ration DM to maintain a milk fat percentage of 3.4 in midlactation Holstein cows. The $peNDF_{DM}$

values in this study ranged from below these recommendations, 16.2% of DM in H1, to meeting the recommendations in diet S1, disregarding the variation in calculation procedures (Table 5). Using the PSPS guidelines, it can be assumed that 60% of a wet TMR passes through to the bottom pan, and 40% remains in the top two sieves; multiplying 40% retained by 34% NDF, which is the minimum recommendation for barley silage based diets, yields a peNDF value of 13.6%. When comparing this calculated peNDF value to the Mertens (1997) recommendations, it becomes evident that the values obtained in this experiment, though lower than the current recommendations, are in fact higher than the accepted literature values.

In experiment 2, the peNDF content of the diet was calculated as the proportion of dietary NDF retained by the 19 and 8 mm screens of the PSPS. The amount of peNDF in a diet has been calculated in several ways. Mertens (1997), calculated peNDF content as the product of the diet's total NDF content, multiplied by a tabular physical effectiveness factor. Yang et al. (2001a) and Beauchemin et al. (2003) calculated peNDF content as the amount of DM retained by the 19 and 8 mm sieves of the PSPS, multiplied by the total dietary NDF content. Both of these calculations make the assumption that NDF content is uniformly distributed amongst the three fractions of the PSPS. NDF distribution amongst the three PSPS fractions measured in this experiment displays significant variation between PSPS fractions (Table 10). That the NDF content of the PSPS fractions differs suggests that when total dietary NDF content is used to determine peNDF content, it is under estimated. Therefore, determining peNDF content on an NDF distribution basis will correct for the fact that significantly the bottom pan of the PSPS retains less NDF.

In both experiments, the particle distribution of the TMR was significantly different from the particle distribution of the orts (Figure 1 and Figure 2). The proportion of particles retained by the 19 and 8 mm PSPS screens was greater for the orts than for the TMR, and did not very between diets, which indicates that cows selected against large feed particles in favour of smaller particles.

Dry matter intake

Substituting chopped alfalfa hay for alfalfa silage resulted in an increase ($P < 0.001$) in DMI in experiment 1. The chopped alfalfa hay had a larger proportion of particles passing through the 19 and 8 mm PSPS screens than the alfalfa silage (Table 4). Due to the fact that, in experiment 1, all three diet's nutrient compositions remained virtually unchanged, it can be assumed that DMI was not limited by a metabolic constraint, but by distension of the reticulo-rumen of diets containing alfalfa silage because of its relative coarse nature (Allen, 2000). There are numerous studies demonstrating a positive relationship between reduction in dietary particle size and particulate passage rate (Martz and Belyea, 1986; Soita et al., 2002). However, Yang et al. (2001a) and Beauchemin et al. (2003) both found that when forage particle size was reduced, no effect was seen on intake. The extent, to which forage particle size affects DMI, through physical constraint, is most likely dependent on the amount of forage in the diet (Beauchemin et al., 2003).

Substituting chopped alfalfa hay for alfalfa silage had no effect on DMI in experiment 2 (Table 12). Yang et al. (2001a), Krause et al. (2002), and Beauchemin et al. (2003) also did not observe that differences in forage particle length affected

DMI in high concentrate diets. In contrast, Beauchemin et al. (2003) found that when the proportion of alfalfa silage to alfalfa hay was increased, DMI significantly increased. Due to the fact that the experimental diets were fairly high in concentrate (50:50 F:C), and that altering dietary particle size had no effect on DMI, it can be assumed that short-term voluntary DMI was not limited by distension of the reticulo-rumen, but rather by a chemical or metabolic constraint (Allen, 2000).

When chopped alfalfa hay was substituted for alfalfa silage, dietary DM content increased significantly in both experiments (Table 1 and Table 2). Higher moisture content of silages relative to hay is reported to be negatively related to DMI (Allen et al., 2000). The change in DM content in these experiments could have resulted in differences in DMI, but that is unlikely because Lahr et al. (1983) found that increasing DM content only affected DMI when diets contained over 55% DM. Holter (1992) also suggested that in cows fed diets ranging between 30-70% DM, DMI was not significantly affected by dietary DM content. Thus, in the current experiment, with DM content ranging from 45.2 – 54.9%, DM content should not affect DMI. Dry matter intake did not respond uniformly to a change in $peNDF_{DM}$ for the 2 experiments, or in the parallel trail (Calberry et al., 2003).

Rumen fermentation

Substituting chopped alfalfa hay for alfalfa silage had no effect on rumen pH in both experiments (Table 6 and Table 11). Yang et al. (2001a) found no significant interaction between reduction of dietary forage particle size and rumen pH. The peNDF contents of all three diets, in experiment 1, greatly exceeded the minimum

recommendation for dietary peNDF inclusion (Mertens, 1997). The effect of peNDF on rumen pH is only observed at lower forage inclusion rates (Mertens, 1997). The effect of peNDF on rumen pH is limited at higher peNDF (>25% DM) levels (Mertens, 1997). This may help explain the minimal effect on rumen pH observed in this experiment. In contrast, Beauchemin et al. (2003) found that when dietary peNDF content was increased from 18.3 to 26.7%, chewing activity and rumen pH also increased. Krause et al. (2002) found that decreasing forage particle size reduced average rumen pH from 6.02 to 5.81. Calberry et al. (2003) fed a higher concentrate level (60% DM) and observed rumen pH decreased when chopped alfalfa hay was substituted for alfalfa silage. The $peNDF_{DM}$ content of the diets in that parallel experiment were 20.1, 21.0, and 23.3% of DM, for diets with the same forage inclusion as diets H2, HS2, and S2 respectively, and were on average, 5.4% DM lower than the values obtained in this experiment. The fact that an effect of peNDF content on rumen pH was observed in the experiment conducted by Calberry et al. (2003), and not in the second experiment indicates the presence of a threshold for effect of peNDF level on rumen pH (Mertens, 1997). This threshold is located below a $peNDF_{DM}$ content of 16.2% of DM, for barley silage based diets, and beloew 20.8% $peNDF_{DM}$ content for corn silage based diets.

Increasing dietary peNDF by substituting alfalfa silage for chopped alfalfa hay had no effect on total ruminal VFA concentration, or individual rumen VFA concentrations in experiment 2. This is not surprising given the lack of effect of increasing dietary peNDF content on DMI and rumen pH. These results are in agreement with Beauchemin et al. (2003), where no effect was observed when forage

particle size was altered by varying inclusion rates of alfalfa silage and alfalfa hay on ruminal VFA concentrations. Yang et al. (2001a) found conflicting results in which total ruminal VFA concentration was affected by forage particle length. This discrepancy may be attributed to the fact that peNDF levels were lower in this experiment, and rumen conditions are more susceptible to changes in peNDF at lower levels (Mertens, 1997).

Rumen fluid samples were collected on 2 separate days, 4 hours after feeding, during sampling weeks, using a Geishauser oral probe for both experiments (Geishauser, 1993). Due to differences exist between rumen fluid sampling techniques, adjustment must be considered when interpreting pH values. Duffield et al. (2000) reported that the pH of rumen fluid samples obtained with an oral probe were on average 0.35 units higher than the pH of rumen fluid samples obtained from the caudal ventral rumen via a rumen cannula of lactating dairy cows. Differences in rumen pH between these two techniques is due to differences in saliva contamination, as well as differences in site of collection in the rumen (Duffield et al., 2000). In this study, the first 50-100 ml of rumen fluid collected was discarded, and saliva contamination was considered minimal. Despite this discount, our pH levels were still high enough not to be considered in the range of SARA.

Milk production and composition

Milk fat percent, and milk fat yield were the highest in the HS1 diet (Table 7). These results contrast work by Beauchemin et al. (1994b) who concluded that effects of particle size on milk fat content are likely to occur when NDF levels are below

minimum NRC recommendations. The NDF levels of the diets fed in the present experiment exceed NRC recommendations (NRC, 2001). Mertens (1997) compiled data from 36 citations and observed that a positive correlation exists between dietary peNDF content, rumen pH and milk fat percentage. Griinari et al. (1998) theorized that when rumen pH is low, there is an increase in the production of trans-fatty acids. Trans-fatty acids have a negative effect on *de novo* milk fat synthesis (Griinari et al., 1998). This is most likely not the case in this experiment because rumen pH, measured four hours post-feeding, was never below 6.5 (Table 6). Yang et al. (2001a) found that when dietary peNDF content, calculated as the %DM retained on the top two sieves of the PSPS, decreased as result of decreasing the amount of dietary fibre, that acetate to propionate ratio significantly decreased. It has been shown that when ruminal propionate production increases, that insulin production increases as well (Griinari et al. 1998). Insulin has been proven to cause preferential channelling of lipid precursors to adipose tissue, away from the mammary gland, thereby reducing milk fat synthesis (Griinari et al., 1998). This is probably not the case in this experiment due to the fact that milk fat percentage was the lowest when $peNDF_{DM}$ content was the highest (Table 5 and Table 7).

