Effects of alfalfa variety and nitrogen status on pasture bloat in ruminants
by Charles Donald MacDonald

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Animal and Range Sciences
Montana State University

Abstract:
Replicated field strips (0.25 ha) of AC Grazeland alfalfa, and Ladak 65 alfalfa, were established in June, 1997 near Bozeman, Montana. Six trials during 1998 and 1999 compared bloat between livestock grazing AC Grazeland or Ladak 65 alfalfa in a randomized complete block with four replications. Four trials compared bloat in livestock grazing AC Grazeland or Ladak 65 alfalfa with or without N fertilizer in a 2 x 2 factorial design in 1999 and 2000.

Forty mixed breed ewes of mixed ages were grazed on the pasture during 1998. Ewes were observed and scored for bloat based on the bloat severity index scale of: BSI, 1 = no bloat, 2 = slight swelling to left side, 3 = moderate swelling to left side, 4 = swollen on both sides, 5 = severely swollen, and 6 = dead.

Twelve two-year old beef heifers were grazed during July 1998. Twenty four to forty yearling ewes were grazed on the pasture during 1999. Forage samples were analyzed for crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF) and NO3.

Two in situ tests compared DMD of Ladak 65 or AC Grazeland alfalfa in cannulated cattle. One in situ test compared DMD of samples that had been refrigerated, frozen or dried. One in situ test compared Ladak 65 and AC Grazeland alfalfa with and without fertilizer.

During the three-year pasture study, sheep and cattle were observed for 3442 and 216 animal-grazing days, respectively.

Livestock grazing Ladak 65 had greater ($P < 0.05$) mean bloat incidence and severity scores overall in five trials, while AC Grazeland had greater mean bloat severity index (BSI, 1= no bloat, 6= dead) scores in one trial. There were no significant differences between varieties in mean BSI in four trials. Livestock grazing N-fertilized alfalfa had greater mean BSI scores than livestock grazing unfertilized alfalfa.

In situ tests between Ladak 65 and AC Grazeland alfalfa had no significant differences in dry matter disappearance at 4-h. There were significant differences between fresh and both frozen and dried samples. Ladak 65 and AC Grazeland with and without N fertilization had small but significant differences 4-h DMD.

Correlation coefficients for percent bloat vs. CP, ADF, NDF, or NO3 were 0.41, -0.31, -0.34 and 0.29, respectively. Correlation coefficients for bloat severity vs. CP, ADF, NDF, or NO3 were -0.11, 0.17, 0.12, and 0.23, respectively Bloat incidence and severity appear to be slightly less on AC Grazeland than Ladak 65. However, the differences were overcome by field fertility, environment and plant maturity. Crude protein and NO3 appear to play a role in bloat. However, better protocol is necessary to accurately define this role.

EFFECTS OF ALFALFA VARIETY AND NITROGEN STATUS ON PASTURE BLOAT IN RUMINANTS

by

Charles Donald MacDonald

A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in

Animal and Range Sciences

MONTANA STATE UNIVERSITY
Bozeman, Montana

August 2001

APPROVAL

of a thesis submitted by

Charles Donald MacDonald

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

Dr. Dennis Cash Dennis Cash 8/30/2001
Date

Approved for the Department of Animal and Range Sciences

Dr. Peter Burfening Peter Burfening 8-30-01
Date

Approved for the College of Graduate Studies

Dr. Bruce McLeod Bruce R. McLeod 8-31-01
Date

STATEMENT OF PERMISSION TO USE

Signature ______________________

Date ______________________

ACKNOWLEDGEMENTS

I would like to thank Dennis Cash for offering me this opportunity, Ron Carlstrom for talking me into it, Reyer Rens and Ross Macdonald for getting me into Colombos, Tammy MacDonald for getting me out and Research Seeds, International for paying the tab.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Replicated field strips (0.25 ha) of AC Grazeland alfalfa, and Ladak 65 alfalfa, were established in June, 1997 near Bozeman, Montana. Six trials during 1998 and 1999 compared bloat between livestock grazing AC Grazeland or Ladak 65 alfalfa in a randomized complete block with four replications. Four trials compared bloat in livestock grazing AC Grazeland or Ladak 65 alfalfa with or without N fertilizer in a 2 x 2 factorial design in 1999 and 2000.

Forty mixed breed ewes of mixed ages were grazed on the pasture during 1998. Ewes were observed and scored for bloat based on the bloat severity index scale of: BSI, 1 = no bloat, 2 = slight swelling to left side, 3 = moderate swelling to left side, 4 = swollen on both sides, 5 = severely swollen, and 6 = dead.

Twelve two-year old beef heifers were grazed during July 1998. Twenty four to forty yearling ewes were grazed on the pasture during 1999. Forage samples were analyzed for crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF) and $N0_3$.

Two *in situ* tests compared DMD of Ladak 65 or AC Grazeland alfalfa in cannulated cattle. One *in situ* test compared DMD of samples that had been refrigerated, frozen or dried. One *in situ* test compared Ladak 65 and AC Grazeland alfalfa with and without fertilizer.

During the three-year pasture study, sheep and cattle were observed for 3442 and 216 animal-grazing days, respectively.

Livestock grazing Ladak 65 had greater ($P < 0.05$) mean bloat incidence and severity scores overall in five trials, while AC Grazeland had greater mean bloat severity index (BSI, 1= no bloat, 6= dead) scores in one trial. There were no significant differences between varieties in mean BSI in four trials. Livestock grazing N-fertilized alfalfa had greater mean BSI scores than livestock grazing unfertilized alfalfa.

In situ tests between Ladak 65 and AC Grazeland alfalfa had no significant differences in dry matter disappearance at 4-h. There were significant differences between fresh and both frozen and dried samples. Ladak 65 and AC Grazeland with and without N fertilization had small but significant differences 4-h DMD.

Correlation coefficients for percent bloat vs. CP, ADF, NDF, or NO_3 were 0.41, -0.31, -0.34 and 0.29, respectively. Correlation coefficients for bloat severity vs. CP, ADF, NDF, or NO_3 were –0.11, 0.17, 0.12, and 0.23, respectively

Bloat incidence and severity appear to be slightly less on AC Grazeland than Ladak 65. However, the differences were overcome by field fertility, environment and plant maturity. Crude protein and NO_3 appear to play a role in bloat. However, better protocol is necessary to accurately define this role.

CHAPTER 1

INTRODUCTION

Alfalfa is highly nutritious and widely adapted forage. In Montana about 700,000 ha of alfalfa are harvested for hay, with an annual value of about $250,000,000 (Montana Agricultural Statistics, 2000). Most of this alfalfa is grazed periodically, and alfalfa hay aftermath is often used as a source of fall feed. Alfalfa has great potential in crop mixtures or as the sole forage in pastures. Unfortunately, bloat is a hazard when ruminants on pasture consume alfalfa forage. No extensive bloat data are available in Montana, however in the intermountain areas of western Canada, bloat was reported on 40% of the farms surveyed (Howarth, 1975).

Many studies have addressed bloat and its causes. Bloat is a complex interaction of plant, animal, microbial and environmental factors. Frothy pasture bloat is type of acute ruminal tympany that results from formation of a stable froth that traps gases in the rumen upon ingestion of certain pasture plants, primarily alfalfa and clover. Approaches to bloat prevention and control have mostly been ineffective or expensive. One approach has been to breed a bloat safe alfalfa plant. Breeders have attempted to manipulate plant factors to reduce bloat incidence and severity.

Bloat is a cost to producers due to loss in productivity, death loss, expensive bloat preventatives, and labor. If the mechanisms of pasture bloat were clearly understood, the increased use of alfalfa could improve pasture quality, livestock performance and production efficiency.

Our objectives were: To evaluate 'AC Grazeland', a variety bred for reduced bloat, to 'Ladak 65', a major dryland alfalfa variety in Montana under "worst case" conditions and to determine if N status plays role in bloat incidence or severity.

CHAPTER 2

LITERATURE REVIEW

Acute ruminal tympany or bloat is a disorder in ruminant animals caused by retention of gases in the rumen. Only ruminants are susceptible to bloat, and livestock species vary in bloat susceptibility. For example, cattle (*Bos taurus*) generally bloat more often than sheep (*Ovir*) (Mendel and Boda, 1961; Clarke and Reid, 1973). It has also been noted that certain animals within a species are more susceptible to bloat than others (Johns et al., 1954; Miller and Frederick, 1966; Cockrem et al., 1983; Morris et al., 1997).

Two major forms are free-gas bloat and frothy bloat. Free gas bloat is generally caused by an obstruction to the esophagus or cardia. This can be the result of injury, disease or blockages from feeds such as turnips. Gas builds up behind the obstruction resulting in bloat (Cheng et al., 1998). Frothy bloat is characterized by a stable proteinaceous froth that traps gas, which then accumulates in the rumen (Howarth et al., 1975).

There are two types of frothy bloat, pasture bloat and feedlot bloat. The major difference between feedlot bloat and pasture bloat is that froth in pasture bloat is primarily caused by factors of plant origin, whereas the factors leading to feedlot bloat are believed to be of microbial origin (Cheng and Costerton, 1975; Cheng et al., 1976; Cheng et al., 1998.) Feedlot bloat can occur when over 50% of the diet is concentrate feeds. It often develops slowly over weeks and may become chronic (Clarke and Reid, 1973). Feedlot bloat is often accompanied by acidosis (Cheng et al., 1998).

Frothy bloat is caused by retention of gases in the rumen. Normally, excess gas in the rumen is simply eructated. In the case of frothy bloat, a viscous fluid is formed when ruminants eat certain legumes. Alfalfa (*Medicago sativa* L.), white clover (*Trifolium repens* L.), and red clover (*Trifolium pratense* L.) are generally considered bloat-inducing legumes, whereas birdsfoot trefoil (*Lotus corniculatus* L.), sainfoin (*Onobrychis viciifoilia* Scop), and cicer milkvetch (*Astragalus cicer* L.) do not cause bloat (Fay et al., 1980; Fay et al., 1981; Howarth et al., 1982). Pasture bloat has also been reported on immature winter wheat (*Triticum aestivum* L); [Branine and Galyean, 1990; Garry, 1990], pasture immature ryegrass (*Lolium perenne* L.; Garry, 1990), maize (*Zea mays* L.; Leek, 1983), and occasionally on alfalfa hay (Howarth, 1975). Like feedlot bloat, pasture bloat is associated with low pH (McArthur and Miltimore, 1969; Garry, 1990).

Carbon dioxide, methane and other gases are formed by fermentation of feed by rumen microflora and by acidification of bicarbonate (Bush and Burton, 1994). In bloating cattle the normal ruminal gases are accompanied by elevated levels of nitrogen gas and oxygen (Moate et al., 1997). Under normal conditions, gas bubbles can coalesce, separate from the rumen contents and be eructated (Howarth, 1975). However in the case of frothy bloat, a stable, proteinaceous froth forms (Howarth, 1975). The froth traps gas and the ruminal contents expand (Clarke and Reid, 1973). This inhibits the action of the cardiac sphincter (Leek, 1983), which normally allows gas to escape the rumen. Unlike feedlot bloat, which may develop slowly over weeks, pasture bloat generally occurs within 2-h of initiation of grazing (Majak et al., 1985). Feedlot bloat characteristically involves individual chronically-bloating animals (Clarke and Reid, 1973), whereas pasture bloat tends to involve "bloat storms" of entire groups of animals (Hall et al., 1984). Many cases of bloat are sub-clinical and show no visible signs (Hall et al., 1988). However, bloating animals can usually be recognized by cessation of grazing and symptoms of obvious discomfort such as panting, kicking, or stomping. The expansion of the ruminal contents creates extreme pressure in the rumen exceeding 1.35 psi (Cheng et al., 1998), and mechanically interferes with breathing and may result in death (Leek, 1983).

Traditional methods of reducing pasture bloat include legume-grass pasture mixes, grazing after the dew is off the vegetation, feeding hay before allowing animals access to the pasture, and waiting to graze until after a "killing frost" (Gomm, 1979b; Guyer and Hogg, 1980). Recently, Majak et al. (1995) established several guidelines for reducing the risk of pasture bloat of cattle: 1) graze after the legume has begun to flower, 2) only move cattle onto new pasture in the afternoon, 3) graze animals continuously with no interruption of grazing, 4) supplement poloxolene, a surfactant that has 100% efficacy in preventing bloat by breaking down froth (Hall et al. 1994a) and, 5) be aware that the bloat potential of alfalfa is not lost after a killing frost.

There currently is increased interest in grazing alfalfa-based pastures in the West. Alfalfa is a widely adapted, highly productive and very nutritious forage species. This investigation was designed to evaluate factors associated with alfalfa pasture bloat.

Ruminant Physiology Related to Bloat

Susceptibility to bloat has been shown to be a heritable trait (Cockrem, 1975, Morris et al., 1997.) Many traits have been considered as possible factors in bloat initiation. Three factors that have consistently been shown to be associated with high bloat susceptibility are: rumen fill volume (Cockrem et al., 1987; Carruthers et al., 1988), rumen clearance rate (Majak et al., 1983; Majak et al., 1986; Carruthers et al., 1988; Okine et al., 1989), and salivary makeup and secretion rate (Mendel and Boda, 1961; Clarke and Reid, 1973; Clark et al., 1974; Bartley, 1976; McIntosh and Cockrem, 1977; Rajan et al., 1996; Wheeler et al., 1998.)

Rumen Fill Volume

Cockrem et al. (1987) increased rumen fill levels of cannulated low susceptibility (LS) cattle to the levels observed in high susceptibility (HS) cattle and succeeded in initiating bloat. Rumen content volume was correlated to the tendency to bloat ($r=.71$, $P < 0.05$; Cockrem et al., 1987). This study suggests that LS cattle may not have rumen content volume levels high enough to produce the amount of gas and foam seen in HS cattle (Carruthers et al., 1988.) Hall et al. (1988) recorded feed consumption levels prior to the initial signs of bloat, and found that cattle which subsequently bloated consumed 18 to 25% less fresh alfalfa forage than non-bloating cattle. Total forage consumption therefore may not be primarily responsible for rumen content volume and subsequent bloat susceptibility. Another explanation for high rumen content volume is low passage rate (Majak et al., 1986.)

Rumen Clearance Rate

In marker studies to measure rumen clearance rates, passage was reported to be slower through HS cattle than LS cattle (Majak et al., 1986; Okine et al., 1989). Foam production was inversely correlated with fractional outflow rates of the marker CoEDTA (Majak et al., 1995). This study suggested that concentrated digesta in the rumen creates more froth than less concentrated digesta. Clarke and Reid (1970) reciprocally exchanged rumen contents between cannulated HS and LS cattle. This exchange of rumen contents resulted in a 24-h exchange of bloat susceptibility. These results indicated that bloat potential is related to dynamic factors in the rumen fluid such as microbial types or numbers, plant constituents, rumen fill volume or rumen fill concentration.

Carruthers et al. (1988) compared cannulated HS and LS cattle fed different diets. The LS cattle had less digesta in the rumen before feeding, and a lower percentage of solid digesta by weight than HS cattle. Water and dry matter (DM) intake were equal, suggesting a higher turnover rate in LS cattle (Carruthers et al., 1988). This indicates that bloat induction may be the result of a threshold concentration of certain bloat-inducing factors in the rumen.

Saliva Production

Ruminants secrete a large amount of serous and mucous saliva from a number of salivary glands. Serous saliva is continuously secreted and mucous saliva is secreted mainly at feeding. Highly bloat-susceptible cattle generally produce less of all classes of saliva than LS cattle (Mendel and Boda, 1961). A protein from bovine saliva, BSP30, has been isolated and negatively correlated to bloat incidence (Rajan et al., 1996; Morris et al., 1997). It has also been found that at low rates of salivation, saliva tends to be more viscid (Froetschel et al., 1986), which could contribute to ruminal froth. Rate of saliva production, especially saliva containing antifoaming agents, may explain bloat susceptibility to some extent.

Cation concentrations in saliva from the parotid glands vary with secretion rate. Low secretion rates are associated with lower concentrations of sodium (Na) and higher concentrations of potassium (K); [Bailey and Balch, 1961; Turner, 1981, Hall et al., 1988; Majak and Hall, 1990]; and elevated levels of calcium (Ca) and magnesium (Mg); [Hall et al., 1988]. Hall et al. (1988) hypothesized that Na or K ions in the rumen might affect the dispersion, aggregation or suspension as a colloid of the chloroplast particles. However, manipulation of these cations with Na supplements, with or without EDTA, an agent that sequesters Mg and Ca, failed to reduce incidence of bloat (Carruthers et al., 1988; Hall and Majak, 1992).

Microbial Factors

In order for an animal to bloat, rumen microbes must rapidly break down forage. This rapid breakdown releases bloat-inducing components, producing gas and slime (Howarth et al., 1978; Howarth et al., 1982). Certain microbes are known to be major factors in feedlot bloat. Production of gas and slime has been attributed to ruminal bacteria (Clarke and Reid, 1973; Kopecny and Wallace, 1982). Gutierrez et al. (1959) reported that *Streptococcus bovis* and P*eptostreptococcus elsdenii* populations in the rumen increased as cattle started to bloat on feedlot diets. Accumulation of froth has been associated with numbers of encapsulated lactic acid streptococci (Gutierrez et al., 1959; Majak et al., 1985; Cockrem et al., 1987). Conversely, entodinia protozoa consume streptococci bacteria, and may reduce bloat susceptibility by reducing the number of bacteria associated with bloat (Shirley, 1986) and *Lactobacillus* sp. may break down slime produced by *S. bovis* (Clarke and Reid, 1973).

Types and numbers of protozoa have been associated with bloat occurrence (Clarke, 1966; Clarke and Reid, 1969; Clarke and Reid, 1973; Katz et al. 1986). Clarke (1966) eliminated holotrich protozoa populations in feedlot cattle fed fresh forage using dimetridazol, and achieved complete bloat protection in cattle for up to eight days. Cattle began to bloat again as holotrich populations began to reach pre-treatment levels (Clarke, 1966). However, Clarke and Reid (1969) failed to achieve complete bloat protection in pasture trials and concluded that while holotrich protozoa seem to increase bloat incidence and severity, these are not necessary for bloat to occur (Clarke and Reid, 1969; Clarke and Reid, 1973).

While feedlot bloat appears to primarily originate from microbial factors (Cheng and Costerton 1975; Cheng et al., 1976; Cheng et al., 1998), differences in bacterial number or species did not appear to be related to pasture bloat (Bryant et al., 1961; Clarke and Hungate, 1971; Katz et al., 1986). In fact, mucinolytic bacteria may be involved in disrupting froth formation (Mishra et al., 1968). Pasture bloat appears to be caused by factors of mostly plant origin (Cheng and Costerton, 1975; Cheng et al., 1976; Cheng et al., 1998).

Plant Components

Protein and Nitrogenous Compounds

Many studies have associated the numerous forms of plant nitrogen (N) content (Fig. 1) with bloat incidence and severity (Howarth et al., 1977; Hall et al., 1984; Hall and Majak, 1991). Total N and levels of other N compounds have been shown to be higher on days of high bloat incidence than when bloat does not occur (Howarth et al., 1977; Hall et al., 1984; Hall and Majak, 1991). Soluble plant proteins have generally been considered the primary foaming agents in the rumen (Mangan, 1959; Howarth, 1975; Hall et al., 1988). Majak et al. (1985) reported that protein N levels were elevated on days when bloat occurred compared to when it did not. Hall et al. (1988) isolated ruminal proteins and generated stable foams *in vitro* over a range of alfalfa protein concentrations. The volume of foam increased with increased protein concentration, however, the soluble protein (SP) concentrations did not show any consistent relationship to the frequency of frothy ruminal contents (Majak et al., 1985). These researchers concluded that alfalfa SP is a component of the frothy complex, but not completely responsible for the immediate onset of bloat (Majak et al., 1985). Howarth et al. (1977) found a significant correlation between soluble non-protein nitrogen (SNPN) and bloat incidence. Mean soluble protein nitrogen (SPN) over three years of testing was not significantly correlated to bloat, however, SNPN was correlated to bloat ($r = 0.28$, $P<0.05$,), suggesting both soluble forms of N may play a role in bloat.

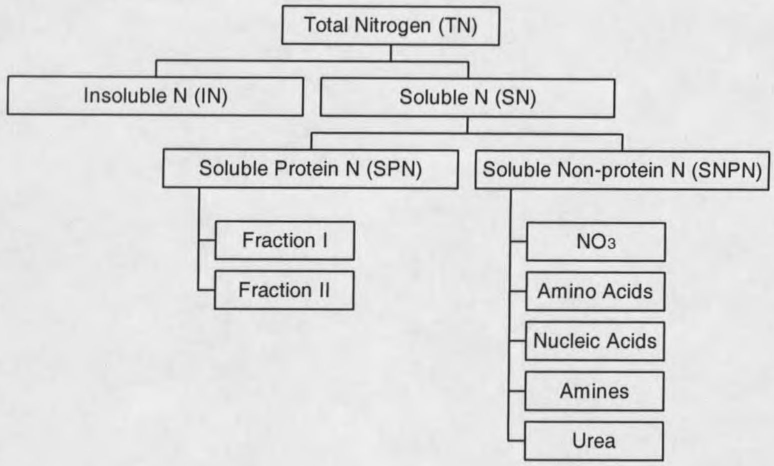

Figure 1. Plant nitrogen fractions (adapted from Howarth et al., 1977).

