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Introduction

Forage grass production is a major component to profitable dairying in the Pacific Northwest. Grazing forage grasses with both milk cows and heifers is a well-established practice. In recent years, intensive grazing and mechanical harvesting of grasses are also common. To remain profitable, dairy producers have invested heavily in their own systems to provide forage needed for their herd.

Grass cultivars are normally selected for yield and resistance to disease and pests. For optimum milk production, quality, palatability, and intake are very important. Dry matter (DM) intake must be at a level where the animal can meet her physiological requirements and other production goals such as milk volume and reproduction. We really know very little about the animal's preference, and are just beginning to see an interest by researchers to learn more about a cultivar's palatability and intake potential. The need to understand performance differences between cultivars is of growing importance.

A relatively new (commercially available) analysis used to evaluate grass forage quality is measurement of **total nonstructural carbohydrates** (TNC). Carbohydrates drive the efficiency of digestion in the rumen of the cow and the ensiling process. In the rumen, increasing TNC increases the use of rumen degradable protein, consequently increasing microbial protein production. During the ensiling process, increasing TNC increases the rate of fermentation, which increases the preservation of the ensiled nutrients (Woolford, 1984).

The overall efficiency of grass nitrogen use for milk production tends to be low, due partly to the slow rate of release of energy in the rumen. Up to 40 percent of the dietary nitrogen in fresh forages may be lost as rumen ammonia, because the microbial population in the rumen is unable to incorporate much of the nitrogen released due to a lack of available carbohydrate (Scollan et al., 1998). This reduces the efficiency of capture of rapidly degradable plant proteins by the rumen microbial population. When additional sugars are introduced to the rumen, microbial protein is increased (Rooke et al., 1987).

Plants vary diurnally in concentrations of TNC because the export of photosynthate does not keep pace with the rate of carbon fixation during the photoperiod. This is why the highest concentrations of TNC have been observed in forages cut late in the afternoon (Fisher et al., 1999). Plants accumulate sugars during the day and use them up at night. Forage cut during the late afternoon (p.m.) captures much of this extra sugar and has higher feed values than morning-cut (a.m.) feed (Shewmaker et al., 1999). Researchers at USDA-ARS in Idaho, in collaboration with others, have conducted several trials measuring animal preference for p.m. cut forages over forages cut in the morning (Mayland et al., 2000; MacKay et al., 2003).

Very little work has been done regarding cultivars' natural variations in sugar content. Tava et al. (1995) reported that three tall fescue varieties having 13.3 percent water-soluble carbohydrates were considered more palatable to cattle than three others having

only 10.8 percent. Similar observations were made by Shewmaker et al. (1999) in their study of eight different fescues.

Researchers at the Institute of Grassland and Environmental Research (IGER) in the United Kingdom have begun to look at selecting ryegrasses for higher total nonstructural carbohydrates. One study of forages bred for higher TNC reported that dairy cows eating them had higher dry matter intakes and increased milk production by 8 percent over typical ryegrasses (Miller et al., 1999). They also observed differences in efficiency of use of feed nitrogen, as indicated by changes in nitrogen excretion in the urine (Miller et al., 2001). Authors theorized that this was primarily due to differences in the microbial capture of rumen degradable nitrogen. This data also suggests selecting forages for higher TNC not only increases animal performance, but also may have the potential to increase nitrogen utilization and reduce nitrogen excretion.

Studies on other species have been conducted indicating that high-sugar grasses also can significantly improve live weight gain in grazing animals (Lee et al., 1999).

Oregon's livestock industry produces forage on approximately 2 million acres of pasture annually. The combined gross sales of cattle, calves, sheep, and dairy products were estimated at over $1.2 billion in 2006, making this the second-largest agriculture industry in the state (Oregon Agricultural Statistics Service, 2006–07). The ability of these industries to compete depends primarily on their capacity to produce and convert low cost forages into high value agricultural commodities.

Understanding the variation in carbohydrate levels of forage varieties used in Oregon should have significant impacts on livestock profitability. For example, this past year Tillamook County dairy farmers produced milk worth $80 million. If forages were developed that would increase productivity 8 percent, the potential impact to Tillamook County dairy farmers is $6.4 million a year. There are many assumptions inherent in these numbers, but the advances in our understanding of the TNC in grasses have the potential to make a significant economic impact on the livestock industries of the West Coast.

