AN ABSTRACT OF THE THESIS OF

Annette C. Buyserie for the degree of Master of Science in Animal Science presented on September 21, 2004.

Title: Perennial Ryegrass Nonstructural Carbohydrates in Dairy Cattle Nutrition.

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Ruminal microorganisms require nitrogen and energy for microbial crude protein (MCP) synthesis. High-quality grass provides an excess of readily available proteins relative to available carbohydrates which reduces the conversion efficiency of grass protein to MCP. Nonstructural carbohydrates (NSC) are the primary source of readily available energy. Objectives of trial 1 were to study the effects of perennial ryegrass NSC on milk yield and composition, dry matter intake (DMI), and rumen fermentation in dairy cows. Two perennial ryegrasses, one with a relatively high NSC content (HNSC; Elgon®) and one commonly grown in Oregon (CNSC; Linn) were fed as green chop. Twelve Holsteins and two Jerseys were blocked by milk yield and assigned at random to a treatment. Cows were supplemented with a total mixed ration (TMR) for 1 h twice daily. Grasses were cut, sampled, and offered ad-libitum twice daily after the TMR. Individual grass and TMR intake and milk yield were collected twice daily for 21 d. Milk samples were collected d 0 of the treatment adaptation period and d 7 and 21 of the treatment period. On d 9 and 21 of the treatment period, rumen samples were collected at 0, 1, 2, 3, 4, 6, 8, 10, and 12 h relative to each TMR feeding and analyzed for pH, volatile

fatty acids (VFA), and ammonia (NH_3). Data were analyzed with the MIXED procedure of SAS. For grass DMI, treatment by wk interaction was significant ($P<0.01$). For HNSC, grass DMI was greater wk 2 ($P<0.01$) and tended to be greater wk 3 ($P<0.10$). Total mixed ration DMI tended to be greater for HNSC treatment ($P=0.06$). Milk yield and yield of milk fat and protein were greater for the HNSC treatment ($P<0.05$). Milk urea nitrogen and ruminal VFA and NH_3 did not differ between treatments. Grass composition was different than expected. High NSC grass was lower in NSC ($P<0.05$) and higher in crude protein ($P<0.01$). Grass neutral detergent fiber and acid detergent fiber were similar. In this study, milk and component yields for HNSC were greater than CNSC treatment; however, effects were not due to grass NSC.

Well-preserved grass silage is the result of the controlled fermentation of fresh grass; characterized by low pH, high lactic acid, and low NH_3. Nonstructural carbohydrates are the primary fermentation substrate. Objective of trial 2 was to determine if differences exist between fermentation characteristics of three high NSC grasses and one control NSC grass ensiled in vacuum sealed bags. Perennial ryegrasses, three with a relatively high NSC concentration (HNSC; AberAvon®, AberDart®, and Elgon®) and one commonly grown in Oregon, control NSC (CNSC; Linn) were selected. Three replicates of each grass were ensiled at the a.m. and p.m. harvests. Each bag was packed, vacuum sealed, and ensiled for 60 d. Fresh grass samples were taken from each bag. Fresh grass NSC was greater for HNSC grasses versus Linn. Final pH was lower, total acids was higher, and lactic acid tended to be higher for HNSC grasses. Final pH,

lactic acid, acetic acids, total acids, and NH_3 were lower for p.m. versus a.m. cutting. Ensiling was most efficient for HNSC grass varieties harvested at the p.m. cutting.

Perennial Ryegrass Nonstructural Carbohydrates in Dairy Cattle Nutrition.

by

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CONTRIBUTION OF AUTHORS

Troy Downing assisted in design, data collection, interpretation of the data, and writing of Chapters 3 and 4.

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Chapter 1
Introduction

In dairy production systems, the decision to use a specific forage is dependent on key factors such as available land base, manure management, soil type and topography, climate, and availability of purchased forages and feeds (Harrison et al., 1994). In Europe, New Zealand, and Australia, grass forages are commonly used as the primary source of nutrients for lactating dairy cattle. In the United States, use of grass forage for lactating dairy cattle has been varied. In the Pacific Northwest, grass is an important source of nutrients for dairy cattle. Pasture, green chop, and silage are common forms of grass forages. In the United States, research on the utilization of grass forages for dairy production has increased in the last 20 years.

Well-managed grass forages are an economical source of nutrients for lactating dairy cattle. Perennial ryegrass is an important perennial forage in animal production systems around the temperate regions of the world. In the Pacific Northwest, perennial ryegrass is the primary temperate grass forage used as a feedstuff for lactating dairy cattle. Perennial ryegrasses have high yield and nutritive value. In animal production systems, perennial ryegrass is also valued for agronomic properties such as ease of establishment, rapid regrowth, and ability to use high levels of soil nutrients.

Nonstructural carbohydrates (NSC) and water-soluble carbohydrates (WSC) are the more readily available carbohydrates in grasses. In temperate grasses, the primary

NSC are glucose, fructose, sucrose, starch, and fructans and the primary WSC are glucose, fructose, sucrose, and fructans (Smith, 1973). In the rumen, microorganisms use NSC and produce microbial crude protein (MCP) and volatile fatty acids used by the ruminant. During ensiling, microorganisms use NSC and produce acids which reduce the pH and preserve the forage.

For dairy cattle consuming high-quality fresh grass, energy is the limiting nutrient for milk production. It was estimated that energy supplementation is required to achieve milk yields greater than 30 kg/d for cows consuming high-quality grass pastures (Kolver and Muller, 1998). Fresh, high-quality grass provides an excess of readily available proteins relative to available carbohydrates which reduces the conversion efficiency of grass protein to MCP. Extensive research has been conducted to determine the optimal type, rate, and timing of carbohydrate supplementation to improve the utilization efficiency of grass forage for milk production (Bargo et al., 2002; Delahoy et al., 2003; Meijs, 1986; Soriano et al., 2000; Trevaskis et al., 2004; Valk et al., 1990; vanVuuren et al., 1986).

In an effort to improve utilization efficiency of grass nutrients for production, perennial ryegrasses that accumulate elevated levels of WSC have been developed (Humphreys, 1989 a, b, c). Miller et al. (2001b) hypothesized that an increase in WSC concentration would improve the balance of readily available energy and protein, increase the conversion of grass proteins to MCP, and decrease nitrogen excretion. High WSC grass forages have improved growth in lambs and milk production in dairy cattle (Lee et al., 2001; Miller et al., 2001b; Miller et al., 1999). As WSC are the primary

fermentation substrate during ensiling, grasses with elevated WSC may also improve the efficiency of nutrient preservation in ensiled forages.

Objectives of the research were to compare milk yield and composition, dry matter intake, and rumen fermentation of a high NSC grass and a control NSC grass fed fresh to lactating dairy cows and fermentation characteristics of three high NSC grasses and one control NSC grass.

Chapter 2
Review of literature

Grass-based systems

Researchers in Western Europe, New Zealand, and other countries have extensively studied grass-based systems, focusing on understanding grass physiology, sward dynamics, and grazing management to increase milk component production per unit of grassland (Hodgson and Illius, 1996; Pearson and Ison, 1987). The future of grass for dairy cattle, highlighting economic, social, and environmental impacts of grass-based dairy systems has been discussed. Grass-based dairy systems can enhance profitability and sustainability of plant and animal agriculture and have positive impacts on air, climate, water, soil, and biodiversity (Fick and Clark, 1998).

Generally, in temperate climate grass-based systems, perennial ryegrass is a component of a dynamic mixture of species. This thesis will focus on perennial ryegrass and specific cultivars of perennial ryegrass.

Perennial ryegrass

Perennial ryegrass (*Lolium perenne L.*) is an important temperate perennial forage grass in world animal production systems. In temperate regions, perennial ryegrass is the preferred forage grass. In the United States, primary regions in which perennial ryegrass is grown are the northwest and northeast (Balasko et al., 1995). Perennial ryegrass is primarily grown as forage for lactating dairy cattle (Evers et al., 1996).

Perennial ryegrass is well adapted to cool, moist climates and is most productive in early spring and fall. Growth rate is affected by temperature, moisture, photoperiod, and soil fertility. Optimal temperatures for growth are 20-25° C (Spedding and Diekmahns, 1972). Even with adequate moisture, growth rates significantly decrease when day and night temperatures are greater than 30 and 25° C, respectively (Evers et al., 1996). Perennial ryegrass is relatively intolerant of temperature extremes and drought. Perennial ryegrasses are classified into three maturity categories: early, intermediate, and late. Maturity classification is on a continuum and affected by temperature and photoperiod, therefore classifications are imprecise (Cooper, 1957; Silsbury, 1965).

Perennial ryegrass is adapted to a wide range of soil compositions and pH. Perennial ryegrass is often grown in heavy, wet soils. It tolerates soils within a pH range of 5.0-8.3 (Cropper, 1997; Hall, 1992; Miller and Reetz, 1995). Perennial ryegrass is tolerant of close, frequent defoliation, but regrowth is dependent on growing conditions, plant carbohydrate reserves, amount of leaf remaining after defoliation, and preservation of growing points.

Perennial ryegrasses are valued for high yields, palatability, digestibility, and nutritive value. Perennial ryegrass has the highest digestibility compared to other temperate perennial grasses (Jung et al., 1976; Pysher and Fales, 1992).

Nitrogen (N) fertilization significantly improves dry matter (DM) yield and nutritive value including N concentration and digestibility of perennial ryegrass. To maximize DM yield and quality, fertilize at the onset of the growing season and after each harvest with the exception of the final harvest of the season (Castle and Reid, 1968;

Wedin, 1974; Whitehead, 1995). While perennial ryegrasses require significant amounts of N to support growth, the economical rate of application varies. Evers et al. (1996) discussed various methods used to estimate the economical rate of N application. In temperate regions, perennial ryegrasses are present and may grow continuously during the year. Therefore, given appropriate environmental and soil conditions, manure nutrients can be applied to and used by perennial ryegrasses the entire year. In addition, the extensive, shallow, fibrous root systems of ryegrasses reduce soil erosion and surface losses of nutrients (Evers et al., 1996).

Perennial ryegrass is naturally diploid (2n) with one set of chromosome pairs. Tetraploid (4n) perennial ryegrasses have been developed. Tetraploids are used, in part, due to their improved nutritive value. In general, tetraploids are more palatable, have higher digestibility, and have higher concentration of water-soluble carbohydrates (WSC) (Castle and Watson, 1971; Dent and Aldrich, 1963; Wilkins, 1991).

As tetraploidy is associated with an increase in cell size, the increase in nutritive value may be the result of increased ratio of cell contents:cell walls (Smith et al., 2001; Wilkins and Sabanci, 1990). Tetraploid cultivars have a higher proportion of cell contents:cell walls (Castle and Watson, 1971). In a study conducted to evaluate tetraploid versus diploid ryegrasses, tetraploids were significantly lower in DM, neutral detergent fiber (NDF), acid detergent fiber (ADF), and higher in WSC (Mayne and Patterson, 1998). Use of distantly related cultivars may confound tetraploid versus diploid research (Smith et al., 2001). In animal production studies, production is often greater for animals

fed tetraploid versus diploid varieties (Castle and Watson, 1971; Davies et al., 1989, 1992; Vipond et al., 1992, 97).

Feeding value of grass

Feeding value of grass is based on its ability to provide nutrients for maintenance and production. The three primary components of feeding value are intake, nutrient composition, and nutrient availability. Nutrient composition and availability are the two components of nutritive value. Nutrient availability is the ability of the animal to digest and absorb the nutrients. For forages, nutrient availability depends on the rate and extent of microbial fermentation in the rumen.

Nutritionally, grass DM may be divided into plant cell walls and plant cell contents which is the basis of the detergent fiber system (Van Soest and Wine, 1967). Cell walls primarily consist of structural polysaccharides (i.e. hemicellulose and cellulose) and lignin and availability varies and depends on structure and composition. Cell contents are the primary source of proteins, peptides, nucleic acids, lipids, organic acids, sugars, and starch in the plant and are highly digestible and readily available.

Intensively managed grass forage has a high feeding value. Energy is the primary limiting nutrient in use of high-quality grass forages for milk production. It was estimated that energy supplementation is required to achieve milk yields greater than 30 kg/d for cows consuming high-quality grass pastures (Kolver and Muller, 1998). Average chemical composition (on a DM basis) of intensively managed temperate grasses is presented in Table 2.1 (National Research Council, 2001).

Table 2.1 - Average chemical composition of intensively managed temperate grasses.[1]

Item[2]	
DM, %	20.1
CP	26.5
NDICP[3]	3.9
ADICP[4]	1.1
NDF	45.8
ADF	25.0
Lignin	2.1
Ether extract	2.7
Ash	9.8

[1]National Research Council, 2001

[2]With exception of DM, reported as % DM

[3]Neutral detergent fiber insoluble CP

[4]Acid detergent fiber insoluble CP

Beever et al. (2000) discussed the feeding value of grasses. Nutritive value varies with species, stage of maturity, environmental (i.e. temperature, light intensity, moisture) and soil (i.e. fertility) conditions, and defoliation timing and severity. Stage of maturity at harvest is the primary factor affecting nutrient composition and availability of grasses. Figure 2.1 describes the effect of maturity on the nutrient composition of grasses (Beever et al., 2000). As grass matures, as a proportion of DM, plant cell walls increase and plant cell contents decrease. One exception is fructans which is accounted for in the sugars fraction (Figure 2.1). As grass matures, nutrient and DM digestibility decrease.

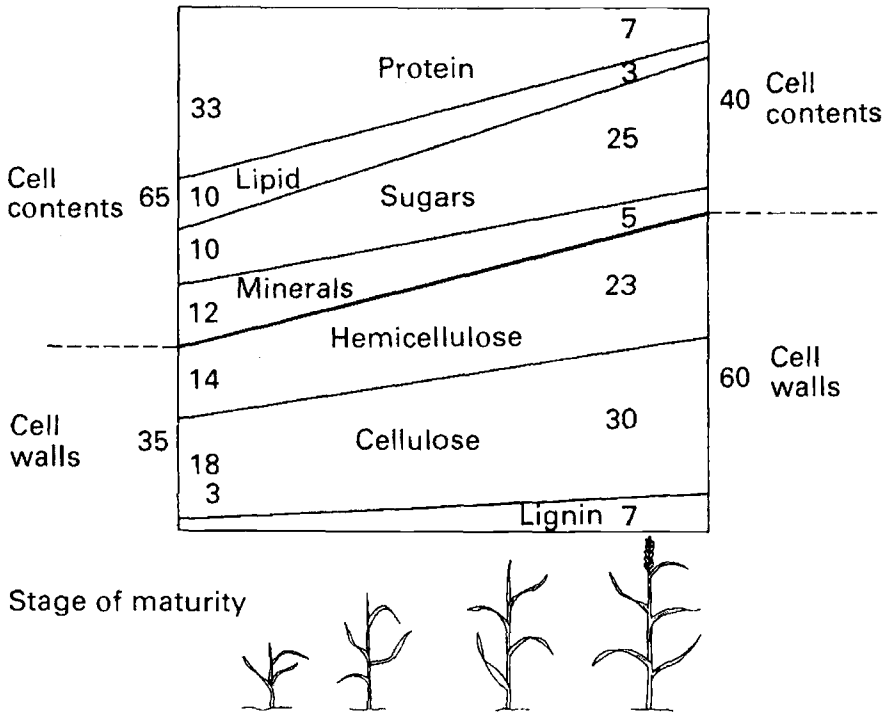

Figure 2.1 – Representation of the effect of maturity on the chemical composition of grasses. Values as a proportion of dry matter. (Beever et al., 2000).

Environmental conditions that affect feeding value include temperature, moisture, and intensity and duration of light. Seasonal and year to year variation of the feeding value of grass forages is one of the major challenges associated with grass forages as feedstuffs.