Soluble protein N is generally accepted as the primary source of SP that stabilizes froth in the rumen (Howarth et al., 1977; Hall et al., 1984; Majak et al., 1985), however, there are conflicting results between studies. In one study during 1977, SPN was higher on days of high bloat incidence than non-bloat days, but in 1978 the same study resulted in no significant relationship between SPN and bloat incidence (Majak et al., 1985.) In 1978, frothiness in the rumen was actually associated with lower levels of SPN in the rumen (Majak et al., 1980, 1985.) Levels of SN or TN have been correlated to bloat, however, these have failed to account for the extreme variation in number and severity of bloat cases (Howarth et al., 1977).

Miltimore et al. (1970) reported a high correlation between bloat occurrence and Fraction I protein, a fraction of protein determined with centrifugal separation. Miltimore et al. (1974) studied the distribution and heritability of Fraction I protein within populations of alfalfa. Less than 3% of alfalfa plants had levels of Fraction I protein below the hypothesized bloat threshold. Further, alfalfa plants had low heritability of

Fraction I protein, and after N fertilization the differences in Fraction I levels in plants diminished. Jones and Lyttleton (1969, 1972) disputed that Fraction I protein alone was responsible for bloat initiation and presented evidence that Fraction II protein also had the potential to cause bloat. Subsequent research showed no significant association between Fraction I and bloat incidence (Howarth et al., 1977). Howarth et al. (1977) estimated that a 50% reduction in SP in alfalfa would be necessary to make it bloat safe and that all SP should be considered as potential agents in causing pasture bloat. Coulman et al. (1999) concluded that because Fraction I chloroplast proteins are important in photosynthesis, it may have been difficult to achieve the estimated 50% reduction in SP needed for bloat safety.

Growth Stage

Bloat is most likely to occur when the alfalfa is in its vegetative to mid-bud stages and is growing rapidly (Hall et al., 1994b). As plant maturity increases bloat incidence decreases (Howarth et al., 1991, Majak et al., 1995). This may be due to the corresponding decrease in SP and carbohydrate, and reduced rate of plant cell rupture in the rumen (Hall et al. 1984).

Saponins

Alfalfa saponins have been implicated as factors having a role in bloat (Lindahl et al., 1957). However, in a study comparing alfalfa bred for high or low saponin levels, Majak et al. (1980) found no significant difference in bloat potential. These workers concluded that alfalfa saponins did not contribute to bloat.

Condensed Tannins

Several non-bloating legumes such as birdsfoot trefoil and sainfoin contain high levels of condensed tannins. Some researchers have attributed bloat resistance to these condensed tannins (McMahon, 1999). Condensed tannins may reduce bloat incidence by binding to proteins and reducing the rate of digestion (McMahon et al., 1999). However Miltimore et al. (1974) noted positive correlation with condensed tannin levels to bloat. Other bloat safe legumes such as cicer milkvetch do not contain tannins (McMahon et al., 1999), indicating that bloat-safety may be due to other factors. Bloat safety in cicer milkvetch as well as birdsfoot trefoil and sainfoin, has been attributed to a slower initial rate of digestion (IRD; Kudo et al., 1985).

Initial Rate of Digestion

A rapid breakdown of plant cells in the rumen is a contributing factor in the initiation of bloat (Howarth et al., 1978; Lees et al., 1981). Bloat-inducing legumes have a high initial rate of digestion compared to legumes that do not cause bloat (Howarth et al., 1978; Fay et al., 1981; Howarth et al., 1982). Rate of fresh forage digestibility in cannulated cattle was higher on days of high-bloat incidence than on days with no bloat (Hall et al., 1994a). Howarth et al. (1982) hypothesized that an alfalfa cultivar with 20 to 30 percent lower rates of digestibility within the rumen over the first 6 to 8 h might be safe and proposed the "cell rupture hypothesis". This hypothesis is that bloat-inducing legumes are less resistant to mechanical rupture than bloat safe legumes (Howarth et al., 1978), due to thicker epidermal or mesophyll cell walls (Lees et al., 1981; Lees et al., 1982; Lees, 1984). Rapid rupture of leaf mesophyll cells of bloat-inducing legumes results in rapid release of chloroplast fragments and SP (Howarth et al., 1977, 1978). These components are exposed to rapid microbial colonization and breakdown, contributing to bloat (Howarth et al., 1979).

Environmental Factors

Weather

Anecdotal evidence suggests that bloat occurs under nearly every weather condition. Occurrence of bloat is higher following days with low temperature (Hall et al., 1984), and temporarily increases after the first frost of fall (Hall and Majak, 1991; MacAdam et al., 1996). Concentrations of SPN, the plant constituent most highly related to bloat (Howarth et al., 1977) are higher with decreasing soil moisture and increased shading (Walgenbach et al., 1981; Walgenbach and Martin, 1981). Yearly proportion of days on which bloats occurred had a significant negative correlation with precipitation. Bloat was not significantly correlated with daily hours of sunshine, or maximum and minimum temperature (Hall et al., 1984). Hall et al. (1984) concluded that bloat is not due to a single unique weather variable.

Development of a Reduced-Bloat Cultivar

Researchers at the Saskatoon Research Center of Agriculture and Agri-Food Canada initiated a breeding program to develop an alfalfa cultivar with thicker mesophyll and epidermal cell walls more resistant to rupture and with a slower IRD (Goplen et al., 1992; Berg et al., 1999). A wide array of samples from existing cultivars was screened for IRD. No cultivar with an abnormally low IRD was found (Howarth et al., 1991). Howarth et al. (1982) found consistent differences in cell wall thickness in 11 clones of 'Beaver' alfalfa and suggested that alfalfa could be bred for low initial rate of digestion (LIRD). Low IRD and high IRD plants were identified using a modified nylon bag *in situ* trial with fresh chopped alfalfa forage in fistulated sheep. Resulting low and high IRD populations following one cycle of selection were grown on dryland alfalfa plots at Saskatoon, SK and fed to fistulated sheep. Several chemical components were measured in rumen fluid sampled at 0, 2, and 4-h post-feeding. Rumen fluid from sheep fed low IRD alfalfa had significantly lower levels of chlorophyll, SP, soluble carbohydrates, and volatile fatty acids than the high IRD variety at all time periods (Kudo et al. 1985), implying that breeding for IRD could be effective.

Cycle 1 LIRD (LIRD-1)

A nursery containing 1200 plants of Beaver, 'Vernal', 'Anchor' and 'Kane' alfalfa was established at Saskatoon, SK in 1979. Each plant was individually screened for IRD in 1980 with a 4-h nylon bag technique. From these plants, the 5% with the lowest IRD were selected. These plants were intercrossed in a greenhouse in 1980-1981. The LIRD-1 alfalfa population showed a 6 % reduction in IRD compared to the mean IRD of the control variety, Beaver ($P < 0.05$; Goplen, 1992.)

Cycle 2 LIRD (LIRD-2)

A field nursery of 39 polycross progenies from LIRD-1 was established. Each plant was screened for IRD in its second year at the pre-bud to mid-bud stage of growth. The 5% with the lowest IRD was selected and intercrossed in a growth chamber in 1982-1983.

Cycle 3 LIRD (LIRD-3)

Polycross seed from 1982-1983 was used to create a field nursery. One thousand fifty plants were screened for IRD, and the lowest 20% were selected and intercrossed in 1984-1985. A replicated progeny trial was created in 1985, and results of IRD screening in 1986 were used to select a total of 20 plants with the lowest mean IRD, and a field increase of LIRD-3 was produced for field testing. The LIRD-3 population had 15% reduction of IRD compared to the parent variety. Numerous bloat evaluations were conducted with LIRD-3 in Canada (Goplen et al., 1992; Hall et al., 1994a; Berg et al., 1999). Canadian pasture trials showed no difference in bloat incidence or severity between LIRD-3 and Beaver. Further, 'LIRD-3' was only significantly lower in bloat incidence in one of three feedlot trials. The deviation in bloat incidence when it occurred in these trials could not be explained by differences in feed composition (Hall et al., 1994a).

Cycle 4 LIRD (LIRD-4)

A field nursery of LIRD-3 selections was established in 1987. One thousand six hundred plants were screened during 1988. The lowest 12% IRD were selected. These plants were intercrossed in 1988, and these were used to establish a progeny test for IRD in 1989. Parental clones were selected and intercrossed in the winter of 1990-1991. Field seed increase of LIRD-4 was initiated in 1991. The LIRD-4 generation was widely tested, and subsequently released in 1997 as a bloat-reduced variety named 'AC Grazeland' (Coulman et al., 1999).

Extensive testing in Canada has shown that the effectiveness of AC Grazeland in reducing bloat incidence has been variable, from no significant effect to 84% reduction (Berg et al., 1997). In 1991, there were no significant differences in IRD between AC Grazeland and the control alfalfa, Beaver (Goplen et al., 1992). Conversely, Berg et al. (1996) found that when grazing lush immature forage, ruminal distention in cattle occurred 30% less with AC Grazeland alfalfa than with Beaver, the control variety. Severe bloat was 56% less frequent. These workers also found that maturity was more important than cultivar in preventing bloat (Berg et al., 1996).

Plant maturity and environmental conditions may mask the effect of the LIRD character (Goplen et al., 1992). When grown under irrigation, 4-h dry matter disappearance (DMD) of AC Grazeland was 89 to 96% of that of Beaver. AC Grazeland appears to have more bloat protection on dryland than under irrigation (Hall et al., 1994a). The improved IRD characteristics diminish at mature growth stages (Goplen et al., 1992).

While bloat protection in AC Grazeland does not appear to be complete, any bloat reduction could be useful in production under proper management. Successful breeding of an alfalfa variety for reduced bloat is a significant step toward an effective solution to bloat problems.

Objectives

The objectives of this research project were to: 1) evaluate the bloat hazard of AC Grazeland to determine if this alfalfa variety bred for lower bloat incidence was in fact superior to a conventional alfalfa variety, Ladak 65 and 2) determine if alfalfa N status or forage quality play a role in bloat.

The goal was to graze alfalfa under "worst case" conditions in Montana, during the growing season and in the fall, typical of many ranch operations. Alfalfa was grazed at all growth stages from vegetative to mature with sheep and cattle fasted overnight. This was the first evaluation of AC Grazeland in the United States. Ladak 65 was used as a control variety as it is the most widely grown dryland variety in Montana.

The hypotheses tested were: 1) AC Grazeland and Ladak 65 have equivalent tendencies to cause pasture bloat, and 2) plant N levels and forage quality during grazing are unrelated to bloat.

CHAPTER 3

MATERIALS AND METHODS

Pasture Comparison of Bloat Potential of AC Grazeland and Ladak 65

Site Description

Replicated field strips of AC Grazeland alfalfa, Ladak 65 alfalfa and 'Tretana' birdsfoot trefoil were established 25 June, 1997 near Bozeman, Montana. Prior to planting, 135 kilograms of 11-52-0 (N-P-K) fertilizer and Eptam herbicide were incorporated. Each strip was 0.25 ha, arranged in a randomized complete block with four replications (Fig. 2). During 1997, abundant precipitation was received allowing for excellent stand establishment. The plots were harvested once in 1997 to remove dead material before initiation of grazing. In June 1998, holding pens, fences and water tanks were installed and each strip was sectioned into 20 grazing cells with electric fence. Each pen had an individual water tank and salt and mineral supplement fed ad libitum. This pasture system was used for 10 separate grazing trials from May 1998 to September 2000 (Table 1).

Trial 1, 1998 Sheep

On June 10, 1998, 40 mixed purebred ewes were transported to the site. These ewes were of mixed ages and had all been grazing primarily grass pasture. Prior to

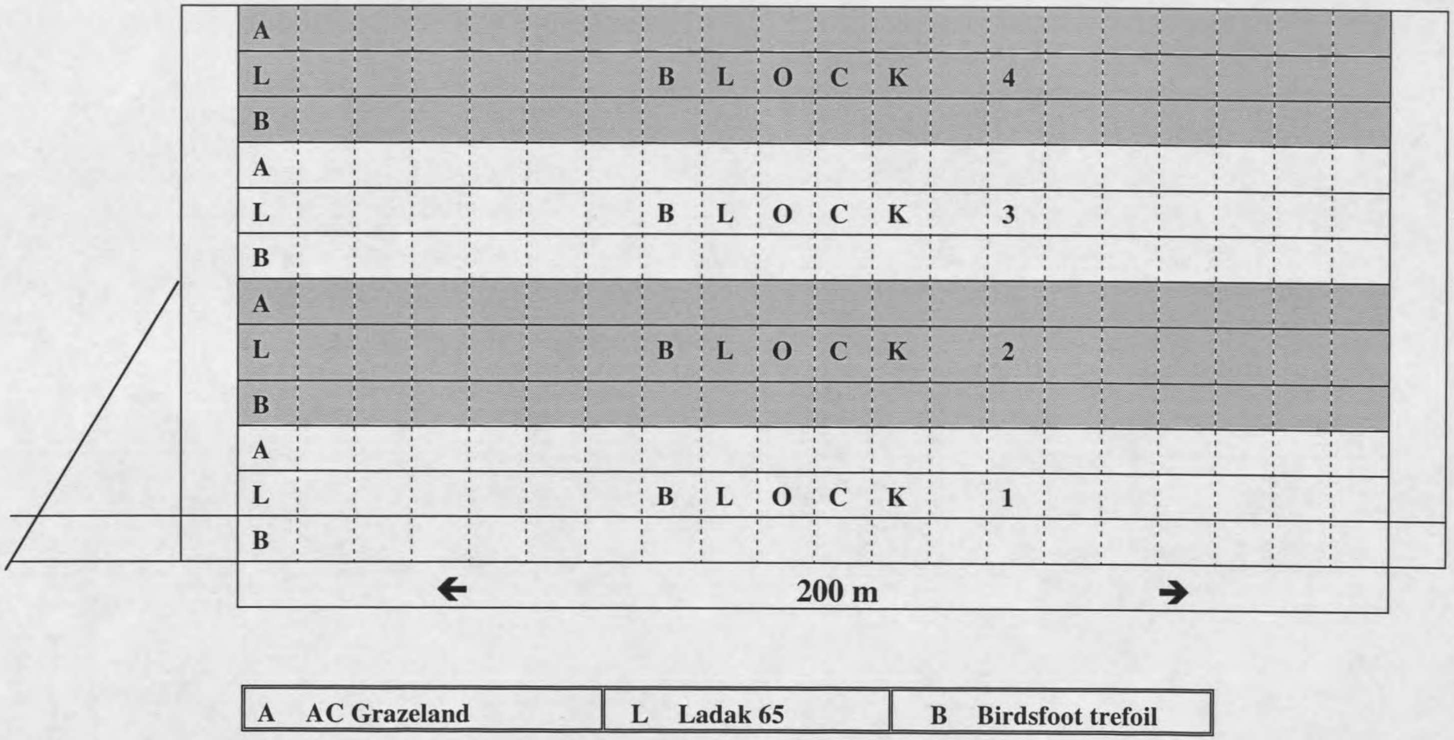

Figure 2. Schematic of grazing trial established June 1997 at Bozeman, MT.

Table 1. Details of grazing trials at Bozeman, MT.

Trial Number	Trial Description	Date	Grazing Days	Number of Animals
1	1998 Sheep 1st half	6/10/-7/3/98	24	32
2	1998 Sheep 2nd half	7/8/-8/12/98	25	32
3	1998 Cattle	6/29/-7/8/98	7	8
4	1999 Sheep 1st half	6/9/-7/13/99	25	40
5	1999 Sheep 2nd half	8/2/-8/15/99	12	32
6	1999 Cattle	8/11-8/19,9/8, 9/9/99	10	8
7	1999 Sheep +/- N fertilization	9/3,9/8-9/10/99	4	24
8	1999 Cattle +/- N fertilization	9/20/-9/22/99	3	8
9	2000 Sheep +/- N fertilization	5/29/00-6/19/00	16	24
10	2000 Cattle +/- N fertilization	9/9/00-9/16/00	7	8

moving, the ewes were weighed (mean weight 61 kg) and scored for body condition. Based on these data, four ewes were assigned to each replicated strip of Ladak 65 and AC Grazeland alfalfa and two to each strip of birsdsfoot trefoil. The ewes had ear tags and were marked with paint to maintain identity. Information was maintained on each ewe throughout the grazing season. Initially, the intent was to fast the livestock for 14 h at night, then observe grazing for 8 to 10 hours each day. However, after the first week, it was apparent that acute bloat or death was unlikely, so all ewes were given 24-h access to the forage. Prior to grazing, a stocking density of four sheep per 100 m^2 was calculated so that livestock would consume the forage in each cell daily, and then rotate through the strips in a three-week period. However during June and early July, forage growth was excellent, exceeding sheep consumption for the planned rotation rate through the strips. Therefore, ewes were moved to a new cell every two to three days. After 10 cells in each strip were grazed, these cells were mown and irrigated in order to provide bloat-potent immature forage throughout the season. In the first half of the 1998 grazing season 40 sheep grazed 24 days (Trial 1). Eight sheep were grazed on birdsfoot trefoil (no bloats, data not shown) and 32 were grazed on alfalfa. Bloat severity scores (BSI, 1 = no bloat, 6 = dead) were assigned by subjective assessment and these became fairly repeatable among observers. Bloats pushes the sub-lumbar triangle outward and upward, while ruminal distention due to fill rarely fills the triangle and does not push it upward. On some animals, particularly fistulated animals, it can be difficult to ascertain bloat by observing the sub-lumbar triangle. However, bloat can be evaluated by comparing distention between animals. Flanks on a bloating animal distend far more rapidly than those of it's unbloated peers. A bloated animal also shows signs of distress such as ceasing to graze, panting, stomping or kicking, and frequent urination and defecation. Animals were observed for >8 h each day and scored repeatedly throughout the day. The highest daily score for each animal was used for data analysis.

Observation began at 0800 each weekday and ended at 1700. The following data were collected: daily livestock condition, climatic conditions, bloat severity index score and plant condition. A bloat severity index (BSI) was used: 1 = no bloat, 2 = slight swelling on one side, 3 = moderate swelling on one side, 4 = severe swelling on one side, 5 = severe swelling on both sides, 6 = dead. A bloat incident or event was recorded for an animal scoring higher than a 1. Scores were recorded throughout the day and the highest score for each ewe for each day was used for statistical analysis.

On weekends, the ewes grazed the stems from the previous cells or were moved to birdsfoot trefoil pasture. During 1998, the ewes were maintained on Ladak 65 or AC

Grazeland for 24 days (Trial 1) then grazed the opposite variety (Trial 2) for 25 days. Data for bloat comparison were stored in Microsoft Excel spreadsheets (Microsoft Corporation, www.microsoft.com). Daily BSI scores and percent bloated ewes on Ladak 65 and AC Grazeland were analyzed by ANOVA. The experimental design was a randomized complete block with four blocks. A group of four sheep in one cell was used as the experimental unit for each day. Across the trial, mean BSI scores and percent of ewes bloated were compared with paired t-tests.

Trial 3, 1998 Cattle

Twelve two-year old beef heifers were introduced to the grazing plots in July 1998. Two heifers were grazed on each alfalfa cell and one heifer was grazed on each cell of birdsfoot trefoil. They were grazed on plots previously grazed by sheep for three days, before being introduced to the new cells. Observations were taken in the same manner as the sheep trials. Data for bloat comparison were stored in Microsoft Excel spreadsheets. Daily BSI scores and percent bloated cows on Ladak 65 and AC Grazeland were analyzed by ANOVA. The experimental design was a randomized complete block. One cow in one cell was used as the experimental unit for each day. Across the trial, mean BSI scores and percent of cows bloated were compared with paired t-tests.

Trials 4 and 5, 1999 Sheep

Minor modifications were made to the sheep pens in 1999 so that sheep were exposed to fresh forage only. Birdsfoot trefoil strips were only used for overflow pasture on weekends. Sheep numbers were increased to 56 ewes to approach a stocking density that would consume the forage in each cell daily, then rotate through the strips in a 3-week period. The ewes were 40 yearlings and 16 older ewes of similar breeding and background. Prior to the trial, all ewes were weighed (mean weight = 50 kg) and scored for body condition. Ewes were penned at night and re-randomized daily and grazed from 0800 until 1600. Animals were scored for bloat severity throughout the day and the highest daily score for each animal was used for statistical analysis. Information was maintained on each individual ewe throughout the grazing season. Plant samples and forage data were collected. Plant height was measured, maturity was assessed and grab samples from the top 15 cm of 10 plants were taken daily from the area immediately surrounding each of the cells that the sheep were grazing. The samples were transported to the laboratory and frozen in a conventional household freezer without a thaw cycle for storage. Pre- and post-grazed samples were taken from a 0.3 m^2 quadrat at ground level. One sample from each strip was from fresh forage (pre-grazed) and one was taken from the previous day's grazed forage (post-grazed). These samples were taken three times per week to estimate livestock consumption. The pre and post-grazed samples were weighed fresh, dried then re-weighed. All samples were taken between 0900 and 1200h. The samples were analyzed for CP, acid detergent fiber (ADF), and neutral detergent fiber (NDF) by near infrared spectroscopy (NIRS). A representative group of samples were analyzed for CP, ADF, and NDF with AOAC (Association of Official Analytical Chemists, 2000) approved methods for NIRS calibration, and nitrate (NO_3).