When chopped alfalfa hay was substituted for alfalfa silage in experiment 1, milk protein percent, and milk protein yield tended to decrease (Table 7). Dietary CP content did not significantly vary between experiment 1 diets (Table 1). Soluble protein content was higher in diets containing alfalfa silage, which is to be expected. This increased solubility, along with a possible increase in rumen retention time due to the relative coarseness of alfalfa silage compared to alfalfa hay, may have allowed

for improved microbial crude protein (MCP) synthesis. Yang et al. (2001a) also found that when dietary particle size was reduced, milk protein content tended to decrease as in the current experiment. In experiment 1, the trend for higher milk protein percentage in diets containing alfalfa silage may have been due to a change in the ratio of RUP and RDP, when chopped alfalfa hay was substituted; RUP and RDP were not determined in this experiment. It is unlikely that MCP would have been impaired in diet H1 because of a low pH or lack of sufficient N, because pH never dropped below 6.5, and dietary CP percentage was not different between diets. Rumen ammonia values obtained in this study exceeded the recommended optimal level of 10 mg/100 ml (Van Soest, 1994).

No effect was exhibited on milk yield when chopped alfalfa hay was substituted for alfalfa silage in the two experiments (Table 7 and Table 12). No effect was exhibited on milk fat percentage, milk fat yield, milk protein percentage, and milk protein in the second experiment (Table 12). Yang et al. (2001a) and Soita et al. (2000) both found that particle size reduction of barley silage based diets had no effect on milk yield. Beauchemin et al. (2003) found that milk yield is not affected by dietary peNDF content when dietary DM ranges from 21.9 to 41.3%. Krause et al. (2002a) also found that diets having a mean particle size between 3.0 mm and 6.3 mm did not affect milk production. Particle size was determined in the previously mentioned experiment differently than in the current experiment, and is, therefore difficult to compare. However, over 60% DM of the experimental diets in Krause et al. (2002a) was concentrate, and it can be inferred that these diets were less coarse, and higher in energy.

The lack of effect on milk yield when chopped alfalfa hay was substituted for alfalfa silage is somewhat surprising given that this substitution yielded an increase in DMI in the first experiment. This may be attributed to a change in dietary digestibility when chopped alfalfa hay was substituted for alfalfa silage. Yang et al. (2001a) found that when peNDF content increased, by varying dietary fiber content, ruminal digestibility significantly decreased from 48.3 to 39.6%.

In experiment 2, average rumen pH never dropped below 6.27 (Table 11). The threshold for SARA is stated to be below a pH of 5.6 (Cooper and Klopfenstein, 1996). It can, therefore, be inferred that, in addition to the fact that dietary contents of NDF and starch (Table 2) were sufficient to negate any risk of ruminal acidosis (NRC, 2001), the cows in this experiment did not experience SARA. However, an inversion of milk fat and protein percentage was observed (Table 12), and is sometimes considered an indication of ruminal acidosis (NRC, 2001). Several factors lead us to believe that SARA did not cause this inversion. All diets contained 4.2% sunflower seeds, which contained 41.9% unsaturated fat, and added over 1.76% of unsaturated fat to the diet. Griinari et al. (1998) found that addition of unsaturated fats to high concentrate diets can cause milk fat depression. The selection program used by the Glenlea Research Station has selected for high protein content in dairy cattle for many years, and this has had a negative impact on milk fat:protein ratios. These two factors can in part explain the low milk fat observed in this experiment. In a companion experiment conducted by Calberry et al. (2003), milk fat percentage averaged 2.52 across 3 diets averaging 65:35 concentrate: forage; substantially lower than 2.88% recorded in this experiment. The diets in the companion experiment,

having more DM passing through the 19 and 8 mm sieves of the PSPS, facilitated the production of ruminal propionate more so than the current experiment, by way of increasing rumen passage rate and digestion, as well as depressing rumination (Van Soest, 1994). Griinari et al. (1998) observed a positive relationship between rumen propionate production and insulin production. Insulin has been proven to cause preferential channelling of lipid precursors to adipose tissue, away from the mammary gland, thereby reducing milk fat synthesis (Griinari et al., 1998). This could possibly explain the reduced amount of milk fat observed in the study carried out by Calberry et al. (2003).

CONCLUSIONS

Replacing 13.6% DM chopped alfalfa hay in the first experiment, and 9.8% DM in the second, with alfalfa silage in a total mixed ration containing barley grain and coarse barley silage or corn silage increased dietary $peNDF_{DM}$ and reduced dietary DM content in both experiments, as well as $peNDF_{NDF}$ in the second experiment. Dietary contents of CP, NDF, ADF, and minerals were not affected in both experiments. Replacing chopped alfalfa hay with alfalfa silage significantly decreased DMI, but no effect was exhibited on milk yield in the first experiment. This could be due to an improved rumen and total tract digestibility when alfalfa silage was fed. No effect was seen on DMI in the second experiment. Replacing chopped alfalfa hay with alfalfa silage had no effect on rumen pH or ammonia concentration in both experiments, as well as VFA profile in the second. Milk fat percentage was significantly higher in the alfalfa hay/alfalfa silage diet. No effect was observed on milk production or composition in the second experiment. The fact that no effect was observed on most parameters in the second experiment could indicate that the effect of dietary particle size is limited to either very fine diets, high concentrate diets, or to very coarse, high forage diets. The minimum amount of physically effective fibre required in a diet to prevent SARA is less than 16.2% $peNDF_{DM}$ in barley silage based diets, and less than 20.8% $peNDF_{DM}$, or 25.4% $peNDF_{NDF}$ for corn silage based diets. Additional research into the interactions between these factors on production and health of dairy cows is required before these guidelines can be finalized.

MANUSCRIPT II

Effects of barley silage chop length on productivity and rumen conditions of lactating dairy cows fed a total mixed ration

ABSTRACT

Barley silage, cut at the early dough stage, was chopped short (10 mm, SC) or long (19 mm, LC), ensiled, and incorporated into total mixed rations (TMR). The TMRs contained (DM basis) either 58.0% or 41.4% concentrate with either short or long chopped barley silage. Reducing chop length of barley silage decreased the proportion of TMR particles retained by the 8 and 19 mm screens of the Penn State Particle Separator (PSPS) from 60.4 to 51.8%, and decreased dietary physically effective fiber (peNDF), calculated as the NDF retained by the screens of the PSPS from 32.1 to 27.9% across concentrate levels. Dietary NDF, starch, and mineral contents were not affected by chop length. Average dietary DM content was higher (39.5% vs. 36.7%) and average dietary CP level was lower (17.7% DM vs. 19.2% DM) in diets containing SC barley silage compared to diets containing LC barley silage. Rumen fluid pH and VFA concentrations were 6.43 and 93.2 mM L^{-1}, respectively, across diets and were not affected by chop length. Reducing the chop length increased ($P < 0.01$) DMI from 18.1 to 18.9 kg d^{-1} across concentrate levels, but did not affect milk yield and milk composition. Increasing the concentrate inclusion rate reduced ($P < 0.001$) rumen pH from 6.52 to 6.35, did not affect total VFA concentration, reduced ($P < 0.001$) acetate to propionate ratio from 3.1 to 2.7, increased ($P < 0.001$) milk yield from 28.7 to 31.3 kg d^{-1}, reduced ($P < 0.001$) milk fat content from 3.48 to 2.94%, and increased ($P < 0.01$) milk protein content from 3.11 to 3.27%.

Keywords: chop length, barley silage, physically effective NDF, dairy cows

Abbreviation key: ADIP = acid detergent insoluble protein, **HSC** = higher concentrate, short chop diet, **HLC** = higher concentrate, long chop diet, **LSC** = lower concentrate, short chop diet, **LLC** = lower concentrate, long chop diet, **SC** = short chop barley silage, **LC** = long chop barley silage, **MPL** = mean particle length, **peNDF** = physically effective NDF, $\mathbf{peNDF_{DM}}$ = proportion of DM retained by the 19 and 8 mm PSPS screens multiplied by dietary NDF content, $\mathbf{peNDF_{NDF}}$ = proportion of dietary NDF retained by the 19 and 8 mm PSPS screens, **PSPS** = Penn State Particle Separator **TLC** = theoretical length of chop.

INTRODUCTION

Energy demands of lactating dairy cows have been increased dramatically as genetic potential for performance improves. High concentrate, low fiber diets are fed in order to meet these increasing nutritional demands. Energy dense diets have limitations to the ratio of non-structural carbohydrate (NSC) to structural carbohydrates, as a minimum amount of fiber is required for salivary rumen buffering (Beauchemin and Rode, 1997). Insufficient rumen buffering capacity, potentially instigated by maximizing dietary NSC content, will have a detrimental effect on the rumen leading to a variety of metabolic disorders, including ruminal acidosis (NRC, 2001). Conversely, including too much fiber in high producing dairy cattle rations can compromise intake and, therefore, productivity as a result of rumen fill from fiber bulk (Allen, 2000). Not all fiber sources are equal in their rumen buffering capacity (Mertens, 1997). The amount of, as well as the physical and chemical characteristics of fiber in a diet affects animal performance (Mertens, 1997). It is, therefore, imperative that a validated unit or measure be established for the buffering capacity provided by a diet (Mertens, 1997).

Several methods have been employed to assess the capacity of a diet to stimulate saliva production and buffer the rumen. Physically effective NDF (peNDF) is a measure that reflects the ability of physical characteristics of fiber, mainly particle size, to stimulate chewing and saliva buffering in the rumen (Mertens, 1997). The amount of peNDF in a diet is based on forage chop length, concentrate: forage

ratio, and dietary NDF content (Mertens, 1997). The suggested minimum requirements for peNDF content in lactating dairy cattle diets is 22% of ration DM, for maintenance of an average rumen pH of 6.0, and 20% of ration DM for maintenance of a milk fat percentage of 3.4 in midlactation Holstein cows (Mertens, 1997). The National Research Council (NRC) (2001) recommends a minimum of 25% DM as NDF, of which 75% must be from forage sources. Differences in rumen buffering capacity between forages due to differences in particle size and intrinsic rumen buffering capacity are not considered in this recommendation. The NRC (2001) provides no recommendations for peNDF inclusion, due to the lack of a standard, validated technique to quantify the physically effective properties of fiber in a diet. This suggests a need for one.