Palatability is a complex response affected by plant, animal, and environmental factors (Sheaffer et al., 1998). Wilman et al. (1996) suggested the high palatability of perennial ryegrass may be the result of high carbohydrate concentrations. For temperate grass forages, the more readily available carbohydrate fraction (i.e. nonstructural and

water-soluble carbohydrates) is positively correlated to preference and intake (Jones and Roberts, 1991; Mayland et al., 2000).

Carbohydrates

In the United States, carbohydrates in feedstuffs are commonly classified as structural carbohydrates (SC) and nonstructural carbohydrates (NSC). Nonstructural carbohydrates are associated with plant cell contents and are the more readily available carbohydrates. In temperate grasses, the primary NSC are glucose, fructose, sucrose, starch, and fructans (Smith, 1973).

In Europe and Australia, the term WSC is used to quantify the more readily available carbohydrates in grasses. In temperate grasses, the primary WSC are glucose, fructose, sucrose, and fructans (Smith, 1973). Of the temperate grasses, ryegrass has the highest WSC concentration (Henderson, 1973; Waite and Boyd, 1953). For traditional perennial ryegrasses, WSC concentrations less than 5% and greater than 40% have been reported. For 191 samples of perennial ryegrass, WSC concentration ranged from 4.6-31.5 % of DM, with a mean of 17.0 % of DM, and standard error of 0.38% of DM (Henderson, 1973).

In temperate grasses, the predominant monosaccharides are glucose and fructose, present at 1 to 3% of DM, in approximately a 1:1 ratio (Smith, 1973). In general, the concentration of glucose and fructose are similar in the leaf and stem and as a plant matures (Waite and Boyd, 1953). Sucrose is the most abundant oligosaccharide, present at 2 to 8% of DM (Smith, 1973). Sucrose is a disaccharide of glucose and fructose.

Sucrose concentration is similar in the leaf and stem and varies with maturity, peaking at approximately the time of primary growing point development (Waite and Boyd, 1953). Glucose, fructose, and sucrose comprise total free sugars. Free sugars are metabolic intermediates, and are present in relatively low concentrations. Sugars are soluble in water and ethanol solution and may be separated from nonstructural polysaccharides with specific concentrations of ethanol. In ryegrass, a 90% ethanol solution is used to separate total free sugars from fructans (Smith, 1973).

Starches are storage carbohydrates; polysaccharides of glucose molecules arranged as amylose and amylopectin polymers. Amylose is a linear molecule linked via α-1-4 linkages and amylopectin is a more complex molecule with both α-1-4 and α-1-6 linkages. Amylose is water-soluble while amylopectin is not water-soluble. Enzymatic hydrolysis is one method used to determine starch. In temperate grasses, starch is a minor component; approximately 2 to 4% of DM (Smith, 1973).

In temperate grass forages, fructans are the primary storage carbohydrate (Smith, 1973). Fructans are nonstructural polysaccharides; polymers of fructose with a terminal sucrose. In temperate grasses, the primary fructans are short-chain molecules and fructans occur as levans; β-2-6 linked polymers with a terminal sucrose. Fructans are water-soluble and differentially soluble in ethanol (Cairns, 2003; Smith, 1973). For temperate grasses, an 80% ethanol solution is used to extract and determine total sugars and fructans (Wylam, 1954). In temperate grass forages, fructans are the primary WSC and the only important fermentable, water-soluble, polysaccharide (McDonald, 1981; Waite

and Boyd, 1953). In one study, McGrath (1988) reported perennial ryegrass WSC averaged 20% of DM for the season and fructans accounted for 70% of the WSC.

Water-soluble carbohydrate concentration and the proportion of each WSC component are highly variable. Accumulation rate of WSC is a function of photosynthesis, growth, and respiration rates. In temperate grasses, fructans account for the majority of the variation in WSC. Primary factors affecting concentration of WSC are forage species, cultivar, stage of maturity, time of day, temperature, light intensity, and fertilizer application (McDonald, 1981). Water-soluble carbohydrate concentration also varies by plant part (McGrath, 1988).

As grasses mature, WSC concentration increases; generally, peaking prior to or at the time of inflorescence (Smith, 1973). In temperate grasses, WSC is affected by the ratio of stem:leaf tissue. As plants mature the stem:leaf ratio increases and WSC is greater in stem versus leaf tissue (Smith, 1973). Increase in WSC is primarily due to an increase in fructans. Stage of maturity at which WSC peaks varies with environmental conditions (Jung et al., 1976).

WSC concentration undergoes diurnal variation; levels are at a minimum immediately before dawn, increase throughout the day to a maximum in the late afternoon, and then decrease during hours without daylight. The majority of diurnal variation appears to be due to changes in sucrose. Waite and Boyd (1953) reported sucrose concentration peaked in the late afternoon at 7.0% of DM. Diurnal variation of glucose and fructose appear to be minimal. Daily variation is greater on warm, sunny

days versus cold, cloudy days (Smith, 1973). Diurnal variation in fructans has not been well established (Smith, 1973; Waite and Boyd, 1953).

Temperature and WSC are inversely correlated. In a controlled study, Deinum (1966) identified the difference in WSC for day/night temperatures of 15/10° C to 25/20° C were 33.2 and 21.2% DM, respectively. Temperatures prior to harvest may have the most significant effect on WSC at harvest (Smith, 1970). Environmental temperatures appear to affect the concentration of fructans (Smith, 1973). Light intensity and WSC are correlated. In the same study, Deinum (1966) reported a three to four-fold increase in WSC grown in high versus low light intensity. Deinum (1966) concluded WSC in perennial ryegrass is maximized under high light intensity and low temperature growing conditions. Pettersson and Lindgren (1990) reduced the WSC concentration by shading grass three days prior to harvest.

In grasses, N fertilization reduces WSC as a result of increased growth. During plant growth, WSC are used for protein synthesis and growth. It is well established that WSC and N are inversely correlated which appears to be due to a decrease in fructan concentration (Nowakowski, 1962; Smith, 1973; Waite and Boyd, 1953).

Water stress also affects WSC. Initially, drought stress causes an increase in WSC (Arcioni et al., 1985; Thomas and James, 1999). However, prolonged water-limiting conditions decrease WSC (Thomas, 1991; Thomas and James, 1999).

Proteins

Plant proteins may be classified as seed proteins or leaf proteins. For grass forages, the study of protein focuses on leaf proteins. The majority of leaf proteins are metabolic proteins (i.e. associated with growth and biochemical functions of cells) and are present in the cytoplasm of the leaf cell (Lyttleton, 1973). The majority of leaf cell proteins are present in organelles; the most relevant are the chloroplasts, mitochondria, and nucleus. In forage, approximately 75% of the total leaf protein is chloroplastic in origin (Thomson, 1982). The three primary classes of fresh forage proteins are: 1) Fraction I leaf protein; 2) Fraction II proteins; and 3) chloroplast membrane proteins (Mangan, 1982). Additional minor nitrogenous components of fresh forages are nucleic proteins, mitochondrial proteins, extensin, free amino acids, nucleic acids, and nitrate (Mangan, 1982).

Fraction I leaf protein is approximately 35% of the total leaf protein (Mangan, 1982) and approximately 70% of the true protein of forage. In the plant, Fraction I is responsible for the initial stages of photosynthesis and photorespiration. Fraction II protein comprises approximately 25% of the total leaf protein (Mangan, 1982). Fraction II protein is chloroplastic and cytoplastic in origin and is a very complex mixture of proteins. Chloroplastic membrane protein is insoluble in water (Mangan, 1982).

For temperate regions, grass crude protein (CP) is commonly 15-20% of DM (Lyttleton, 1973). In highly productive regions, CP of temperate grasses may be greater than 30% of DM, with a true protein concentration to 25% of DM (Barnicoat, 1957;

Bryant and Ulyatt, 1965). In less than optimal growing conditions (i.e. moisture or temperature limitations) CP is commonly 7-15% (Lyttleton, 1973).

In grasses, CP concentration is variable. Primary factors affecting grass CP concentration are stage of maturity, environmental conditions (i.e. temperature and moisture), and soil conditions(i.e. fertility); stage of maturity is the primary factor (Lyttleton, 1973). As grasses mature, CP concentration decreases due to the decreasing proportion of leaf:stem ratio and decreasing concentration of CP in the leaf (Mowat et al., 1965). Leaf blades have approximately twice the CP compared to the leaf sheath and stem (Minson, 1990). For example, in one study with perennial ryegrass, CP was 12.6 and 5.4% DM, for leaf and stem, respectively (Johnston and Waite, 1965).

Primary factors affecting nitrogenous composition of fresh forage are plant physiology, anatomy, maturity, fertilization, climate, and light intensity (Mangan, 1982). In fresh grass forages, approximately 75-90% of the total N is true protein and the balance is non-protein nitrogen (NPN) compounds including peptides, free amino acids, amides, amines, nucleotides, and nitrate (McDonald, 1981). Nitrate concentration is variable and depends on the level of N fertilization (Henderson and McDonald, 1975).

Amino acid profiles of herbage are similar across species (Lyttleton, 1973). In a study of five grasses and six samples of alfalfa, arginine, lysine, aspartic acid, glutamic acid, alanine, leucine, and glycine accounted for 63% of the amino acid N recovered (Wilson and Tilley, 1965).

Carbohydrates and proteins in the ruminant

Carbohydrates in the rumen

In the rumen, the primary source of energy (i.e. adenosine triphosphate (ATP)) is carbohydrates. For grazing ruminants, NSC are the primary source of readily available energy and are rapidly and completely digested in the rumen (Bouden et al., 2002). If sufficient quantities of NSC are unavailable, rumen microorganisms must rely on SC with a slower rate of energy availability as their primary energy source.

Rumen digestion and metabolism of carbohydrates may be divided into two phases. The initial phase is the degradation of dietary carbohydrates and the second phase is the microbial use of substrates to produce ATP to meet microbial energy requirements (Beever et al., 2000).

In the rumen, mono- and oligosaccharides are rapidly fermented and the primary fermentation products are lactate and propionate. Limited data is available on fructans. Bacteria and protozoa ferment fructans to lactic acid (Ziolecki et al., 1992). In the rumen, grass WSC are released over a 6-8 h period (Miller, unpublished).

Proteins in the rumen

In the rumen, significant proportions of perennial ryegrass proteins undergo rapid and extensive degradation (van Vuuren, et al., 1991). Extent of ruminal protein degradation is a function of the rate of protein hydrolysis and time (Buttery and Lewis, 1982). For rumen bacteria, proteolytic activity is primarily associated with the cell wall and proteolysis requires binding of the protein to the bacteria (Nugent and Mangan,

1981). Quantity, nature, and distribution of SC may alter the rate and extent of forage protein digestion (Thomson, 1982).

Proteins are hydrolyzed to peptides and amino acids which may be used by the microflora or further degraded. Amino acids are readily fermented (Lewis, 1955). Amino acids are deaminated and degraded to yield primarily ammonia (NH_3), volatile fatty acids (VFA), and carbon dioxide (Buttery and Lewis, 1982). In a study in which energy was limiting, Scheifinger et al., (1976) reported the majority of amino acids were degraded versus being directly incorporated into bacteria. In vivo, the rate of amino acid breakdown varies between amino acids (Chalupa, 1976). In perennial ryegrass, the initial stages of proteolysis may be, in part, the result of plant proteases (Zhu et al., 1999).

Fresh grass leaf proteins are highly soluble and rapidly degraded by plant and microbial proteases in the rumen releasing peptides and NH_3. In the study of degradation of Fraction I leaf proteins, Nugent and Mangan (1981) reported peptides were absent and amino acid concentration was minimal, with the exception of four amino acids, and concluded proteolysis of Fraction I protein was the rate limiting step. Ruminal degradation of Fraction II protein has not been investigated.

Interaction of carbohydrates and proteins

In the rumen, digestion and metabolism of proteins and carbohydrates is highly interdependent. Dietary proteins and carbohydrates are the primary nutrients required for microbial crude protein (MCP) synthesis; proteins supply N and carbohydrates supply energy to the microorganisms. Efficient MCP synthesis requires a balanced supply of

NPN (i.e. amino acids and NH_3) and energy (i.e. carbohydrates) available to the rumen microorganisms. Buttery and Lewis (1982) stated the factors affecting the efficiency of MCP synthesis are energy source, N supply, feeding frequency, intake level, and passage rate. Across dairy production systems, carbohydrates are the primary factor regulating MCP yield (Nocek and Russell, 1988).

Generally, MCP component accounts for a large proportion of total protein available to the animal and is an important source of amino acids for lactating dairy cows. Microbial crude protein will provide adequate amino acids to support approximately 30 kg of milk. Microbial crude protein available to the animal is primarily bacterial CP. In terms of nutritive value, bacterial protein is the most valuable component of the bacterial cell. On a DM basis, rumen bacteria average 50% protein. Microbial crude protein has a high biological value and digestibility and bacterial amino acid composition usually is constant (Buttery and Lewis, 1982).

Protein synthesis requires energy; ATP produced via fermentation of dietary carbohydrates supports microbial synthesis of amino acids and proteins. The primary source of ATP is via carbohydrate fermentation. Quantity of ATP synthesized is regulated by available energy and utilization efficiency of available energy. Given adequate quantities of ATP, amino acids are incorporated into microbial protein. Energy is also required for bacterial cell maintenance.

In addition, readily available carbohydrates are metabolized to VFA. Insufficient quantities of readily available carbohydrates in grass forage produce an acetate-dominant fermentation which results in reduced supplies of MCP and net energy to the ruminant

(Corbett et al., 1966; Blaxter et al., 1971). An increase in WSC may result in more efficient rumen fermentation with higher VFA yields and higher propionate:acetate ratio (Grimes et al., 1967; Beever et al., 1978).

Efficient MCP synthesis requires an adequate N supply. The majority of rumen bacteria use NH_3-N as their N source. Primary sources of NH_3 are degradation of dietary true protein, dietary NPN compounds, hydrolysis of recycled urea, and degradation of microbial CP (Owens and Zinn, 1988).

For optimal efficiency of MCP production, degraded dietary N must be incorporated into microbial N. Excess NH_3 accumulates in the rumen and reduces efficiency of MCP synthesis. Excess NH_3 is absorbed through the rumen wall, enters the blood stream, is converted to urea in the liver, and excreted via the urine. Urinary N represents an inefficiency of conversion of grass N to microbial N. As rumen NH_3 concentration increases, absorption of NH_3 through the rumen wall increases (Owens and Zinn, 1988).

Traditionally, immature, perennial ryegrass forages provide an excess of readily available proteins relative to available carbohydrates which reduces the utilization efficiency of protein for MCP synthesis, decreases the quantity of N used for productive purposes (i.e. milk and muscle), and increases the quantity excreted in the urine. For fresh grass, up to 35-40% of dietary N may be lost as NH_3 (Lee et al., 2001). For immature grass forages, urinary N accounts for substantial losses of grass N (Tamminga, 1992).

Extensive research has been conducted to study the effects of carbohydrates and proteins on microbial protein synthesis. Research has been conducted to identify the

effects of various methods to synchronize available grass protein and supplemental energy (Carruthers and Neil, 1997; Kolver et al., 1998; Lee et al., 2002; Lee et al., 2003). Efficacy varies.

Traditional supplementation of fresh grass

Optimization of a grass-based system requires supplementation of the grass nutrients. Bargo et al. (2003) reviewed the production (i.e. dry matter intake (DMI) and milk yield and components) and digestion (i.e. ruminal and postruminal) of supplemented dairy cows on pasture. Literature evaluating energy (i.e. NSC and SC), rumen undegradable protein, and fat supplementation was reviewed. For dairy cattle consuming high-quality grass forages, energy is the primary limiting nutrient for milk production. Extensive research has been conducted to determine the optimal type, rate, and timing of carbohydrate supplementation to improve the utilization efficiency of grass forage for milk production (Bargo et al., 2002; Delahoy et al., 2003; Meijs, 1986; Soriano et al., 2000; Trevaskis et al., 2004; Valk et al., 1990; vanVuuren et al., 1986).