Nonstructural carbohydrate studies at Oregon State University—variety selection

Interest in nonstructural carbohydrates in grasses began at Oregon State University in 2000. The overall objective was either to find varieties of grasses that had more energy than traditional varieties or to manage existing grasses for increased productivity. There was little data on natural variations in cool-season grass populations. The first year, a number of cool-season grasses in use in Oregon were tested to determine how much variation in TNC there is between varieties. Several of the grasses tested were developed in New Zealand, some in Europe, and a few were grown in Oregon.

The objectives of the first studies were:

1. Sample TNC and DM yield of cool-season forage grasses throughout the growing season.
2. In year 1, measure seasonal and diurnal variations in TNC and observe potential variations in the relationship between the level of TNC and DM yield among species and varieties of cool-season forage grasses.
3. In year 2, determine total sugar production throughout the season in 10 new varieties that have potential to be high in sugars, including varieties studied in grazing trails in the UK.

Year 1

Eleven perennial ryegrasses, four orchardgrasses, one festolium, and one prairie grass were planted in 4' x 25' field plots in Tillamook, Oregon. Three replicates of each variety were planted. Each of the 51 field plots was harvested on 6 sampling dates in the 2001 growing season. For each of the six dates, DM yield of each field plot was recorded. For three harvest dates, April, June, and October, forage samples were collected for TNC analysis in both the early morning and late afternoon. Immediately after cutting, samples were placed on dry ice to reduce respiration losses and subsequently frozen. Later, samples were dried in an oven at 50°C. TNC analyses were performed at Dairy One Lab, Ithaca, NY.

Year 2

Ten perennial ryegrasses were identified as being possible high-sugar grasses from forage breeders. Surprisingly, very few grass seed companies could provide varieties they knew were high in TNC. *AberDart* and *AberAvon* from IGER in the UK were planted. All cultivars were planted in 4' x 25' field plots and replicated three times (similar to year 1). Each of the 30 field plots was harvested on 6 sampling dates in the 2002 growing season. Immediately after cutting, samples were placed on dry ice to reduce respiration losses. Samples were dried in an oven at 50°C. TNC analyses were performed at Dairy One Lab, Ithaca, NY.

Results and Discussion

Nonstructural carbohydrates in cool-season grasses significantly vary between varieties, species, from a.m. to p.m., and seasonally. While there was enormous variation, this project demonstrated that certain cultivars and varieties consistently were higher in nonstructural carbohydrates. Table 1 shows the nonstructural carbohydrate data for year 1. A few varieties appeared to vary more from a.m. to p.m. than others. However, all varieties demonstrated some variation. Varieties are listed by average nonstructural carbohydrate concentration from the highest to lowest. The value in the far right column labeled "Total" is actually an index rating of varieties. The highest varieties are all ryegrasses. *Matua* is a prairie grass and averaged in the middle of those varieties tested. The festolium tested was *Barfest*, averaging below all the ryegrasses and just above the orchard grasses. All four varieties on the bottom of the table are orchard grasses. Figure 1 illustrates the relationship observed between nonstructural carbohydrates and dry matter yield.

Table 1. Nonstructural carbohydrate % of dry matter for a.m./p.m. harvested cultivars in 2001.