On weekends, the ewes grazed the stems from the previous cells or were moved to birdsfoot trefoil pasture. After the first 10 cells were grazed, they were mowed back for irrigation and subsequent regrowth and grazing. After July, sheep numbers were reduced to 24, and sheep were condensed to Blocks 3 and 4. At this point the alfalfa was mature. Ewes were observed and scored for bloat and plant samples were collected for the remainder of the summer. Data for bloat comparison were stored in Microsoft Excel spreadsheets. Daily BSI scores and percent bloated ewes on Ladak 65 and AC Grazeland were analyzed by ANOVA. The experimental design was a randomized complete block with four (Trial 4) or two (Trial 5) blocks. A group of four sheep in one cell was used as the experimental unit for each day. Across the trial, mean BSI scores and percent of ewes bloated were compared with paired t-tests.

Trial 6, 1999 Cattle

During August, eight mature cannulated beef cows were grazed on the cells. The cattle had previously been grazing primarily grass pasture. They were acclimated on alfalfa stubble for three days before observations were taken. Each morning the cows were randomized and moved to fresh forage within the cells. The cows were grazed from 0800 to 1600 each day. Cattle were observed and BSI scores were taken. When cattle bloated severely, cannula plugs were removed to relieve pressure. Data for bloat comparison were stored in Microsoft Excel spreadsheets. Daily BSI scores and percent bloated cows on Ladak 65 and AC Grazeland were analyzed by ANOVA. The experimental design was a randomized complete block (RCBD) with four blocks. One cow in one cell was used as the experimental unit for each day. Across the trial, mean BSI scores and percent of cows bloated were compared with paired t-tests.

Effect of N Fertilization and Variety on Pasture Bloat

Trial 7, 1999 Sheep

In order to evaluate the effects of alfalfa N status on bloat incidence, 12 matching cells (six Ladak 65 and six AC Grazeland) were selected in Blocks 1 and 2. Half of the cells were fertilized with ammonium nitrate fertilizer (333 kg ha^{-1} N) during August 1999. Beginning on 16 August, the cells were irrigated. By September, the alfalfa had regrown to approximately 20 cm, and were grazed by 24 mixed breed ewes. The ewes were grazed on alfalfa pasture until this trial began. This trial was timed to include the first forecasted hard seasonal frost. Grazing began at 0800 and ended daily at 1600 The 24 sheep were randomly allocated to the cells and grazed for three days. Animals were observed and bloat severity index scores were recorded throughout the day. Forage samples were collected from the top 15 cm of ten plants in each of the plots. Bloat incidence and severity in sheep on Ladak 65 and AC Grazeland alfalfa with and without fertilizer were compared. Data for bloat comparison were stored in Microsoft Excel spreadsheets. Analysis of variance templates were created in Microsoft Excel to analyze the factorial data of varieties (V), fertilizer level (F) and the V x F interaction. The experimental design was a 2 x 2 factorial design in a RCBD with two blocks. Cells of sheep (n = 4) were considered experimental units. Daily BSI scores were compared with ANOVA templates.

Trial 8, 1999 Cattle

Eight mature cannulated beef cattle were grazed on N-fertilized and non-fertilized cells of AC Grazeland and Ladak 65 alfalfa in September. These cattle grazed grass pasture until the first hard seasonal frost, at which point they were grazed on alfalfa. Each day the cows were randomized and moved to fresh forage. The cattle were grazed from 0800 to 1600 daily. Cattle were observed and bloat severity index scores were recorded. When cattle had severe bloat, cannula plugs were removed to relieve pressure. Plant samples were taken as outlined previously. Data for bloat comparison were stored in Microsoft Excel spreadsheets. Analysis of variance templates were created in Microsoft Excel to analyze the factorial data of V, F and the V x F interaction. The experimental design was a 2 x 2 factorial design in a RCBD with two blocks. One cell with one cow was considered the experimental unit. Daily BSI scores were compared with ANOVA templates.

Trial 9, 2000 Sheep

In April 2000, alternate cells in each alfalfa strip were fertilized with ammonium nitrate (175 kg ha^{-1}). In May 2000, 24 mixed purebred yearling ewes of similar background were transported to the site. The ewes were grazed on grass pasture until this trial began. Eight mobile pens were built to maintain pasture stocking density without the problems inherent with electric fence. Eight pens, each approximately 25 m^2 held three sheep each. There were two replications of each variety by fertilizer combination. The pens were moved daily at 0800 to the opposite fertilizer status from the previous day. Throughout the day, these pens were moved to maintain fresh forage of the same variety and fertilizer status. Sheep completely grazed the cells at least once per day, and the sheep remained in the pens at night until they were moved to fresh forage at 0800. Sheep were observed throughout the day, and bloat severity index scores were recorded at 1000, 1200, 1400 and 1600. Plant maturity and condition was assessed. Ten random forage samples were taken from the top 15 cm of plants adjacent to each of the cells being grazed. These samples were taken concurrent with sheep being moved to fresh forage and were immediately transported to the laboratory and placed in a dryer at 65 ^{o} C (Association of Official Analytical Chemists AOCA, 2000). Sheep were randomized weekly to mobile pens which were moved daily between fertilizer levels on one variety. On weekends, sheep were grazed on birdsfoot trefoil pasture.

Data were stored in Microsoft Excel spreadsheets. Analysis of variance templates were created in Microsoft Excel to analyze the factorial data of V, F and the V x F interaction. The experimental design was a 2 x 2 factorial design in a RCBD with two blocks. Cells of sheep (n = 3) were considered experimental units. Daily BSI scores were compared with ANOVA templates.

Trial 10, 2000 Cattle

In July and August, the pasture was irrigated. During August the pasture was mown, and cordoned off with electric fence. From 9 September to 16 September, eight mature cannulated beef cows were grazed on regrowth of Ladak 65 and AC Grazeland alfalfa with or without N fertilizer. The cattle were grazed on grass pasture until this trial began. Cattle were dry-lotted overnight and randomly assigned to cells the following morning. Cattle were grazed from 0800 to 1600 and bloat scores were recorded. Forage samples were collected as previously described. When cattle bloated severely, cannula plugs were removed to relieve pressure. Days were separated into three periods each in order to accumulate enough data for statistical analysis. Data for bloat comparison were stored in Microsoft Excel spreadsheets. Analysis of variance templates were created in Microsoft Excel to analyze the factorial data of V, F and the V x F interaction. The experimental design was a 2 x 2 factorial design in a RCBD with two blocks. One cell with one cow was considered the experimental unit. Daily BSI scores were compared with ANOVA templates.

In Situ Tests

1999 *In Situ* Tests

Two *in situ* DMD trials of AC Grazeland and Ladak 65 digestibility were conducted with the protocol used in the development of AC Grazeland (Goplen et al., 1992). On 7 July 1999, entire plants were taken fresh from the pasture. The samples were cut into one-cm lengths and digested for four hours *in sacco* (Goplen et al., 1992). Five kg of alfalfa was selected from each variety and from two stages of growth: fully bloomed first growth and pre-bud regrowth. As the samples were collected they were placed in paper bags. The paper bags were placed into a large black plastic bag and transported directly to the laboratory (approximately 400 m from the pasture). Because of the bulk of the samples and the proximity of the laboratory, no further storage precautions were taken. At the laboratory all samples except the samples being processed were refrigerated. Plants were cut into 1 cm lengths with scissors. Only the upper half of each plant was used in order to approximate the forage animals would select. After thorough mixing, duplicate 10 g samples of chopped material were placed in pre-weighed 10 x 20 cm nylon bags with pore size of 50 μm. Bags were connected with a fishing stringer, placed in plastic freezer bags and refrigerated overnight. The following morning, The bags were separated into eight groups with one bag of each variety x maturity combination for each time frame. The nylon bags were placed in nylon mesh laundry bags and soaked in 39° C water for five minutes to facilitate initial digestion. Each group of bags was placed in the rumen of one of eight cannulated cows. The cows had been fed fresh chopped alfalfa during and for 3 days prior to the trial. The bags were mixed thoroughly with rumen fluid and pushed beneath the fiber mat in the rumen. Bags were removed at: 0 (control), 0.5, 1, 2, 3, and 4 h in one trial to determine initial rate of digestion and at 0, 1, 4, 12, 24, 48, 32, and 72 h in another to determine rate of digestion. After samples were removed from the cattle they were washed thoroughly with water. They were then placed in a dryer at 65° C for 72 h, then removed and weighed. Dry matter percent was calculated by subtracting the weight of the nylon bag from fresh and dry 0-h samples. The dry forage weight was then divided by the fresh weight. Dry matter disappearance (DMD) was calculated by subtracting bag weights from each sample, and dividing the dry weight of the fresh sample by the dry weight of the sample after digestion. This value was subtracted from one and the resulting value multiplied by 100 for percent DMD. Mean dry matter disappearance for each variety was averaged across bags in a cow and compared by ANOVA as a randomized complete block with each of eight cows considered as a replication.

1999 Fresh, Frozen and Dried Comparison

In situ evaluation of fresh forage material is very labor intensive for a limited number of samples. Further there is considerable risk of inaccuracy of fresh weights during sample preparation. For these reasons, evaluation of frozen or dried material might improve convenience, accuracy and number of samples. However, the effects of freezing or drying samples for storage on 4-h DMD are not known. In July 1999, clippings of AC Grazeland and Ladak 65 alfalfa were cut at ground level and sealed in nylon bags as described previously. The bags and fresh forage were then weighed. Two bags of each combination of variety and storage method were weighed, then dried and re-weighed for a DM percentage estimate. The bags were then refrigerated for 24-h, dried or frozen. The bags were then separated into five duplicate groups, one set for each of five cows. The cows were fed alfalfa hay during the trial and for 3 days prior to the trial. Nylon bags were connected with a fishing stringer and placed in a nylon mesh laundry bag. One set, consisting of three bags of pre-bud alfalfa of each variety by treatment combination and three bags of mature alfalfa of each variety by treatment combination was placed into each of the rumens of five cannulated cows. The bags were mixed thoroughly with rumen fluid and pushed beneath the fiber mat in the rumen. Each cow had one bag of each combination of variety, maturity, and refrigerated (fresh), frozen or dried samples. The bags were removed at 4-h. They were washed thoroughly with cold water and placed in a dryer at 65^{o} C for 72h, then weighed to calculate DMD. The 4-h DMD of each treatment was analyzed with a single factor ANOVA with bags in individual cows as the experimental units.

2000 *In situ* DMD of alfalfa leaves

Additional *in situ* tests were conducted in 2000 to evaluate the effects of N fertilization on bloat incidence and severity. The trial was conducted as a 2 x 2 factorial design (varieties and fertilizer levels) with bags of forage as the experimental unit. Several procedural changes were implemented in order to reduce potential error.

Forage samples were collected from regrowth from fertilized or unfertilized plots of Ladak 65 and AC Grazeland alfalfa in September 2000. The pasture had been mown three weeks earlier and plants were all in the pre-bud stage and approximately the same height. Entire plants were clipped in the pasture, placed in paper bags, which were placed in a black plastic bag and transported immediately to the laboratory. All of the plant material except for the material being processed was refrigerated. The leaves were cut from the stems with scissors then weighed to 10 g and placed in pre-weighed 10 x 20 cm nylon bags with pore size of 50 μm. There were three bags for each time period for each of three cannulated cows. The bags were then heat-sealed and weighed with the forage. Three bags of each variety by fertilizer status group of forage were then dried and weighed to estimate DM.

Nylon bags were handled and placed in cannulated cows as described previously. The cows were fed alfalfa hay during the trial and for 3 days prior to the trial. Bags were removed at 0, 2, 4, 6, and 24 h. After samples were taken from the cow they were immediately placed in ice water to halt further digestion. They were rinsed in cold water until rinse water was not discolored and the bag and its contents no longer felt viscous. The bags were then placed in a dryer at 65° C for 72 h. Each set of bags for each cow and time period contained an empty bag. The empty bag was dried with the sample bags at 65° C for 72h, weighed, then placed in a forced-air oven at 100° C for 48h. After removal from the oven it was re-weighed. These bags were used to estimate percent weight gain from trapped fibers and rumen fluid ((1 - (original dry bag weight / post digested bag weight)) x 100).

Forage Quality Analyses of Pasture During Grazing

1999

During 1999, all samples were analyzed for crude protein (CP), acid detergent fiber (ADF), and neutral detergent fiber (NDF) by near infrared spectrography (NIRS) (Appendix). NIRS calibration and validation sets, and NO_3 samples were analyzed by AOAC approved methods (AOAC, 2000).

Daily forage samples were collected from the top 15 cm of 10 plants from all plots. These samples were placed in 10x10 cm sealable plastic bags, transported to the laboratory and frozen. The samples were later dried at 65° C methods (AOAC, 2000) and ground to pass through a 1mm screen. Analysis of variance for CP, ADF, NDF, and NO_3 were calculated using templates in Microsoft Excel. Data from these samples were compared with ANOVA (RCBD, four blocks).

Pre- and post-graze samples were clipped at ground level from 0.3 m^2 quadrats, dried at 65° C, weighed and ground to pass through a 1mm screen. These samples were analyzed as a 2 x 2 (variety and grazing treatments) factorial design by ANOVA. Means were compared by a protected LSD ($P < 0.1$).

2000

During 2000, only samples from the top 15 cm of the plant were collected. Pre- and post-grazed samples were not collected because there was no forage remaining in the mobile cells after they were moved. Samples were collected daily upon initiation of grazing. The samples were from the top 15 cm of ten plants from each cell and were taken to the laboratory and immediately dried at 65° C. They were placed in dry storage until mid-winter then ground to pass a 1 mm screen and analyzed for TN, ADF, and NDF by NIRS, and for NO_3 with AOAC approved methods (AOAC, 2000). These samples were analyzed as a 2 x 2 factorial design by ANOVA. Means were compared by a protected LSD ($P < 0.1$).

Relationship Between Bloat and NO_3, TN, Fiber and Variety

Daily values of NO_3, TN, ADF and NDF as well as variety and mean BSI scores were entered into Rweb (www.http//math.montana.edu/Rweb). Linear relationships were calculated and multiple correlation coefficient between 30 pairs of samples taken during Trial 9, 2000 were calculated. A linear model was developed using the highest correlation to bloat. Residual error for this model was entered as another category and paired with the remaining factors. The factor with the highest correlation to the residual was added to the previous model. This process was repeated until additional factors in the model did not produce P values less than 0.1.

CHAPTER 4

RESULTS AND DISCUSSION

Crop Growing Conditions and Alfalfa Pasture Productivity

Grazing commenced in May 1998, when 40 ewes were allocated to the alfalfa paddocks. Grazing observation days began 10 June 1998. The first two weeks of Trial 1 were wetter than normal, (Fig. 3) and cooler than normal (Fig. 4), with 150 mm of rainfall received. Alfalfa was initially in the vegetative stage and had advanced to late bloom by early July. Both varieties had similar rates of maturity (Fig. 5). Available forage from the same period advanced from about 2000 to 5500 kg ha $^{-1}$ (Fig. 6). Productivity was estimated from forage cut at ground level from 0.3 m^2 quadrats. During this rapid growth period, the stocking rate was too low, and required 35 days to graze through the 20 cells. After 10 of the 20 cells per strip in Trial 1 had been grazed (15 July, 1998) all remaining biomass was mowed with a flail mower, and the plots were irrigated with approximately 75 mm of water using hand-line sprinklers.

In 1999, Ladak 65 alfalfa production estimated from forage cut at ground level from 0.3 m^2 quadrats (Fig. 6) increased from about 3000 to 6700 kg ha $^{-1}$, while production from AC Grazeland increased from 3000 to 5800 kg ha^{-1} between 9 June and 6 July. Temperatures were near average during June and August, but were higher than the 30 year average for the first two weeks of July, and lower than average for the second two weeks (Fig. 4). The first 10 cells had been grazed and were mown on 25 June.

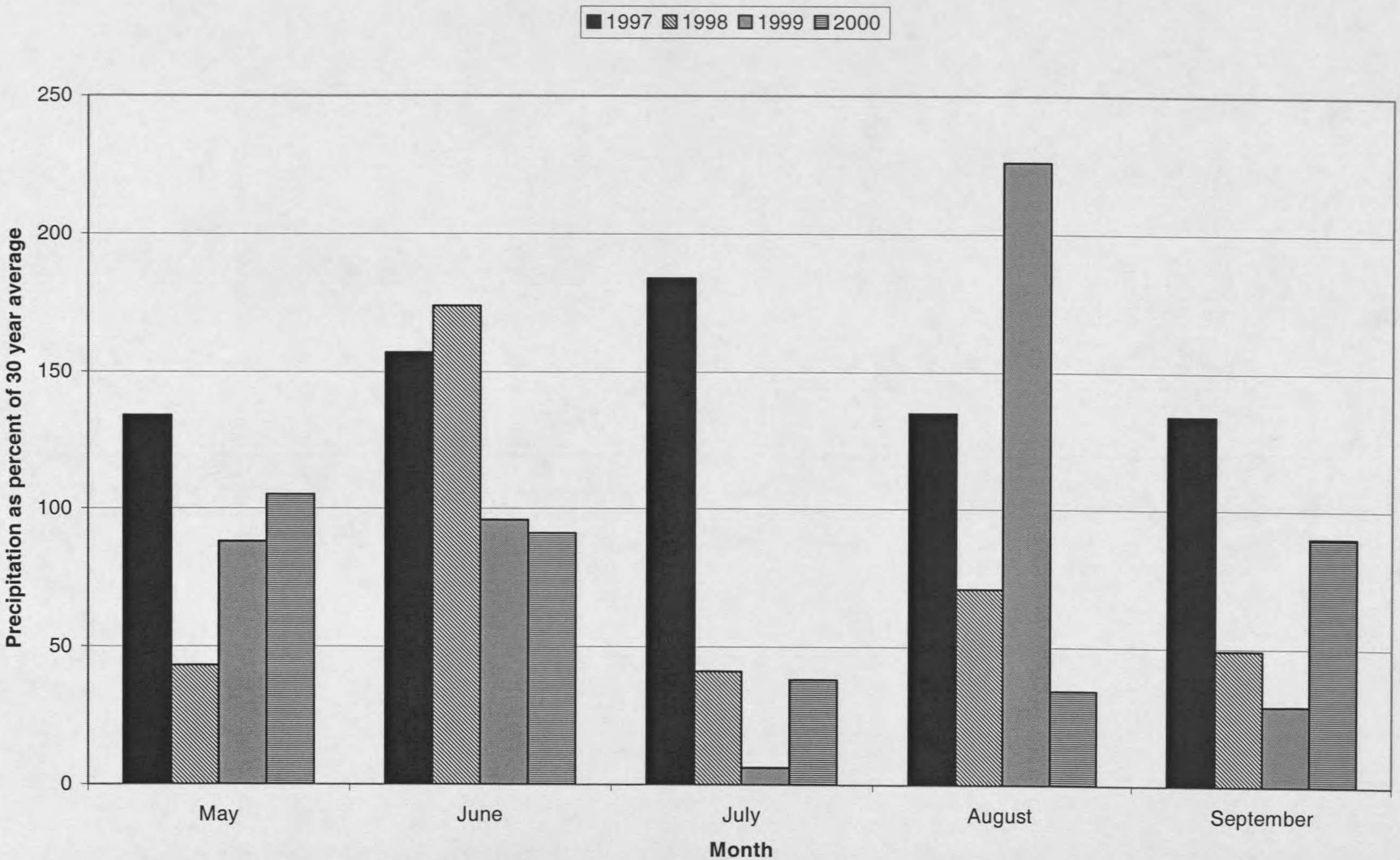

Figure 3. Monthly precipitation as a percent of 30-year average at MSU, Bozeman.