High water-soluble carbohydrate grass forages

Breeding of perennial grass forages may be divided into two categories: 1) adaptation, production, and agricultural fitness; and 2) forage nutritional value (Casler, 1998). Adaptation, production, and agriculture fitness are the predominant consideration. Given a cultivar is well-adapted, an increase in nutritive value is one of the most economical means to increase animal production (Casler, 1998). Generally, increases in

forage nutritive value via genetics are consistent across environments and management and harvesting systems (Wilman et al., 1992).

One measure of nutritive value of grass forages is WSC concentration. Since energy is the limiting nutrient in use of grasses for milk production and WSC are the primary energy source in grasses, increasing the WSC concentration of the forage should increase utilization efficiency of grass nutrients for milk production.

A research group based in the United Kingdom (U.K.) has led the research and development of perennial ryegrass cultivars that accumulate elevated levels of WSC (Humphreys, 1989a, b, c). In perennial ryegrasses, WSC can be consistently improved via breeding (Humphreys, 1989a, b). AberAvon, AberDart, AberDove, Aurora, and Cariad are examples of high WSC perennial ryegrasses. In perennial ryegrass, induction of tetraploidy on a diploid bred for elevated WSC does not further increase the WSC concentration (Smith et al., 2001).

Limited data has been presented to determine the mechanism responsible for the elevated WSC phenotype. Smith et al. (2002) suggested reduced growth rates, improved efficiency of photosynthesis or respiration, altered carbon partitioning (i.e. structural to nonstructural), or altered carbon partitioning (i.e. roots to shoot) may be responsible for elevated shoot WSC. Available data does not consistently support any of the alternatives.

Humphreys (1989c) identified WSC and DM digestibility (DMD) were positively correlated, WSC and CP were negatively correlated, and WSC and DM production were essentially not correlated. Correlations between DMD and DM production were small.

Humphreys (1989c) concluded high-yielding forages with high WSC and high DMD may be developed.

In a multi-year Australian study, high WSC cultivars bred in the U.K. and standard New Zealand and Australian cultivars were studied (Smith et al., 1998). At all three sites and for the majority of harvests, high WSC grasses had higher WSC and lower DM yields. WSC varied with season and site. For harvests with similar DM yields, high WSC grasses maintained higher WSC, which also indicates it may be possible to develop high-yielding, high WSC concentration cultivars. For more than half of the harvests, elevated WSC grasses had significantly greater CP and DMD and lower NDF. Authors suggested the increase in WSC was associated with a decrease in NDF. In another Australian study, Radojevic et al. (1994) also identified high WSC grasses had higher WSC than standard cultivars and WSC peaked in the summer. Grass WSC and N were negatively correlated which was primarily an environmental (i.e. seasonal) effect. Multiple linear regression identified season explained 72% and WSC explained less than 1% of the variance in N. Higher WSC grasses also improved summer in vitro dry matter digestibility (IVDMD) 2-6%.

Lee et al. (2003) evaluated the effects of addition of WSC to fresh grass on microbial fermentation in-vitro. Basal grass WSC was 25.0% DM and N at 2.1% DM. As WSC level increased, pH, NH_3-N, and acetate decreased and propionate increased. Total microbial N production and microbial protein synthesis efficiency (g N/kg organic matter apparently digested (OMAD)) increased at 1.25x and 1.5x levels and decreased at 1.75x. As WSC level increased, fiber digestion decreased and residual WSC increased,

indicating a shift in microbial population; residual N was similar. Authors concluded that high WSC grasses may improve productivity via improvements in efficiency of N and energy utilization and production of energy-yielding VFA.

Higher WSC increased lamb live weight daily gain and total lamb production per hectare of suckling lambs by approximately 12 and 23%, respectively (Lee et al., 2001). Increase in total lamb production was due to increased live weight gains and carrying capacity. Elevated WSC grass had higher WSC and IVDMD and lower NDF/ADF; N was similar. Live weight gain and total lamb production were positively correlated to WSC and negatively correlated to NDF. Additional production trials support high WSC perennial ryegrasses increasing lamb production per unit area (Davies et al., 1989; Davies et al., 1992; Munro et al., 1992).

Lee et al. (2002) studied the effects of higher WSC ryegrasses on rumen metabolism and N utilization in steers. Elevated WSC grass was significantly higher in DM, WSC (24.3 vs. 16.1% DM), lower in NDF/ADF, and similar in N (1.66 vs. 1.59% DM). Dry matter intake, organic matter (OM) intake, and N intake were greater for steers fed high WSC grass. Nitrogen utilization efficiency was similar; believed to be the result of insufficient dietary N. Steers fed high WSC had a lower rumen NH_3 and acetate, and higher propionate; total VFA were similar. It appears elevated WSC grass increased the quantity of nutrients via an increase in DMI, but did not increase efficiency of MCP synthesis.

Miller et al. (2001a) evaluated N utilization efficiency of lactation dairy cows grazing high WSC perennial ryegrass. High WSC grass was higher in WSC (23.6 vs.

16.6% DM), lower in CP (12.8 vs. 17.6% DM), and NDF was similar. Forage DMI, milk yield, and milk component yields were similar between treatments. Animals consuming elevated WSC consumed 35% less dietary N and more efficiently converted dietary N to milk protein (26.0 vs. 21.5%). Authors suggested the increased utilization efficiency of N was due to improved ruminal N utilization and/or increased propionic acid.

Miller et al. (1999) studied milk production and N-partitioning in dairy cows fed high WSC perennial ryegrass as green chop. The high WSC grass had higher WSC (20.1 vs. 12.9% DM); DM and CP (9.17 vs. 10.60% DM) were similar between treatments. NDF was not reported. Animals fed high WSC grass consumed more DMI (12.5 vs. 10.8 kg DM/d), produced more milk (15.3 vs. 12.6 kg/d), and exhibited an increase in N-utilization efficiency. Animals fed high WSC grass partitioned a greater proportion of N towards productive purposes (i.e. milk N and N balance) and away from excretion. Authors concluded high WSC grasses increase DMI, milk yield, and N utilization efficiency.

In a similar trial, DM, WSC (16.5 vs. 12.6% DM), and DMD were higher and CP (9.2 vs. 10.6% DM) and NDF were lower for the high WSC grass. DMI and N intake were similar between treatments. Animals fed elevated WSC grass had a higher DDM intake which is known to affect nutrient supply and control milk yield and composition (Sutton and Morant, 1989; DePeters and Cant; 1992). Milk yield (15.3 vs. 12.6 kg/d) and milk protein yield were greater for animals consuming high WSC. Ratio of milk yield to digestible DM was similar. Treatment animals excreted less urinary N and more milk N. Miller et al. (2001b) concluded the increase in milk yield was the result of an increased

nutrient supply which may be the result of an improvement in utilization efficiency of protein in the rumen.

Silage production

Well-preserved grass silage is the result of the controlled fermentation of fresh grass. Optimally, lactic acid bacteria (LAB) dominate fermentation. Lactic acid bacteria ferment sugars and produce lactic acid to effectively reduce the pH to inhibit competing microorganisms. Water-soluble carbohydrates are the primary substrates for microbial growth. Three critical factors affecting silage production are: 1) an adequate level of fermentable substrate (i.e. WSC); 2) DM concentration greater than 20%; and 3) a relatively low buffering capacity (McDonald, 1981).

Plant enzymes

Immediately after harvest, microbial population on forages is minimal (Stirling and Whittenbury, 1963). Therefore, plant enzymes are responsible for respiration and proteolysis in the initial stages of ensiling (McDonald, 1981).

In the silo, plant respiration continues. Respiration rate is affected by temperature, oxygen and carbon dioxide concentrations, pH, DM concentration, and wounding of the plant tissue (McDonald, 1981).

Proteolysis also continues in ensiled forage. Primary products are peptides, free amino acids, and amides (Pettersson, 1989). In grass, NPN concentration can increase from 20 to 40% of total N within 12-24 h. Optimal pH for plant proteases is 5.0-6.0

(Tracey, 1948). Macpherson (1952) reported proteolysis ceased at a pH value of 4.3. However, others have concluded a reduction in pH only reduces the rate of proteolysis (Tracey, 1948; Carpintero et al., 1979).

Microorganisms

Generally, the majority of microorganisms on plants are strict aerobes (Gibson and Stirling, 1959). During the aerobic phase, the majority of epiphytic microorganisms multiply; extent depends on the degree of cell damage at harvest and duration of the aerobic phase (Pettersson, 1989). Generally, clostridia and LAB on growing plant tissue are minimal (Stirling and Whittenbury, 1963). Forage may be inoculated with clostridia and LAB at harvest. Nutrient-dense plant cell contents are an ideal medium for growth. At harvest, LAB rapidly grow and multiply (McDonald, 1981). Release of cell contents is required for lactic acid fermentation, and in turn, acidity of the fermentation products affects rate of release (Pettersson, 1989).

With the establishment of anaerobic conditions, facultative and obligate anaerobes grow exponentially and dominate (Pettersson, 1989). Initially, enterobacteriaceae and LAB undergo rapid growth. Given adequate conditions (i.e. lactic acid counts, substrate, temperature, etc.), LAB quickly dominate fermentation and within one or two days, LAB are a significant proportion of viable microorganisms. Rapid establishment and growth of LAB inhibits enterobacteriaceae growth. A fermentation dominated by enterobacteriaceae is indicated by high pH, extremely high NH_3-N, and a high acetic acid (Pettersson, 1989).

Lactic acid bacteria dominate an effective fermentation. Lactic acid bacteria are facultative anaerobes. Lactobacilli are classified as homofermentative or heterofermentative. Homofermentative lactic acid bacteria ferment glucose and fructose to lactic acid. Products of fermentation of glucose and fructose by heterofermentative lactic acid bacteria include lactate, ethanol, acetate, and mannitol. Optimal pH is approximately 6.0, although Lactobacilli tolerate a range of 4.0-6.8 and some strains will grow at 3.5 (Wilson and Miles, 1975). Lactobacilli have minimal proteolytic activity and fermentation of amino acids is limited (McDonald, 1981).

Clostridia are gram-positive bacteria. Clostridia grow in anaerobic conditions and can be classified as saccharolytic and proteolytic. Saccharolytic primarily ferment carbohydrates and organic acids and proteolytic primarily ferment proteins (Beck, 1978). pH and moisture are two primary factors affecting clostridia growth. Acidic conditions inhibit growth and, therefore, lactic acid production is critical to reduce the pH and inhibit clostridia growth (Pelczar and Reid, 1972). Generally, growth is inhibited at moisture levels less than 70%. Further, pH and moisture are interrelated; as moisture concentration increases, critical pH for inhibition of growth decreases (Weissbach et al., 1974). Wet silages are at high risk for clostridia fermentation. In wet silages, clostridia may grow at pH values as low as 4.1.

Clostridia activity is critical after initial fermentation. If the decrease in pH was not sufficiently rapid or the final pH value was not sufficiently low, clostridia initiate secondary fermentation. During secondary fermentation, clostridia ferment sugars and lactate producing butyric acid, carbon dioxide, and hydrogen. Clostridia fermentation of

lactic acid raises pH via two modes: 1) butyric acid is a weaker acid than lactic acid; and 2) only one mole of butyric acid is produced for two moles of lactic acid fermented. The pH increase promotes subsequent microbial growth. In silages dominated by clostridia, the primary acid is butyric, acetic acid may be present in significant quantities, and minimal quantities of lactic acid present (McDonald, 1981). Butyric acid is an indicator of silage quality; values less than 0.1% are acceptable (Woolford, 1984). As the primary product of N fermentation in high pH clostridia silages, NH_3 is an accurate indicator of the extent of proteolytic activity (Hughes, 1971; McDonald, 1981).

In grass silages, yeasts and molds are primarily responsible for aerobic deterioration. Low pH values do not inhibit yeasts. Many yeasts grow at pH values of 3.5-3.8 and some tolerate pH values of 2.0 or lower (Pelczar and Reid, 1972; Woolford, 1972). Yeasts respire sugars to carbon dioxide and water. Anaerobic conditions and low pH inhibit mold growth. Molds respire sugars and lactic acid.

Carbohydrates

Immediately after harvest, aerobic conditions affect WSC (McDonald, 1981). In the initial hours after harvest, Wylam (1953) identified a significant decrease in sucrose and fructose. As a result of metabolism of glucose and fructose and hydrolysis of sucrose and fructans, the glucose and fructose concentrations oscillate in the aerobic conditions after harvest (Clark, 1974). Overall, total WSC concentration decreases (Pettersson, 1989).

In a sealed structure, oxygen is used rapidly by plants and, therefore, minimal quantities of WSC are lost during the aerobic phase in the silo (McDonald and Whittenbury, 1973). During the anaerobic phase, glucose and fructose are the primary substrates. In the first few days of ensiling, components of sucrose and fructans are available for fermentation (Pettersson, 1989).

Forage DM affects the quantity of available carbohydrates required for effective fermentation. Low DM forages require greater quantities of available carbohydrates to achieve well-fermented silage. For fresh forages, approximately 25 g WSC per kg of fresh material were required to achieve a well-fermented silage (i.e. pH of 4.09 and NH_3-N of 7.4% of total N (TN)) (Pettersson, 1989). Other authors have recommended greater concentrations of 30-35 g WSC per kg fresh material (Haigh, 1990; Haigh and Parker, 1985; Parker and Crawshaw, 1982). In low sugar forages, efficient wilting reduces substrate required and increases lactic acid production (Pettersson, 1989).

Proteins

During the initial phases of ensiling, plant enzymes degrade proteins and amino acids. In the anaerobic phase, proteolysis continues and the extent depends on time required to establish acidic conditions.

Amino acids are fermentation substrates. Specific microorganisms, primarily enterobacteriaceae and clostridia, use amino acids as an energy source (Pettersson, 1989). Proteolytic microorganisms also have the ability to deaminate and decarboxylate amino acids, resulting in production of NH_3. Microorganisms are primarily responsible for the

deamination of amino acids to form NH_3. Ammonia-N, as a % of TN, is an indicator of silage quality; an acceptable NH_3-N concentration is less than or equal to 8.0% of TN (Breirem and Homb, 1970).

Moisture

Forage moisture concentration is critical in silage production. Microorganisms responsible for fermentation require moisture. However, excess moisture may be detrimental to the nutritive value of silage.

McDonald (1981) discussed the reasons moisture concentration is critical. Critical pH at which clostridia growth is inhibited is directly related to moisture concentration (i.e. the higher the moisture, the lower the pH required for inhibition). Therefore, ensiling high moisture forages, with the exception of forages high in available carbohydrates, promotes clostridia fermentation. Forage DM also affects the effluent quantity and as effluent contains readily available carbohydrates, effluent losses may significantly affect subsequent fermentation.

Wilting to increase forage DM has no substantial effect on the growth of LAB (Pettersson, 1989). However, wilting has a marked effect on clostridia; generally, growth is restricted at DM greater than 30 % (Woolford, 1984).

As DM concentration increases, microbial fermentation is increasingly restricted, indicated by reduced concentrations of lactic, acetic, butyric acid and NH_3-N and increased pH (Jackson and Forbes, 1970; Pettersson, 1989). Morgan et al. (1980) studied the effects of DM (DM of wilted and unwilted at 16.9 and 35.9%, respectively) on

fermentation of a high WSC (19.3% DM) ryegrass-clover. Wilting decreased lactic acid (3.4 vs. 16.5% DM) and NH_3-N (8.0 vs. 10.9% of TN) and increased pH (5.09 vs. 4.00). In contrast, addition of water stimulates bacterial growth, especially LAB. Provided extremely high concentrations of available carbohydrates, high moisture crops can be effectively ensiled; LAB will be very active and dominate fermentation, resulting in low pH and high lactic acid (McDonald, 1981).