The peNDF value of a feedstuff has been calculated by Mertens (1997) as the product of the NDF content of the feed, and its physical effectiveness factor. This factor is a tabular value, between 0 and 1.00, where long grass hay is equal to 1.00, and is fully effective in stimulating chewing activity, and where the NDF of a feedstuff with a value of 0 will be ineffective in promoting chewing (Mertens, 1997). Yang et al. (2002) measured peNDF as the proportion of DM retained by the top two PSPS sieves, multiplied by total dietary NDF content. Earlier, Yang et al. (2001a) determined peNDF as the NDF content of a TMR multiplied by the percent of DM remaining on a 1.18 mm screen, using a dry sieving technique. It is assumed that DM passing through a 1.18 mm screen would not stimulate chewing activity (Mertens, 2000).

The peNDF value of a feed has also been determined as the amount of NDF retained on the top two sieves of the Penn State Particle Separator (PSPS) (Lammers

et al., 1996), multiplied by the respective DM% of the individual sieves. This method takes into account the variation in distribution of NDF throughout the PSPS correlated with saliva production (Beauchemin et al., 2003).

A recent survey of 40 randomly selected dairy farms across the province of Manitoba indicated that there is a shift to increased use of annual crops, predominantly barley, for silage in Western Canada (Plaizier et al., 2003). Also, most recommendations for optimum fiber levels and physical effectiveness have been developed using corn grain based, and not barley grain based diets (Beauchemin et al., 1991; Beauchemin and Rode, 1997; Soita et al., 2002). It is, therefore, important to establish these guidelines for barley-based diets due to relatively large numbers of farms incorporating it in this province.

There are several differences between barley and corn grain, such as a higher NDF content of barley (19 to 25%) than that of corn (7%) (Beauchemin and Rode, 1997). Also, barley starch is more rapidly fermentable than corn (McCarthy et al., 1997). As a result, Beauchemin (1991) recommended that diets based on barley grain should contain 34% NDF in order to maintain a milk fat content of 3.5%, which exceeds the amount recommended for corn grain diets (Mertens, 1997). This suggests that minimum peNDF requirements should be higher for barley grain diets versus corn grain diets.

The objectives of this experiment were to study the effect of varying barley silage chop length on dietary peNDF, DM intake, rumen fermentation and milk production when used with two barley grain inclusion rates.

MATERIALS AND METHODS

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 14-d of adaptation to the experimental diet and 7-d of data collection. Animals were cared for in accordance with the Canadian Council for Animal Care (CCAC) guidelines. Upon commencement of the experiment cows averaged 104 ± 33.6 days in milk (DIM), had an average body condition score (BCS) of 3.3 ± 0.26, and had an average body weight (BW) of 620 ± 62.8 kg.

Cows were assigned one of four total mixed rations (TMR) (higher concentrate, short chop (10 mm) barley silage = HSC; higher concentrate, long chop (19 mm) barley silage = HLC; lower concentrate, short chop (10 mm) barley silage = LSC; and lower concentrate, long chop (19 mm) barley silage = LLC) during each experimental period. The higher concentrate diets contained (DM basis) 43.3% barley grain concentrate, 14.7% protein supplement, and 42.0% of either short (HSC) or long chop (HLC) barley silage (Tables 14 and 15). The lower concentrate diets contained (DM basis) 32.1% barley grain concentrate, 9.3% protein supplement, and 58.6% of either short (LSC) or long chop (LLC) barley silage.

A Robust cultivar barley silage was harvested at the early dough stage, and chopped at two lengths (10 mm and 19 mm) using a New Holland Forage Harvester, model 790, from the same field on the same day. The diets were mixed using a Data

Table 13. Ingredient composition of energy supplement and protein supplement (% DM).

Ingredient	Energy supplement	Protein supplement
Rolled barley	54.0	—
Luprosil salt	0.2	—
Protein pellet[1]	1.8	—
Dairy supplement[2]	40.0	—
Tallow	4.0	
Dried distillers grain	—	42.0
Fish meal	—	7.0
Canola meal	—	22.8
Soybean meal	—	20.0
Beet molasses	—	3.2
Niacin	—	0.3
Sodium bicarbonate	—	5.0

[1]Protein pellets contains 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% dynamate, 0.3% pellet binder, 1.0% cane molasses, 3.7% calcium carbonate, and 10.0% corn gluten meal.

Table 14. Nutrient composition of the forages included in the experimental diets[1].

	Barley Silage			
Chemical component	Long chop	Short Chop	SE	*P* Value
DM %	20.4	25.0	0.55	<0.001
	% of DM			
NDF	61.1	61.1	1.51	0.99
ADF	37.9	38.3	1.23	0.82
CP	13.8	11.7	0.70	0.08
SP	8.9	7.0	0.96	0.21
ADIP	0.95	1.1	0.15	0.52
Fat	3.03	2.68	0.11	0.07
Ca	0.53	0.61	0.06	0.36
P	0.39	0.32	0.02	0.03
K	3.04	2.72	0.12	0.11
Mg	0.35	0.40	0.02	0.11
Na	0.15	0.09	0.01	<0.002

[1](n = 4) for treatment analyzed by ANOVA.

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

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 cow ort samples were obtained daily during the collection period and pooled by weight and period. Forages were sampled once per collection period and pooled across collection periods. DM content of pooled diets, forages and ort samples were determined by drying at 60°C for 48 hr (AOAC, 1990). 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, 1990), NDF (National Forage Testing Association, 1993) using alpha-amylase, ADF (AOAC, 1990), rumen undegradable protein (RUP, Licitera et al., 1996), soluble protein (SP) using the method described by Licitra et al., (1996), ether extract (AOAC, 1990), and starch (McRae and Armstrong, 1968). Calcium, P, K, Mg, and Na were measured by inductively coupled plasma emission spectroscopy (AOAC, 1990) using an Atom Scan 25 plasma spectrometer (Thermo Jarrell Ash Corp, Grand Junction, CO) after acid digestion. Acid detergent lignin was

determined in forage samples according to AOAC (1990). The pH of the TMR was determined according to Buchanan-Smith and Yao (1981), 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).

Particle size distributions were determined for all TMR, pooled refusals, and forage samples using the Penn State Particle Separator (PSPS) (Heinrichs 1996; Lammers et al., 1996). The PSPS has two screens and a bottom pan. The diameters of holes of the screens were 19 and 8 mm for the top and middle screen, respectively. Approximately 150 g wet sample was placed on the top screen of the PSPS. The PSPS was shaken a total of 40 times (5 times in each direction, twice) (Heinrichs 1996). The contents of each fraction were weighed and analyzed for DM and NDF as described earlier. The $peNDF_{DM}$ was determined as the proportion of DM retained by the 19 and 8 mm PSPS screens multiplied by dietary NDF content. The $peNDF_{NDF}$ was determined as the proportion of NDF retained by the 19 and 8 mm PSPS screens.

Milk yield and composition analysis

Cows were milked twice daily and milk production was determined using Tru Test regulation meters (Westfalia Surge, Mississauga, ON). Milk samples were collected from four subsequent 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 Manitoba Milk Producers

(Winnipeg, MB) by near infrared analysis using the Milk-O-Scan 303AB (Foss Electric, Hillerød, Denmark).

Rumen pH measurement

Rumen fluid was sampled twice during each collection period (Tuesday and Thursday afternoons) at 4 to 5 hr post-feeding. Approximately 50 ml of fluid was aspirated using a Geishauser oral probe (Geishauser, 1993; Green et al., 1999). 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.

VFA and ammonia analysis

Frozen rumen fluid samples were thawed at room temperature and 1 ml of a 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, with initial and final column temperatures were 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).

Statistical analysis

Analysis of variance for weekly averages of rumen fluid, milk, and intakes was conducted using the SAS Mixed models procedure (SAS, 1990). The effects of chop length and level of concentrate inclusion were considered random effects. The effects of cow and period were considered random. Analysis of variance for physical and chemical 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 value of equal to or less than 0.05. Differences between treatment means were established using Tukey's multiple range test (SAS, 1990). Reported SE are those used for the comparison of treatment means.

RESULTS

Dry matter content was greater in the SC barley silage compared to the LC barley silage ($P < 0.0001$) (Table 14). As expected, the higher concentrate diets also had higher DM contents ($P < 0.0001$) (Table 15). Increasing chop length increased dietary CP, and SP contents. Increasing level of concentrate in the diet increased CP, starch, Ca, P, K, and Na contents, decreased NDF and ADF contents, but had no effect on SP or Mg contents.