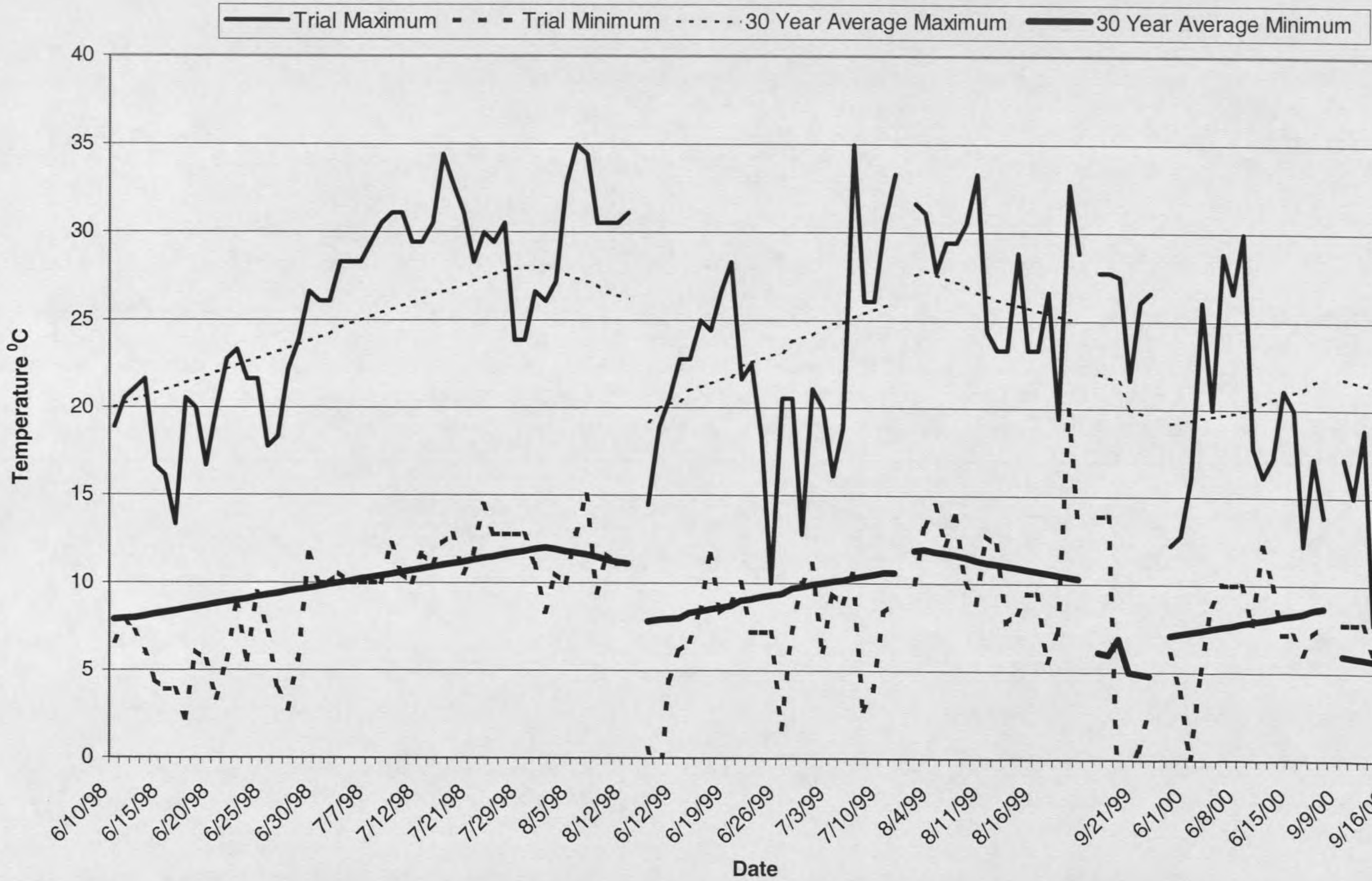

Figure 4. Daily maximum and minimum temperatures during 1998-2000 trial periods.

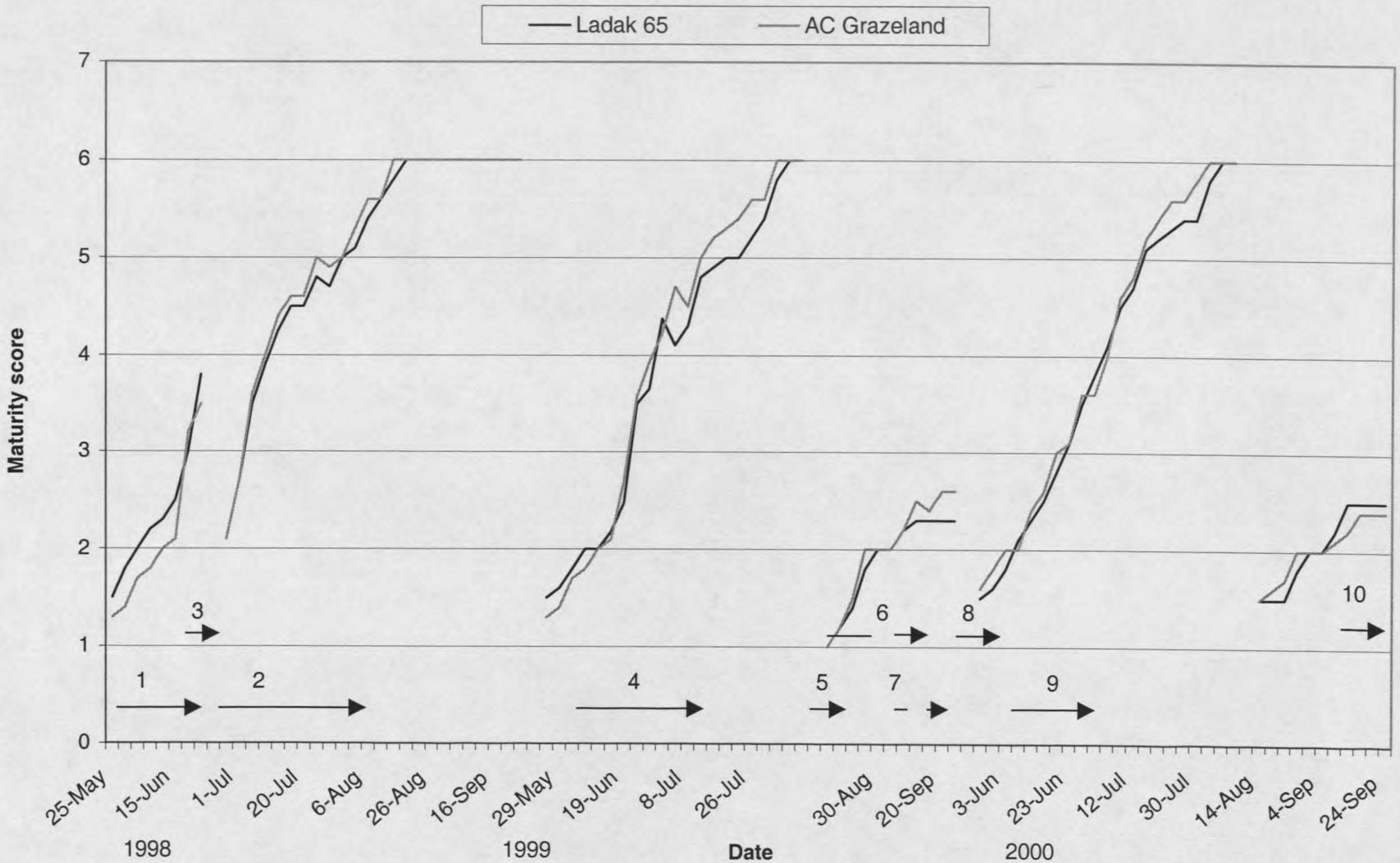

Figure 5. Alfalfa maturity scores (1=vegetative, 6 = fully bloomed) of AC Grazeland and Ladak 65 alfalfa during the 1998, 1999, and 2000 growing seasons. Numbers and arrows denote individual grazing trials in Table 1 upper = cattle, lower = sheep).

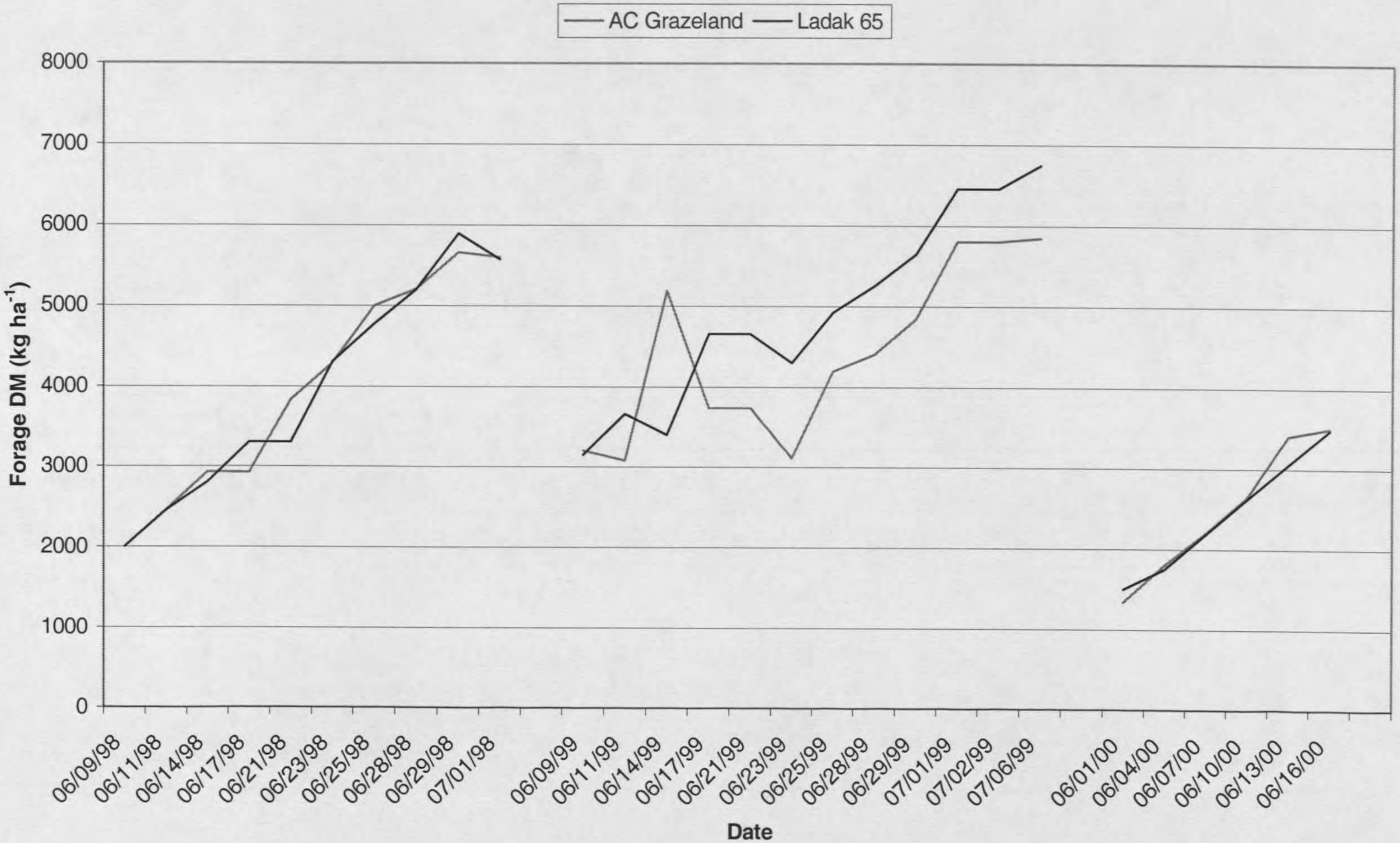

Figure 6. Available forage DM during the first growth of alfalfa in 1998, 1999, and 2000.

Irrigation began on 28 June with hand line sprinklers. During July the cells were irrigated after they were grazed. Adequate moisture was applied for optimum regrowth. Alfalfa was grazed before and after the last frost of spring and the first two frosts of autumn during 1999. Neither maturity (Fig. 5) nor available forage (Fig. 6) were significantly different between varieties during 1999.

In 2000, production of Ladak 65 increased from 1500 to 3500 kg ha $^{-1}$ while AC Grazeland increased from 1400 to 3500 kg ha $^{-1}$ between 29 May and 16 June (Fig. 6). The pasture was mown and irrigation with hand lines began on 11 August. Temperatures were higher than the 30 year average during the end of June, July and August (Fig. 4) and precipitation was below normal (Fig. 3). The pasture was irrigated again beginning 29 August with hand line sprinklers. One main line transected the pasture and two secondary lines were rotated along the main line. The portion of the pasture that contained the secondary lines received approximately 30 percent of the moisture that the mainline did. In portions of the pasture irrigated with the secondary lines, regrowth with Ladak 65 alfalfa was abundant while AC Grazeland failed to regrow. But both alfalfa varieties growing in the portions of the pasture adequately irrigated with the mainline had equivalent maturity (Fig. 5), and available forage (Fig. 6). Despite early initiation and late cessation of grazing, no frost dates were included in this portion of the grazing study.

Bloat Results

From 1998 through 2000, 10 grazing trials with sheep or cattle were conducted. During this study, sheep and cattle were observed for 3442 and 216 animal-grazing days, respectively.

Trial 1, sheep on first growth, 1998

Across this 24-day trial, there were 125 bloat events (BSI > 1) on Ladak 65 and 65 bloat events on AC Grazeland. Mean BSI for sheep grazing Ladak 65 was 1.45, (SE = 0.31) and 1.17 (SE = 0.23) for sheep grazing AC Grazeland (Fig. 7). Across the trial dates, Ladak 65 had significantly higher ($P< 0.05$) bloat severity than AC Grazeland by paired t-tests. Bloat severity scores for Ladak 65 were higher than those of AC Grazeland on six of 24 days ($P < 0.1$). Percent of animals bloated on Ladak 65 was higher on 12 days ($P< 0.1$) [Fig. 8]. Mean percent of ewes bloated per day across Trial 1 was 30 and 14 for Ladak 65 and AC Grazeland, respectively ($P< 0.05$). An average of 25 to 60% of the ewes were affected (BSI >1.0) each day.

Trial 2, sheep on regrowth, 1998

During 25 grazing days of Trial 2, there were 121 bloat events on Ladak 65, and 101 bloat events on AC Grazeland. Across Trial 2, mean BSI for Ladak 65 was 1.53 (SE = 0.26) and 1.41 (SE = 0.23) for AC Grazeland (Fig. 9). By paired t-tests across the dates, bloat severity was significantly higher in Ladak 65 than on AC Grazeland ($P< 0.05$). AC Grazeland had higher BSI ($P < 0.1$) on 11 July, and Ladak 65 had higher BSI on 28 July. Percent of animals bloated (Fig. 10) was greater for AC Grazeland on three

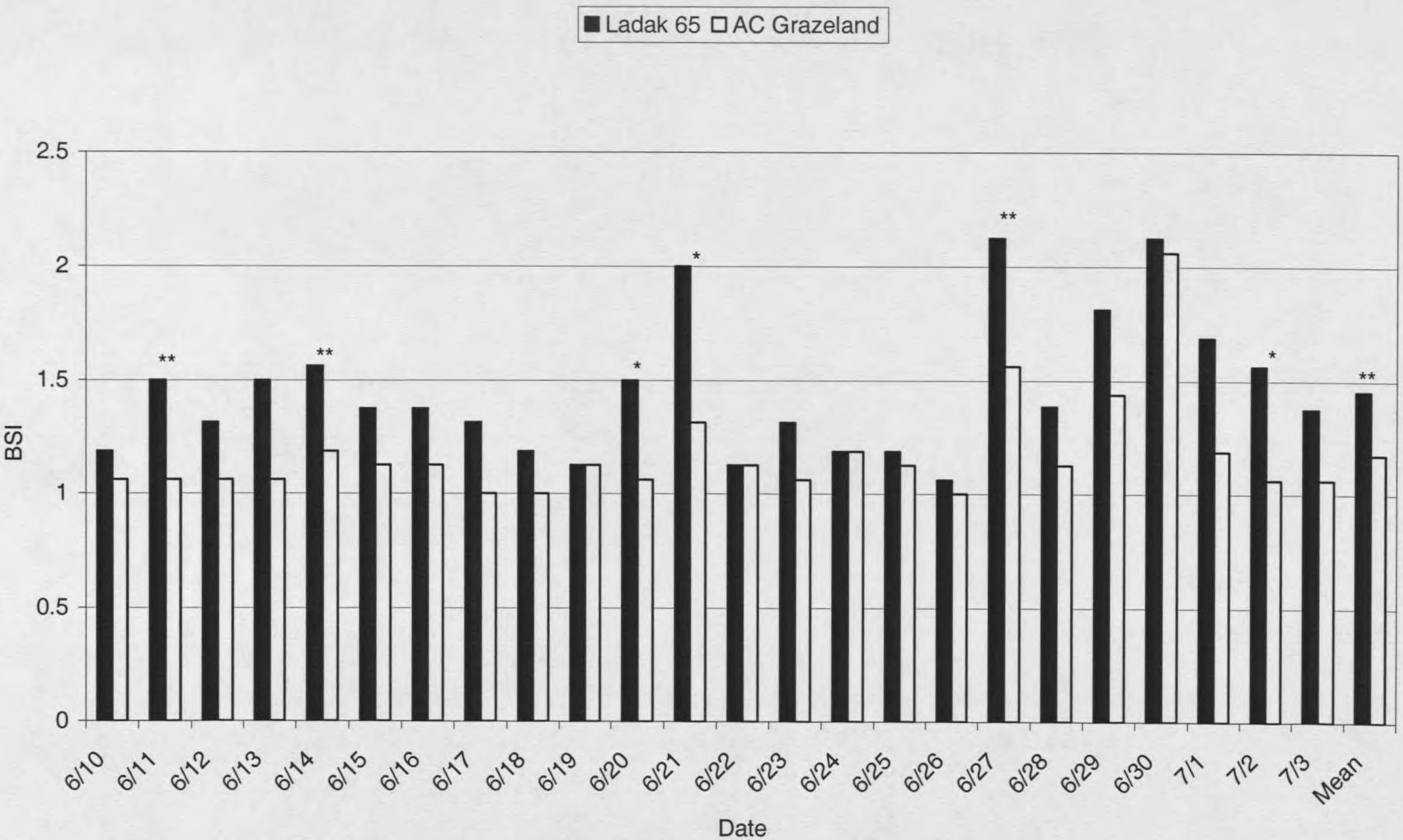

Figure 7. Mean bloat severity index (BSI, 1 = no bloat, 6 = dead) of 32 mixed purebred ewes grazing Ladak 65 or AC Grazeland alfalfa in Trial 1, 1998. * and ** denote significant differences at P< 0.1 or 0.05, respectively. Values based on four replications of each variety.

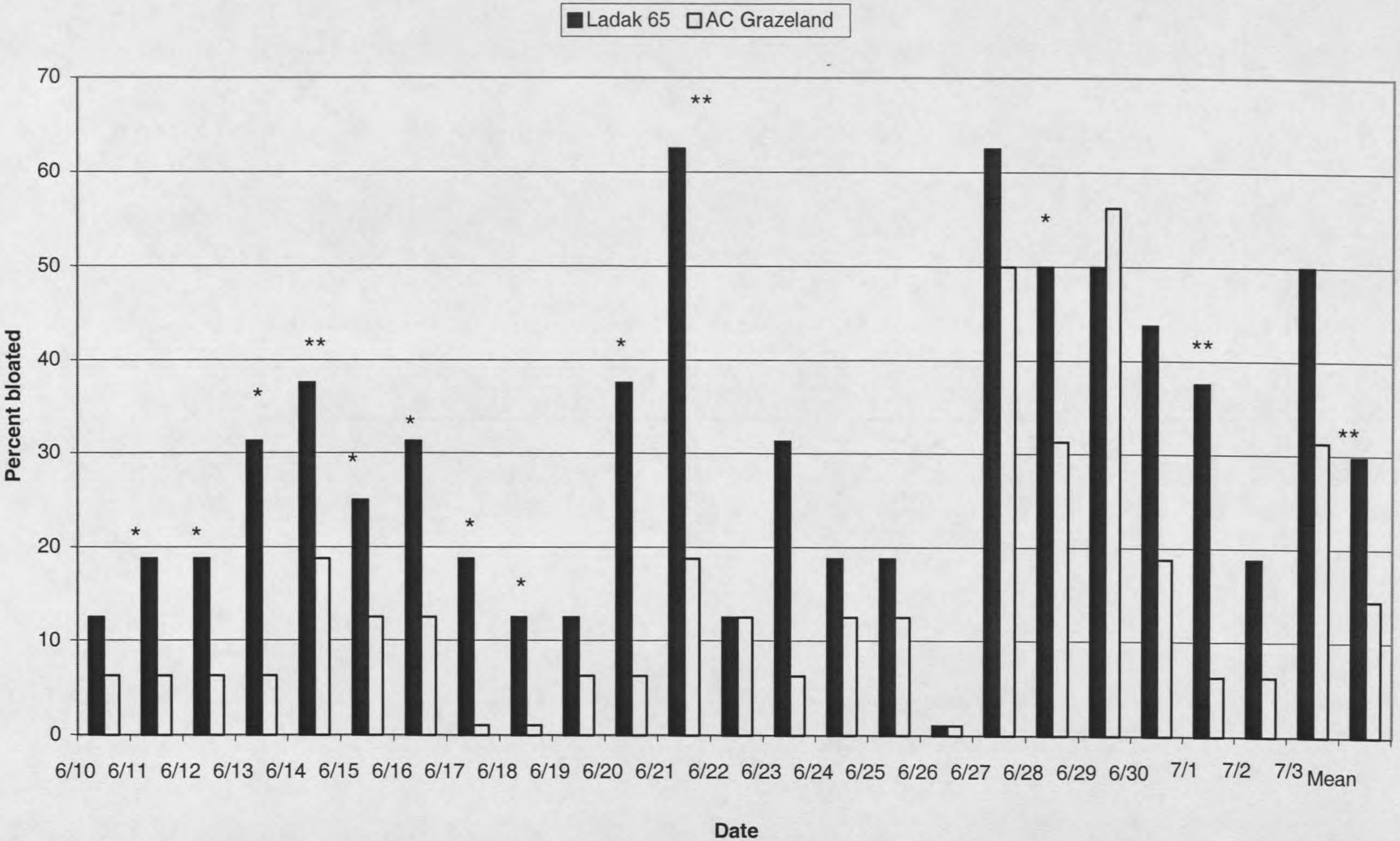

Figure 8. Percent of 32 mixed purebred ewes bloated (BSI >1) while grazing Ladak 65 or AC Grazeland alfalfa in Trial 1, 1998. * or ** denote significant differences at P< 0.1 and 0.05, respectively. Values based on four replications of each variety.