Oxygen

Establishment and maintenance of an anaerobic environment is required for LAB to dominate fermentation. Oxygen promotes growth of enterobacteriaceae, clostridia, yeasts, and molds and inhibits LAB (Langston et al., 1962; Ohyama et al., 1975; Pettersson, 1989).

Provided the silo has been adequately sealed, oxygen in the silo is used rapidly. Utilization of oxygen is more rapid in direct-cut forages. During the initial aerobic phase, aerobic and facultative anaerobic microorganisms metabolize plant cell nutrients. Respiration rate is affected by temperature, forage DM, and pH. As temperature increases, respiration increases. As DM concentration increases, respiration decreases. As pH decreases, respiration decreases; respiration ceases below pH of 3.0 (Virtanen, 1933). Extension of the aerobic phase decreases the quantity of substrate available for fermentation, potentially resulting in insufficient lactic acid to inhibit clostridia (Takahashi, 1968; Takano et al., 1977).

Packing to exclude oxygen promotes the release of cell contents and growth of LAB (Weise, 1968). Plant cell breakdown and release of plant contents via plasmolysis is required for the establishment of LAB and infiltration of oxygen delays plasmolysis and initiation of pH reduction (Greenhill, 1964). McDonald et al. (1960) identified a well-packed ryegrass silage had a lower pH (3.7 v. 4.1), NH_3-N (7.3 v. 12.5% TN), butyric acid (0.18 v. 2.3% DM), and DM loss (17.4 v. 34.6%) and higher lactic acid (11.5 v. 5.6% DM) than a poorly-packed silage.

During feed-out, aerobic conditions are reinstated. Aerobic, dormant microorganisms grow and multiply, deteriorating silage quality (McDonald, 1981). Aerobic deterioration results in nutrient losses. Primary energy sources for microorganisms are lactic acid, acetic acid, and soluble carbohydrates and products of oxidation are carbon dioxide, water, and heat. Amino acids are also catabolized. Respiration of lactic and acetic acid and production of NH_3 result in an increase in pH.

Temperature

To a point, a temperature rise increases the rate of enzymatic activity and microbial metabolism. At 10-24° C, lactic acid fermentation is dominant. Lactic acid bacteria do not compete well at high temperatures (Woolford, 1984). Temperatures higher than 30° C favor clostridia growth (Woolford, 1984). Concentration of available substrate affects the significance of high temperatures; as available substrate increases, effects of high temperatures may be overcome.

Buffering capacity

Buffering capacity is the ability to resist change in pH. Silage production requires a pH reduction; fresh forage has a pH of approximately 6.0 and preserved silage has a pH of approximately 4.0. During ensiling, production of fermentation acids increases the buffering capacity (McDonald and Whittenbury, 1973).

pH

Rapid substrate fermentation reduces pH and inhibits the growth of competing microorganisms. Rate of pH decline is more important than the final pH of the silage (Whittenbury et al., 1967). Rate of pH decline is related to rate of lactic acid production. However, final pH is commonly used to measure silage quality. For silage with a DM concentration below 25%, 4.2 is satisfactory (Pettersson, 1989). As DM concentration of the silage increases, acceptable pH value increases. For DM concentrations 25-35%, the pH of a quality silage may be as high as 4.5. For DM concentrations greater than 35%, pH value is not an accurate measure of silage quality.

Lactate silages

Based on the principal fermentation characteristics, McDonald (1981) classified silages into six categories: 1) lactate; 2) acetate; 3) clostridial; 4) wilted; 5) additive inhibited; and 6) aerobically deteriorated. In lactate silages, fermentation is dominated by LAB. Lactate silages have a high concentration of lactic acid; in grasses, generally, 8.0-12.0% DM (McDonald, 1981). However, lactic acid concentrations may be higher if wet

crops with high concentrations of soluble carbohydrates are ensiled. Appreciable quantities of acetic acid are also present; the majority is the product of fermentation of sugars by homofermentative LAB and enterobacteriaceae. Frequently, small quantities of propionic and butyric acid are present in the silage and the quantity of butyrate is affected by the rate of lactic acid production. Lactate silages are characterized by a low pH; generally, 3.7 to 4.2 (McDonald, 1981).

Lactate silages are characterized by low quantities of available carbohydrate and high quantities of available protein. After fermentation, minimal sugars remain; generally, less than 2.0% of DM. In lactate silages, the nitrogenous compounds are as NPN and protein-N; the majority of NPN compounds are amino acids.

Lactate silages are produced from crops with high concentrations of soluble carbohydrates (McDonald, 1981). A typical composition of lactate silage from perennial ryegrass with a pre-ensiling soluble carbohydrate concentration of 17.7% DM is presented in Table 2.2 (Henderson et al., 1972).

Table 2.2 - Composition of lactate silage from perennial ryegrass with a pre-ensiling soluble carbohydrate concentration of 17.7% DM[1].

pH	3.9
DM, %	19.0
Lactic acid, % DM	10.2
Acetic acid, % DM	3.6
Propionic acid, % DM	0.2
Butyric acid, % DM	0.1
Ammonia, % of total N	7.8
CP, % DM	14.4

[1]Henderson et al., 1972

Chapter 3
Effect of perennial ryegrass nonstructural carbohydrates on production and rumen fermentation of dairy cows

Abstract

Objectives were to study the effects of perennial ryegrass nonstructural carbohydrates (NSC) on milk yield and composition, dry matter intake (DMI), and rumen fermentation in dairy cows. Two perennial ryegrasses, one with a relatively high NSC content (HNSC; Elgon®) and one commonly grown in Oregon (CNSC; Linn) were planted in the fall of 2002 and fed as green chop in the June and July of 2003. Twelve Holsteins and two Jerseys were blocked by milk yield and assigned at random to treatment. Cows were supplemented with a total mixed ration (TMR) that was offered for one h twice daily at 0500 h and 1630 h. Grasses were cut, sampled, and offered ad-libitum twice daily after the TMR. Individual grass and TMR intake and milk yield were collected twice daily for 21 d. Milk samples were collected d 0 of the treatment adaptation period and d 7 and 21 of the treatment period. On d 9 and 21 of the treatment period, rumen samples were collected at 0, 1, 2, 3, 4, 6, 8, 10, and 12 h relative to each TMR feeding and analyzed for pH, volatile fatty acids (VFA), and ammonia (NH_3). Data were analyzed with the MIXED procedure of SAS. For grass DMI, the treatment by wk interaction was significant ($P<0.01$). For HNSC, grass DMI was greater for wk 2 ($P<0.01$) and tended to be greater during wk 3 ($P<0.10$). Total mixed ration DMI tended to be greater for HNSC treatment ($P=0.06$). Milk yield and yield of milk fat and milk protein were greater for HNSC treatment ($P<0.05$). Milk urea nitrogen (MUN) and ruminal VFA and NH_3 did not differ between treatments. Grass nutrient composition was

different than expected. High NSC was lower in NSC ($P<0.05$) and higher in crude protein ($P<0.01$). Grass neutral detergent fiber and acid detergent fiber were similar. In this study, milk and component yields for HNSC were greater than CNSC treatment; however, effects were not due to grass NSC.

Introduction

For dairy cattle consuming high-quality fresh grass, energy is the primary limiting nutrient for milk production. Immature, perennial ryegrass provides an excess of readily available protein relative to available carbohydrate, which reduces efficiency of protein utilized for microbial crude protein (MCP) synthesis, decreases the amount of nitrogen (N) used for productive purposes (i.e. milk and muscle), and increases the amount of N excreted in urine. For lactating dairy cows consuming grass, up to 35-40% of dietary N may be lost as ammonia (NH_3) (Lee et al., 2001). Nonstructural carbohydrates (NSC) and water-soluble carbohydrates (WSC) are used to quantify the more readily available carbohydrates in grasses. In temperate grasses, the primary NSC are glucose, fructose, sucrose, starch, and fructans and the primary WSC are glucose, fructose, sucrose, and fructans (Smith, 1973).

Miller et al. (2001b) proposed that an increase in WSC concentration of grass forage increases energy value of grass and, therefore, utilization efficiency of grass nutrients for milk production. Perennial ryegrasses that accumulate elevated concentrations of WSC have been developed (Humphreys, 1989a, b, c). High WSC grass increased lamb live weight gain and total lamb production which was attributed to an

increase in WSC, decrease in neutral detergent fiber (NDF) and acid detergent fiber (ADF), or a combination (Lee et al., 2001). Dairy cows grazing high WSC grass, consumed less dietary N and were more efficient in conversion of dietary N to milk protein (Miller et al., 2001a). Authors suggested the increased utilization efficiency of dietary N was due to improved ruminal N utilization and/or increased propionic acid. In a green chop study, dairy cows fed high WSC grass consumed more forage DMI, produced more milk, and exhibited an increase in N-utilization efficiency (Miller et al., 1999). In a similar study, dairy cows fed elevated WSC consumed more digestible dry matter, produced more milk, excreted less urinary N, and secreted more milk N (Miller et al., 2001b). Objectives were to study the effects of perennial ryegrass NSC on milk yield and composition, dry matter intake (DMI), and rumen fermentation in lactating dairy cows.

Materials and methods

Forage management

Two perennial ryegrasses, one with a relatively high NSC (HNSC; Elgon®) and one commonly grown in Oregon, control NSC, (CNSC; Linn), were selected. Elgon® is a tetraploid perennial ryegrass, which was developed approximately 15 years ago by Advanta Seeds® in Holland. Linn is a diploid perennial ryegrass, which was released by Oregon Agriculture and Experiment Station in 1961 and is representative of Oregon perennial ryegrass (Oregon State Univ. Department of Crop and Soil Sciences, 1996). In the fall of 2002, approximately 1.2 ha of each grass was planted at the Oregon State University dairy research facility in Corvallis, Oregon at a rate of 45 kg/ha. In May 2003,

the first cutting of the forage was removed. June and July 2003, the second and third cuttings of the forage were used for the green chop trial. After removal of each cutting, grasses were fertilized with 52 kg/ha of N. June 2003 and July 2003, grasses were irrigated once per week and water was not limiting.

Cows

Eighteen Holsteins and two Jerseys were initially grouped and fed CNSC for 7 d. Holstein cows were blocked by average daily milk yield collected during the 7 d initial grass adaptation period and assigned at random to one grass treatment in a randomized complete block design. The two Jerseys were paired and assigned at random to grass treatment. After the initial grass adaptation period, cows were fed assigned treatments and trained to use Calan® gates (American Calan Inc., Northwood, NH) for a 7 d treatment adaptation period. Average parity and days in milk were 2.1 and 175 for CNSC and 1.7 and 173 for HNSC.

Cows were housed in one group in a freestall barn. For the 7 d initial grass adaptation period, cows were group fed along a feed bunk. During the treatment adaptation and treatment periods, Calan® gates were used to collect individual intake data. Cows were milked twice daily at approximately 0430 h and 1600 h. Body weight (BW) of each animal was measured at 1630 h on d 1 and 21 of the treatment period.

Feeding management

Grasses were chopped twice daily with a flail forage plot harvester (Swift

Current, Saskatchewan, Canada); chop height was approximately 10 cm and chop length was dependent on forage height. Forage was fed as green chop to facilitate estimates of DMI and development of relationships between grass composition, intake, and production responses. Results of the green chop trial may not be directly applicable to grazing (Miller et al., 2001b). During the 7 d treatment adaptation and 21 d treatment period, the chopping and feeding order were alternated daily between CNSC and HNSC grasses. Forages were fed at approximately 0630 h and 1800 h. During the treatment period, forage orts were removed and weighed at 0430 h and 1600 h.

Cows were supplemented with a total mixed ration (TMR) twice daily at 0500 h and 1630 h, which was comprised of (DM basis) 19.0% alfalfa hay, 20.6% corn silage, 48.8% corn/barley (C/B), 3.8% soybean meal/dried distiller's grains (SBM/DDG), 3.8% whole cottonseed, and 4.0% mineral-vitamin premix. The ratio for C/B and SBM/DDG mixtures were 1:1.

Total mixed ration orts were removed and weighed at approximately 0600 h and 1730 h. Forage and TMR orts were removed, but not weighed during treatment adaptation period. Total DMI was the sum of grass DMI and TMR DMI.

Sample collection and laboratory analysis

Samples of each treatment grass were collected twice daily. Immediately after distribution of green chop, two random grab samples were collected from the Calan® gate for each cow. Samples were immediately sealed and frozen at -10° C until laboratory analysis.

Grass samples were freeze-dried with a Freeze Mobile 12 (Virtis Co., Gardiner, NY). Samples were ground using a Thomas Wiley® Mill (Thomas Scientific, USA) with a 1 mm screen. Each of the 84 grass samples was analyzed for dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), sugars, starch, and ash. Nonstructural carbohydrates were calculated as the sum of sugars and starch. Dry matter, CP, and ash were analyzed according to AOAC (1995). To determine DM concentration, subsamples were oven-dried at 100° C for 12 h. Kjeldahl N analysis was used to determine CP. Oven dried samples were heated in a furnace to 600° C for 4 h to determine ash. Samples were sequentially analyzed for NDF and ADF using an Ankom® Fiber Analyzer (Ankom Technology Corporation, Fairport, NY). Solutions used for NDF and ADF are as described in Van Soest et al. (1991) and AOAC (1995), respectively. Analysis and calculation of sugars, starch, and NSC was completed by Dairy One Forage Laboratory (Ithaca, NY). Sugars were analyzed according to procedures by W.H. Hoover and T.K. Miller Webster which is described by Hall et al. (1999).

In addition, freeze-dried and ground grass samples were pooled by week and analyzed for 30 h NDF digestibility and via the Cornell-Penn-Miner (CPM) Plus package by Cumberland Valley Analytical Services, Inc. (Maugansville, MD). Data was used in diet evaluation with CPM Dairy (version 2.0.24) software.

Total mixed ration feedstuffs were sampled weekly. Samples were oven dried at 60° C for 48 h. Samples were ground to 1 mm using a Thomas Wiley® Mill (Thomas Scientific, USA). Each feedstuff was analyzed via the CPM Plus package by Cumberland Valley Analytical Services, Inc. (Maugansville, MD). Data was used to evaluate the diet

in CPM Dairy. Guaranteed analysis on the mineral-vitamin pre-mix was used. Composition of TMR feedstuffs is presented in Table 3.1. Values are the average for the 3 wk treatment period.

Table 3.1 – Composition of TMR feedstuffs and TMR.