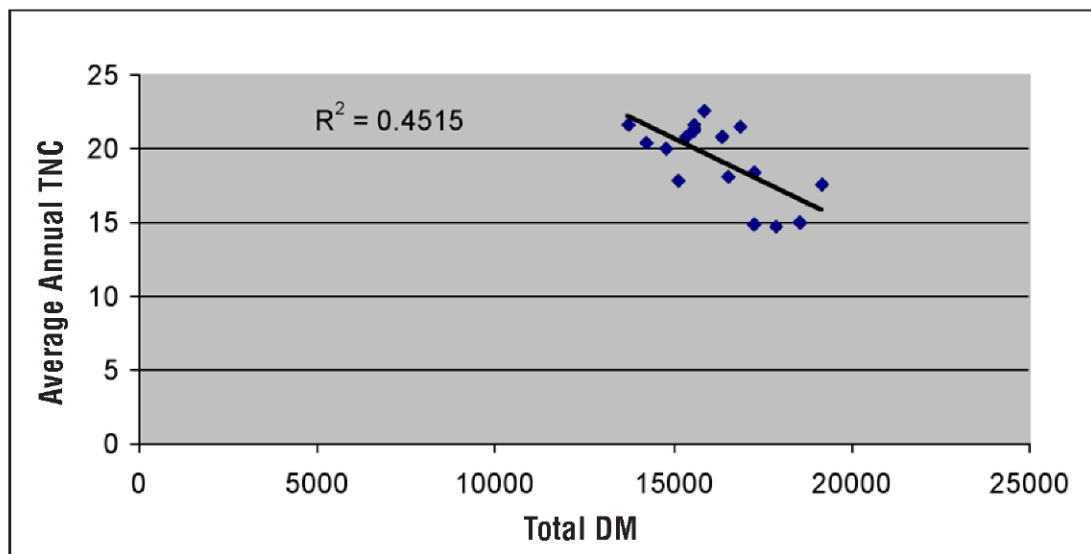|	Percent total nonstructural carbohydrate in dry matter						
	4/20 a.m.	4/20 p.m.	6/28 a.m.	6/28 p.m.	10/1 a.m.	10/1 p.m.	Total
Elgon	14.3	13.7	18.2	25.3	21.0	23.9	19.4
Tetralite	14.8	14.7	16.3	25.1	19.4	23.9	19.0
Herbie	11.5	13.3	19.2	25.9	19.6	21.7	18.5
BG-34	12.6	16.2	17.2	19.4	17.1	27.3	18.3
Tonga	15.8	21.7	16.0	20.6	15.2	20.0	18.2
Glenn	13.8	17.6	17.0	19.1	19.5	21.8	18.1
Bison	12.1	12.4	18.4	24.5	15.9	22.9	17.7
Matua	12.2	22.6	14.5	21.9	15.5	18.7	17.6
Barfort	14.4	18.0	14.0	23.5	13.8	20.3	17.3
Flanker	12.9	13.6	17.7	21.0	15.2	23.5	17.3
Belramo	12.5	14.1	14.6	19.2	18.3	17.5	16.0
Bronsyn	9.0	15.1	17.6	21.2	13.0	16.5	15.4
Barfest	11.7	15.9	13.5	12.2	13.3	21.8	14.7
Orion	14.3	11.9	17.1	22.4	10.2	11.1	14.5
Pizza	10.0	12.4	12.3	19.6	8.2	12.5	12.5
Cambria	9.7	14.8	9.1	15.6	10.3	12.8	12.0
Baridana	10.8	11.6	8.8	14.0	9.0	16.5	11.8

Figure 1. The relationship between nonstructural carbohydrates and dry matter yield throughout all sampling periods.

In year 2, data collection focused on documenting the total pounds of sugars or non-structural carbohydrates produced. Figure 2 illustrates the actual TNC data by cutting. *Amazon* ryegrass averaged the highest percent nonstructural carbohydrates throughout the season at 20.9 with *Impact* the lowest at 16.3 percent. The ryegrass variety from the UK, *AberAvon*, was the highest in total nonstructural carbohydrates produced per acre at 2,306 pounds (Figure 3). This is a variety bred at IGER in the UK to be higher in nonstructural carbohydrates than most ryegrasses.
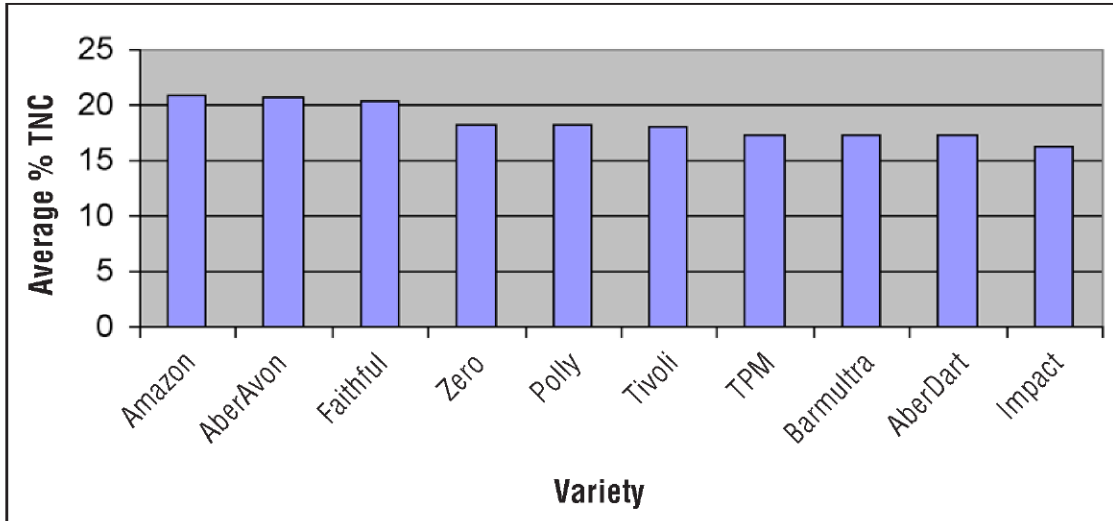