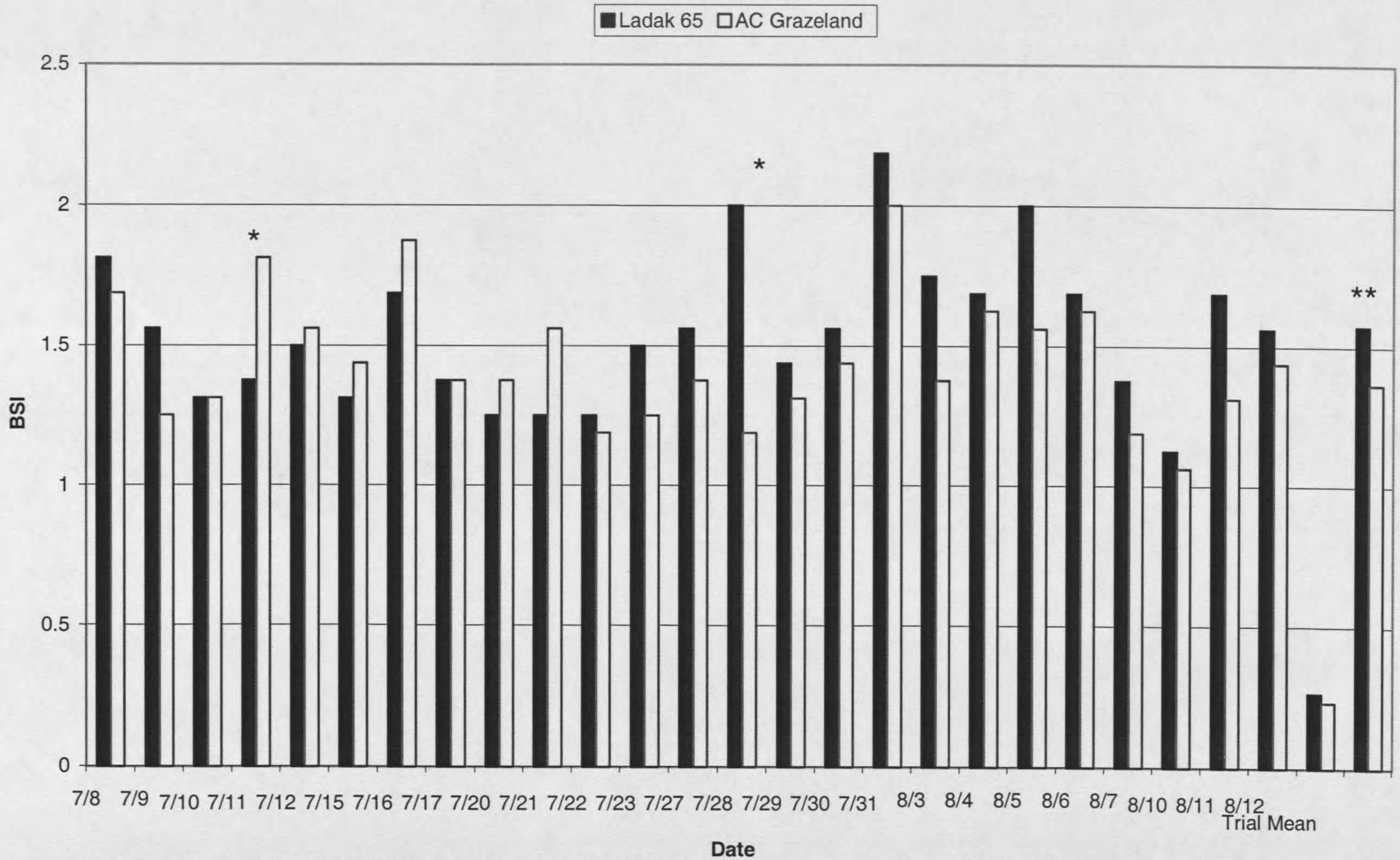

Figure 9. Bloat severity index (BSI, 1=no bloat, 6=death) of 32 mixed purebred ewes grazing Ladak 65 or AC Grazeland alfalfa in in Trial 2, 1998. * and ** denote significant differences at P< 0.1 or 0.05, respectively. Values based on four replications of each variety.

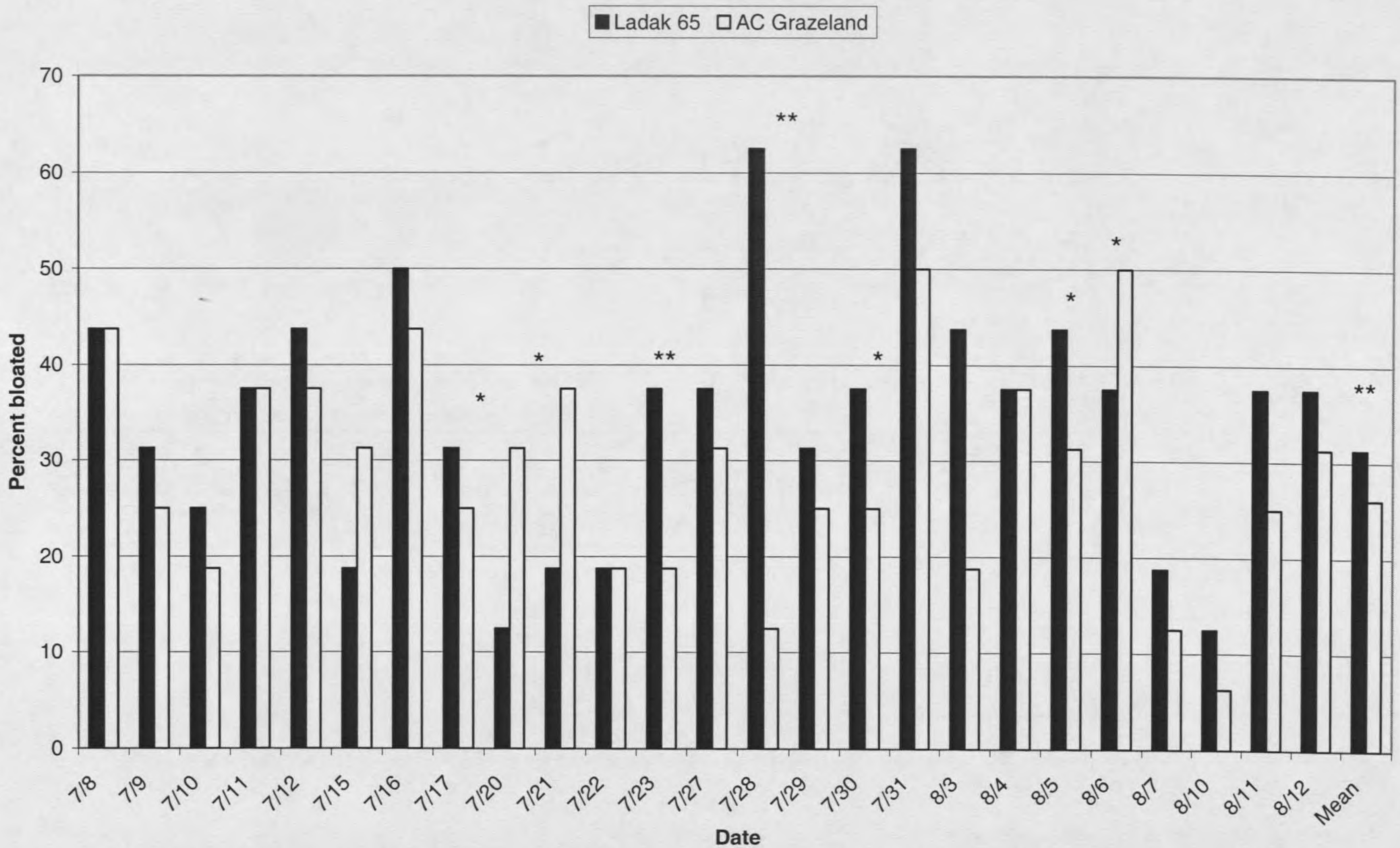

Figure 10. Percent of 32 mixed purebred ewes bloated (BSI > 1) while grazing Ladak 65 or AC Grazeland alfalfa in Trial 2, 1998. * and ** denote significant differences at P< 0.1 or 0.05, respectively. Values based on four replications of each variety.

days (P <0.1) and greater for Ladak 65 on four days (P< 0.1). Across the 25-day period, 35% of the ewes bloated on Ladak 65 and 28% bloated on AC Grazeland (P < 0.05).

Trial 3, cattle on mature first growth, 1998

The heifers were observed while grazing mature alfalfa for seven days in Trial 3. There were 11 bloat events on Ladak 65, and six (2 deaths) on AC Grazeland. The two severe bloat deaths occurred within 20 minutes of initiation of grazing on 8 July. The rapid onset and severity of these two bloat events precluded any attempts to save these heifers. Therefore, this portion of the trial was terminated. Mean BSI for Ladak 65 (2.75, SE = 0.96) was higher than that of AC Grazeland (1.55, SE = 1.38) [Fig. 11]. Across the seven-day trial, bloat severity of AC Grazeland was significantly less (P < 0.05) than that of Ladak 65 by paired t-tests. Percent of cattle bloated was greater on Ladak 65 for two days (P < 0.1) [Fig. 12]. Mean percent cattle bloating in Trial 3 was 45 and 21 percent for Ladak 65 and AC Grazeland, respectively. Despite bloat incidence and severity being higher across the trial for Ladak 65, both heifers that died of acute bloat were grazing AC Grazeland. This trial illustrated the variable nature of bloat as well as the speed with which acute bloat develops.

Trial 4, sheep on first growth, 1999

For the first segment of the 1999 grazing season, 40 yearling ewes and 16 mature ewes grazed on alfalfa for 25 days. There were 131 bloat events (BSI > 1) on Ladak 65 and 122 on AC Grazeland. Across Trial 4, mean BSI of Ladak 65 and AC Grazeland were 1.46 (SE = 0.7) and 1.37 (SE = 0.7) respectively (Fig. 13) [P < 0.05]. There were

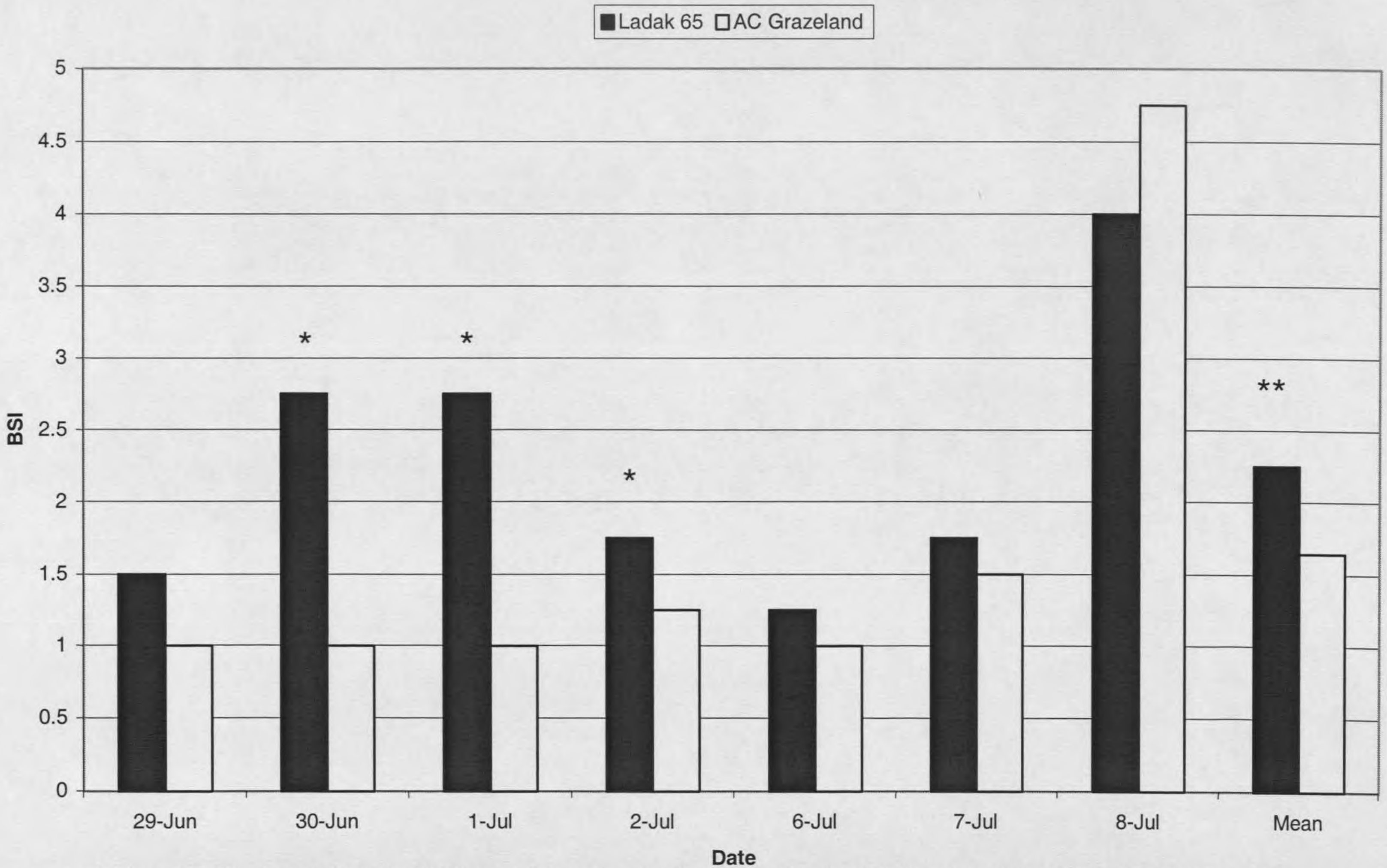

Figure 11. Bloat severity index (BSI 1=no bloat, 6 = dead) of 8 yearling beef heifers grazing mature Ladak 65 or AC Grazeland alfalfa in Trial 3. * or ** denote significant differences at $p<0.1$ and 0.05, respectively. Due to two deaths on 8 July on AC Grazeland, the trial was terminated. Values based on four replications of each variety.

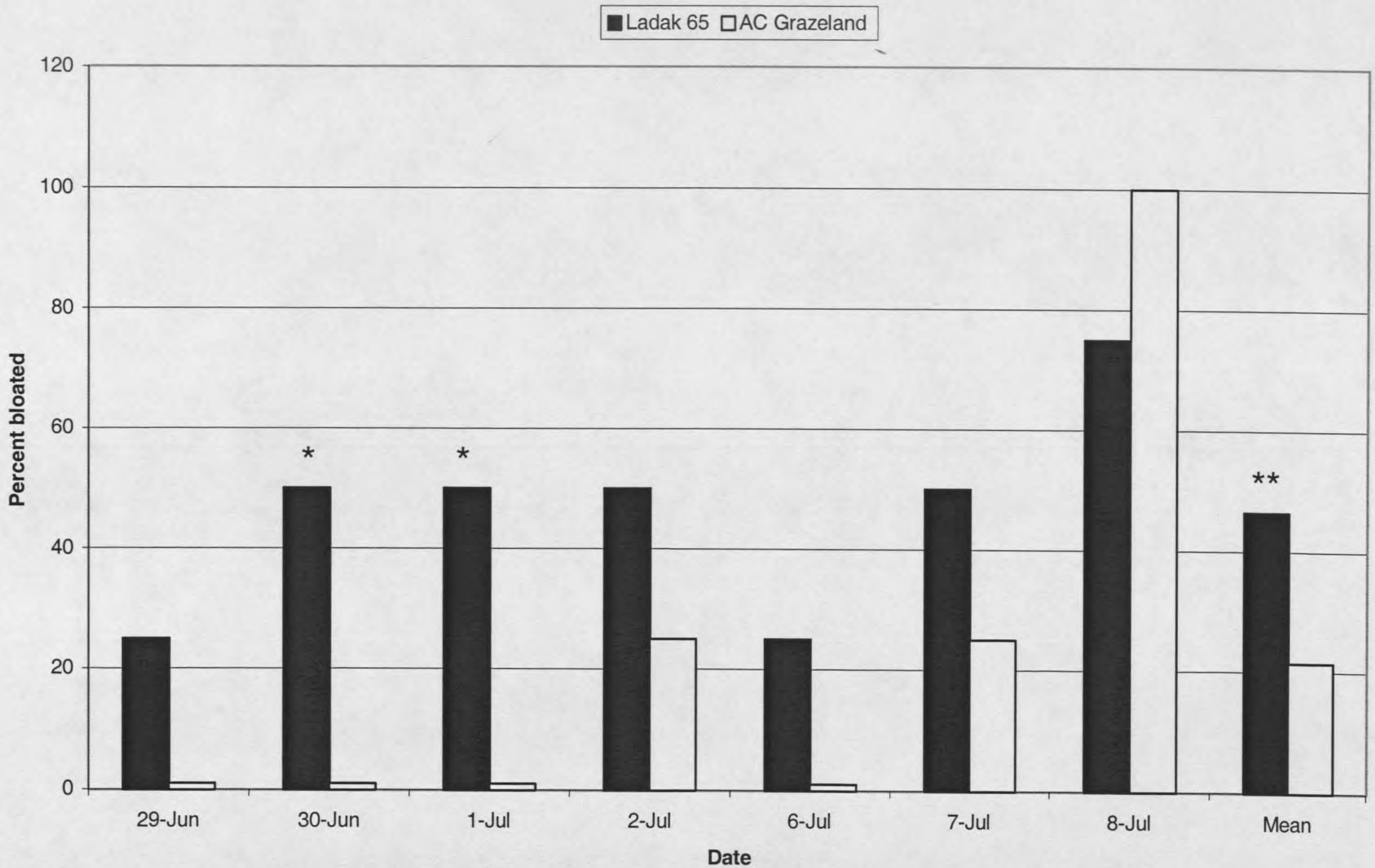

Figure 12. Percent of 8 yearling beef heifers bloated while grazing mature Ladak 65 or AC Grazeland alfalfa in trial 3. * or ** denote significant differences at P<0.1 or 0.05, respectively. Values based on four replications of each variety.

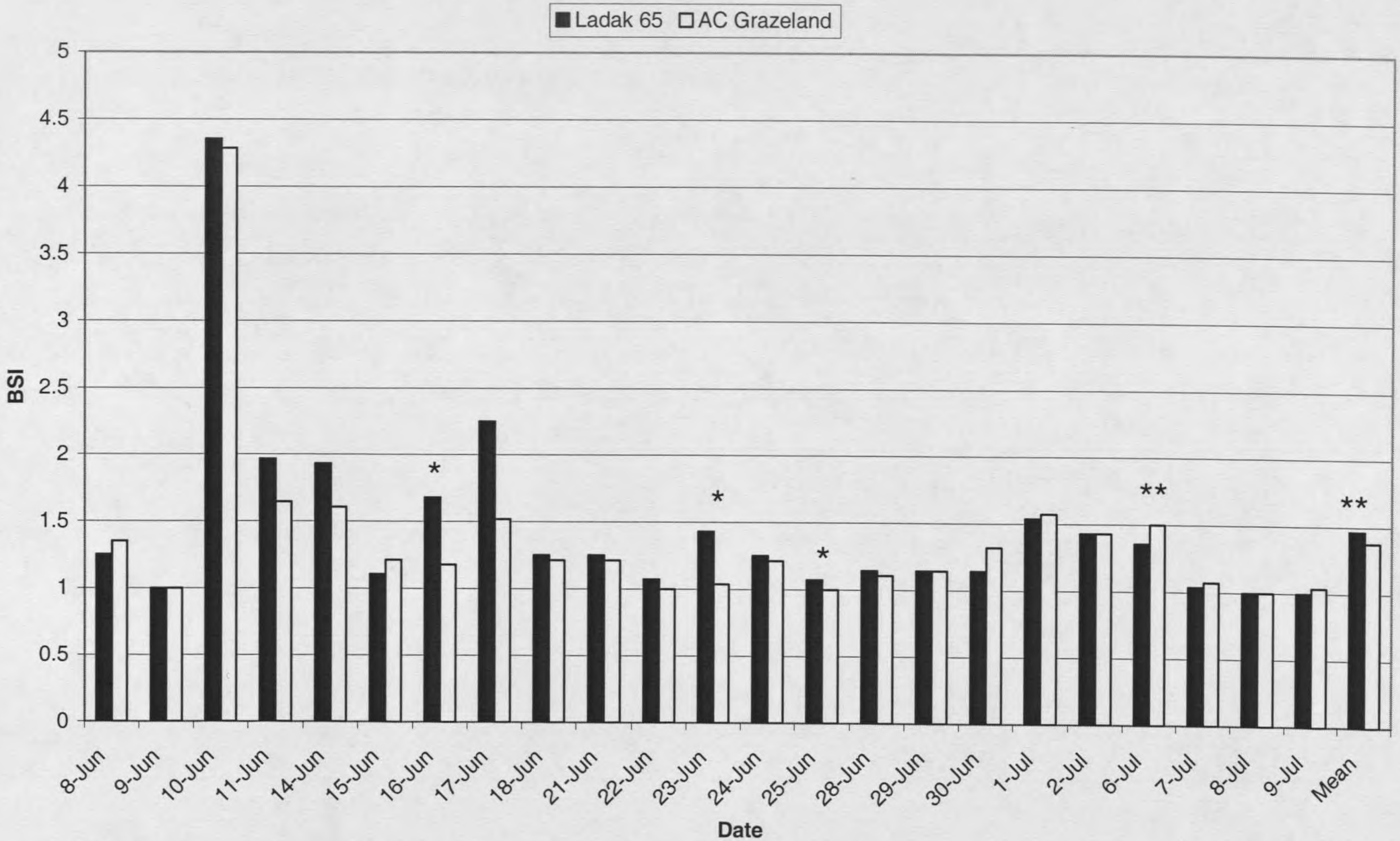

Figure 13. BSI (1 = no bloat, 6 = dead) of 40 mixed purebred ewes grazing Ladak 65 or AC Grazeland alfalfa in trial 4, 1999. * or ** denote significant differences at p<0.1 and 0.05, respectively. Values based on four replications of each variety.

four days of significant difference in BSI between varieties; Ladak 65 was higher (P< 0.1) than AC Grazeland on three days. Across Trial 4, mean percent bloated per day were 12 and 10 for Ladak 65 and AC Grazeland, respectively (P < 0.05) [Fig. 14]. A bloat "storm" occurred on 10 June, when over 90% of the ewes bloated. This event coincided with the last hard seasonal frost, which may have released plant components related to bloat (MacAdam et al., 1995).