Long chop barley silage had considerably fewer particles passing through the 8mm screen of the PSPS to the bottom pan than did the SC barley silage (Table 16). As a result, diets that incorporated the SC barley silage had more DM passing through the 8 mm screen of the PSPS ($P < 0.01$) and less DM retained by the 19 mm screen ($P < 0.001$) (Table 17). Increasing the level of dietary concentrate increased the amount of DM passing through the 8 mm screen to the bottom pan ($P < 0.01$), and decreased the amount of DM retained on the top (19 mm) screen ($P < 0.001$) (Table 17). Long chop silage doubled DM retained in the top PSPS screen for the higher concentrate diet; the increase being even greater for the lower concentrate diet (Table 17). Dietary NDF content differed between diets with level of concentrate, but was unaffected by chop length (Table 13). Dietary NDF contents averaged across diets were 57.3, 44.8, and 33.6% DM in the 19 mm screen, 8 mm screen, and bottom pan of the PSPS, respectively (Table 17). The NDF content of the experimental diets differed significantly among PSPS fractions. Decreasing barley silage chop length increased the amount of NDF passing through to the bottom pan ($P < 0.0005$) (Table 17).

Table 15. Ingredients and nutrient composition of experimental diets with higher (H) and lower (L) forage:concentrate ratios, and short (SC) and long (LC) chop length of barley silage[1].

	Diet (% of DM)[2]					Effect	
Diet ingredients	HSC	HLC	LSC	LLC	SE	Chop	Conc
Long chop barley silage	0	42.0	0	58.6	—	—	—
Short chop barley silage	42.0	0	58.6	0	—	—	—
Energy supplement	43.3	43.3	32.1	32.1	—	—	—
Protein supplement	14.7	14.7	9.3	9.3	—	—	—
Nutrient Composition[3]							
DM	43.2	40.4	35.8	33.0	0.40	<0.0001	<0.0001
CP, % DM	18.0	20.1	17.3	18.2	0.31	<0.0005	<0.002
SP, %CP	4.8	5.4	5.2	6.9	0.48	<0.05	0.06
NDF, % DM	42.3	44.1	48.1	47.1	0.88	0.67	<0.0005
ADF, % DM	24.0	23.6	28.0	27.1	0.63	0.32	<0.0001
Starch, % DM	18.0	17.0	14.7	13.5	0.56	0.21	<0.002
Ca, % DM	1.25	1.29	1.05	1.04	0.06	0.84	<0.003
P, % DM	0.62	0.68	0.55	0.55	0.02	0.20	<0.003
K, % DM	1.85	1.85	2.21	2.19	0.10	0.92	<0.003
Mg, % DM	0.41	0.38	0.40	0.38	0.02	0.17	0.66
Na, % DM	0.50	0.55	0.39	0.42	0.03	0.11	<0.002

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

[2]HSC = Higher concentrate, short chop; HLC = higher concentrate, long chop; LSC = lower concentrate, short chop; LLC = lower concentrate, long chop.

[3]Nutrient compositions of experimental diets were calculated from ingredient analyses

Table 16. Penn State particle size analysis of long and short chop barley silages.

	Barley Silage	
PSPS sieving	Long chop	Short Chop
	% retained, as fed basis	
Top screen (> 19 mm)	37.1	12.9
Middle screen (> 8 mm)	55.9	60.3
Bottom pan	7.0	26.8
	% retained, DM basis	
Top screen(> 19 mm)	34.3	9.0
Middle screen (> 8 mm)	60.8	68.6
Bottom pan	4.8	22.4

Table 17. Penn State Particle Separator analysis of experimental diets higher (H) and lower (L) forage:concentrate ratios, and short (SC) and long (LC) chop length of barley silage.

	Diet[1]					Effect		
	HSC	HLC	LSC	LLC	SE	Chop	Conc	Chop x Conc
PSPS distribution[2]	% retained, as fed basis							
Top screen (>19 mm)	9.7	21.5	12.2	26.7	1.03	<0.0001	<0.005	0.25
Middle screen (>8 mm)	42.9	45.4	48.7	52.8	2.26	0.17	0.02	0.73
Bottom pan	47.3	33.1	39.1	25.2	0.82	<0.0001	<0.0001	0.84
	% retained, DM basis							
Top screen (>19 mm)	7.7	15.5	9.9	22.5	0.97	<0.001	<0.001	<0.05
Middle screen (>8 mm)	41.4	39	44.6	43.7	1.87	0.4	0.06	0.72
Bottom pan	51.0	45.5	45.4	33.8	1.95	<0.01	<0.01	0.15
	NDF retained in fraction % DM							
Top screen (>19 mm)	57.7	56.2	59.4	56.0	1.46	0.12	0.61	0.52
Middle screen (>8 mm)	41.1	42.7	46.6	48.6	0.89	0.06	<0.0001	0.82
Bottom pan	33.4	29.5	38.4	33.1	0.94	<0.0005	<0.001	0.47
$peNDF_{DM}$[3], % DM	18.9	21.2	24.6	29.5	0.94	<0.005	<0.0001	0.20
$peNDF_{NDF}$[4], % DM	25.2	29.2	30.6	34.9	0.98	<0.01	<0.01	0.86

[1]HSC = Higher concentrate, short chop; HLC = higher concentrate, long chop; LSC = lower concentrate, short chop; LLC = lower concentrate, long chop.

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

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

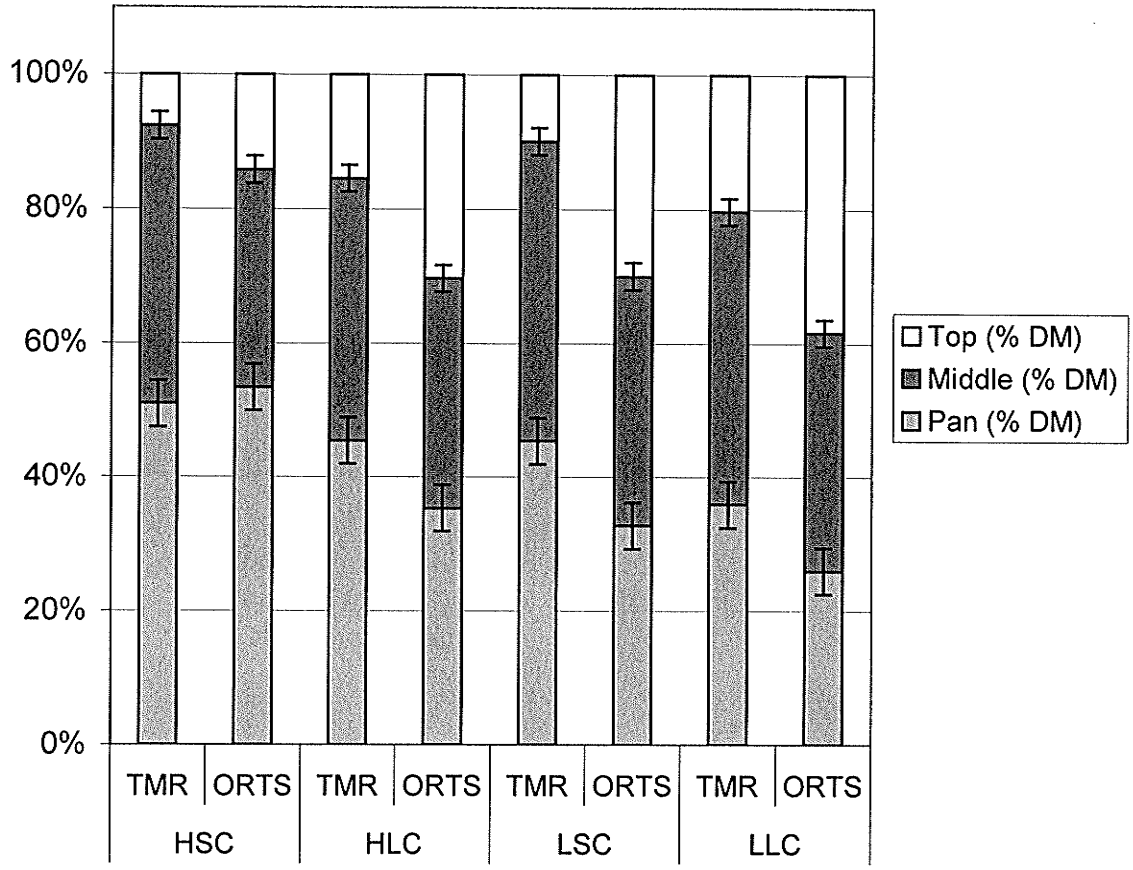
[4]$peNDF_{NDF}$ = proportion of NDF retained by the 19 and 8 mm PSPS screens.

Increasing level of concentrate decreased the amount of NDF retained, and passing through the 8 mm PSPS screen ($P < 0.0001$, and $P < 0.001$ respectively) (Table 17). Analysis of particle size distribution of the TMR and orts is shown in Figure 3. The orts had a greater ($P < 0.0001$) proportion of particles that were retained by the 19 mm screen of the PSPS, compared to the experimental diets (Figure 3). The feed refusals for diet HSC had a higher percentage of DM passing through the 19 and 8 mm screens of the PSPS than the treatment TMR.

Decreasing barley silage chop length increased ruminal propionate concentration ($P < 0.05$) and ammonia concentration ($P = 0.005$), decreased acetate:propionate (A:P) ratio ($P = 0.001$) but had no effect on pH, total VFA, acetate, butyrate, or other VFA content (Table 18). Increasing level of concentrate decreased ruminal pH ($P < 0.0001$), A:P ($P < 0.0001$), and ammonia concentration ($P = 0.02$), and increased propionate content ($P < 0.0005$), but had no effect on acetate, butyrate, or other VFA content (Table 18).

Decreasing barley silage chop length increased DMI ($P < 0.05$), but did not affect milk yield, or milk composition (Table 19). Increasing level of concentrate increased DMI, milk yield, protein yield and protein percentage (Table 19). An interaction trend between chop and concentrate interaction was observed on milk fat percentage (Table 19).