Figure 2. Percent nonstructural carbohydrates for cultivars harvested in 2002.
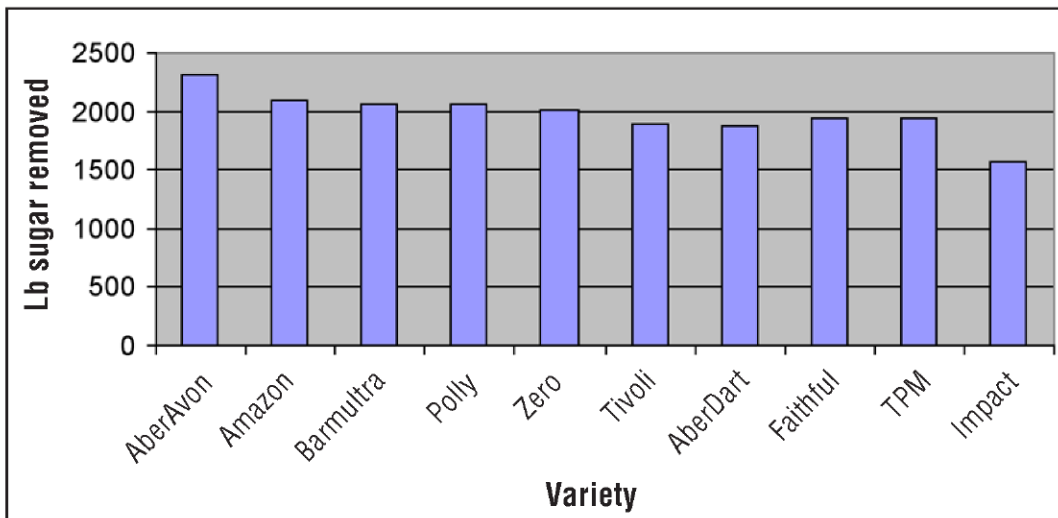

Figure 3. Total pounds of water-soluble carbohydrate or sugar removed per acre.

TNC influence on animal intake, performance, and silage quality

The third major project done on grass TNC looked at the effects of nonstructural carbohydrates on fermentation characteristics of ensiled perennial ryegrass. This study was organized to show the potential for using high-sugar ryegrass for silage production.

Introduction

Miller et al. (2001) proposed that an increase in water-soluble carbohydrate (WSC) concentration of grass forage increases energy value of grass and, therefore, efficiency of grass nutrients for milk production. Perennial ryegrasses that accumulate elevated concentrations of WSC have been developed (Humphreys, 1989a, b, c). Lamb live weight gain and total lamb production increases were attributed to an increase in WSC, as well as decreases in neutral detergent fiber (NDF) and acid detergent fiber (ADF), or a combination of all (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., 2001). Authors suggested the increased efficiency of dietary N was due to improved ruminal N use and/or increased propionic acid. In a green chop study, dairy cows fed high WSC grass consumed more forage DM, produced more milk, and exhibited an increase in N-use 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., 2001).

Well-preserved silage is 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, sucrose, starch, and fructans are the primary nonstructural carbohydrates (Smith, 1973).

Rapid substrate fermentation reduces pH and effectively inhibits competing microorganisms. The 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. A final pH of 4.2 is satisfactory for silage with a dry matter (DM) concentration less than 25 percent (Pettersson and Lindgren, 1989; Breirem and Homb, 1970).

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 are present, low DM forages may be effectively ensiled. Lactic acid bacteria will be active and dominate fermentation (McDonald, 1981). Pettersson and Lindgren (1989) demonstrated that final pH and NH^3-N decrease as available fermentation substrates increase.