Trial 5, sheep on regrowth, 1999

For the second session in 1999, there were only two bloat events during 12 days of observation (Fig. 15). Across all 12 days, varieties did not differ (P > 0.1) for BSI or percent bloated (Fig. 16). During this grazing period, the alfalfa was mature and heavily infested with thrips. These could have accounted for limited bloat incidence compared with a similar time period during 1998, as well as for the lack of measurable difference between varieties.

Trial 6, cattle on regrowth, 1999

During 10 grazing days with eight mature cannulated beef cows, there were 15 bloat events (BSI > 1.0) on Ladak 65, and 10 bloat events on AC Grazeland. Across Trial 6, mean BSI was 1.20 (SE = 0.22) for cattle grazing Ladak 65 and 1.23 (SE = 0.35) for cattle grazing on AC Grazeland (P < 0.1) [Fig. 17]. There were two days in which cattle grazing AC Grazeland had higher BSI scores than cattle grazing Ladak 65 (P < 0.1). On 11 and 19 August, over half of the cows bloated and across Trial 6 mean percent of cattle bloated did not differ between varieties (P > 0.1) [Fig. 18]. These results

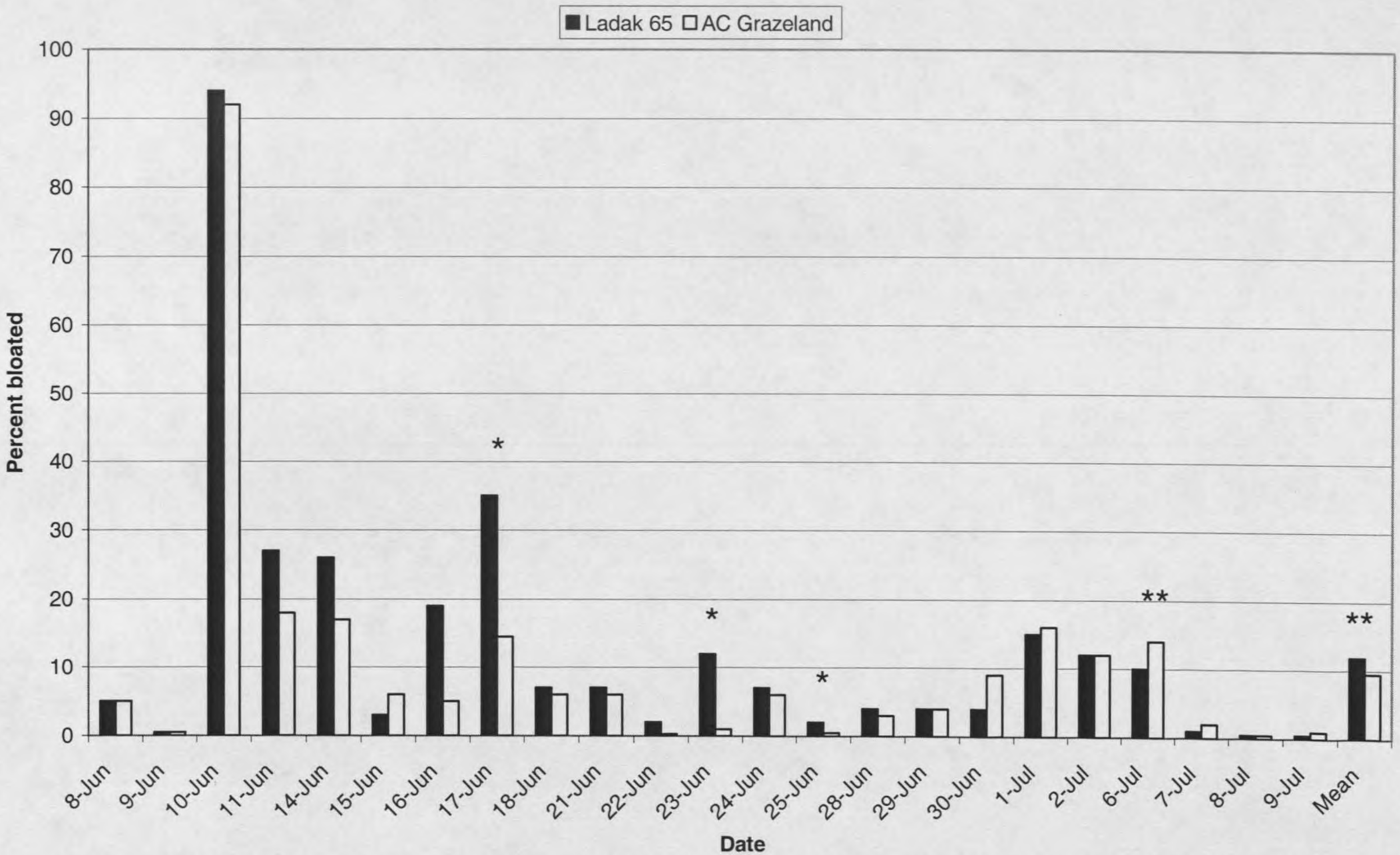

Figure 14. Percent of 40 mixed purebred ewes bloated (BSI >1) while grazing Ladak 65 or AC Grazeland alfalfa in Trial 4 in 1999. * or ** denote significant differences at $p<0.1$ and 0.05, respectively. Values based on four replications of each variety.

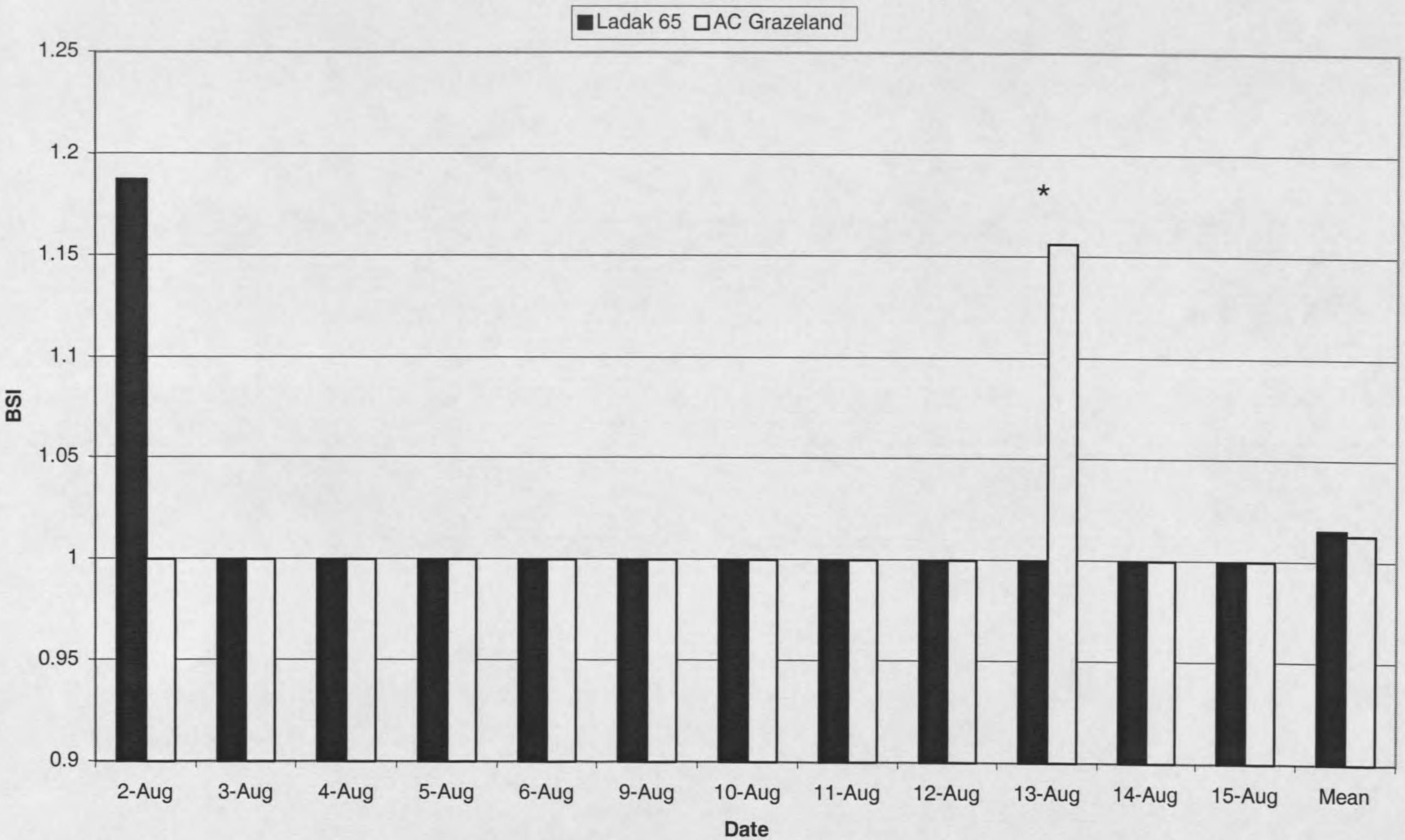

Figure 15. Mean bloat severity index (BSI, 1=no bloat, 6=death) of 32 mixed purebred ewes grazing Ladak 65 or AC Grazeland alfalfa in Trial 5 in 1999. * denotes significant difference at P<0.1. Values based on four replications of each variety.

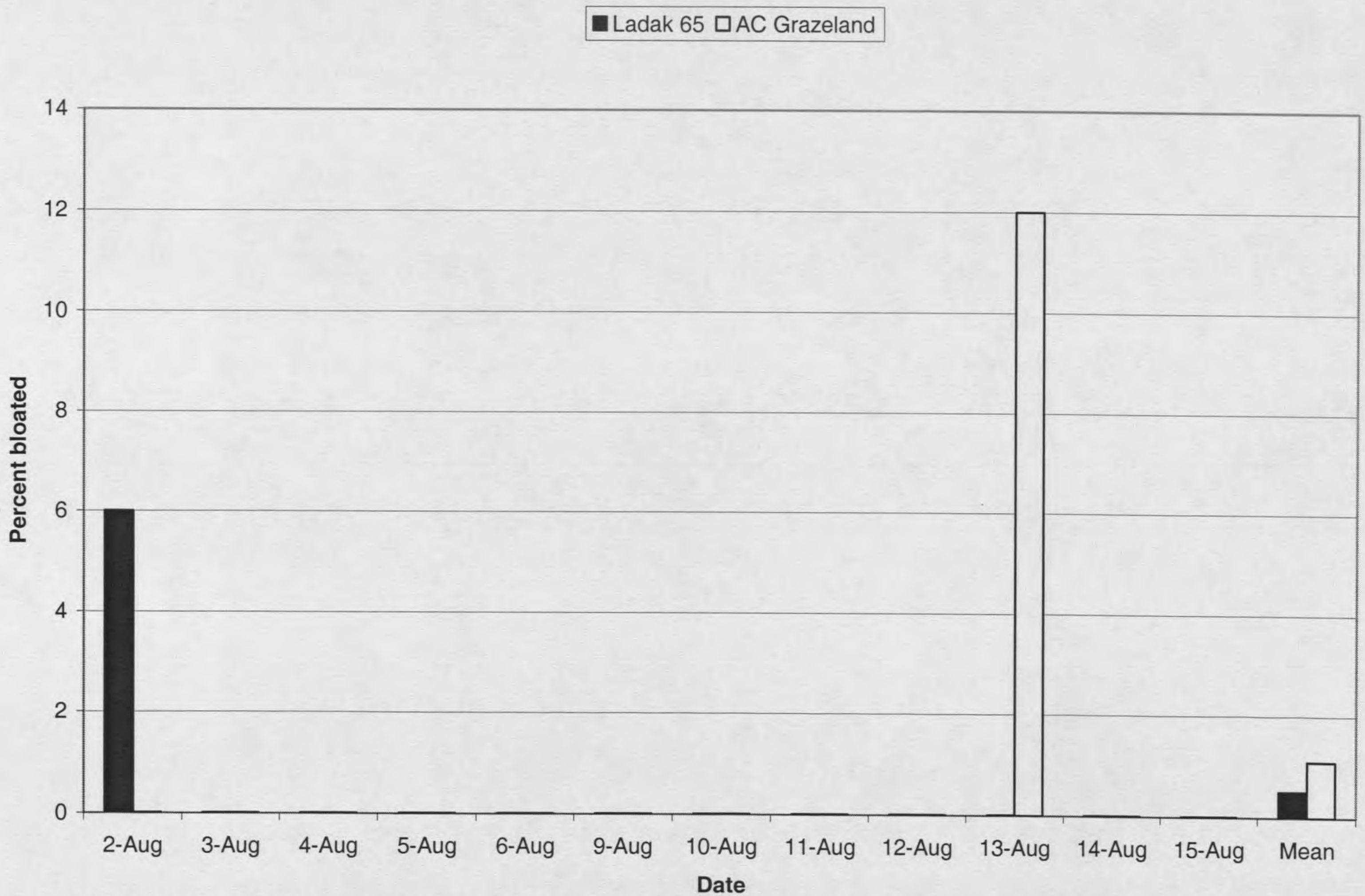

Figure 16. Percent of 32 mixed purebred ewes bloated (BSI > 1) while grazing Ladak 65 or AC Grazeland during Trial 5, 1999. Values based on four replications of each variety.

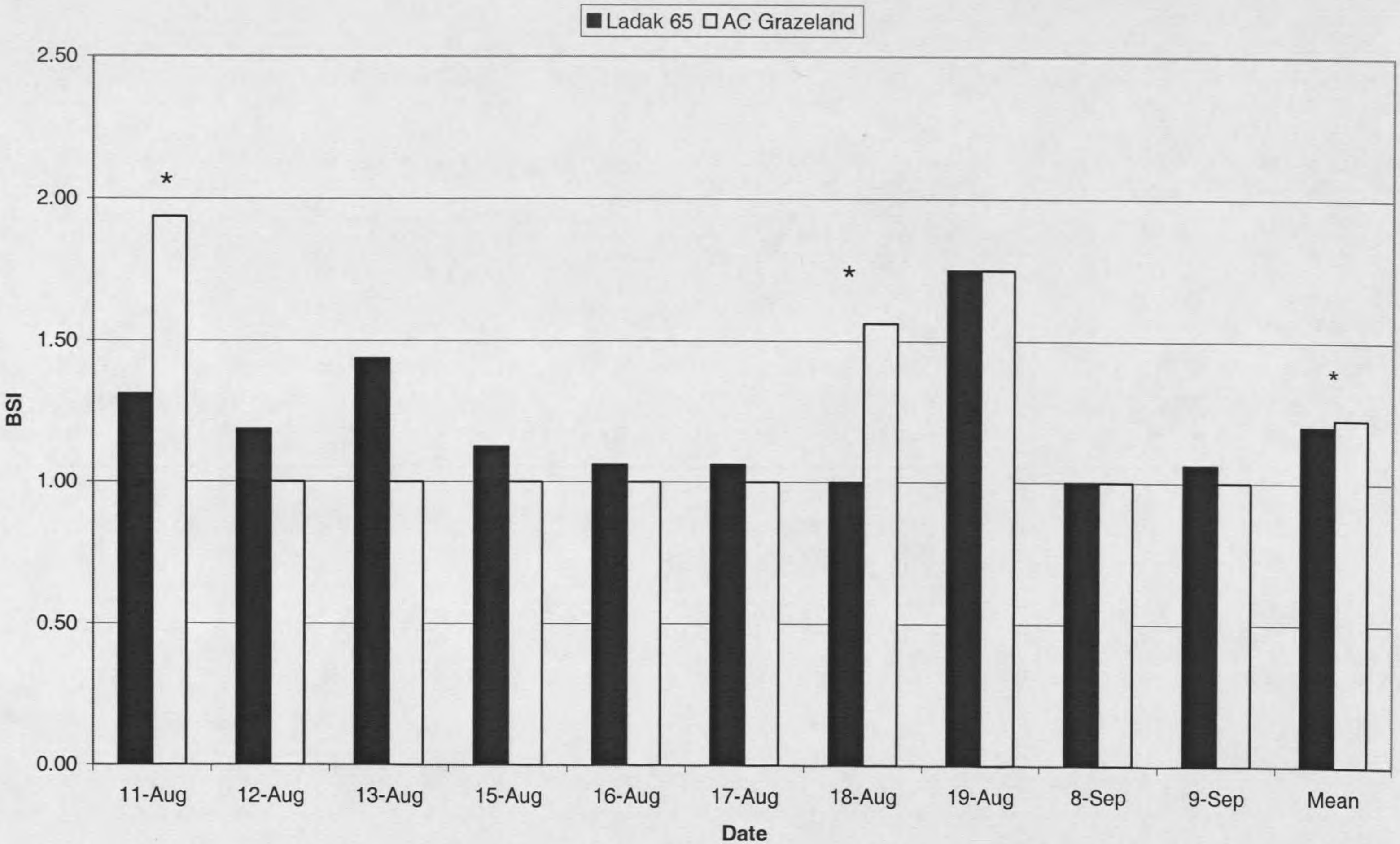

Figure 17. Mean bloat severity Index (BSI, 1=no bloat, 6=dead) of 8 mature beef cows grazing Ladak 65 or AC Grazeland alfalfa during Trial 6, 1999. * denotes significant differences at P<0.1. Values based on four replications of each variety.

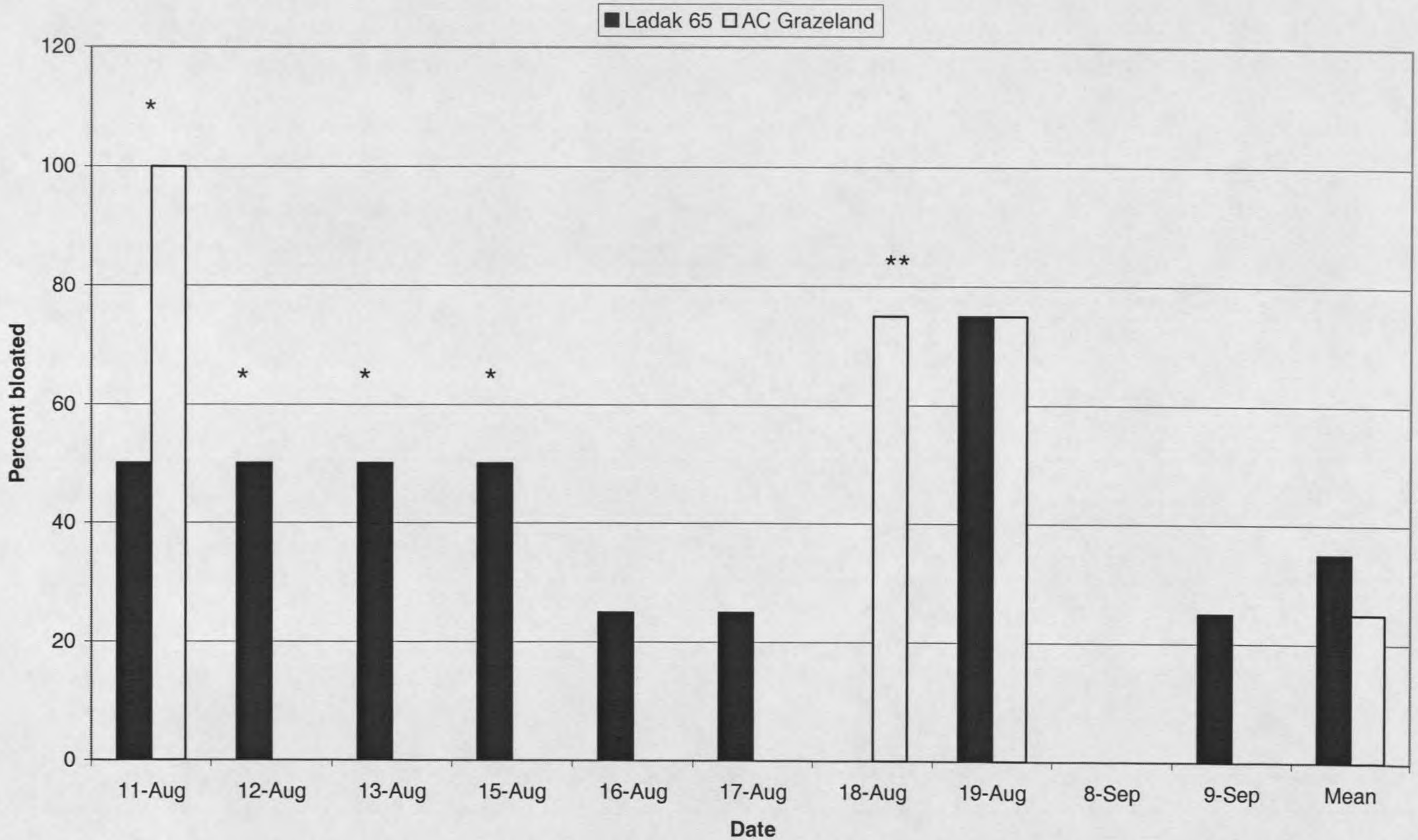

Figure 18. Percent of 8 mature beef cows bloated (BSI > 1) while grazing Ladak 65 or AC Grazeland alfalfa in trial 6, 1999. * and ** denote significant differences at P<0.1 and 0.05, respectively. Values based on four replications of each variety.

are in contrast to the previous year's severe bloat with cattle. The cattle during 1998 were yearling heifers without cannulas while cattle in 1999 were mature cannulated cows. Perhaps characteristics of cattle maturity, such as rumen size, or characteristics of cannulation, such as air leakage, reduced the effects of bloat. Variable plant factors may also have played a role.