Figure 3. Penn State Particle Size Distribution of Diets[1] and Orts (DM Basis) where diets contained equal inclusion rates of energy supplement and protein supplement within each level of concentrate and varying inclusion rates of barley silage at long and short chops.



[1]HSC = Higher concentrate, short chop; HLC = higher concentrate, long chop; LSC = lower concentrate, short chop; LLC = lower concentrate, long chop.

Table 18. Rumen fluid composition for cows fed experimental diets with fixed inclusion rates of energy supplement and protein supplement within each level of concentrate and varying inclusion rates of barley silage at long and short chops.

	Diet[1]					Effect		
Item[2]	HSC	HLC	LSC	LLC	SE	Chop	Conc	Chop x Conc
pH	6.37	6.35	6.51	6.53	0.03	0.97	<0.0001	0.38
VFA, m*M* L^{-1}								
Total	94.4	92.9	91.8	93.6	4.34	0.79	0.60	0.93
Acetate (A)	56.8	55.5	58.5	58.4	3.17	0.99	0.63	0.95
Propionate (P)	22.1	20.9	20.0	17.7	0.68	<0.05	<0.01	0.49
Butyrate	12.6	12.7	12.1	12.7	0.60	0.37	0.38	0.98
Other	1.8	3.0	15.5	4.1	6.66	0.39	0.28	0.34
A:P	2.6	2.7	2.9	3.3	0.11	0.001	<0.0001	0.06
Ammonia (mg dL^{-1})	11.1	14.5	14.0	16.3	1.14	<0.05	<0.01	0.50

[1]HSC = Higher concentrate, short chop; HLC = higher concentrate, long chop; LSC = lower concentrate, short chop; LLC = lower concentrate, long chop.
[2]Rumen fluid composition variables were averaged for each animal during each period (n= 12) for each treatment and analyzed by analysis of variance.

Table 19. Milk production and feed intake of cows fed experimental diets with equal inclusion rates of energy supplement and protein supplement within each level of concentrate and varying inclusion rates of barley silage at long and short chops.

	Diet[1]					Effect		
Item[2]	HSC	HLC	LSC	LLC	SE	Chop	Conc	Chop x Conc
DMI, kg d^{-1}	20.1	19.4	17.7	16.9	0.34	<0.05	<0.0001	0.85
Orts, % of feed provided	9.77	8.02	7.10	7.03	0.73	0.23	0.03	0.27
Milk yield, kg d^{-1}	31.1	31.4	28.7	28.7	0.53	0.81	<0.001	0.78
Milk Components								
Fat, %	3.05	2.82	3.37	3.56	0.09	0.87	<0.0001	0.09
Fat yield, kg d^{-1}	0.94	0.88	0.97	1.02	0.03	0.88	0.01	0.08
Protein, %	3.24	3.29	3.10	3.11	0.03	0.37	<0.0001	0.47
Protein yield, kg d^{-1}	1.01	1.03	0.89	0.89	0.02	0.59	<0.0001	0.60

[1]HSC = Higher concentrate, short chop; HLC = higher concentrate, long chop; LSC = lower concentrate, short chop; LLC = lower concentrate, long chop.
[2]Feed intake and milk production variables were averaged for each animal during each period (n= 12) for each treatment and analyzed by analysis of variance.

DISCUSSION

Chemical and physical compositions of forage ingredients and experimental diets

Decreasing chop length and increasing the level of concentrate inclusion increased the proportion of particles passing through the two screens of the PSPS and the dietary peNDF content calculated as the percentage of dietary DM retained by the PSPS screens ($peNDF_{DM}$) as well as the proportion of dietary NDF retained by the PSPS screens ($peNDF_{NDF}$). As NDF content varied significantly among PSPS fractions, $peNDF_{NDF}$ was thought to be a more accurate measure for physically effective fibre than $peNDF_{DM}$ (Table 17). NRC (2001) provides minimal recommendations for amount, as well as physical quality of dietary fiber. The diets used in this experiment exceeded the NRC (2001) minimum NDF recommendation of 25% dietary DM, with 75% of NDF from forage. The diets used in this experiment also exceeded the current recommendations for minimum NDF content, 34% DM, in barley silage based diets established by Beauchemin (1991).

One recommendation for TMR particle size distribution on a wet basis is 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 40 to 60%, respectively (Heinrichs, 1996). In the current experiment, all diets were coarser than these guidelines, with the exception of diet HSC, which was on par with these recommendations. Mertens (1997) recommends that dietary peNDF content should be 22% of ration DM in order to maintain an average rumen pH of 6.0, and 20% of ration DM to maintain a milk fat percentage of

3.4 in midlactation Holstein cows. However, the calculations performed by Mertens (1997) use tabular physical effectiveness factor values, and differ from the peNDF calculation in this study. The $peNDF_{DM}$ values in this study ranged from below 18.9% in HSC, to well in excess, 29.5% in LLC, of these recommendations (Mertens, 1997). Comparing particle size distribution of TMRs and their orts, show that orts had a larger percentage of DM retained by the 19 mm screen of the PSPS TMR (Figure 3). This was also observed by Calberry et al. (2003), and demonstrates that cows selected against large feed particles in favour of small feed particles.

Khorasani (1999) recommends that a high moisture barley crop be wilted to a moisture content of 60 to 65% prior to ensiling; the barley used in this study was ensiled at a higher moisture content. The SC barley silage used in this experiment had a significantly higher DM content than the LC barley silage (Table 15), the result of a slightly longer wilting time as the LC barley was chopped and ensiled first. The differences in DM content between the HSC and HLC diets and between the LSC and LLC diets were less than 3%. Lahr et al. (1983) varied DM content in diets that otherwise did not differ in composition from 40% to 78% and found that DMI increased from 19.4 kg d^{-1} to 22.3 kg d^{-1}. Holter (1992) also suggested that in cows fed diets ranging between 30-70% DM, DM content should not affect DMI. Thus, in our study the difference in DM between TMRs with shortly chopped and coarsely chopped barley silage would only have had a minimal effect on DMI.

The high moisture content of the barley silage did not result in poor quality silage. An indicator for silage quality is the dietary acid detergent insoluble protein (ADIP) content. High ADIP contents are an indication of low moisture content promoting excessive heating and aerobic fermentation, resulting in a reduced

availability of dietary nitrogen (Khorasani, 1999). ADIP contents for short and long chop barley silages were 9.6 and 7.0% CP, respectively. These are lower than the recommended upper limit of 10% CP (Khorasani, 1999). Crude protein content was higher in the diets containing long chop barley silage compared to diets containing short chop barley silage (Table 15). The CP content met or exceeded NRC (2001) recommendations in all diets. This might explain why differences in dietary CP content did not affect milk production.

The barley silage used in this experiment was chopped at 10 mm and 19 mm in length. Khorasani (1999) recommends a theoretical chop length (TLC) for barley silage of 10 – 13 mm; therefore, the silage used in this experiment exceeded recommended chop lengths. A recent survey of dairy farms across the province of Manitoba showed that on average 42.5% DM passed through the 19 and 8 mm screens of the PSPS, and 49.9% DM was retained on the 8 mm screen of the PSPS, of the farms that included barley silage in their rations (Plaizier et al., 2003). In the current study, the long chop barley silage forage had 4.8% DM passed through both PSPS screens, and 60.8% DM was retained by the 8 mm screen, and the short chop barley silage forage had 22.4% DM passed through both PSPS screens, and 68.6% DM was retained by the 8 mm screen (Table 16). This shows that even though the middle screen of the PSPS retained the majority of particles at both chop lengths, on average the barley silages used in this study were of a coarser nature that what is commonly seen across Manitoba. In a study conducted by Yang et al. (2001a), barley silage was also used as a forage source at two particle lengths. The longer particle length had 37.0% DM passing through the 19 mm and 8 mm screens of the PSPS, and 57.4% DM was retained on the 8 mm screen; the shorter particle length had 38.3%

DM passing through the 19 mm and 8 mm screens, and 61.4% DM was retained on the 8 mm screen (Yang et al., 2001a). The barley silages used in the current experiment were not as fine, and spanned a wider range of lengths than those used by Yang et al. (2001a), explaining the differences between results observed in both studies.

Table 20 displays a comparison of several studies in which the effect on milk production and rumen conditions were observed by varying dietary particle length. The particle distribution of the TMR used in the current experiment overlapped in terms of particle size with those used by Kononoff et al (2000) and Kononoff and Heinrichs (2003b), and was generally coarser than the rest of the studies documented in this table.

Dry matter intake

Decreasing dietary peNDF content, by chopping barley silage short increased DMI from 19.4 kg d^{-1} to 20.1 kg d^{-1} at the high level of concentrate and from 16.9 to 17.7 kg d^{-1} at the lower level of concentrate inclusion (Table 19). Of the studies included in Table 20, reducing particle chop length increased DMI in the studies from Soita et al. (2002) and Kononoff and Heinrichs (2003a), decreased DMI in the study from Kononoff et al. (2000), but did not affect DMI in the studies from Yang et al.