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, 2003). The trial's objective 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

Four cool-season, perennial ryegrasses, three with a relatively high NSC concentration (*AberAvon, AberDart, Elgon*) and one commonly grown in Oregon (*Linn*), were selected as treatments and a control. *AberAvon* and *AberDart* are diploid perennial ryegrasses that have been selected for elevated NSC. *Elgon* is a tetraploid perennial ryegrass that exhibited high levels of NSC in field plot trials in Oregon (Downing et al., 2004). *Linn* is a diploid perennial ryegrass representative of Oregon perennial ryegrass.

Grasses were harvested with a flail forage plot harvester (Swift Current, Saskatchewan, Canada) in the vegetative stage. Chop height and length were approximately 6 cm and 15 cm, respectively. Three replicates of each grass were harvested at 0900 h and 2000 h. After harvest, an 800-g sample of each was placed in an individual 3-mil Zublon® plastic bag (Triume Enterprises, CA) and sealed with a Roschermatic VM-21® vacuum sealer (Roscherwenke GMBH, Germany). Harvesting, sampling, and sealing were completed within 2 hours. Bags were stored in a cool place for the 60-day ensiling period. After the 60 days, bags were frozen at -10°C and kept 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). The 24 fresh grass samples were individually analyzed for DM, crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), sugars, starch, and ash. Ensiled forage samples were analyzed for percent DM, pH, lactic acid, acetic acid, 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). A contrast statement was used to compare the high NSC 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.

Results and discussion

Composition of the fresh grass samples by treatment and cutting time is shown in Table 2. Dry matter concentration was lower in the high NSC grass varieties versus *Linn* ($P<0.01$) and lower in the a.m. versus p.m. cutting ($P<0.01$). NDF and ADF were lower in the high-NSC varieties ($P<0.01$) and in the p.m. versus a.m. cutting ($P<0.01$). Crude protein was higher in the high-NSC grass varieties ($P<0.01$) and lower in the p.m. versus a.m. cutting ($P<0.05$). Starch was higher in high-NSC versus *Linn* ($P<0.05$) and lower in the a.m. versus p.m. cutting ($P<0.01$). For variables listed in Table 3, treatment by cutting interaction was not significant.

Total NSC were higher in the high-NSC varieties versus *Linn* ($P<0.01$). For NSC concentrations, treatment by time of cutting interaction was significant ($P<0.05$). Figure 4 illustrates the NSC levels of fresh grass for all treatments and both the a.m. and p.m.

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cuttings. For *Linn*, NSC were similar for the a.m. and p.m. cuttings. For the high-NSC grasses, NSC was 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 the high-NSC grass varieties and *Linn* appears to be due to grass variety.

Fermentation data for all varieties and both harvest times is shown in Table 3. For final pH, the interaction of treatment by time of cutting was significant (P< 0.01). For *Linn* and *Elgon*, 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 *AberAvon* and *AberDart* were the same for both a.m. and p.m. cuttings.

Lactic acid concentration tended to be higher in the high-NSC versus *Linn* (P<0.10) and was lower in p.m. versus a.m. cuttings (P<0.05). Acetic acid concentration was similar for the high NSC varieties and *Linn* and lower in p.m. versus a.m. cutting (P<0.01). Acetic acid concentration was less for *AberAvon*, *AberDart*, and *Linn* compared to *Elgon* (P<0.01). Lactic:acetic acid ratio was similar for *Linn* versus the high-NSC varieties and greater for the p.m. versus a.m. cutting (P<0.01). Greater lactic: acetic 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 *AberAvon* and *AberDart* versus *Elgon* (P<0.01), which was due to lower acetic acid concentrations for *AberAvon* and *AberDart*. Total acids concentration was higher in the high-NSC grasses versus *Linn* (P=0.05) and lower in 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.

Ammonia is an indicator of protein degradation via plant and microbial proteases prior to establishment of pH values that stabilize the ensiled forage. Ammonia was lower in *AberAvon* and *AberDart* versus *Elgon* (P<0.05) silage. Crude protein concentration was higher in the high-NSC grasses versus *Linn* (P<0.05) and similar between a.m. and p.m. cuttings. Figure 5 illustrates the final pH of grass silages across all treatments.

Based on lower pH, higher total acids, and a tendency for higher lactic acid, the high-NSC 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 high-NSC grass varieties will maximize ensiling efficiency.