Trials 7-10, AC Grazeland and Ladak 65 alfalfa without N fertilizer

Trial 7, sheep on regrowth, 1999

During four days with 24 sheep there were five bloat events on each variety. There were no significant differences in either mean BSI (Fig. 19) or percent bloated between AC Grazeland and Ladak 65 (Fig. 20) [P > 0.1]. However, it is interesting to note that all cases of bloat occurred immediately after frost (10 September) when over 80% of ewes on both varieties bloated.

Trial 9, sheep on first growth, 2000

During 15 observed grazing days with 24 sheep, there were 28 bloat events on Ladak 65 and 36 on AC Grazeland. Across Trial 9, there were no significant differences (P > 0.1) between varieties in mean BSI (Fig. 21), however, mean percent bloated ewes was higher (Fig. 22) for AC Grazeland (24%) than for Ladak 65 (17%) [P < 0.1].

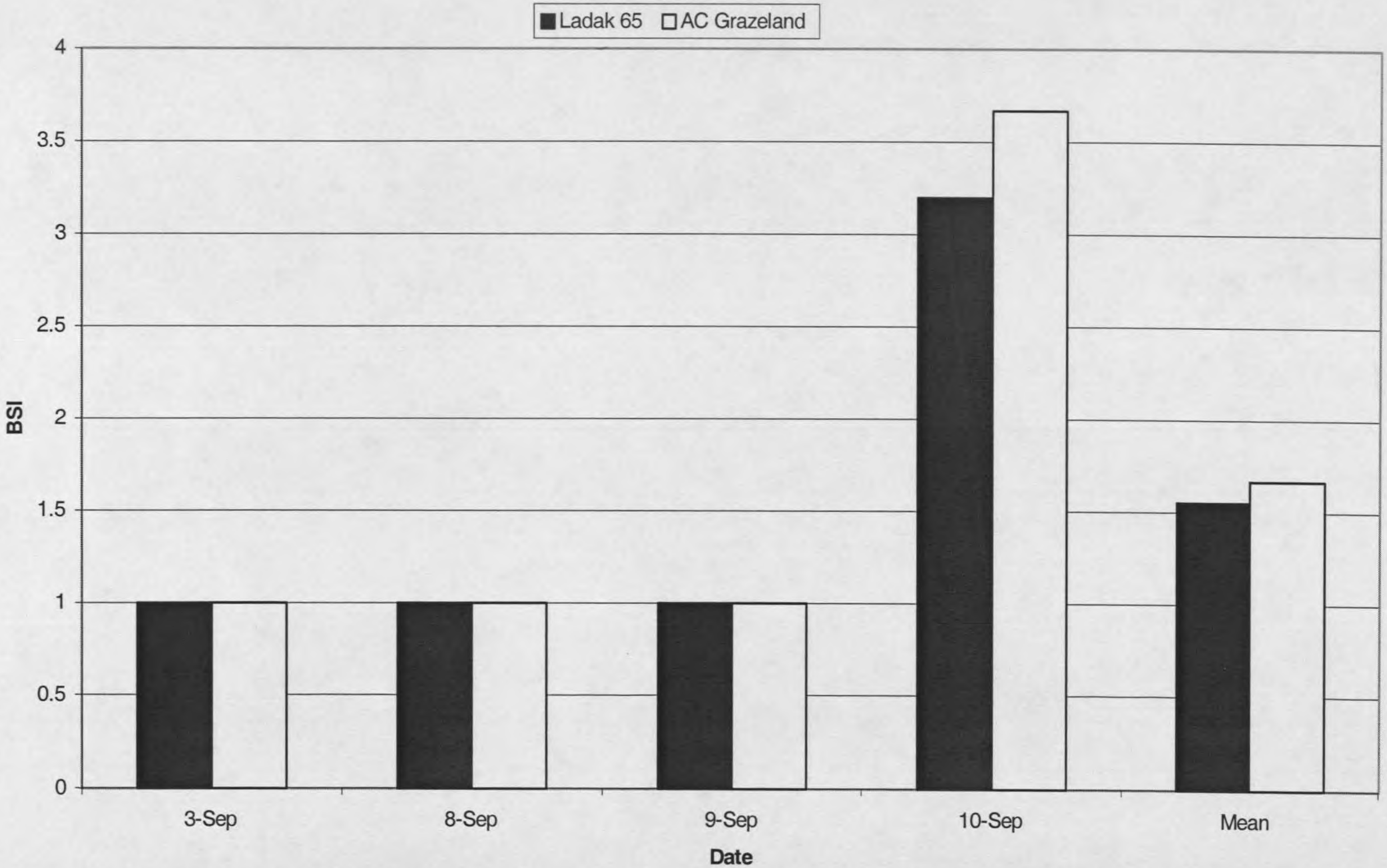

Figure 19. Mean bloat severity scores (BSI, 1=no bloat, 6=dead) of 24 mixed purebred ewes grazing Ladak 65 or AC Grazeland alfalfa in Trial 7, 1999. Values based on four replications of each variety.

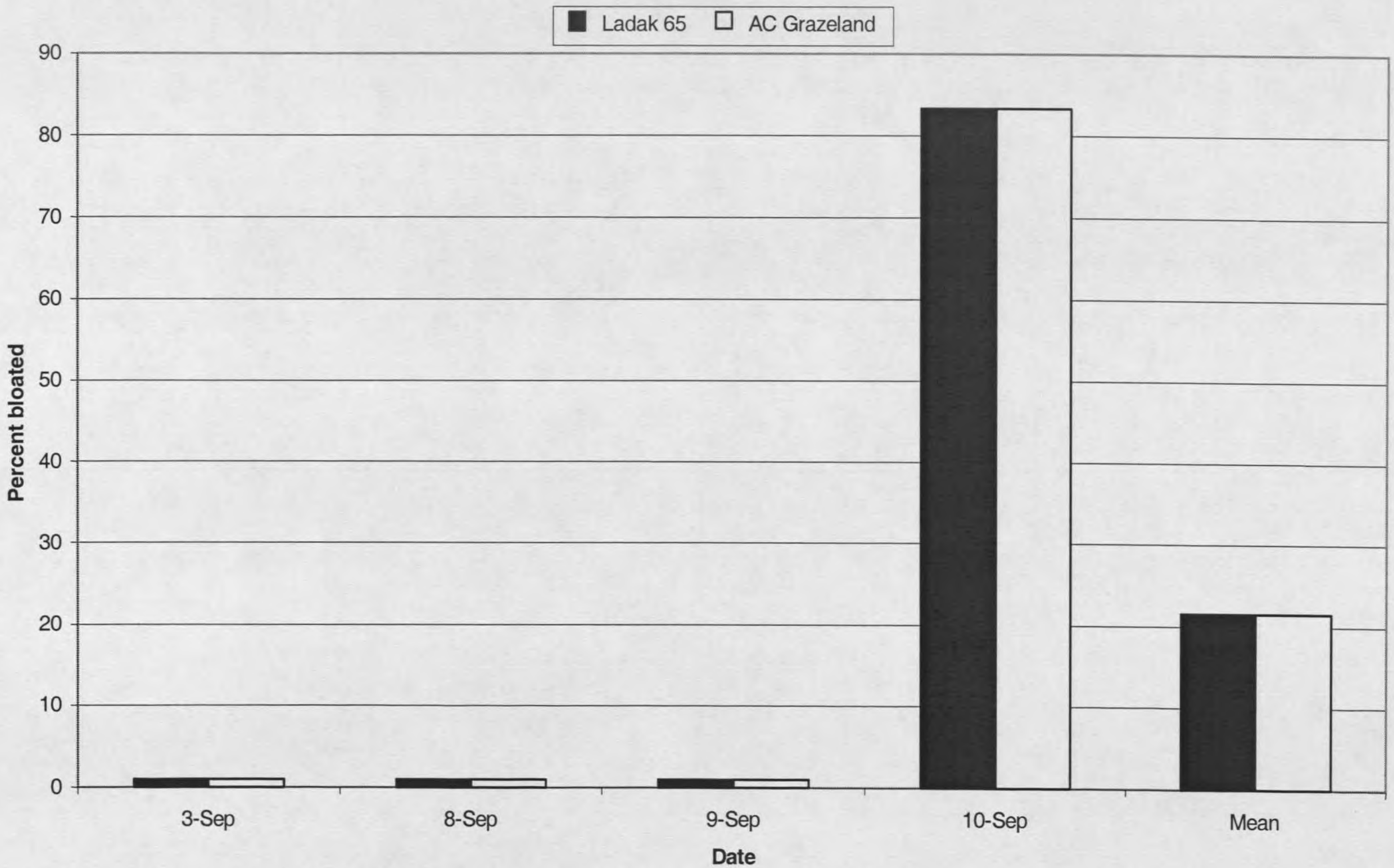

Figure 20. Mean percent of 24 mixed purebred ewes bloated while grazing Ladak 65 or AC Grazeland alfalfa in Trial 7, 1999. Values based on two replications of each variety.

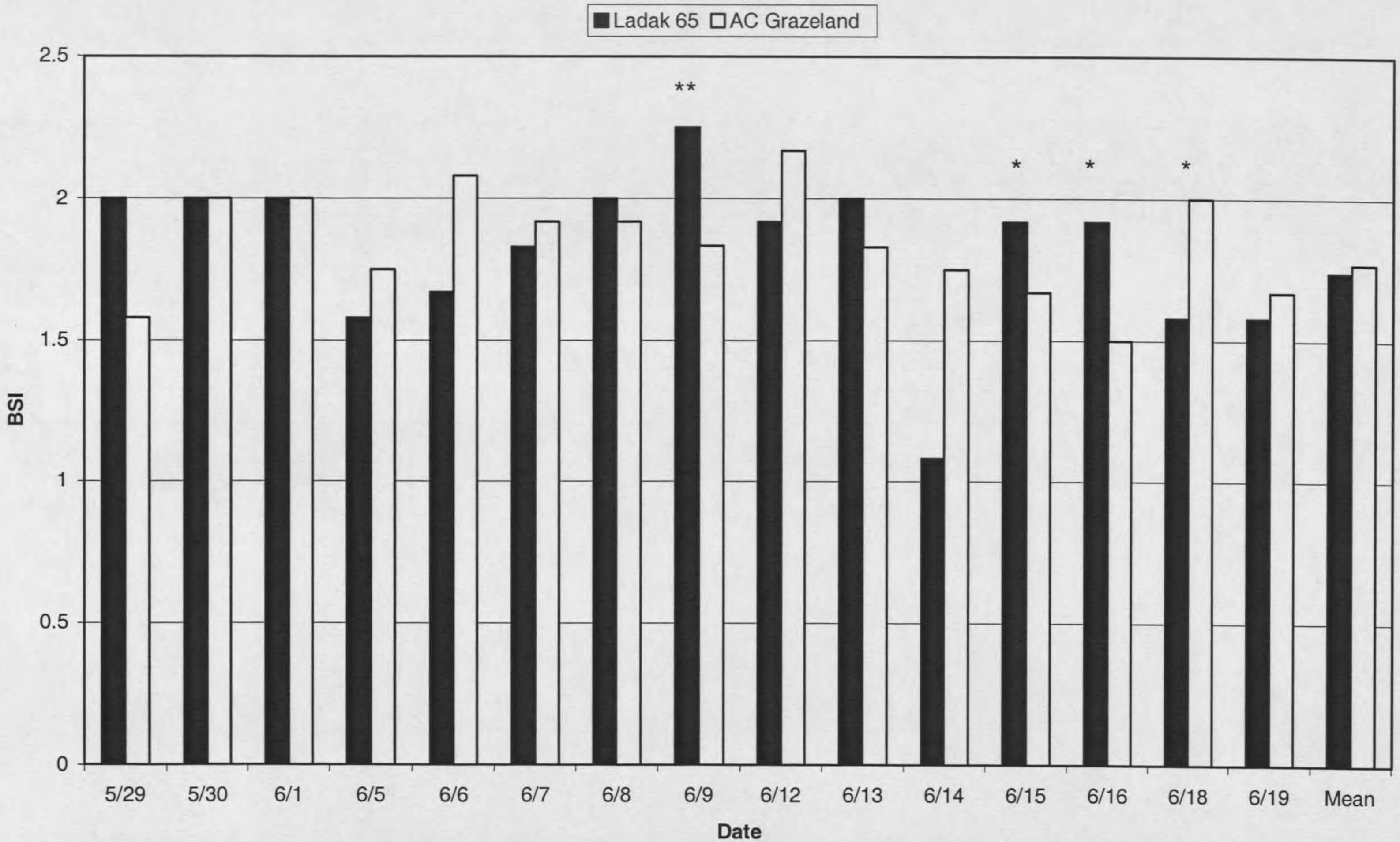

Figure 21. Mean bloat severity index (BSI, 1 = no bloat, 6 = dead) of 24 mixed purebred ewes grazing Ladak 65 or AC Grazeland alfalfa in Trial 9, 2000. * and ** denote significant differences at P < 0.1 and 0.05, respectively. Values based on two replications of each variety.

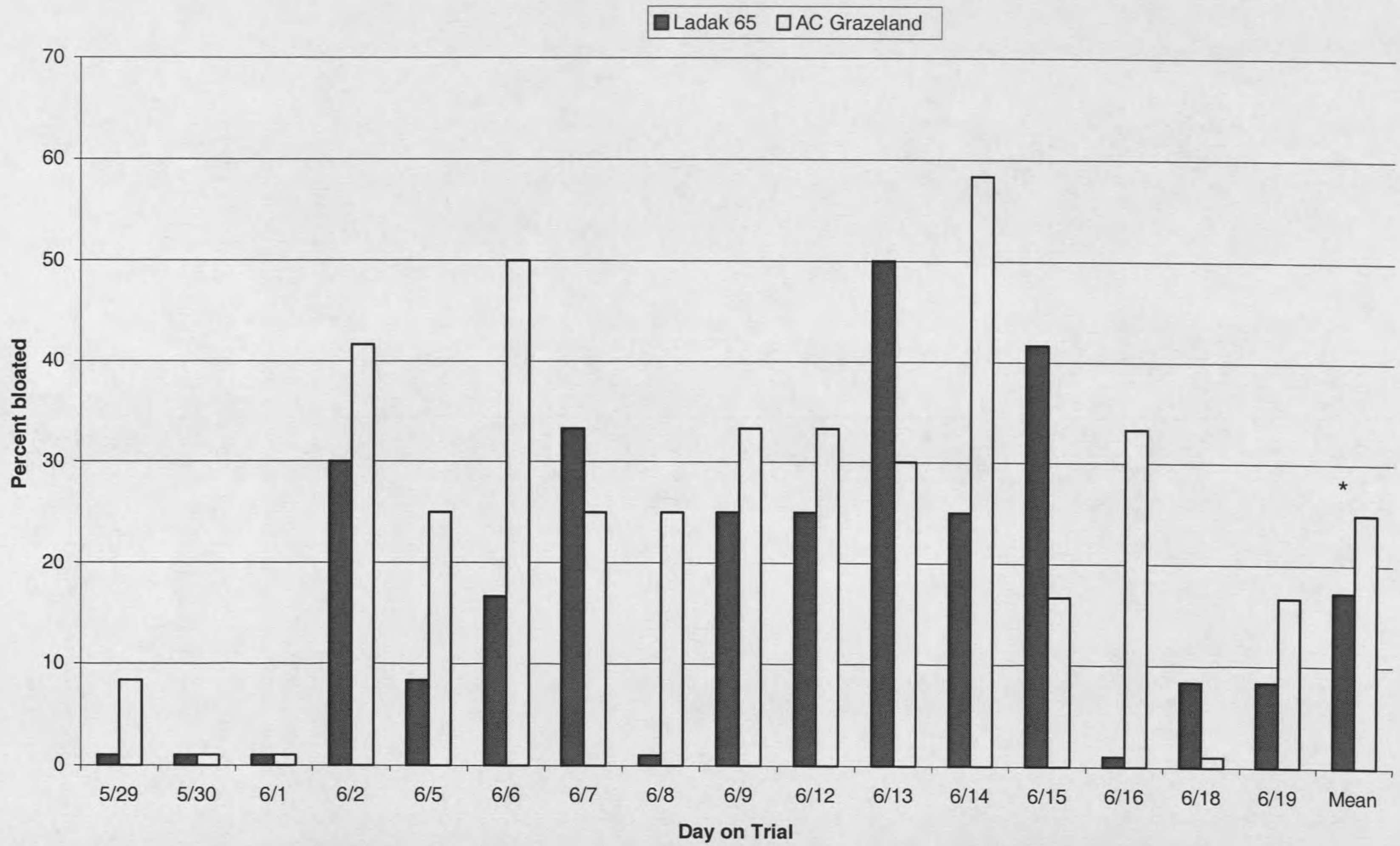

Figure 22. Percent of 24 mixed purebred ewes bloated (BSI > 1) while grazing Ladak 65 or AC Grazeland alfalfa in Trial 9, 2000. * denotes significant difference at $P < 0.1$. Values based on two replications of each variety.

Trial 10, cattle on fall regrowth, 2000

During 15 periods in six days there were no significant differences (P> 0.1) between varieties in mean BSI (Fig. 23) or percent bloated (Fig. 24).

Trials 7-10, AC Grazeland and Ladak 65 alfalfa with and without N fertilizer

Trial 7, sheep on regrowth, with or without N fertilizer, 1999

During this four-day trial, 24 sheep were grazed on AC Grazeland and Ladak 65 alfalfa with or without N fertilizer. This trial was intentionally timed to include the first hard seasonal frost. Grazing began on 3 September, however, the forage base was not adequate, and grazing of these cells was temporarily halted until frost was forecast. The sheep were grazed for two hours each morning that frost was forecast (3, 8, 9 and 10 September). Overall, there were 23 bloat events on fertilized Ladak 65, 5 bloat events on non-fertilized Ladak 65, 19 bloat events with fertilized AC Grazeland and 5 with non-fertilized AC Grazeland (Table 2). No ewes bloated on either variety without N fertilizer before the frost. With N fertilizer, both varieties resulted in significantly ($P < 0.05$) higher BSI than without fertilizer. High levels of soluble proteins and forms of N have been implicated in bloat (Hall et al., 1984; Hall and Majak, 1991; Howarth et al., 1977). This appears to be the first report in which alfalfa pasture bloat was induced by N fertilization. After the frost (10 September), 80% of sheep on all treatments of alfalfa bloated, with no significant differences among treatments ($P > 0.1$). There were no significant V x F interactions on any day.

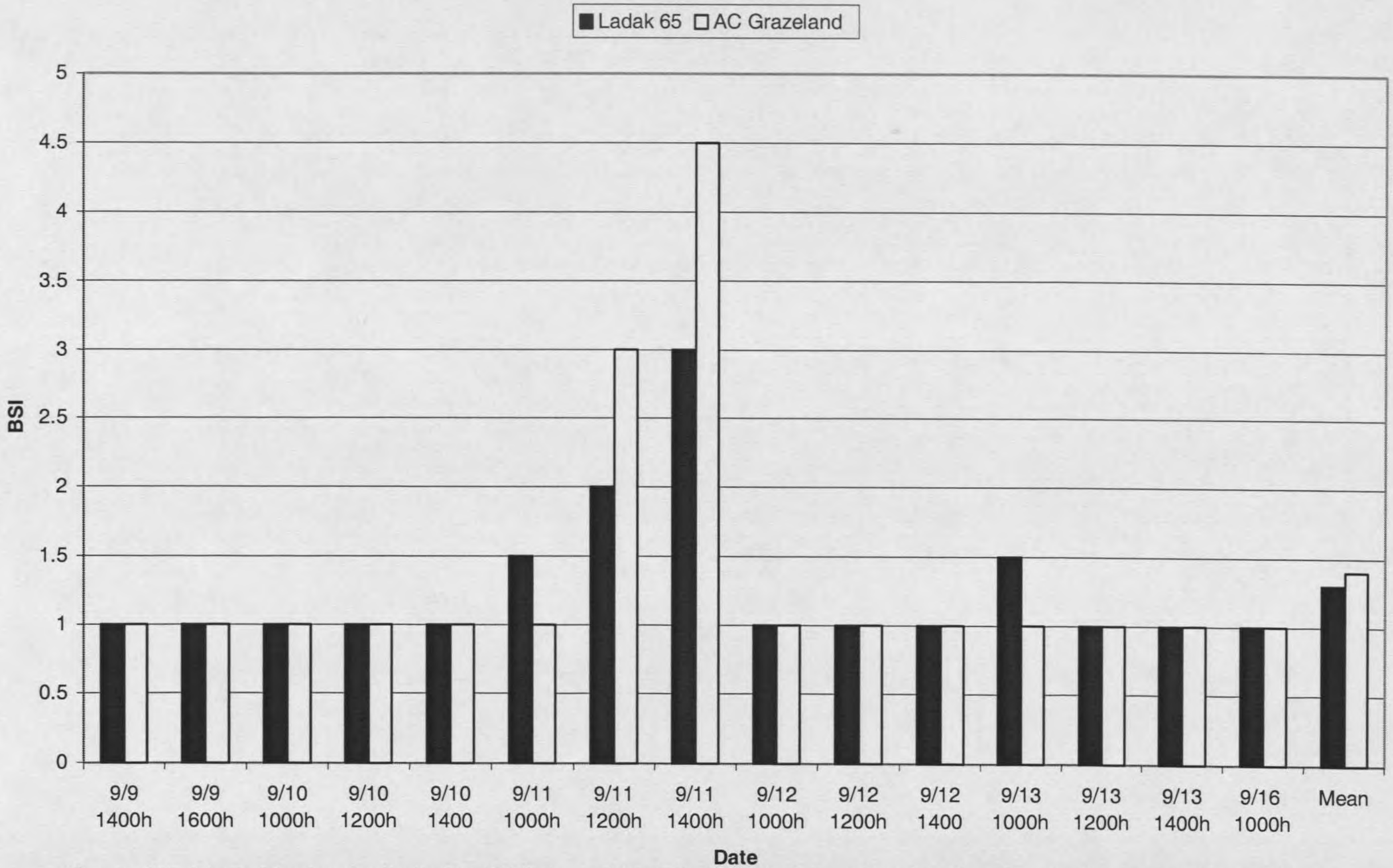

Figure 23. Mean bloat severity index (BSI, 1=no bloat, 6=death) of 8 mature beef cows grazing Ladak 65 or AC Grazeland alfalfa in Trial 10, 2000.