	Feedstuff						
Item	**Alfalfa hay**	**Corn silage**	**C/B[1]**	**SBM/ DDG[2]**	**WCS[3]**	**Min-Vit[4]**	**TMR[5]**
DM, %	91.3	33.0	90.0	91.7	91.7	88.5	
CP, % DM	20.2	8.93	10.2	40.8	25.4	2.7	13.3
ADICP[6], % DM	1.17	0.97	1.03	3.97	1.83		1.14
NDICP[7], % DM	2.07	1.63	1.37	6.60	3.17		1.77
Soluble CP, % DM	8.03	5.17	1.90	6.33	5.13		3.95
NE_L, Mcal/kg	1.28	1.56	1.96	1.94	2.14		1.68
NFC[8], % DM	28.0	38.5	69.9	24.2	2.10	10.9	48.9
NDF, % DM	35.9	44.9	15.7	26.2	48.5	3.50	26.7
Lignin, % DM	6.33	3.13	0.93	3.67	11.1		2.86
Crude fat, % DM	2.43	3.13	3.17	8.47	21.9		3.80
Ca, % DM	1.41	0.22	0.04	0.33	0.17	15.0	0.95
P, % DM	0.31	0.19	0.27	0.75	0.74	3.00	0.41
Mg, % DM	0.56	0.17	0.12	0.37	0.43	3.00	0.35
K, % DM	3.77	1.14	0.37	1.52	1.13	3.00	1.35
S, % DM	0.32	0.12	0.13	0.56	0.28	1.50	0.24
Na, % DM	0.17	0.02	0.02	0.17	0.02	7.50	0.35
Cl, % DM	0.79	0.18	0.09	0.19	0.11	9.00	0.60
Fe, ppm	744	226	52.3	134	83.0	1,200	270
Mn, ppm	84.0	59.7	10.7	41.3	23.3	2,400	132
Zn, ppm	15.0	39.7	26.7	67.3	47.0	2,400	124
Cu, ppm	12.0	5.00	4.33	12.3	14.3	600	30.4
Se, ppm						18.0	
Co, ppm						6.00	
Vitamin A, KIU/kg						529,000	
Vitamin D, KIU/kg						198,000	
Vitamin E, KIU/kg						4,630	

[1]Corn/barley mix; 1:1 ratio

[2]Soybean meal:dried distiller's grains; 1:1 ratio

[3]Whole cottonseed

[4]Mineral-vitamin premix

[5]TMR average

[6]Acid detergent insoluble CP

[7]Neutral detergent insoluble CP

[8]Nonfiber carbohydrates

Compositions of feedstuffs were similar to predicted values. Due to soil contamination, ash concentration of alfalfa hay was high during approximately wk 1 and 2 of the treatment period.

Total diet (i.e. grass and TMR) was evaluated with CPM software. For each treatment, rations were evaluated by week. Balance of minerals and vitamins was not evaluated. Average total diet composition for the 3 wk treatment period is presented in Table 3.2.

Table 3.2 - Average composition of total diet for control NSC (CNSC) and high NSC (HNSC) grasses during 3 wk treatment period[1].

	Treatment	
	CNSC	**HNSC**
CP, % DM	13.2	13.4
Available CP, % DM	12.2	12.3
ADICP[2], % DM	1.00	1.02
NDICP[3], % DM	1.90	1.93
Adjusted CP, % DM	13.1	13.3
Soluble CP, % DM	4.09	4.16
Degradable CP, % DM	6.02	6.13
TDN, % DM	70.2	71.1
NE_L, Mcal/kg	1.54	1.56
NE_M[4], Mcal/kg	1.65	1.67
ADF, % DM	23.3	23.6
NDF, % DM	37.8	38.3
Lignin, % DM	3.16	3.21
Lignin:NDF ratio	8.13	8.27
Crude fat, % DM	3.76	3.81
Ash, % DM	7.62	7.74
Starch, % DM	22.5	22.8
Sugars, % DM	4.62	4.67
NFC, % DM	37.5	37.9
Enzymatic NSC, % DM	27.1	27.4
Ca, % DM	0.75	0.76
P, % DM	0.38	0.39
Mg, % DM	0.28	0.29
K, % DM	1.87	1.90
S, % DM	0.24	0.24
Na, % DM	0.25	0.25
Cl, % DM	1.00	1.01
Fe, ppm	220	224
Mn, ppm	101	102
Zn, ppm	86.3	87.1
Cu, ppm	27.0	27.4
Se, ppm	0.43	0.44
Co, ppm	0.14	0.15
Vitamin A, IU/kg	12,742	12,878
Vitamin D, IU/kg	4,778	4,829
Vitamin E, IU/kg	111	113

[1]Samples pooled by week and analyzed

[2]Acid detergent insoluble CP

[3]Neutral detergent insoluble CP

[4]Net energy for maintenance

Milk yield was electronically recorded at each milking via a Westfalia® milk meter system and downloaded to a computer. Milk samples were collected for four consecutive milkings centered on d 0 of the treatment adaptation period for use as a covariate and d 7 and 21 of the treatment period. Milk samples were analyzed for fat, true protein, lactose, solids non-fat, and milk urea nitrogen (MUN) at the Central Valley Dairy Laboratory (Tulare, CA).

Dry matter intake of each grass and TMR feeding was measured for the 21 d treatment period. To determine individual DMI, amount fed and orts were recorded for each cow at each feeding and multiplied by the respective DM concentration. Change in BW was calculated as d 1 BW subtracted from d 21 BW.

Rumen samples were collected via rumen cannula for one Holstein and two Jerseys and via esophageal tube for one Holstein centered on d 9 and 21 of the treatment period. Rumen samples were collected 0, 1, 2, 3, 4, 6, 8, 10, and 12 h relative to feeding of TMR at 1630 h on d 8 and 20 and 0500 h on d 9 and 21 of the treatment period. Rumen fluid was immediately analyzed for pH. Procedures adapted from Broderick and Kang (1980) were used to determine rumen NH_3 and procedures adapted from Baumgardt (1964), Erwin et al. (1961), and Simkins (1965) were used to determine rumen VFA (i.e. acetic, propionic, and butyric). Per respective procedures, immediately following rumen fluid collection, 5 ml sulfuric acid was added to 100 ml rumen fluid and 1 ml metaphosphoric acid was added to 5 ml rumen fluid for NH_3 and VFA analysis, respectively. Samples were immediately sealed and frozen at -10° C.

Statistical analysis

Data were analyzed with the MIXED Procedure of SAS (SAS User's Guide, 1998). Significance was determined at $P \leq 0.05$ and trends were determined at $0.05 < P < 0.10$.

Grass composition was analyzed by week and cutting. Model for grass composition by week was:

$$Y_{ijk} = \mu + T_i + W_j + M_k + (T \times W)_{ij} + (T \times M)_{ik} + (W \times M)_{jk} + (T \times W \times M)_{ijk} + E_{ijk}$$

where

Y_{ijk} = dependent variable,

μ = overall population mean,

T_i = effect of treatment i (i = HNSC or CNSC),

W_j = effect of week j (j = 1, 2, 3),

M_k = effect of time k (k = a.m. or p.m.),

$(T \times W)_{ij}$ = effect of interaction between treatment i and week j,

$(T \times M)_{ik}$ = effect of interaction between treatment i and time k,

$(W \times M)_{jk}$ = effect of interaction between week j and time k,

$(T \times W \times M)_{ijk}$ = effect of interaction between treatment i, week j, and time k,

E_{ijk} = residual error term.

Model for grass composition by cutting was:

$$Y_{ijk} = \mu + T_i + C_j + M_k + (T \times C)_{ij} + (T \times M)_{ik} + (C \times M)_{jk} + (T \times C \times M)_{ijk} + E_{ijk}$$

where

Y_{ijk} = dependent variable,

μ = overall population mean,

T_i = effect of treatment i (i = HNSC or CNSC),

C_j = effect of cutting j (j = 2 or 3),

M_k = effect of time k (k = a.m. or p.m.),

$(T \times C)_{ij}$ = effect of interaction between treatment i and cutting j,

$(T \times M)_{ik}$ = effect of interaction between treatment i and time k,

$(C \times M)_{jk}$ = effect of interaction between cutting j and time k,

$(T \times C \times M)_{ijk}$ = effect of interaction between treatment i, cutting j, and time k,

E_{ijk} = residual error term.

Total mixed ration DMI was analyzed by day. For grass and total DMI, treatment and day interaction was significant. To identify the effect of treatment, daily grass and total DMI were condensed and analyzed by week. Effect of treatment by week was determined via analysis for slice effects in SAS. The model for TMR DMI was:

$$Y_{ijk} = \mu + B_i + T_j + D_k + (T \times D)_{jk} + E_{ijk}$$

where

Y_{ijk} = dependent variable,

μ = overall population mean,

B_i = effect of block i (i = 1, 2, 3, …, 7),

T_j = effect of treatment j (j = HNSC or CNSC),

D_k = effect of day k (k = 1, 2, 3,…., 21),

$(T \times D)_{jk}$ = effect of interaction between treatment j and day k,

E_{ijk} = residual error term.

The model for grass and total DMI was similar to the model for TMR DMI; effect of day was replaced with week (i.e. effect of week l (l = 1 or 3)).

Data analysis of milk yield and components used covariate and repeated measures analysis. The model for milk yield was:

$$Y_{ijkl} = \mu + COV_i + B_j + T_k + D_l + (T \times D)_{kl} + E_{ijkl}$$

where

Y_{ijkl} = dependent variable,

μ = overall population mean,

COV_i = covariate adjustment for pretreatment performance,

B_j = effect of block j (j = 1, 2, 3, …, 7),

T_k = effect of treatment k (k = HNSC or CNSC),

D_l = effect of day l (l = 1, 2, 3, …, 21),

$(T \times D)_{kl}$ = effect of interaction between treatment k and day l,

E_{ijkl} = residual error term.

The milk yield model was used for milk components and MUN; effect of day was replaced with week (i.e. effect of week l (l = 1 or 3)).

The model for BW change analysis was:

$$Y_{ij} = \mu + B_i + T_j + E_{ij}$$

where

Y_{ij} = dependent variable,

B_i = effect of block i (i = 1, 2, 3, …, 7),

T_j = effect of treatment j (j = HNSC or CNSC),

E_{ij} = residual error term.

Data analysis of rumen fermentation parameters used time-series analysis. Model for analysis of rumen fermentation parameters was:

$$Y_{ijk} = \mu + B_i + T_j + H_k + (T \times H)_{jk} + E_{ijk}$$

where

Y_{ijk} = dependent variable,

μ = overall population mean,

B_i = effect of block i (i = 1, 2),

T_j = effect of treatment j (j = HNSC or CNSC),

H_k = effect of hour k (k = 0, 1, 2, 3, 4, 6, 8, 10, or 12),

$(T \times H)_{jk}$ = effect of interaction between treatment j and hour k,

E_{ijk} = residual error term.

Results and discussion

Forage composition

Table 3.3 – Least squares means and standard errors for chemical composition of fresh grasses by treatment and week.

	Treatment[1]			Week				
Item[2]	CNSC	HNSC	SE	1	2	3	SE	P[3]
DM	22.4	19.4	0.41	19.2	23.8	19.7	0.50	T**,W**
NDF	52.8	52.1	0.57	53.8	56.4	47.2	0.69	W**
ADF	30.0	29.8	0.36	30.5	32.9	26.3	0.44	W**
CP	11.1	13.0	0.34	10.0	9.8	16.3	0.41	T**,W**
NSC	18.2	15.7	0.41	19.9	15.1	15.8	0.50	T**,W**
Sugars	15.9	13.9	0.36	18.0	13.0	13.8	0.44	TxW*
Starch	2.2	1.8	0.15	1.9	2.1	2.0	0.19	T*

[1]Control NSC (CNSC); high NSC (HNSC)

[2]With exception of DM, reported as % DM

[3]T=Treatment; W=Week; TxW=Treatment x week

*P < 0.05

**P<0.01

The composition of grasses is shown in Table 3.3. Dry matter concentration was greater for CNSC versus HNSC (P<0.01). Crude protein concentration was greater for HNSC versus CNSC (P<0.01). Neutral detergent fiber and ADF concentrations were similar between treatments. Starch and NSC concentrations were greater for CNSC (P<0.05 and P<0.01, respectively).

Dry matter was greater wk 2 versus wk 1 and 3 (P<0.01). Crude protein concentration was greater wk 3 compared to wk 1 and 2 (P<0.01). Neutral detergent fiber and ADF concentrations were less wk 3 versus wk 1 and 2. Changes in CP, NDF, and ADF over the 3 wk treatment period are consistent with composition changes due to maturity (Beever et al., 2000). Weeks 1 and 2 corresponded with second cutting and advanced maturity; seed heads emerged during wk 1. Third cutting was initiated wk 3

and the grasses were vegetative and less mature than wk 1 and 2. Nonstructural carbohydrates were greater for wk 1 versus wk 2 and 3 ($P<0.01$), which is consistent with changes due to maturity (Smith, 1973). Starch concentration was similar for wk 1, 2, and 3.

For sugars, the interaction between treatment and week was significant ($P<0.05$). For other variables in Table 3.3, the interaction between treatment and week was not significant. Figure 3.1 illustrates the least squares mean and standard errors for sugars for CNSC and HSNC for wk 1, 2, and 3. For wk 1, concentration of sugars was greater for CNSC than HNSC ($P<0.05$), which corresponds to the wk with the highest NSC concentration. However, sugars were similar wk 2 and 3.

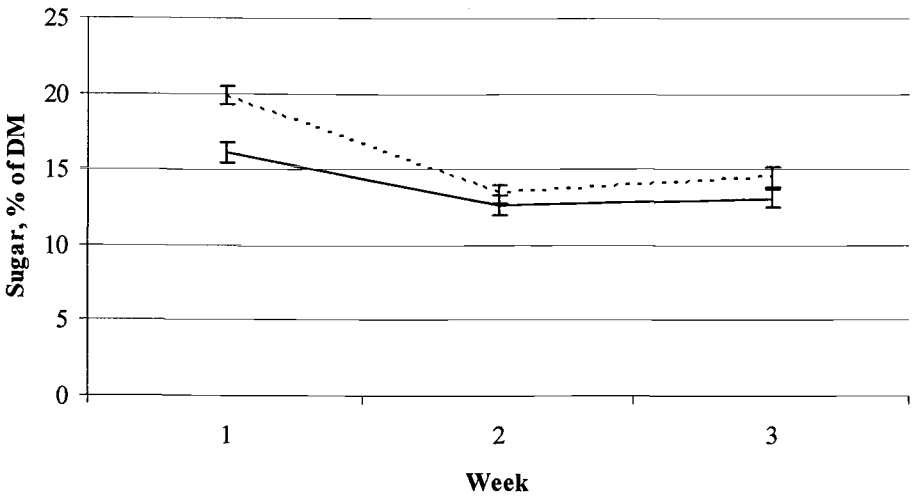

Figure 3.1 – Least square means and standard errors (vertical bars; SEM=0.62) for concentration of sugars for control NSC (----) and high NSC (——) grasses by week during 3 wk treatment period. Treatment by week interaction was significant ($P<0.05$).

Table 3.4 shows the 30 h NDF digestibility of CNSC and HNSC grasses. Values are expressed on a DM basis, as a % of NDF. Thirty h NDF digestibility was similar for CNSC and HNSC treatments. Neutral detergent fiber digestibility by week follows maturity and chemical composition of grasses.

Table 3.4 –30 h NDF digestibility for CNSC and HNSC fresh grasses by wk during 3 wk treatment period[1].

	Treatment[2, 3]	
	CNSC	**HNSC**
Week 1	66.5	67.9
Week 2	57.9	61.5
Week 3	67.8	69.8

[1]DM basis, % of NDF

[2]Control NSC (CNSC); high NSC (HNSC)

[3]Effect of treatment: P>0.05

Fresh grass composition by cutting is shown in Table 3.5. Cutting 2 is approximately wk 1 and 2 and cutting 3 is approximately wk 3 of the treatment period. To assure adequate 3rd cutting forage was available the last three days of the treatment period, both treatment and control animals were fed 2nd cutting grass at the p.m. feeding on d 17 and the a.m. feeding on d 18.

Table 3.5 – Least square mean chemical composition of fresh grasses by cutting during 3 wk treatment period.