Table 2. Composition of fresh grasses by treatment and cutting time.

Item	Treatment[1]					Cutting			P[2]
	Linn	AberAvon	AberDart	Elgon	SE	AM	PM	SE	
DM	19.9[a]	18.2[b]	18.6[b]	17.3[c]	0.30	17.5	19.6	0.21	C**, CS**
WSC	14.9[a]	18.5[b]	17.8[b]	17.3[b]	0.28	14.4	19.9	0.23	C*, CS**
NDF	40.0[a]	33.6[b]	34.5[bc]	35.0[c]	0.37	37.0	34.5	0.26	C**, CS**
ADF	23.6[a]	18.9[d]	19.7[c]	20.3[b]	0.20	21.5	19.8	0.14	C**, CS**
CP	22.1[a]	24.5[c]	23.0[ab]	23.8[bc]	0.41	23.9	22.8	0.29	C*, CS**

[1]Control WSC (*Linn*); high WSC (*AberAvon, AberDart, Elgon*). Means within the same row with different superscripts differ at P <0.05.

[2]C=Cutting; CS=Contrast statement (the three high WSC varieties compared to Linn)

*P < 0.05

**P < 0.01

Table 3. Fermentation profile of ensiled grasses by treatment and cutting time.

Item	Treatment[1]					Cutting			P[2]
	Linn	AberAvon	AberDart	Elgon	SE	AM	PM	SE	
DM, %	19.3[c]	17.7[b]	18.1[bc]	16.3[a]	0.40	16.3	19.4	0.28	C**
Final pH	4.3[a]	4.0[b]	4.0[b]	4.1[b]	0.12	4.5	4.1	0.11	C* CS**
Lactic acid	13.9[a]	14.7[ab]	15.5[c]	15.2[bc]	0.42	15.3	14.3	0.30	C*, CS*
Acetic acid	2.45[a]	2.44[a]	2.47[a]	3.05[b]	0.09	2.99	2.22	0.06	C**
Lactic:Acetic[3]	5.77[b]	6.10[bc]	6.40[c]	5.13[a]	0.15	5.18	6.52	0.11	C**
Total acids	16.4[a]	17.2[ab]	17.9[bc]	18.2[c]	0.47	18.3	16.6	0.34	C** CS*
Ammonia[4]	1.87[a]	1.78[a]	1.83[a]	2.16[b]	0.08	2.31	1.51	0.06	C**
CP	22.5[a]	24.1[b]	23.5[ab]	24.2[b]	0.39	24.0	23.2	0.28	CS*
Ammonia[5]	8.17[ab]	7.33[a]	7.83[ab]	8.83[b]	0.40	9.67	6.42	0.28	C**

[1]Control NSC (*Linn*); high WSC varieties, *AberAvon, AberDart,* and *Elgon.* Means within the same row with different superscripts differ at P <0.05.

[2]C=Cutting; CS=Contrast statement (the three high WSC varieties compared to *Linn*)

[3]Lactic:Acetic acid ratio

[4]Crude protein equivalent percent
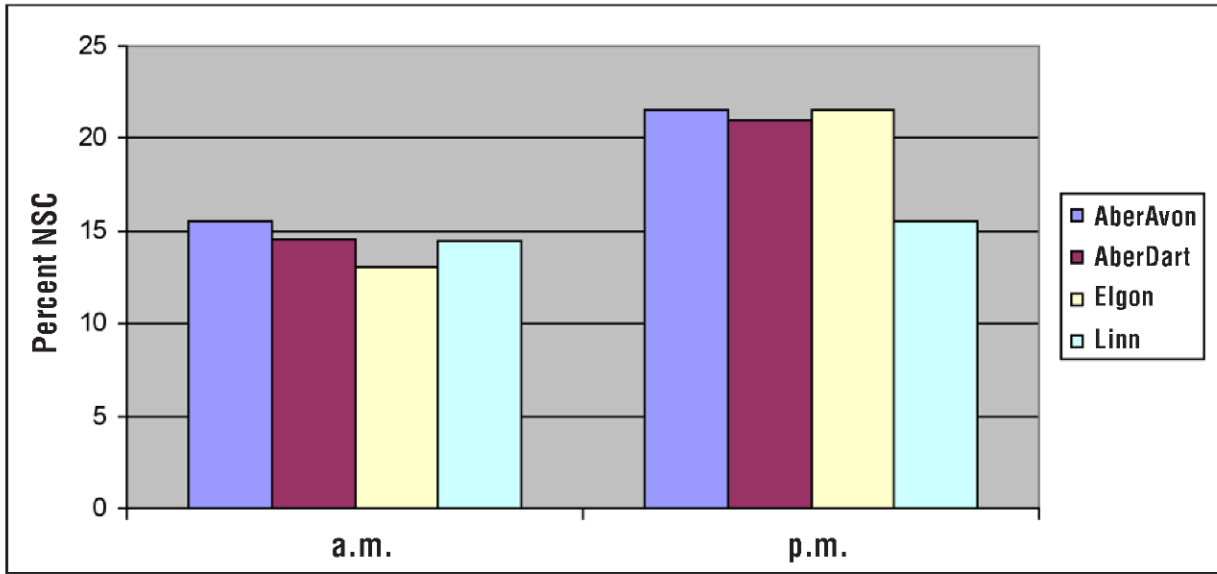
[5] N, % of total N