(2001a), Krause et al. (2002a), Beauchemin et al. (2003), Kononoff and Heinrichs (2003b), and Calberry et al. (2003). Of all studies in Table 20, the study from Soita et al. (2002) had the lowest concentrate content, and the concentrate inclusion rate in the study from Kononoff and Heinrichs (2003a) was also low compared to other studies. When high forage diets are fed to lactating dairy cows, DMI can be limited by distension of the reticulo-rumen (Allen, 2000). In this case, a reduction of particle size, resulting in higher rumen passage rate, would allow for greater feed intake (Allen, 2000). Soita et al. (2002) indeed observed that a reduction in forage particle size increased liquid outflow rate and rumen particulate passage rate. However, Yang et al. (2001b) and Kononoff and Heinrichs (2003a) did not find such an effect. Physical fill could be limiting feed intake at the lower concentrate inclusion, but at the higher concentrate inclusion, a metabolic, rather than a physical constraint on feed intake, can be expected to be rate limiting (Allen, 2002). Diets in our study were also relatively coarse compared to studies in which no effect of dietary particle size on feed intake was observed, which would explain why reducing dietary particle size resulted in a small increase in feed intake in our study. Barley silage based diets containing greater that 22% $peNDF_{DM}$ appear to limit intake.

Rumen fermentation

Altering barley silage chop length did not affect rumen pH at both levels of concentrate inclusion (Table 18). Rumen pH never dropped below 5.6 (Keunen et al., 2002), indicating that SARA was not induced. Of the studies in Table 20, Yang et al.

(2001a) and Kononoff and Heinrichs (2003b) also did not find an effect of dietary particle size on rumen pH, whereas Soita et al. (2002), Krause et al. (2002b), Beauchemin et al. (2003), and Calberry et al. (2003) observed that reducing dietary particle size reduced rumen pH. In the study from Kononoff and Heinrichs (2003a), a quadratic, but not a linear effect of dietary particle size on rumen pH was found. This disparity between studies can be explained by presence of a threshold, only below which, peNDF and dietary particle size affects rumen pH. As determined by our studies, this threshold exists below 18.2% $peNDF_{DM}$ and below 25.2% $peNDF_{NDF}$ for barley silage based diets. This explains why reduction in particle size affected rumen pH in fine diets, but not in coarse diets. Calberry et al. (2003) also found an effect on reducing particle size in a corn silage based TMRs on rumen pH, despite the fact that the diets used were coarser than the alfalfa silage based TMRs used by Krause et al. (2002b) and Beauchemin et al. (2003). This might be caused by factors other than particle size, such as intrinsic buffering capacity, that affect rumen buffering. Corn silage has a lower intrinsic buffering capacity than alfalfa silage (McBurney et al., 1983), facilitating a lower rumen pH, thus explaining the effect seen by Calberry et al. (2003) despite using coarser diets. The diets used by Kononoff and Heinrichs (2003b) also used corn silage, but diets were coarser than those used by Calberry et al. (2003). This explains why in the former study no effect of dietary particle size on rumen pH was seen. The high dietary inclusion rate of alfalfa could also explain why, despite small dietary particle sizes, rumen pH levels indicative of subacute ruminal acidosis (SARA), e.g rumen pH below 5.6 (Keunen et al., 2002), were not observed by Calberry et al. (2003).

Discrepancies in the effects of dietary particle size on rumen pH between studies, displayed in Table 20, might also be due to differences in the monitoring of rumen pH. Of the studies in Table 20, Yang et al. (2001a), Krause et al. (2002ab) and Beauchemin et al. (2003) used continuous rumen pH measurement in the ventral sac of the rumen. Kononoff and Heinrichs (2003a, 2003b) collected rumen contents for pH measurement from different sites in the rumen at 2 to 3 hr intervals. In the current study rumen fluid was collected using an oral probe at 4 hr after feeding. Rumen fluid sampling through a cannula is preferred to rumen fluid collected using an oral probe, but the availability of rumen fistulated animals often constrains the number of animals available for the experiment. Continuous pH monitoring is preferred over less frequent determination of rumen pH. However, in-dwelling pH probes are normally positioned in the ventral sac of the rumen. Hence, continuous rumen pH does not take variation between pH in different sites of the rumen into account (Duffield et al., 2003).

Chop length and level of concentrate had no effect on total ruminal VFA, but increased ruminal propionate and decreased the acetate to propionate ratio (Table 18). As seen in Table 20, reducing dietary particle size increased total VFA and reduced acetate to propionate ratio in the studies from Krause et al. (2002b) and Kononoff and Heinrichs (2003a), decreased total VFA without affecting acetate to propionate ratio in the study from Yang et al. (2001a), but had no effect on total VFA and acetate to propionate ratio in the studies from Beauchemin et al. (2003) and Kononoff and Heinrichs (2003b). These disparities might be explained by the many factors that affect rumen VFA, such as level of concentrate, forage source, concentrate source, rumen volume and flow rate, as well as animal-to-animal variation (Van Soest, 1994),

and interactions between these factors and dietary particle size. The absence of an effect of chop length on total rumen VFA could be interpreted as a sign that chop length did not affect the degree of rumen digestion of carbohydrates. However, rumen concentration of VFA do no truly reflect production of VFA, and rumen VFA concentration is regulated by the balance between production and absorption of VFA, as well as rumen pool size and turn regulates rumen concentration of VFA (Van Soest, 1994). Reduction in dietary particle size decreases liquid passage rate and volume of liquid digest in the rumen due to reduction in saliva production (Yang et al., 2001a, Yang et al. 2001b; Krause et al., 2002a; Yang et al., 2002).

A decrease in rumen ammonia concentration was found when barley silage chop length was reduced from 19 mm to 10 mm (Table 18). Crude protein and soluble protein content were significantly higher in the long chop barley silage, as well as in diets that contained long chop barley silage (Table 13; Table 15). This could also account for the increase in rumen ammonia concentration when long chop barley silage was fed. Yang et al. (2001a), Beauchemin et al. (2003), Kononoff and Heinrichs (2003a), and Kononoff and Heinrichs (2003b) found no effect on rumen ammonia when forage particle size was reduced. As a result, it is believed that differences in protein contents between diets, rather than differences in dietary particle size were responsible for differences in rumen ammonia. Rumen ammonia concentration increased as concentrate inclusion rate decreased (Table 18). Dry matter intake increased when the higher concentrate diets were fed. As feed intake increases, as does passage rate, and rumen digestion, more specifically, protein digestion decreases, resulting in diminished ruminal ammonia production (NRC, 2001). Increased levels of bypass protein were fed in the high concentrate diets

compared to the lower concentrate diets, resulting in more protein escaping ruminal degradation. Khorasani and Kennelly (2001) also noted that rumen ammonia concentration decreased with increased levels of concentrate.

Milk production and composition

Table 20 shows a comparison of studies measuring the effect of varying forage and dietary particle length on milk yield and composition. In our study dietary particle size did not affect milk yield and milk composition. This confirms the results from Krause et al. (2002a) and Beauchemin et al. (2003) who also did not find such an effect. Also, in the study of Kononoff and Heinrichs (2003a), dietary particle size did not affect milk yield and milk fat percent, and had a quadratic effect on milk protein percentage was observed. In the study from Yang et al (2001a), forage particle length had no effect on milk yield and tended to reduce milk fat percentage and milk protein percentage. Kononoff and Heinrichs (2003b) found that a reduction in dietary particle size did not affect milk yield, but reduced milk fat percentage and increased milk protein percentage. Kononoff et al. (2000) found that a reduction in barley silage cut length did not affect milk yield and milk composition. Calberry et al. (2003) found that increasing dietary particle size numerically increased milk fat concentration, but did not affect milk yield and milk protein percentage. Despite of the discrepancies between these studies, a reduction in particle size and peNDF was expected to reduce milk fat percentage. Mertens (1997) determined the relationship between peNDF and milk fat percentage using data from previous studies and found

this relationship to be curvilinear. In the lower range of peNDF (20-22 % DM) a reduction in peNDF caused a large reduction in milk fat percentage than in higher ranges of peNDF (Mertens, 1997). Our study, and several other studies in which no effect of peNDF on milk fat percentage was observed, dietary peNDF content would be in the upper levels (as calculated by Mertens et al. (1997)). Other reasons that many studies did not observe a significant relationship between peNDF and milk composition, might include that: few animals were used; diets included large amounts of alfalfa silage and alfalfa hay, which have a higher intrinsic buffering capacity than corn silage (McBurney et al., 1983); and that many factors other than dietary particle size and rumen pH, such as dietary fat content and genetics affect milk fat content. (Calberry et al., 2003).

The objective of the study was not to determine the effect of increasing concentration the diet on rumen conditions, but the effect of variation in particle size at two levels of concentrate inclusion. Increasing the inclusion of concentrate in the diet reduced rumen pH, increased rumen propionate, reduced rumen acetate to propionate ratio, increased milk yield, reduced milk fat percentage, increased milk protein percentage, and increased DMI. Such results were expected (NRC, 2001). No interactions between dietary particle size and concentrate level on rumen condition, feed intake, and milk production, with the exception of milk fat percentage, were observed. Reducing forage particle length increased milk fat percentage in the higher concentrate diets, but decreased milk fat percent in lower concentrate diets. The long chop barley silage incorporated in the experimental diets had a higher dietary fat percentage (Table 15). Higher levels of dietary fat content tend to have a negative effect on milk fat production, at higher levels of concentrate inclusions (Griinari et

al., 1998). This could why milk fat content was higher in LLC, because the extent to which dietary fat content affects milk fat production in lower concentrate inclusions is reduced compared to higher concentrate inclusions (Griinari et al., 1998).