	Cutting				
Item[1]	**2**	**SE**	**3**	**SE**	**P**
DM	21.7	0.38	18.5	0.65	***
NDF	55.2	0.32	44.6	0.53	***
ADF	31.7	0.22	24.7	0.37	***
CP	10.0	0.17	17.8	0.28	***
NSC	17.2	0.43	15.9	0.73	NS
Sugars	15.3	0.41	13.9	0.69	*
Starch	2.0	0.13	2.0	0.21	NS

[1]With exception of DM, reported as % DM
***P < 0.01
**P < 0.05
*P < 0.10

Dry matter, NDF, and ADF were greater cutting 2 compared to cutting 3 (P<0.01). Crude protein was less cutting 2 versus cutting 3 (P<0.01). Sugars tended to be greater cutting 2 (P<0.10). Starch and NSC were similar cuttings 2 and 3. Change in CP, NDF, ADF, and sugars between cutting 2 and 3 is consistent with maturity (Beever et al., 2000; Smith, 1973). Due to the change in quality over the 3 wk treatment period, analysis of grass composition by week more accurately described the nutritive value of the grasses and, therefore, was used in ration evaluation.

Forage yield was not measured. Based on observation, forage yield per unit area was greater for HNSC grass. High NSC grass was more established and had a more rapid rate of regrowth.

Grass composition was different than expected; NSC was lower and CP was higher for HNSC grass. Neutral detergent fiber and ADF were similar between treatments. In general, variation in NSC is due to environmental conditions, such as temperature and light intensity, and/or stage of maturity at harvest (McDonald, 1981).

In the current trial, with regard to NSC, HNSC grass did not perform as in a previous field trial (Downing, 2004) and the companion silage trial (Chapter 4). In 2001, HNSC grass was tested in a field plot trial in Tillamook, Oregon. Nonstructural carbohydrates were measured for a.m. and p.m. cuttings. Average NSC concentration, on a DM basis, for April 20, June 28, and October 1 was 15.3, 26.1, and 26.3%, respectively. For the current trial, temperature may have contributed to the relatively low NSC. Temperature and WSC are inversely correlated and, in general, temperature is lower in Tillamook versus Corvallis (Deinum, 1966). However, light intensity is also lower in Tillamook, which is not consistent with the relationship between light intensity and WSC (Deinum, 1966). Further, despite similar environmental conditions and stage of maturity for the green chop trial and companion silage trial, HNSC NSC was less for the green chop trial versus the silage trial. For the silage trial, grasses were harvested wk 2 of the green chop trial and corresponding HNSC NSC was 19.0 vs. 14.9% of DM for the silage and green chop trial, respectively. HNSC NSC cannot be explained with the data collected.

For HNSC and CNSC grasses in the green chop trial, NSC and CP were negatively associated (r^2 = 0.10; $P<0.01$) which has been established (Nowakowski, 1962). For grasses, N fertilization reduces NSC as a result of increased growth.

Nonstructural carbohydrates and CP data and yield observations indicate low NSC for HNSC grass may be the result of a more rapid growth rate than CNSC grass.

HNSC grass selected for the green chop trial is a tetraploid versus a cultivar selected for high NSC. In general, tetraploids exhibit higher NSC than diploids not selected for high NSC. In the Tillamook field plot trial, HNSC exhibited elevated levels of NSC. However, elevated NSC concentrations were not maintained in the green chop trial. Diploids selected for elevated NSC have consistently maintained high NSC in a range of environments and throughout the growing season (Radojevic et al 1994; Smith et al., 1998). Ability to maintain high NSC in a range of environments should be considered in grass selection.

Dry matter intake

Daily grass DMI was extremely variable (SE=0.32 kg/cow/d). For grass DMI analyzed by week, the interaction between treatment and week was significant ($P<0.05$). For HNSC treatment, grass DMI was greater wk 2 ($P<0.01$) and tended to be greater wk 3 ($P<0.10$) (Figure 3.2).

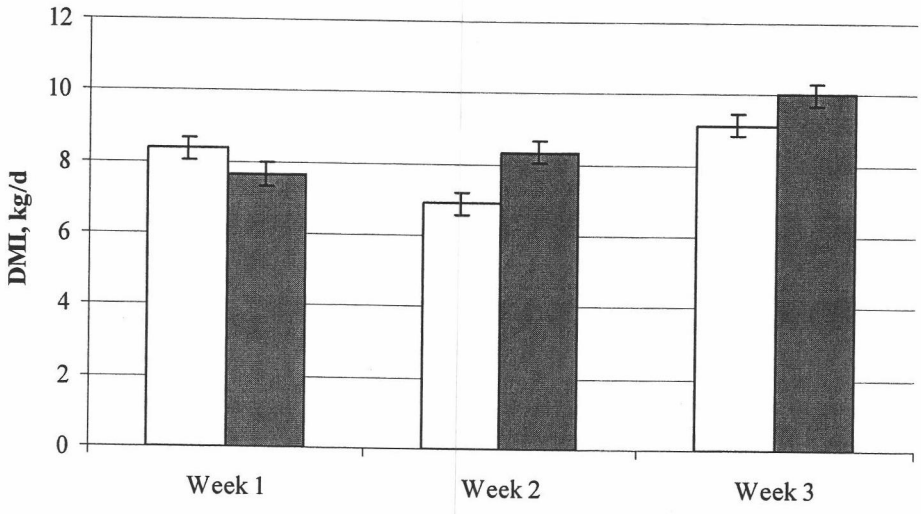

Figure 3.2 – Least squares means and standard errors (vertical bars; SEM=0.32) for average grass DMI per cow per day for control NSC (□) and high NSC (■) grasses by week during the 3 wk treatment period. Treatment by week interaction was significant (P<0.01).

Week 1, numerically, CNSC animals consumed an average of 0.72 kg of grass DM/cow/d more than HNSC animals. In wk 2 and 3, HNSC animals consumed an average of 1.44 and 0.85 kg of grass DM/cow/d, respectively, more than CNSC animals.

For TMR DMI analyzed by day, the effect of treatment tended to be significant (P=0.06). Least squares means for TMR DMI for CNSC and HNSC were 11.65 ± 0.12 and 12.04 ± 0.12 kg/cow/d, respectively. Effect of day was significant (P<0.01). However, the interaction between treatment and day was not significant.

For total DMI analyzed by week, the interaction between treatment and week was significant (P<0.01). For HNSC, total DMI was greater wk 2 (P<0.01) and tended to be greater wk 3 (P<0.10) (Figure 3.3), which is similar to grass DMI.

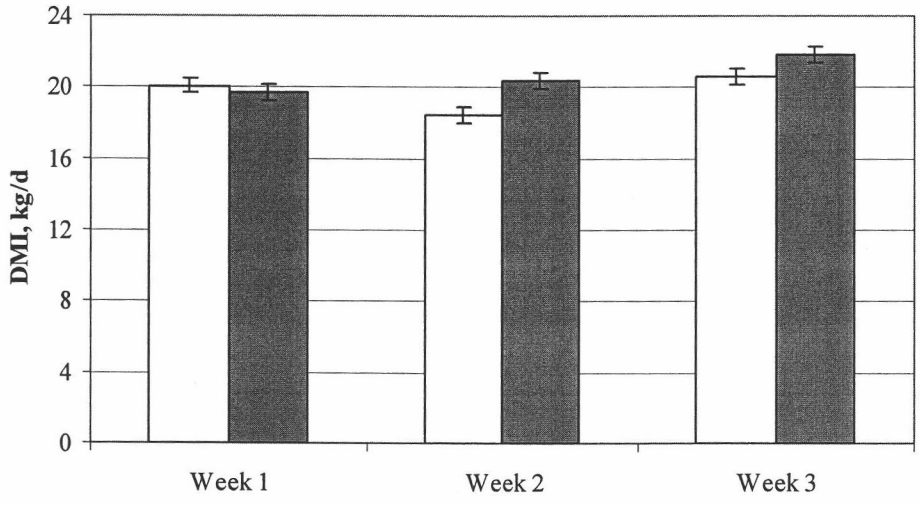

Figure 3.3 – Least squares means and standard errors (vertical bars; SEM=0.44) for average total DMI per cow per day for control NSC (□) and high NSC (■) grasses by week during the 3 wk treatment period. Treatment by week interaction was significant (P<0.01).

Week 1, numerically, CNSC treatment animals consumed an average of 0.35 kg more total DM/cow/d than HNSC treatment animals. In wk 2 and 3, HNSC animals consumed an average of 1.95 and 1.25 kg more total DM/cow/d, respectively.

Milk yield and composition

Milk yield was greater for HNSC treatment (P<0.05). Daily milk yield was 29.1 and 30.2 kg/d for CNSC and HNSC treatments, respectively. Day effect was also significant (P<0.01). The treatment by day interaction was not significant. Figure 3.4 illustrates daily milk yield.

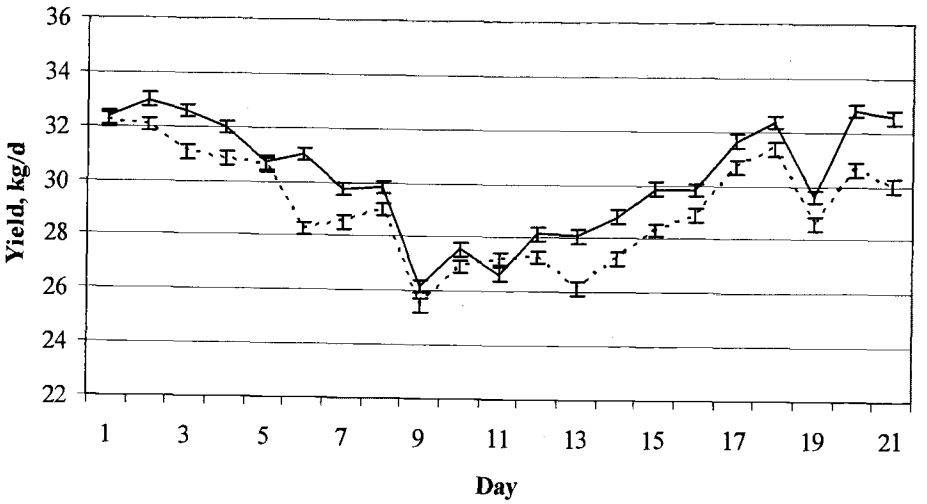

Figure 3.4 – Least square means and standard errors (vertical bars; SEM=0.24) for average daily milk yield per cow for control NSC (----) and high NSC (——) grasses by week during 3 wk treatment period. Effects of treatment and day were significant (P<0.05 and P<0.01, respectively).

Based on the DMI data, the effect of HNSC on milk yield was likely due to an increase in TMR DMI wk 1 and an increase in TMR and grass DMI wk 2 and 3. Milk yield and TMR DMI were consistently greater for the HNSC treatment wk 1, 2, and 3 of the treatment period. For HNSC treatment, grass DMI was similar, greater (P<0.01), and tended to be greater (P<0.10), wk 1, 2, and 3, respectively.

Table 3.6 – Average milk composition and MUN by treatment and sample day during 3 wk treatment period[1].

	Treatment[2]			Sample[3]			
	CNSC	HNSC	SE	Week 1	Week 3	SE	P[4]
Fat, %	3.67	3.72	0.09	3.72	3.67	0.07	NS
Fat yield, kg/d	0.99	1.18	0.04	1.06	1.11	0.03	T**
Protein[5], %	3.18	3.13	0.09	3.13	3.18	0.07	NS
Protein yield[5], kg/d	0.87	1.00	0.04	0.91	0.97	0.03	T**
Lactose, %	4.54	4.61	0.05	4.63	4.52	0.04	S**
Lactose yield, kg/d	1.30	1.49	0.06	1.38	1.41	0.05	T**
SNF, %	8.62	8.62	0.11	8.64	8.59	0.09	NS
SNF yield, kg/d	2.42	2.77	0.10	2.54	2.65	0.08	T**
MUN[1], mg/dl	8.44	8.54	0.20	5.89	11.09	0.23	S***

[1]Milk urea nitrogen

[2]Control NSC (CNSC); high NSC (HNSC)

[3]Samples collected d 7 and 21 of treatment period, denoted week 1 and 3, respectively

[4]T=Treatment; S=Sample; TxS=Treatment x sample

[5]True protein

***P < 0.01

**P < 0.05

Milk components, as a percent, were similar between CNSC and HNSC treatments (Table 3.6). As milk yield was greater for HNSC versus CNSC and components were similar for HNSC and CNSC, yields of milk components (kg/d) were greater for the HNSC treatment (P<0.05). Milk urea nitrogen concentration was similar for HNSC and CNSC treatments (Table 3.6). However, MUN concentration did differ by week (P<0.01); wk 1 was lower than wk 3 (5.89 vs. 11.09 mg/dl). Milk urea nitrogen indicates protein status; target MUN values are 8.5-11.5 mg/dl (Kohn et al., 2002). For the 21 d treatment period, MUN and grass protein had a similar pattern. Weeks 1 and 2 of

the treatment period, grass CP was low (10.0 and 9.8% of DM, respectively) and wk 1 MUN was also low (5.89 mg/dl), which indicates dietary protein was deficient. In wk 3, grass CP increased (16.3% of DM) and MUN also increased (11.09 mg/dl), which indicates dietary protein balance had improved. For variables listed in Table 3.6, the treatment by week interaction was not significant.

Grass composition by week, TMR composition, and milk yield and components by week were evaluated in CPM Dairy. For CNSC and HNSC, wk 1, 2, and 3 of the treatment period metabolizable protein (MP) was the limiting nutrient for milk production. Grass CP and MUN data support MP was the limiting nutrient for milk production. Table 3.7 shows predicted milk yield (as predicted in CPM) actual milk yield, and the difference between predicted milk yield and actual milk yield by treatment and week.

Table 3.7 – Predicted and actual milk yields by treatment group and week (kg/d)[1].

	Treatment					
	CNSC			HNSC		
	Predicted	**Actual**	**Difference[2]**	**Predicted**	**Actual**	**Difference[2]**
Week 1	20.0	30.3	-10.3	22.7	31.5	-8.8
Week 2	18.1	26.9	-8.8	22.2	27.8	-5.6
Week 3	27.0	29.6	-2.6	31.4	31.1	0.3

[1]As predicted in Cornell-Penn-Miner Dairy.

[2]Difference = predicted – actual.

In general, Cornell-Penn-Miner predictions of milk yield were neither accurate nor precise for the diet, which may be, in part, due to grass quality. During wk 1 and 2,

the difference between predicted and actual milk yield was greater than wk 3, which is similar to the pattern of grass quality. Cornell-Penn-Miner predicted milk yields for HNSC were higher than CNSC for wk 1, 2, and 3, which is consistent with actual milk yield data.

As MP was the limiting nutrient for milk production and CP was higher for HNSC grass, the increased yield of milk and milk components for HNSC treatment may be, in part, due to higher grass CP. Grass CP may have enhanced microbial fermentation, which increased MP and improved grass fermentation which increased TMR DMI. In addition, increased TMR DMI for HNSC treatment contributed to the increased yield of milk and milk components via an increased supply of MP and other nutrients for milk production.

Body weight

Initial body weights were 553 kg and 594 kg for the CNSC and HNSC treatments, respectively. Change in body weight over the 21 d treatment period was similar for CNSC and HNSC treatments (Table 3.8).

Table 3.8 – Average BW change for CNSC and HNSC grass-fed groups over 21 d treatment period.

	Treatment				
	CNSC		HNSC		
	LSM	SE	LSM	SE	P
BW change, kg	5.5	3.66	0.4	2.73	NS[1]

[1]P > 0.05

Numerically, change in BW was greater for CNSC versus HNSC treatment animals (5.5 versus 0.4 kg). However, standard errors were 3.66 and 2.73 for CNSC and HNSC, respectively, resulting in a non significant p-value. Range of body weight change over the 21 d treatment period was -18.1 kg to +18.1 kg, with the exception of one outlier value of +105.2 kg. The outlier was a CNSC treatment animal and was omitted from statistical analysis.

Rumen fermentation

Rumen pH is not reported because degrees of freedom was 0. Rumen samples collected via esophageal tube were not valid for pH analysis due to saliva contamination.

Table 3.9 shows rumen NH_3 and VFA for d 9 and 21 of the treatment period

Table 3.9 – Rumen fermentation parameters by sample day for CNSC and HNSC treatments[1].