*P < 0.05

**P < 0.01

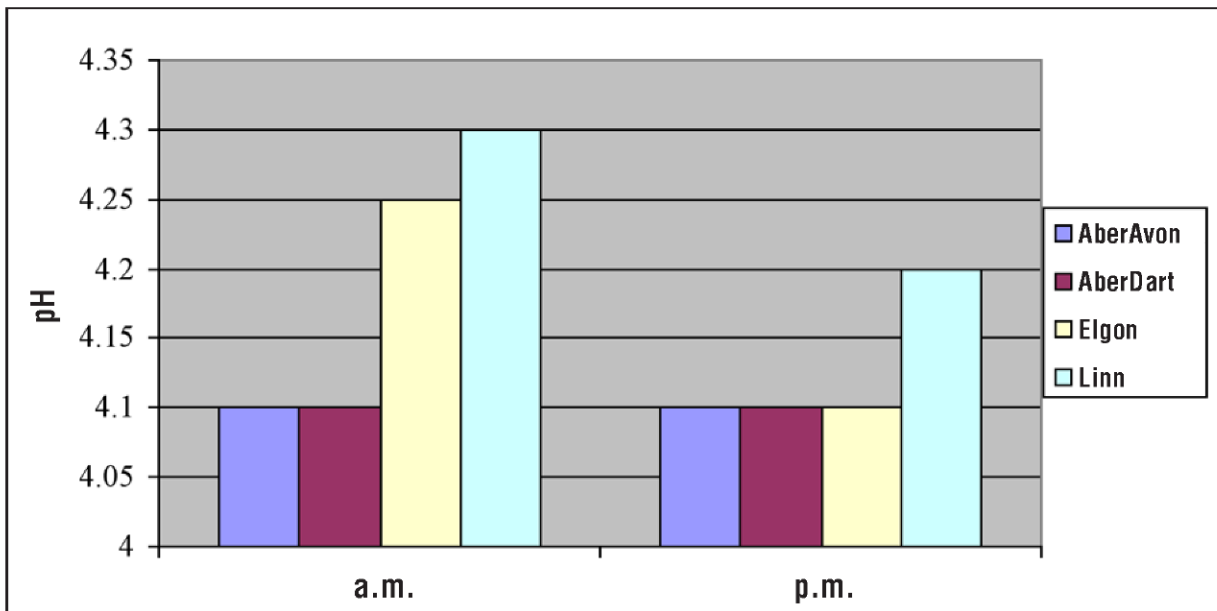Figure 4. Nonstructural carbohydrate levels of fresh grass.

Figure 5. Final pH of grass silages cut in the a.m. and p.m.

Conclusions

This 2-year project has been helpful in characterizing nonstructural carbohydrates in cool-season grasses. We have learned a lot about the normal fluctuations seen across environments and, more specifically, variations due to genetic differences. The main conclusions are:

1. Percentages of nonstructural carbohydrate are highly variable throughout the growing season and between species and varieties of cool-season forage grasses.
2. Growth rate may affect the level of TNC in cool-season forage grasses.
3. Consistently, orchard grasses contained lower levels of TNC and had higher DM yields than ryegrasses.
4. Grasses bred in Europe to emphasize nonstructural carbohydrates are higher in sugars than the average of the population found in the U.S.

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