The increase in DMI due to the shorter chop length did not result in a milk production response (Table 19), which could be due in part to the short duration of the experimental periods not allowing enough time for animals to respond to dietary changes. In the study from Kononoff and Heinrichs (2003a) an increase in DMI resulting from a reduction in dietary particle size that was larger than that found in our study also did not affect milk yield and milk fat percentage. Lack of milk production response due to increased DMI and reduction in dietary particle size might be explained by the short duration of experimental periods, or by reduced rumen digestibility. Studies on the effects of dietary particle size and digestibility are conflicting. Yang et al. (2002) and (Krause et al., 2002a) found that reduction in dietary particle size did not affect total tract digestibility of DM and NDF. Johnson et al. (2003) found that shorter chop length of corn silage reduced total tract digestibility of organic matter and NDF. On the other hand, Soita et al (2002) and Kononoff and Heinrichs (2003a) found that reduction in dietary particle size increased apparent digestibility of DM and NDF. Reduction of feed particle size could reduce rumen digestibility if SARA is induced (Plaizier et al., 2001), which was not the case in our studies as in the other studies referred to in Table 20. Reduction in feed particles size could increase rumen digestion if it increases the access of rumen bacteria to the feed (Van Soest, 1994), unless rumen particle passage rate is so increased that rumen retention time becomes limiting for fiber digestion. Due to the coarseness of the diets in our study, the latter was not expected.

CONCLUSIONS

Reducing the chop length of barley silage from 19 mm to 10 mm reduced the proportion of the TMR passing through the 19 and 8mm screens of the PSPS from 51.0 to 45.5% DM at the higher concentrate inclusion (58.0% DM concentrate) and from 45.4 to 33.8% DM at the lower concentrate inclusion (41.4% DM concentrate). This reduction in chop length increased DMI across concentrate inclusion rates from 18.1 to 18.9 kg d^{-1}, but it did not affect milk yield, milk composition, rumen pH and rumen propionate. Reduction of chop length also increased rumen propionate, and reduced rumen acetate to propionate ratio and rumen ammonia. In concurrence with Khorasani (1999), a recommended TLC for barley silage should be between 10-13 mm in length. Reducing barley silage chop length to 10 mm had no detrimental effects on rumen parameters or production for lactating cows consuming 41.4 to 58.0% of the TMR as a barley grain concentrate.

Table 21. Comparison of studies measuring the effect of varying forage and dietary particle length on milk production and rumen conditions at various forage to concentrate ratios.

Study	Forage type	Concentrate	F:C	Diet				Change to decrease in MPL			
				>19mm[1]	8-19mm[1]	<8 mm[1]	MPL[2]	Rumen pH	DMI	Milk fat	SARA induced
Yang et al. (2001a, 2001b)	Long alfalfa silage, barley silage and alfalfa hay	Coarsely and flatly rolled barley grain	35:65 & 39:61	4.5	34.6	60.9	7.0	No effect	No effect		
	Short alfalfa silage, barley silage and alfalfa hay	Coarsely and flatly rolled barley grain	35:65 & 39:61	0.3	32.2	67.5	5.6				
Krause et al. (2002a, 2002b)	Fine alfalfa silage	High moisture shelled corn	39:61	-	-	-	3.0	Down	No effect	No effect	Yes
	Coarse alfalfa silage	High moisture shelled corn	39:61	-	-	-	6.0				
	Fine alfalfa silage	Dry cracked shelled corn	39:61	-	-	-	2.8				
	Coarse alfalfa silage	Dry cracked shelled corn	39:61	-	-	-	6.3				
Kononoff et al. (2000)	Barley silage TLC 9.0mm	60.7% rolled barley	45:55	11.0[3]	54.2[3]	34.8[3]	8.1	n.a.	Up	No effect	n.a.
	Barley silage TLC 4.8mm	60.7% rolled barley	45:55	3.6[3]	34.1[3]	62.2[3]	6.1				
Kononoff et al. (2003)	Short corn silage (SC)	Ground corn	40:60	2.9	57.5	39.5	7.4	No effect	Up	No effect	No
	1/3 LC, 2/3 SC	Ground corn	40:60	6.7	55.7	37.8	7.8				
	2/3 LC, 1/3 SC	Ground corn	40:60	11.1	53.2	35.8	8.3				
	Long corn silage (LC)	Ground corn	40:60	15.5	50.3	34.2	8.8				
Kononoff and Heinrichs (2003a)	Short alfalfa haylage (SH) TLC 4.8mm	30% ground corn	50:50	3.0	28.3	68.6	4.1	Quadratic	Up	No effect	No
	1/3 LG, 2/3 SH	30% ground corn	50:50	12.3	24.8	62.9	4.8				
	1/3 SH, 2/3 LG	30% ground corn	50:50	21.9	21.1	57.0	5.7				
	Long alfalfa haylage (LH) TLC 22.3mm	30% ground corn	50:50	31.4	17.5	51.1	6.8				

Study	Forage type	Concentrate	F:C	>19mm[1]	8-19mm[1]	<8 mm[1]	MPL[2]	Rumen pH	DMI	Milk fat	SARA induced
Kononoff and Heinrichs (2003b)	Long corn silage	Corn	-	10.9	52.3	36.8	7.9	No effect	No effect	Numeric reduction (P = 0.08)	No
	Long corn silage & cotton seed hulls	Corn	-	6.5	48.2	45.3	6.8				
	Short corn silage	Corn	-	3.3	53.0	43.7	6.8				
	Short corn silage & cotton seed hulls	Corn	-	2.3	47.9	49.8	6.1				
Beauchemin et al. (2003)	Alfalfa silage, chopped alfalfa hay (50:50)	Steam rolled barley grain	40:60	9.4	32.1	58.6	-	Down	No effect	No effect	Yes
	Alfalfa silage, ground alfalfa hay (50:50)	Steam rolled barley grain	40:60	0.8	29.9	69.3	-				
	Alfalfa silage, chopped alfalfa hay (25:75)	Steam rolled barley grain	40:60	11.9	25.8	62.3	-				
	Alfalfa silage, ground alfalfa hay (25:75)	Steam rolled barley grain	40:60	0.2	21.6	78.2	-				
Calberry et al. (2003)	Corn silage, chopped alfalfa hay	Rolled barley grain	40:60	8.7	29.5	61.9	-	Down	No effect	No effect	No
	Corn silage, chopped alfalfa hay/alfalfa silage	Rolled barley grain	40:60	9.4	31.3	59.3	-				
	Corn silage, alfalfa silage	Rolled barley grain	40:60	12.6	32.2	55.2	-				
Krause et al. (2003)	Coarse alfalfa silage	Dry cracked shelled corn	39:61				3.5	No effect	Down	Down	Yes
	Coarse alfalfa/corn silage	Dry cracked shelled corn	39:61				3.6				
	Fine alfalfa silage	Dry cracked shelled corn	39:61				2.5				
	Fine alfalfa/corn silage	Dry cracked shelled corn	39:61				2.6				
	Coarse alfalfa/corn silage	Ground high moisture shelled corn	39:61				4.2				
	Fine alfalfa/corn silage	Ground high moisture shelled corn	39:61				2.8				
Soita et al. (2002)	Short barley silage, TLC 4.7 mm	None	100:0	1.5	22.2[4]	76.3[5]	-	Down	Up	n.a.	No
	Long barley silage, TLC 18.8mm	None	100:0	2.5	51.2[4]	46.3[5]	-				

[1]DM basis [2]Mean particle length [3]As fed basis [4]6.3 – 19 mm

GENERAL DISCUSSION

High yielding dairy cows are fed high concentrate diets to meet the nutrient demands placed on them by high levels of production. Consequently, these diets are low in fibre and high in starch. It is critical to balance these diets with physical fibre to promote rumen buffering and prevent ruminal acidosis. Traditionally, rations were balanced for NDF to provide sufficient rumen buffering. However, not all NDF is equally effective in stimulating chewing and contributing to rumen buffering. Therefore, lactating dairy cattle diets need to be balanced to contain adequate physically effective fibre (peNDF), i.e fibre that stimulates rumination and production of salivary buffers, so that a healthy rumen environment is maintained and cows can achieve their production potential. However, diets with excessive peNDF can limit intake through distension of the reticulo-rumen (Allen, 2000).

The amount of peNDF in a diet is dependent on a number of factors: forage chop length, forage to concentrate ratio, and inclusion rate of various forages with different chop lengths. Variation in these factors might affect peNDF and rumen fermentation differently, which might help to explain the inconsistency of the effects of peNDF on metabolism and milk production amongst previous studies. Therefore, comparison of studies on the effect of particle size distribution on various aspects of dairy nutrition is quite difficult. The variety of techniques for assessing peNDF, particle size distribution, as well as variation in forage source, disallows for adequate comparison. A standard validated technique for measuring particle size distribution is required to facilitate generation of recommendations for dietary peNDF requirement.

Three experiments were conducted to determine the effect of varying particle size and peNDF on rumen conditions, dry matter intake, and milk production of lactating dairy cows. The chemical and physical parameters of the diets where chosen to represent the range of peNDF observed on farms in Manitoba. Forage ingredients were not ground, as done in previous studies to obtain very low levels of peNDF, as this is not common practice on Manitoba farms.