	D 9 sample			D 21 sample		
	Treatment			Treatment		
Item	**CNSC**	**HNSC**	**SE**	**CNSC**	**HNSC**	**SE**
NH_3N, mM	6.26	5.60	0.53	10.53	11.40	0.58
Acetate, mM	51.97	54.32	1.72	51.89	58.10	1.56
Propionate, mM	12.64	14.43	0.34	13.89	18.80	0.99
Butyrate, mM	9.98	9.71	0.29	9.03	10.08	0.46

[1]All tested NS for treatment; P >0.05.

For each sample day, fermentation parameters were similar between HNSC and CNSC treatments. Rumen NH_3 concentration peaked at h 2 (not shown), which corresponded 2 h after TMR supplementation and declined as time progressed indicating TMR was the primary source of rumen NH_3. For rumen VFA, no pattern was identified and variation was often marked. Mean rumen NH_3 concentrations were numerically greater for sample d 21 versus d 9, indicating a difference in protein status which is consistent with grass CP and MUN data.

Conclusions

For HNSC grass, CP was higher and NSC was lower than CNSC grass. Neutral detergent fiber and ADF were similar between treatments. For HNSC grass, NSC was different than expected. For HNSC treatment, grass DMI was greater wk 2 and tended to be greater wk 3 and TMR DMI tended to be greater wk 1, 2, and 3. Daily yields of milk

and components were greater for HNSC treatment. Milk urea nitrogen differed by week. Based on grass CP data, MUN data, and CPM evaluation, MP was the limiting nutrient for milk production. It appears HNSC increased yields of milk and components via an increase in grass CP and grass and TMR DMI.

Chapter 4
Effect of perennial ryegrass nonstructural carbohydrates on fermentation characteristics of ensiled forages

Abstract

Objective was to determine if differences exist between fermentation characteristics of three grasses with high nonstructural carbohydrates (NSC) and one control NSC grass ensiled in vacuum sealed bags. Four perennial ryegrasses, three cultivars with a relatively high NSC concentration (HNSC; AberAvon®, AberDart®, and Elgon®) and one cultivar commonly grown in Oregon, control NSC (CNSC; Linn) were selected. In the fall of 2002, one plot of each cultivar was planted at the Oregon State University dairy research facility in Corvallis, Oregon. July 2003, three replicates of each grass were ensiled at the a.m. and p.m. harvests. Each bag was packed, vacuum sealed, and ensiled for 60 d. Fresh grass samples were taken from each bag. Fresh grass NSC was greater for HNSC grasses versus Linn. Final pH was lower, total acids was higher, and lactic acid tended to be higher for HNSC grasses versus Linn. Final pH, lactic acid, acetic acid, total acids, and NH_3 were lower for p.m. versus a.m. cutting. Ensiling was most efficient for HNSC grass varieties harvested at the p.m. cutting.

Introduction

Well-preserved grass silage is the result of the controlled fermentation of fresh grass; characterized by low pH, high lactic acid concentration, and low ammonia (NH_3) concentration. Nonstructural carbohydrates (NSC) are the primary fermentation substrate. In temperate grass forages, glucose, fructose, and sucrose, starch, and fructans are the

primary nonstructural carbohydrates (Smith, 1973). Water-soluble carbohydrates (WSC) is also a term used to describe the more readily available carbohydrates. In temperate grass forages, the primary WSC are glucose, fructose, sucrose, and fructans.

Optimally, lactic acid bacteria (LAB) dominate fermentation. Lactic acid bacteria ferment readily available carbohydrates and reduce pH to inhibit the growth of detrimental microorganisms. Homofermentative LAB ferment hexoses primarily to lactic acid while heterofermentative LAB ferment hexoses to lactic acid and other products such as ethanol and acetic acid (McDonald, 1981). Generally, LAB are non-proteolytic.

Rapid substrate fermentation reduces pH and effectively inhibits competing microorganisms; rate of pH decline is more important than the final pH of the silage (Whittenbury et al., 1967). However, final pH is commonly used to measure silage quality; final pH of 4.2 is satisfactory for silage with a dry matter (DM) concentration less than 25% (Pettersson, 1989; Breirem and Homb, 1970).

Adequate percent of dry matter, establishment and maintenance of an anaerobic environment, and effective packing are critical to silage production. Wet silages are at high risk for clostridia fermentation, indicated by elevated pH, butyric acid, and NH_3 (McDonald, 1981). Provided high concentrations of available carbohydrates, low DM forages may be effectively ensiled; LAB will be active and dominate fermentation (McDonald, 1981). In grass silage with a DM concentration of 18%, final pH and NH_3-N decrease as available fermentation substrate increases (Pettersson, 1989). Establishment and maintenance of an anaerobic environment is required for a LAB-dominated fermentation. Packing promotes the release of cell contents and growth of LAB (Weise,

1968). Well-packed ryegrass silage had a lower pH, lower NH_3-N, lower butyric acid, higher lactic acid, and lower DM loss than a poorly-packed silage (McDonald et al., 1960).

Silage production research uses scaled-down silos to facilitate data collection from multiple treatments and replications. As the fermentation process may differ from laboratory silos to field-scale silos, direct application of results may be inappropriate and Cherney et al. (unpublished) recommends additional tests be conducted in field-scale silos prior to commercial application. Nonetheless, laboratory silos are an accepted method in silage production research. Although limited data is available on the ensiling technique in which bags are packed and vacuum-sealed, vacuum-sealed bags have been used effectively in silage research (Cherney et al., 2003). Objective of the trial was to determine if differences exist between fermentation characteristics of three high NSC grasses and one control NSC grass ensiled in vacuum sealed bags.

Materials and methods

Forage management

Four cool-season, perennial ryegrasses, three with a relatively high NSC concentration (HNSC; AberAvon® (1HNSC), AberDart® (2HNSC), Elgon® (3HNSC)) and one commonly grown in Oregon, control NSC, (CNSC; Linn), were selected as treatments. AberAvon® (1HNSC) and AberDove® (2HNSC) are diploid perennial ryegrasses that have been selected for elevated NSC (Chapter 2). Elgon® is a tetraploid perennial ryegrass that exhibited high levels NSC in a field plot trial in Tillamook,

Oregon (Downing, 2004). Linn is a diploid perennial ryegrass, which is commonly grown in Oregon and is representative of Oregon perennial ryegrass (Oregon State Univ. Department of Crop and Soil Sciences, 1996). For Linn, NSC concentration had not been identified. In the fall of 2002, one 10 x 10 m plot of each grass was planted at the Oregon State University dairy research facility in Corvallis, Oregon. Silage plots were cut and forage removed May 7 and June 19, 2003. Grasses were fertilized with 52 kg/ha of N after the removal of each cutting. Plots were irrigated once per week and water was not limiting.

Harvesting and ensiling management

On July 3, 2003, grasses were harvested with a flail forage plot harvester (Swift Current, Saskatchewan, Canada) in the vegetative stage. Chop height and length was approximately 6 and 15 cm, respectively. Three replicates of each grass were harvested at 0900 h and 2000 h. After harvest, 800 g of each sample was placed in an individual 3 mil Zublon® plastic bag (Triume Enterprises, CA) and sealed with a Roschermatic VM-21® vacuum sealer (Roscherwenke GMBH, West Germany). Harvesting, sampling, and sealing were completed within 2 h. Bags were stored in a cool location for the 60 d ensiling period. On September 2, 2003, bags were frozen at -10° C and remained frozen until laboratory analysis.

Sample collection and laboratory analysis

Immediately prior to vacuum sealing, random grab samples of the fresh forage

were collected from each bag. Fresh samples were immediately sealed, frozen at -10° C and remained frozen until laboratory analysis.

Fresh grass samples were freeze-dried with a Freeze Mobile 12 (Virtis Co., Gardiner, NY) and ground through a 1 mm screen using a Thomas Wiley® Mill (Thomas Scientific, USA). Each of the 24 fresh grass samples was individually analyzed for DM, crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), sugars, starch, and ash using the same procedures used for the 84 grass samples in the green chop trial (Chapter 3).

Ensiled forage samples were analyzed for percent DM, pH, lactic acid, acetic acid, lactic:acetic acid ratio, propionic acid, butyric acid, isobutyric acid, total acids, CP, NH_3, crude protein equivalent percent (CPE %), and NH_3-N, percent of total N (TN), by Dairy One Forage Laboratory (Ithaca, NY). Lactic acid concentration was reanalyzed to confirm results.

Statistical analysis

Data were analyzed as a completely randomized design with the MIXED Procedure of SAS (SAS User's Guide, 1998). Significance was determined at $P \leq 0.05$ and trends were determined at $0.05 < P < 0.10$.

Model for analysis of components of fresh and ensiled grasses was:

$$Y_{ij} = \mu + V_i + T_j + (V \times T)_{ij} + E_{ij}$$

where

Y_{ij} = dependent variable,

μ = overall population mean,

V_i = effect of variety i (i = Linn, 1HNSC, 2HNSC, or 3HNSC),

T_j = effect of time j (j = a.m. or p.m.),

$(V \times T)_{ij}$ = effect of interaction of variety i and time j,

E_{ij} = residual error term.

With the analysis, a contrast statement was used to compare HNSC varieties to Linn.

Correlation coefficients were computed using PROC CORR of SAS (SAS User's Guide, 1998) to define the relationship between the more readily available carbohydrates and selected fermentation parameters. The independent variable was NSC and dependent variables were pH, lactic acid, acetic acid, lactic:acetic acid ratio, total acids, NH_3 (CPE %), and NH_3-N (% of TN).

Results and discussion

Fresh grass forage

Composition of the fresh grass samples by treatment and cutting time is shown in Table 4.1.

Table 4.1 - Composition of fresh grasses by treatment and cutting time.

	Treatment[1]					Cutting			
Item[2]	**Linn**	**1HNSC**	**2HNSC**	**3HNSC**	**SE**	**AM**	**PM**	**SE**	**P[3]**
DM	19.9	18.2	18.6	17.3	0.30	17.5	19.6	0.21	T**, C**, CS**
NDF	40.0	33.6	34.5	35.0	0.37	37.0	34.5	0.26	T**, C**, CS**
ADF	23.6	18.9	19.7	20.3	0.20	21.5	19.8	0.14	T**, C**, CS**
CP	22.1	24.5	23.0	23.8	0.41	23.9	22.8	0.29	T**, C*, CS**
Starch	2.5	3.2	3.3	3.7	0.34	2.4	3.9	0.24	C**, CS*

[1]Control NSC (Linn); first high NSC variety (1HNSC); second high NSC variety (2HNSC); third high NSC variety (3HNSC)

[2]With exception of DM, reported as % DM

[3]T=Treatment; C=Cutting; CS=Contrast statement

**$P < 0.01$

*$P < 0.05$

Dry matter concentration was less for HNSC grass varieties versus Linn ($P<0.01$) and less for the a.m. versus p.m. cutting ($P<0.01$). Neutral detergent fiber and ADF were less for the HNSC varieties ($P<0.01$) and less for the p.m. versus a.m. cutting ($P<0.01$). Crude protein was greater for the HNSC grass varieties ($P<0.01$) and less for the p.m. versus a.m. cutting ($P<0.05$). Starch was greater for HNSC versus Linn ($P<0.05$) and less for the a.m. versus p.m. cutting ($P<0.01$). For variables listed in Table 4.1, treatment by cutting interaction was not significant.

As identified by the contrast statement, NSC and sugars were greater for HNSC varieties versus Linn ($P<0.01$). For grasses selected for high NSC (i.e. 1HNSC and 2HNSC), the mechanism responsible for NSC accumulation has not been determined (Smith et al., 2002). However, for 3HSNC, the higher NSC may be attributed to ploidy. In general, tetraploids have higher NSC than diploids not selected for high NSC (i.e. Linn). For NSC and sugars concentrations, treatment by time of cutting interaction was significant ($P<0.05$). Figures 4.1 and 4.2 illustrate the NSC and sugars concentrations of the fresh grass samples, respectively.

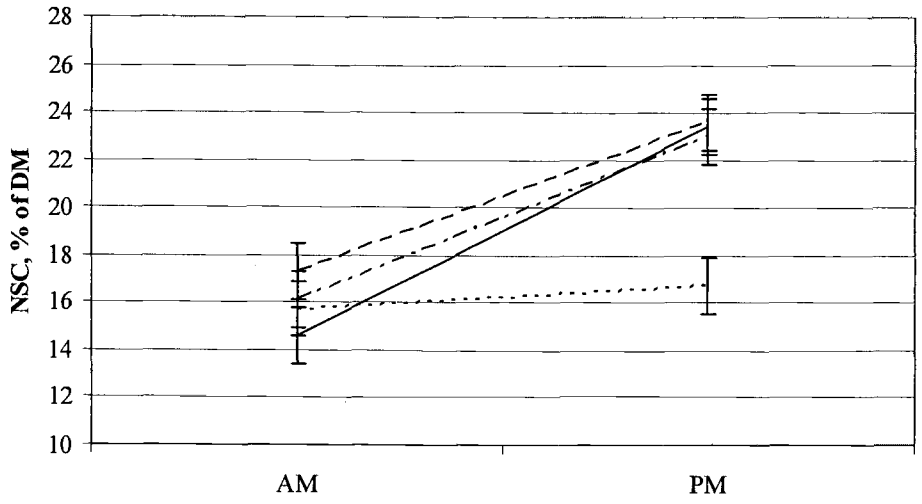

Figure 4.1 –Least square means and standard errors (vertical bars; SEM=1.19) for fresh grass NSC concentrations for one control NSC grass (Linn; - - -) and three high NSC (1HNSC (— —); 2HNSC (— - —); 3HNSC (——)) grasses by cutting time. Treatment by cutting time interaction was significant (P<0.05).

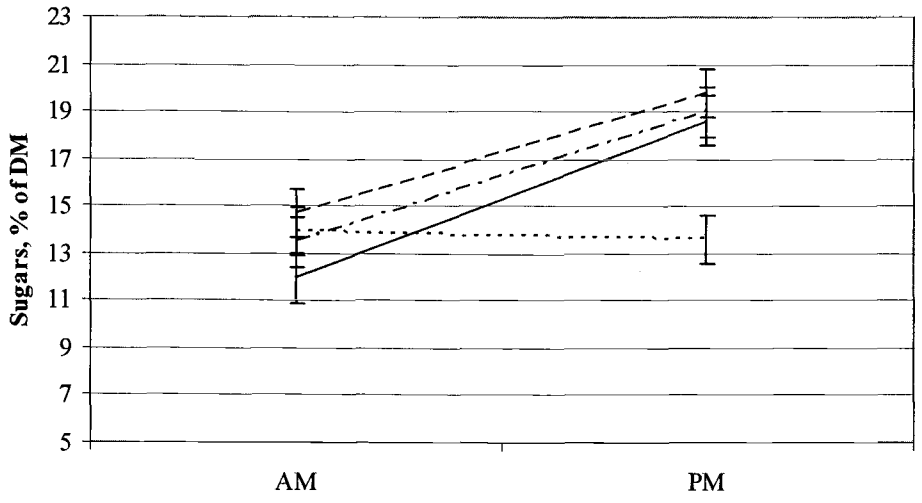

Figure 4.2 - Least square means and standard errors (vertical bars; SEM=1.10) for fresh grass concentrations of sugars for one control NSC grass (Linn; - - -)and three high NSC (1HNSC (— —); 2HNSC (— - —); 3HNSC (——)) grasses by cutting time. Treatment by cutting time interaction was significant (P<0.05).

For Linn, NSC and sugars were similar for the a.m. and p.m. cuttings. For HNSC grasses, NSC and sugars were higher in the p.m. versus the a.m. cutting ($P<0.01$). As environmental and soil conditions and stage of maturity were similar, variation in composition between HNSC grass varieties and Linn appears to be due to grass variety (Chapter 2).