Rumen pH is affected by several dietary and animal factors, such as rumen digestion, VFA production, VFA absorption, supply of inorganic buffers through the saliva, rumen volume, rumen turn over, and intrinsic buffering capacity of feeds (McBurney et al., 1983; Van Soest, 1994). Reduction in dietary particle size has been shown to decrease rumen digestion, increase rumen particulate passage rate, reduce rumen volume, and reduce rumen liquid flow rate (Krause et al., 2002a, Soita et al., 2002, Krause and Combs, 2003, Soita et al., 2003). Some of these effects of peNDF on rumen pH can cancel each other out. Altering peNDF through varying forage particle size did not influence rumen pH in any of the three previously reported trials. Rumen pH was affected when peNDF changed due to a difference in level of concentrate inclusion. In all three experiments, average rumen pH never dropped below 6.27. The threshold for SARA is stated to be below a pH of 5.6 in ruminally fistulated cows (Cooper and Klopfenstein, 1996). Considering orally sampled rumen fluid has a pH that is 0.22 units higher than rumen fluid obtained from rumen fistulated cows, it can be inferred that, in addition to the fact that dietary contents of starch were not excessive and peNDF was sufficient to negate any risk of inducing ruminal acidosis (NRC, 2001), the cows in this experiment did not experience SARA.

Of the studies in Table 21, Yang et al. (2001a) and Kononoff and Heinrichs (2003b) also did not find an effect of dietary particle size on rumen pH, whereas Krause et al. (2002b), Beauchemin et al. (2003), and Calberry et al. (2003) observed that reducing dietary particle size reduced rumen pH. In the study from Kononoff and Heinrichs (2003a), a quadratic, but not a linear effect of dietary particle size on rumen pH was found. This disparity between studies can be explained by presence of a threshold, only below which, peNDF and dietary particle size affects rumen pH (Mertens, 1997). This explains why reduction in particle size affected rumen pH in fine diets, but not in coarser diets like this ones used in these three experiments. Minimum requirements for SARA could not be established in the three experiments conducted because it was never induced.

Ruminal concentration of total VFA depends on rumen digestion, VFA absorption rate, and rumen size and turnover (Van Soest, 1994). The absence of an effect of chop length on total rumen VFA in all studies could be interpreted as a sign that particle size, did not affect any of these factors, or that the effect of one factor was cancelled out by the effect of another factor. In experiment 2, varying dietary particle size had no effect on the ruminal concentrations of individual VFA. This is not surprising given the lack of effect of increasing dietary peNDF content on DMI and rumen pH in this trial. These results are in agreement with Beauchemin et al. (2003), where no effect was observed on ruminal VFA concentrations when forage particle size was altered by varying inclusion rates of alfalfa silage and alfalfa hay. In the third experiment, reducing barley silage chop length increased the ruminal concentration of propionate, and decreased the acetate to propionate ratio.

Varying dietary particle size had no effect on rumen ammonia concentration in the first two experiments. However, in the third experiment, increasing $peNDF_{DM}$ content from 18.9 to 29.5 % DM increased rumen ammonia concentration from 11.1 to 16.3 mg dL^{-1}. The CP content of the diets used in the third experiment had a greater range (2.8%) than the diets used in experiments 1 and 2 (0.4 and 1.0 %, respectively). This could account for significant difference in rumen ammonia concentration when diets in the third experiment were fed. The levels of rumen ammonia measured in these experiments were not excessively high, nor, were they limiting for microbial crude protein synthesis (Van Soest, 1994).

Substituting chopped alfalfa hay for alfalfa silage resulted in a significant increase in DMI in the first experiment, but not in the second. Due to the fact that the diets in both experiments had equal dietary levels of NDF, the difference in DMI is attributed to the broader range of ADF observed in the first experiment (27.0-30.1%) than in the second (23.1-25.3%). Dietary ADF is related to rumen digestibility; thus, physical fill is more likely to limit intake in less digestible diets. In the third experiment, decreasing dietary peNDF content, by chopping barley silage short, increased DMI from 19.4 kg d^{-1} to 20.1 kg d^{-1} at the high level of concentrate and from 16.9 to 17.7 kg d^{-1} at the lower level of concentrate inclusion. The significant change in DMI is attributed to the broad range of dietary peNDF and coarse nature of the some of the diets used in this experiment.

No effect was exhibited on milk yield when varying forage source in the first two experiments, and chop length in the third experiment altered dietary peNDF. The fact that no effect was observed on milk yield in the second experiment is probably

due to the fact that DMI and rumen conditions were not affected. The lack of effect on milk yield when chopped alfalfa hay was substituted for alfalfa silage is somewhat surprising given that this substitution yielded an increase in DMI in the first experiment. This may be attributed to a change in rumen digestibility when chopped alfalfa hay was substituted for alfalfa silage. Yang et al. (2001a) found that when peNDF content increased, by varying dietary fiber content, ruminal digestibility significantly decreased from 48.3 to 39.6%. In the third experiment, the lack of milk production response despite an increase in DMI and reduction in dietary particle size might be explained by reduced rumen total tract digestibility. Studies on the effects of dietary particle size and also digestibility are conflicting. Yang et al. (2002) and (Krause et al., 2002a) found that reduction in dietary particle size did not affect total tract digestibility of DM and NDF. Johnson et al. (2003) found that shorter chop length of corn silage reduced total tract digestibility of organic matter and NDF. In both the first and third experiments the increase in DMI did not result in a milk production response, which could be due in part to the short duration of the experimental periods not allowing enough time for animals to respond to dietary changes.

According to Mertens (1997), milk fat content will increase curvilinearly as dietary peNDF content increases until a point (~25% ration DM) where the effect is minimal. In the first experiment, milk fat percent, and milk fat yield were the highest in the HS1 diet. These results contrast work by Beauchemin et al. (1994) who concluded that effects of particle size on milk fat content are likely to occur when NDF levels are below minimum NRC recommendations. Dietary NDF levels in this

experiment exceeded NRC recommendations (NRC, 2001). Mertens (1997) complied data from 36 citations and observed that a curvilinear relationship exists between dietary peNDF conent, rumen pH and milk fat percentage. Griinari et al. (1998) theorized that when rumen pH is low, there is an increase in the production of trans-fatty acids. Trans-fatty acids have a negative effect on *de novo* milk fat synthesis (Griinari et al., 1998). This is most likely not the case in this experiment because rumen pH was never below 6.5. No effect on milk fat percent, and milk fat yield was observed in experiment 2. In the third experiment, reducing forage particle length increased milk fat percentage in the higher concentrate diets, but decreased milk fat in lower concentrate diets. Despite the fact that both barley silage chop lengths came from the same field and ensiled the same day, the long chop barley silage incorporated in the experimental diets had a higher dietary fat percentage (Table 3). Higher levels of dietary fat content tend to have a negative effect on milk fat production, at higher levels of concentrate inclusions (Griinari et al., 1998). This could explain why milk fat content was higher in LLC, because the extent to which dietary fat content affects milk fat production in lower concentrate inclusions is reduced compared to higher concentrate inclusions (Griinari et al., 1998).

No effect was observed on milk protein percent and yield in experiments 2 and 3. In the first experiment, milk protein percent and yield increased significantly as alfalfa silage was substituted for chopped alfalfa hay. Soluble protein content was higher in diets containing alfalfa silage, which is to be expected. This increased solubility, along with a possible increase in rumen retention time due to the relative coarseness of alfalfa silage compared to alfalfa hay, may have allowed for improved

microbial crude protein (MCP) synthesis. Increasing rumen retention time may have improved rumen digestibility, providing more substrate for MCP synthesis.

FUTURE RESEARCH

- 24 hour continuous monitoring of rumen pH
- Extend length of experimental periods
- Rumen and total tract digestibilities
- Salivary flow rate and rumen volume
- Chewing and saliva production
- VFA profile of feedstuffs
- Broaden range of peNDF fed
- Reduce confounding effects of harvesting and chopping forages
- Use corn grain as a concentrate source

CONCLUSIONS

Variation of dietary peNDF, in a range representative of practice on Manitoba dairy farms, either by altering the relative inclusion of alfalfa silage and chopped alfalfa hay, or chop length of barley silage, had a limited effect on rumen fermentation and milk production. This occurred when peNDF was above a threshold, below which, a decline in peNDF has a large effect on milk production, rumen fermentation and intake. It was observed that excessively coarse diets ($peNDF_{DM}$ > 22% DM) appear to limit intake, and that feeding these diets over a prolonged period could negatively impact on milk production. A standard validated technique for measuring particle size distribution is required to facilitate generation of recommendations. Measuring peNDF content as the amount of NDF retained on the top two sieves of the PSPS appears to such a technique.

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APENDIXES

Appendix 1. Ingredient composition (% of DM) of the energy and protein supplements. Experiments 1 and 2.

Ingredient	Energy supplement	Protein supplement
Rolled barley	54.0	—
Luprosil salt	0.2	—
Protein pellet[1]	1.9	—
Vitamin and mineral mix[2]	40.0	—
Tallow	4.0	
Dried distillers grain	—	42.0
Fish meal	—	7.0
Canola meal	—	22.8
Soybean meal	—	20.0
Beet molasses	—	3.0
Niacin	—	0.3
Sodium bicarbonate	—	5.0

[1]Protein pellets contained 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]Vitamin and mineral mix contained 0.13% vitamin ADE premix (Vit A, 16800 IU/kg; Vit D, 2215 IU/kg; Vit E, 75 IU/kg, DM basis), 0.14% 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% dynamate, 0.3% pellet binder, 1.0% cane molasses, 3.7% calcium carbonate, and 10.0% corn gluten meal.