Ensiled grass forage

Based on the final pH values of the ensiled grasses, 21 of the 24 samples were effectively ensiled. Due to incomplete sealing, three bags of grass were not effectively ensiled and were omitted from data analysis. Of the three bags, one was 3HNSC and two were Linn.

Based on the primary fermentation characteristics, the grass silages can be classified as lactate silages (McDonald, 1981). Lactate silages are produced from crops with high concentrations of soluble carbohydrates. Henderson et al. (1972) provides a typical composition of lactate silage from perennial ryegrass. Fermentation profiles of the ensiled grass samples are shown in Table 4.2.

Table 4.2 – Least squares means, standard errors, and p-values for fermentation profile of ensiled grasses by treatment and cutting time.

	Treatment[1]					Cutting			
Item	**Linn**	**1HNSC**	**2HNSC**	**3HNSC**	**SE**	**AM**	**PM**	**SE**	**P[2]**
DM, %	19.3	17.7	18.1	16.3	0.40	16.3	19.4	0.28	T***, C***, CS***
Lactic acid, % DM	13.9	14.7	15.5	15.2	0.42	15.3	14.3	0.30	C**, CS*
Acetic acid, % DM	2.45	2.44	2.47	3.05	0.09	2.99	2.22	0.06	T***, C***
Lactic:acetic ratio	5.77	6.10	6.40	5.13	0.15	5.18	6.52	0.11	T***, C***
Total acids[3], % DM	16.4	17.2	17.9	18.2	0.47	18.3	16.6	0.34	C***, CS**
Ammonia, CPE[4] %	1.87	1.78	1.83	2.16	0.08	2.31	1.51	0.06	T**, C***
CP, %	22.5	24.1	23.5	24.2	0.39	24.0	23.2	0.28	CS**
Ammonia-N, % of total N	8.17	7.33	7.83	8.83	0.40	9.67	6.42	0.28	C***

[1]Control NSC (Linn); first high NSC variety (1HNSC); second high NSC variety (2HNSC); third high NSC variety (3HNSC)

[2]T=Treatment; C=Cutting; CS=Contrast statement

[3]Total acids is sum of lactic, acetic, propionic, butyric, and isobutyric acids

[4]Crude protein equivalent percent

***$P < 0.01$

**$P \leq 0.05$

*$0.05 < P < 0.10$

Post-ensiling DM concentrations were similar to pre-ensiling DM concentrations. Dry matter was less for HNSC grass varieties versus Linn ($P<0.01$) and less for the a.m. versus the p.m. cutting ($P<0.01$).

Lactate silages are characterized by a final pH value of 3.7-4.2 (McDonald, 1981), which is similar to the current data. Contrast statement identified final pH was less for HNSC versus Linn ($P<0.01$). For final pH, the interaction of treatment by time of cutting was significant ($P< 0.01$) (Figure 4.3). For Linn and 3HNSC, pH was lower in the p.m. versus a.m. ($P<0.01$) which may be attributed to the numerical increase in NSC from a.m. to p.m. cutting. Final pH for 1HNSC and 2HNSC were the same a.m. and p.m. cuttings at 4.1.

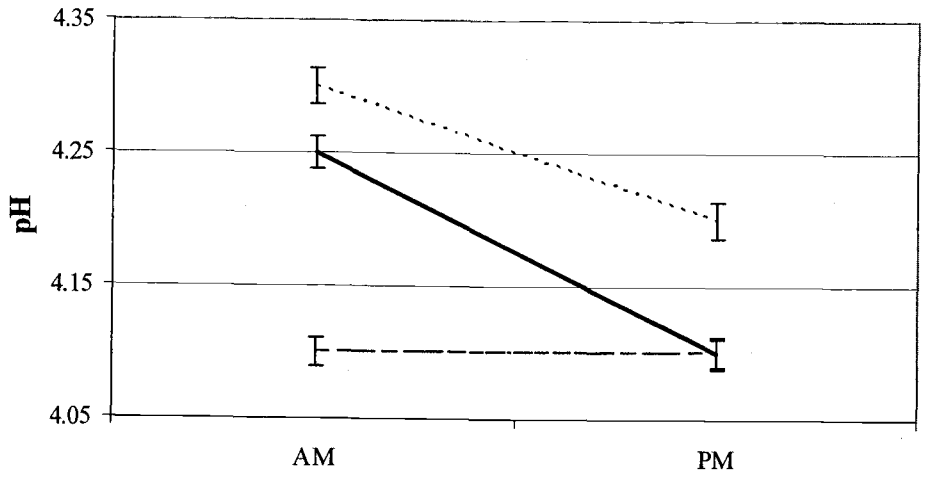

Figure 4.3 - Least square means and standard errors (vertical bars; SEM=0.01) for ensiled grass pH for one control NSC grass (Linn; ----)and three high NSC (1HNSC (— —); 2HNSC (— - —); 3HNSC (——)) grasses by cutting time. Final pH for 1HNSC and 2HNSC were the same a.m. and p.m. and p.m. cuttings at 4.1. Treatment by cutting time interaction was significant (P<0.01).

Lactic acid concentration tended to be greater for HNSC versus Linn ($P<0.10$) and was less for p.m. versus a.m. cutting ($P<0.05$). Lactic acid concentrations were exceptionally high for the ensiled grasses (Sirois, 2004, personal communication). Typically, lactate grass silages have a lactic acid concentration of 8.0-12.0% of DM (McDonald, 1981). Lactic acid concentrations may be higher if wet crops with high concentrations of soluble carbohydrates are ensiled (McDonald, 1981), which is consistent with the current data. Acetic acid concentration was similar for HNSC and Linn and less for p.m. versus a.m. cutting ($P<0.01$). Acetic acid concentration was less for 1HNSC, 2HNSC, and Linn compared to 3HNSC ($P<0.01$). Appreciable quantities of acetic acid are present in lactate silages. Henderson et al. (1982) reported a typical value of acetic acid is 3.6% of DM, which is greater than the current trial and an indicator of the efficiency of the current trial. The majority of acetic acid is the product of fermentation of sugars by homofermentative LAB and enterobacteriaceae (McDonald, 1981). Acetic acid is not as efficient as lactic acid at reducing pH (pK_a of 4.76 vs. 3.86). Lactic:acetic acid ratio was similar for Linn versus HNSC and greater for p.m. versus a.m. cutting ($P<0.01$). Greater lactic:acid ratio for p.m. versus a.m. was due to a lower acetic acid concentration for the p.m. cutting. Lactic:acetic acid ratio was greater for 1HNSC and 2HNSC versus 3HNSC ($P<0.01$), which was due to lower acetic acid concentrations for 1HNSC and 2HNSC. Total acids concentration was greater for HNSC versus Linn ($P=0.05$) and less for p.m. versus a.m. cutting ($P<0.01$). Total acids data with individual acids (i.e. lactic acid and acetic acid) and pH values indicate p.m. cutting fermentation acids were more efficient at reducing pH.

Butyric acid was not detected in any of the 21 properly sealed silages. Normally, small quantities of butyric acid are present in lactate silages. For a lactate silage, Henderson et al. (1972) reported butyric acid at 0.1% of DM. Quantity of butyric acid is affected by the rate of lactic acid production and the absence of butyric acid indicates a rapid rate of lactic acid production.

Ammonia, as CPE %, was similar for HNSC and Linn and less for p.m. versus a.m. cutting ($P<0.01$). Ammonia was less for 1HNSC and 2HNSC versus 3HNSC ($P<0.05$). Ammonia is an indicator of protein degradation via plant and microbial proteases prior to establishment of pH values that stabilize the ensiled forage. Crude protein concentration was greater for HNSC versus Linn ($P<0.05$) and similar between a.m. and p.m. cuttings. Ammonia-N, as % of TN, was similar for Linn and HNSC varieties and less for p.m. versus a.m. cutting ($P<0.01$). Ammonia-N values are acceptable and similar to values for lactate silages (Breirem and Homb, 1970; Henderson et al., 1972; Woolford, 1984).

Based on lower pH, higher total acids, and a tendency for higher lactic acid, HNSC grasses were more efficiently ensiled than Linn. Similarly, based on lower pH, lower lactic, acetic, and total acids, and lower NH_3, p.m. cut grasses were more efficiently ensiled than a.m. cut grasses. Therefore, p.m. cutting of HNSC grass varieties will maximize ensiling efficiency.

Fermentation parameters were regressed on NSC (Table 4.3). Simple linear regressions of NSC versus pH and NH_3 are shown in Figures 4.4 and 4.5, respectively.

Table 4.3 - Coefficients of determination. Independent variable is fresh grass NSC and dependent variables are ensiled grass pH, lactic acid, acetic acid, lactic:acetic acid ratio, total acids, ammonia, and ammonia as a percent of total nitrogen.

Dependent variable	R-squared	P[3]
pH	0.18	0.06
Lactic acid, % DM	< 0.01	0.85
Acetic acid, % DM	0.25	0.02
Lactic:acetic acid ratio	0.36	< 0.01
Total acids[1], % DM	0.04	0.39
Ammonia, CPE[2] %	0.38	< 0.01
Ammonia-N, % total N	0.38	< 0.01

[1]Total acids is sum of lactic, acetic, propionic, butyric, and isobutyric acids

[2]Crude protein equivalent percent

[3]Probability slope is different than zero

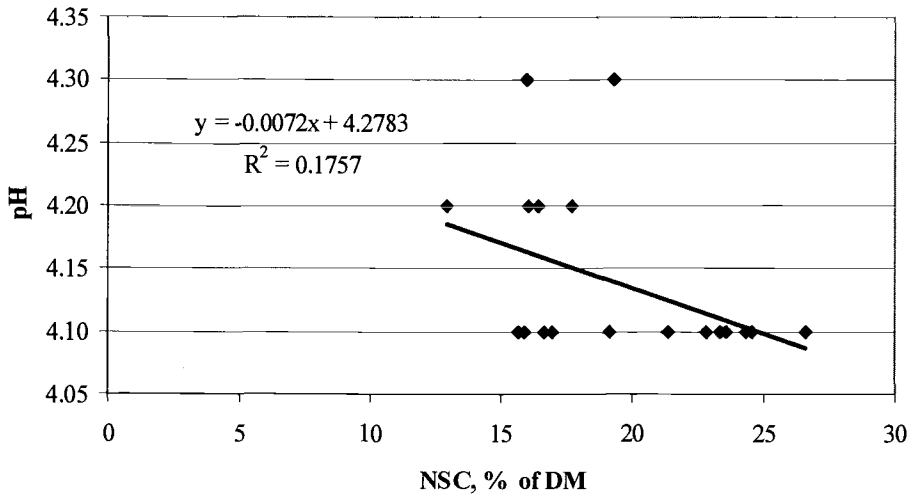

Figure 4.4 – Relationship between pre-ensiling NSC and post-ensiling pH for three high NSC grasses and one control NSC grass.

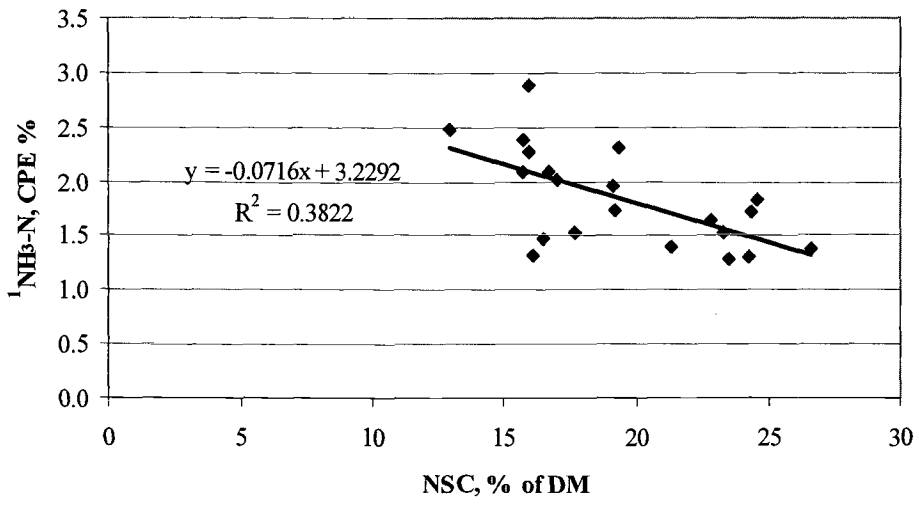

Figure 4.5 – Relationship between pre-ensiling NSC and post-ensiling NH_3 for three high NSC grasses and one control NSC grass. [1]Ammonia-N, as a crude protein equivalent percent.

Final pH, acetic acid concentration, NH_3 (CPE %), and NH_3-N (% total N) were negatively correlated with NSC. Final pH values were reported to 0.1 and were within a small range; increased precision and range may increase the strength of correlation. With the exception of lactic acid and total acid concentration, the nature (i.e. positive or negative) of the relationship was as expected.

Ensiling technique

Limited data is available on the use of vacuum-sealed bags for silage research. Based on the fermentation profiles of the ensiled grasses, vacuum-sealing effectively ensiled 21 of the 24 bags and facilitated ensiling of direct-cut grass forages (DM ≤20%). Bag volume should allow for gas production. During the initial days of ensiling, fermentation gases inflated the bags and the extent of inflation varied from bag to bag.

Original bag volume was approximately four-fold grass volume and gases did not need to be released from the bags.

In a field-scale silo, it is reasonable to assume the magnitude of fermentation parameters will be different than scaled-down laboratory silos. Trials using field-scale silos are required to quantify the effects of grass varieties and evening cutting on ensiling efficiency.

Conclusions

For fresh grasses, DM, NDF, and ADF were less and CP and NSC were greater for HNSC grasses versus Linn grass. Neutral detergent fiber, ADF, and CP decreased from a.m. to p.m. cutting. For HNSC grasses, NSC and sugars increased from a.m. to p.m. cutting. Vacuum sealing effectively ensiled direct-cut grass forages. Final pH was lower, total acids was higher, and lactic acid tended to be higher for HNSC grasses versus Linn. Final pH, lactic acid, acetic acid, total acids, and NH_3 were lower for p.m. versus a.m. cutting. To maximize ensiling efficiency, harvest HNSC grass varieties in the p.m. Future research should be conducted to quantify effects in field-scale silos.

Chapter 5
Conclusion

For HNSC grass in the green chop trial, CP was higher and NSC was lower than CNSC grass. Neutral detergent fiber and ADF were similar between treatments. For HNSC grass, NSC concentration cannot be explained. For the HNSC treatment, grass DMI was greater wk 2 and tended to be greater wk 3 and TMR DMI tended to be greater wk 1, 2, and 3. Daily yields of milk and components were greater for the HNSC treatment. Milk urea nitrogen differed by week. Based on grass CP data, MUN data, and CPM analyses, MP was the limiting nutrient for milk production. It appears HNSC treatment increased yields of milk and components via an increase in grass CP and grass and TMR DMI.

For the fresh grasses in the ensiling trial, DM, NDF, and ADF were less and CP and NSC were greater for HNSC grasses versus Linn grass. Neutral detergent fiber, ADF, and CP decreased from a.m. to p.m. cutting. For HNSC grasses, NSC and sugars increased from a.m. to p.m. cutting. Vacuum sealing effectively ensiled direct-cut grass forages. Final pH was lower, total acids was higher, and lactic acid tended to be higher for HNSC grasses versus Linn. Final pH, lactic acid, acetic acid, total acids, and NH_3 were lower for p.m. versus a.m. cutting. To maximize ensiling efficiency, harvest HNSC grass varieties in the p.m. Future research should be conducted to quantify effects in field-scale silos.

Chapter 6
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