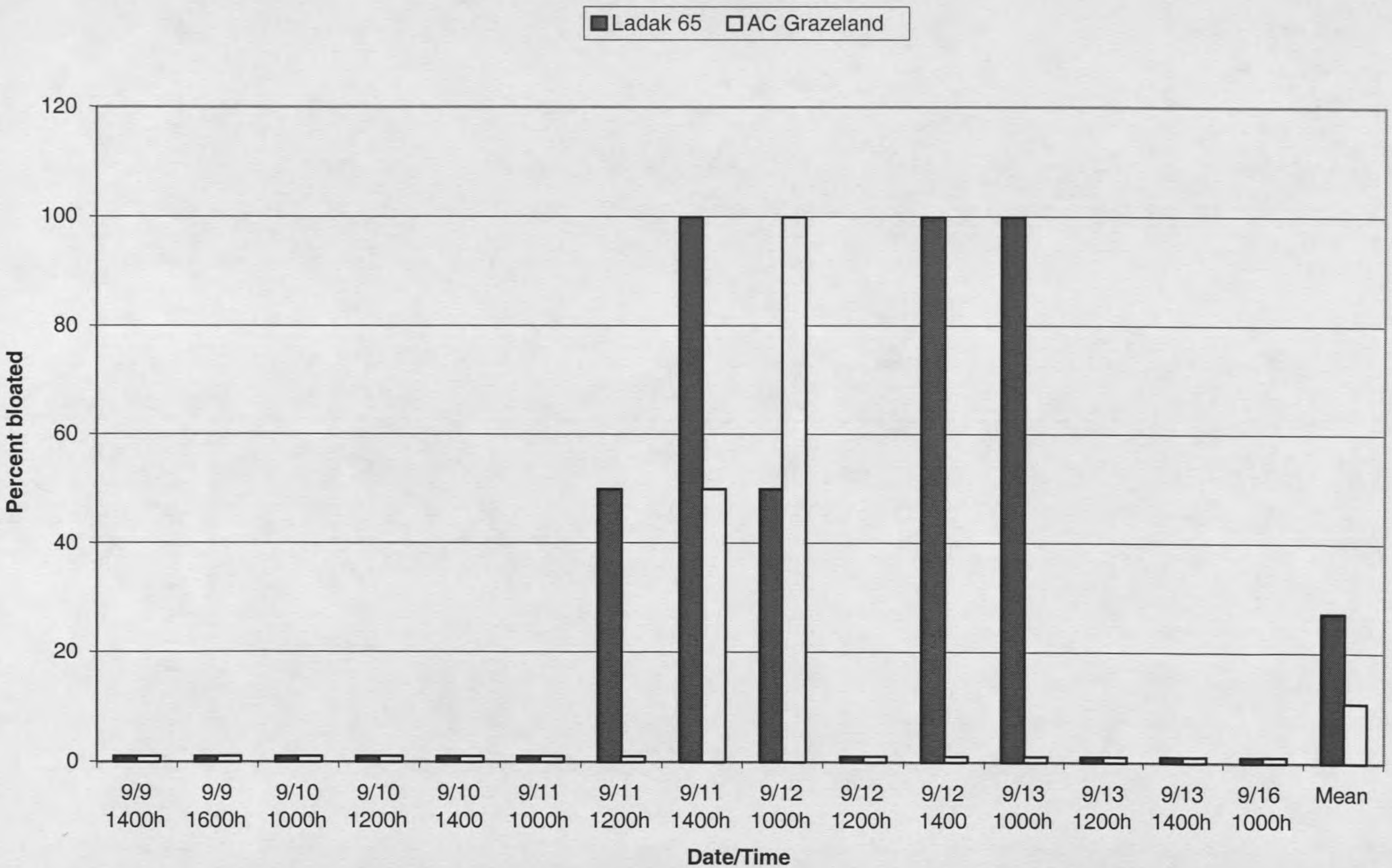

Figure 24. Percent of 8 mature beef cows bloated (BSI > 1) while grazing AC Grazeland and Ladak 65 alfalfa during Trial 10, 2000. Values based on two replications of each variety.

Table 2. Effect of N fertilization on bloat severity index (BSI, 1 = no bloat, 6 = dead) and percent bloated of 24 mixed purebred ewes grazing Ladak 65 or AC Grazeland (ACG) alfalfa in Trial 7, 1999. Values based on two replications of each treatment.

BSI	Without N Fertilizer		With N Fertilizer		LSD		N Fertilizer Status Across Varieties			Varieties (V) Across Fertilizer Status			VxF
DATE	Ladak 65	ACG	Ladak 65	ACG	P=0.05	P=0.1	Without N	With N	P <	Ladak 65	ACG	P <	P <
3 Sept	1.00	1.00	4.40	3.60	0.98	0.66	1.00	4.00	0.05	2.70	2.30	NS	NS
8 Sept	1.00	1.00	3.00	3.43	2.04	1.37	1.00	3.22	0.05	2.00	2.22	NS	NS
9 Sept	1.00	1.00	4.17	3.17	2.49	1.67	1.00	3.67	0.05	2.58	2.08	NS	NS
Pre-frost Mean	1.00	1.00	3.86	3.14	1.47	0.99	1.00	3.63	0.05	2.43	2.20	0.1	NS
10 Sept	3.20	3.67	3.17	2.40	NS	NS	3.43	2.78	NS	3.18	3.03	NS	NS
Post-frost Mean	1.55	1.67	3.68	3.15	1.25	0.84	1.61	3.42	0.05	2.62	2.40	0.1	NS
Mean % Bloated Trial 7	22.0	22.0	96.0	75.0	53.0	36.0	22.0	85.0	0.05	59.0	48.0	NS	NS

The bloat storm after frost was similar to previous reports (Hall et al., 1991; Hall and Majak, 1991). Immediately after frost, plant cell walls are digestible and soluble proteins and carbohydrates are rapidly available. Forage samples were analyzed for NO_3 and CP. Thirty percent of samples contained greater than 6000 ppm NO_3, which is considered a potentially toxic concentration (Edwards and McCoy, 1980). Mean percent bloated was 21 percent for both varieties without fertilizer, 76 percent for AC Grazeland with N fertilization and 95 percent for Ladak 65 with N fertilization.

Trial 8, cattle on fall regrowth, with or without N fertilization, 1999

Eight mature cannulated cattle were grazed in late September on regrowth that appeared to have recovered from the first hard seasonal frost on 10 September. There were no bloat incidences of any cattle grazing alfalfa during this three-day trial (Fig. 25). Typically alfalfa may be safely grazed by ruminants about a week after a hard frost (Gomm, 1979b; Guyer and Hogg, 1980). This effect probably accounts for the absence of bloat. However, the cattle had been taken directly from grass pasture to alfalfa forage immediately post frost. According to Majak et al. (1995), in order for bloat to occur, bloat-inducing plant factors must reach a threshold concentration in the rumen. Absence of bloat may have been due to low ruminal concentrations of alfalfa components causing bloat rather than to an absence of these factors in the plants that these cattle were grazing.

Trial 9, sheep on first growth, with or without N fertilization, 2000

There were 15 observed grazing days with 24 sheep in this period. There were 28 bloat events on non-fertilized Ladak 65, 36 bloat events with non-fertilized AC

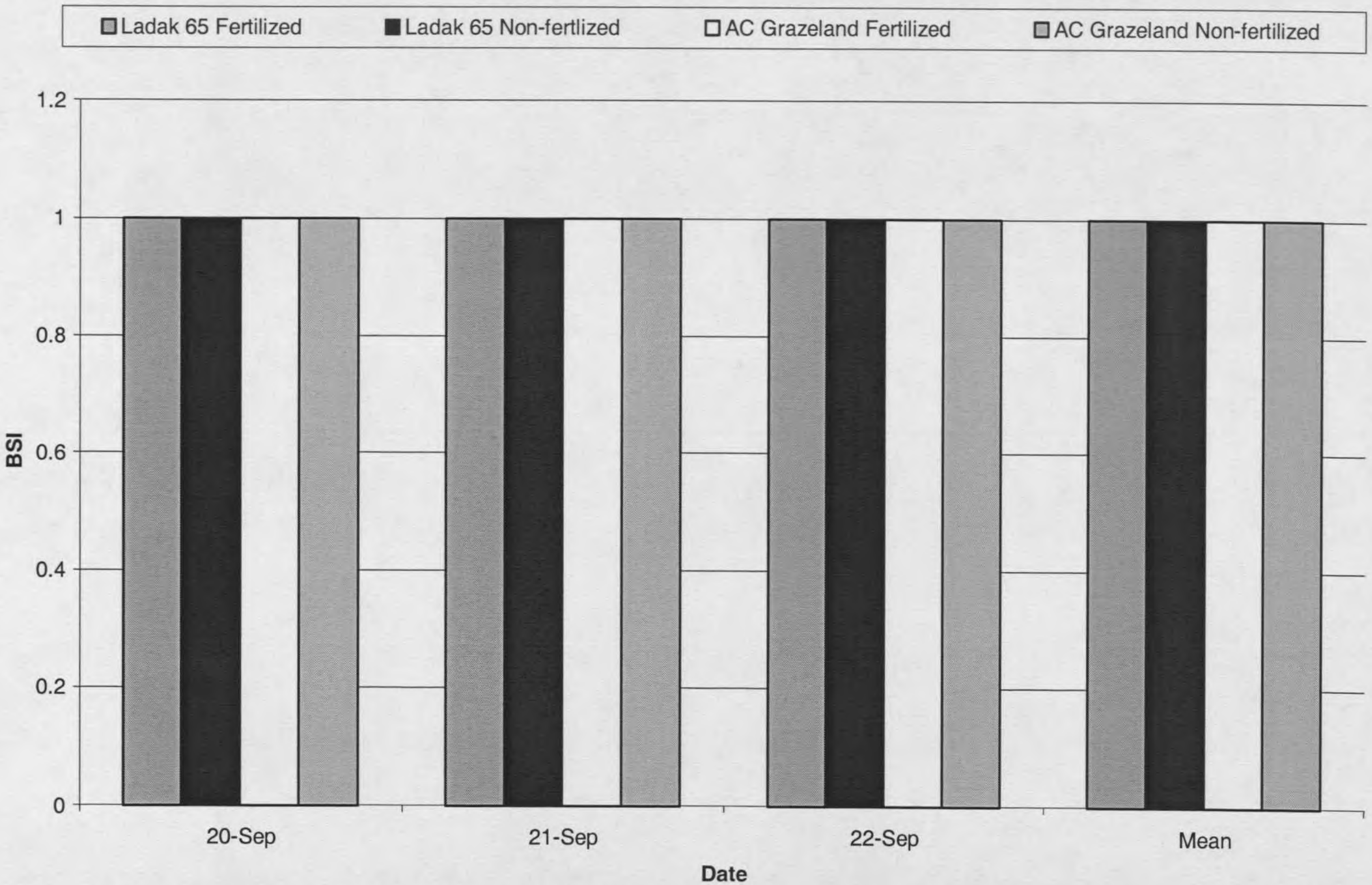

Figure 25. Effect of N fertilization on bloat severity index (BSI, 1=no bloat, 6=dead) of 8 mature beef cows grazing Ladak 65 or AC Grazeland alfalfa in Trial 8, 1999. Values based on multiple observations from four replications of each variety.

Grazeland, 42 bloat events with fertilized Ladak 65 and 42 bloat events with fertilized AC Grazeland (Table 3). There were no significant differences among treatments in bloat severity until the sixth grazing day of the trial, and on this day the variety x fertilization interaction was significant ($P < 0.1$). On 7 and 16 June, BSI scores of AC Grazeland were higher than those of the other three treatments ($P < 0.1$). Across the trial, there were no significant differences between treatments. Averaged across fertilizer status, AC Grazeland had higher mean BSI than Ladak 65 ($P < 0.1$). Averaged across variety, fertilized alfalfa had higher mean BSI than non-fertilized alfalfa ($P < 0.05$).

AC Grazeland, these results indicate that N fertilization of AC Grazeland disrupted any potential bloat safety of this variety.

The differences between Trial 9 and the preceding trials with N fertilization could be due to lower N fertilization levels (175 in Trial 9, vs. 333 kg ha^{-1}), late spring vs. late summer grazing, or from low ruminal concentrations of bloat-inducing factors.

Trial 10, cattle on fall regrowth with or without N fertilization, 2000

During this 6-day trial, eight mature beef cows grazed AC Grazeland and Ladak 65 alfalfa with or without N fertilization (Table 4). Bloat severity was assessed multiple times during each day in order to observe diurnal changes in bloat and corresponding changes in plant factors. There were 7 bloat events on fertilized Ladak 65, 9 events on non-fertilized Ladak 65, 5 events on fertilized AC Grazeland and 4 events on non-fertilized AC Grazeland. There were only two periods with significant differences among the four treatments, and Ladak 65 with N fertilization was greater than both varieties without N fertilization ($P < 0.1$). Overall there were no significant differences

Table 3. Daily mean bloat severity index (BSI, 1 = no bloat, 6 = dead) and percent bloated for 24 mixed purebred ewes grazing Ladak 65 or AC Grazeland (ACG) alfalfa with or without N fertilizer in Trial 9, 2000. Values based on two replications of each treatment.

BSI	WITHOUT N FERTILIZER		WITH N FERTILIZER		LSD		N FERTILIZER STATUS ACROSS VARIETIES			VARIETIES (V) ACROSS FERTILIZER STATUS			VxF
DATE	LADAK 65	ACG	LADAK 65	ACG	P=0.05	P=0.1	WITHOUT N	WITH N	P <	LADAK 65	ACG	P <	P <
29 May	2.0	1.6	1.8	2.0	NS	NS	1.8	1.9	NS	1.9	1.8	NS	NS
30 May	2.0	2.0	2.0	2.0	NS	NS	2.0	2.0	NS	2.0	2.0	NS	NS
1 June	2.0	2.0	1.7	2.0	NS	NS	2.0	1.8	NS	1.8	2.0	NS	NS
5 June	1.6	1.8	1.7	2.2	NS	NS	1.7	1.9	NS	1.6	2.0	NS	NS
6 June	1.7	2.1	2.3	2.5	NS	NS	1.9	2.4	NS	2.0	2.3	NS	0.05
7 June	1.8	1.9	1.8	2.4	0.5	0.3	1.9	2.1	NS	1.8	2.2	NS	0.1
8 June	2.0	1.9	2.3	2.4	NS	NS	2.0	2.4	NS	2.2	2.2	NS	NS
9 June	2.3	1.8	1.7	2.2	0.3	0.2	2.0	1.9	NS	2.0	2.0	NS	0.05
12 June	1.9	2.2	1.7	1.6	0.7	0.5	2.0	1.6	0.1	1.8	1.9	NS	NS
13 June	2.0	1.8	1.9	2.2	0.7	0.5	1.9	2.0	NS	2.0	2.0	NS	NS
14 June	2.1	1.8	2.2	2.3	0.7	0.5	1.9	2.2	NS	2.1	2.0	NS	NS
15 June	1.9	1.7	1.9	1.8	0.3	0.2	1.8	1.9	NS	1.9	1.8	0.1	NS
16 June	1.9	1.5	1.9	2.3	0.4	0.3	1.7	2.1	NS	1.9	1.9	NS	0.05
18 June	1.6	2.0	2.0	1.6	NS	0.4	1.8	1.8	NS	1.8	1.8	NS	0.05
19 June	1.6	1.7	1.6	2.0	NS	NS	1.6	1.8	NS	1.6	1.8	NS	NS
Mean BSI	1.9	1.9	1.9	2.1	NS	NS	1.8	2.0	.05	1.9	2.0	0.1	NS
Mean % Bloated Across Trial	17.3	25.0	29.3	35.7	NS	NS	21.1	32.5	0.1	NS	NS	NS	NS

Table 4. Mean BSI for mature beef cows grazing Ladak 65 or AC Grazeland (ACG) alfalfa with or without N fertilization, in Trial 10, 2000. Values based on replications of each treatment.

BSI	WITHOUT N		WITH N		LSD		N FERTILIZER STATUS ACROSS VARIETIES			ACROSS FERTILIZER STATUS			VxF
DATE/TIME	LADAK 65	ACG	LADAK 65	ACG	P=0.05	P=0.1	WITHOUT N	WITH N	P <	LADAK 65	ACG	P <	P <
9 Sept/ 1400h	1.0	1.0	1.0	1.0	NS	NS	1.0	1.0	NS	1.0	1.0	NS	NS
9 Sept /1600h	1.0	1.0	1.0	1.0	NS	NS	1.0	1.0	NS	1.0	1.0	NS	NS
10 Sept / 1000h	1.0	1.0	1.0	1.0	NS	NS	1.0	1.0	NS	1.0	1.0	NS	NS
10 Sept / 1200h	1.0	1.0	1.0	1.0	NS	NS	1.0	1.0	NS	1.0	1.0	NS	NS
10 Sept/ 1400h	1.0	1.0	1.0	1.0	NS	NS	1.0	1.0	NS	1.0	1.0	NS	NS
11 Sept/ 1000h	1.5	1.0	1.0	1.5	NS	NS	1.2	1.3	NS	1.3	1.3	NS	NS
11 Sept/ 1200h	2.0	3.0	1.0	3.5	NS	NS	2.5	2.3	NS	1.5	3.3	NS	NS
11 Sept/ 1400h	3.0	4.5	5.3	3.0	NS	NS	3.8	4.1	NS	4.1	2.4	NS	NS
12 Sept/ 1000h	1.0	1.0	1.0	1.0	NS	NS	1.0	1.0	NS	1.0	3.8	NS	NS
12 Sept/ 1200h	1.0	1.0	1.0	1.0	NS	NS	1.0	1.0	NS	1.0	1.0	NS	NS
12 Sept/ 1400h	1.0	1.0	3.5	1.8	1.4	1.0	NS	NS	NS	NS	NS	NS	0.1
13 Sept/ 1000h	1.5	1.0	3.5	2.5	2.2	1.4	1.3	3.0	.05	1.0	1.4	NS	NS
13 Sept/ 1200h	1.0	1.0	1.0	1.0	NS	NS	1.0	1.0	NS	1.0	1.0	NS	NS
13 Sept/ 1400h	1.0	1.0	1.0	1.0	NS	NS	1.0	1.0	NS	1.0	1.0	NS	NS
16 Sept/ 1000h	1.0	1.0	1.0	1.0	NS	NS	1.0	1.0	NS	1.0	1.0	NS	NS
Mean BSI	1.3	1.4	1.6	1.5	NS	NS	1.3	1.5	0.1	1.5	1.5	NS	NS
Mean % Bloated	29.0	12.0	22.0	15.0	NS	NS	20.0	19.0	NS	26.0	13.0	NS	NS

between treatments. Averaged across variety, fertilized alfalfa had greater mean BSI than non-fertilized alfalfa ($P < 0.1$).

Summary of pasture trials 1998-2000

During 10 grazing trials, AC Grazeland alfalfa had lower mean BSI scores than Ladak 65 ($P < 0.05$) [Table 5] in five trials. In one trial Ladak 65 had lower mean BSI scores than AC Grazeland. During four of the 10 trials there were no statistical differences between varieties. It appears that AC Grazeland may have slightly less tendency to induce bloat than Ladak 65 in Montana. However, this effect was overcome on days of frost or with N fertilization.

Table 5. Summary of mean BSI during 1998, 1999 and 2000.

	BSI Comparison between Varieties	BSI Comparison between N Fertilizer Levels
Trial 1	Ladak 65 > AC Grazeland (P< 0.05)	-
Trial 2	Ladak 65 > AC Grazeland (P< 0.05)	-
Trial 3	Ladak 65 > AC Grazeland (P< 0.05)	-
Trial 4	Ladak 65 > AC Grazeland (P< 0.05)	-
Trial 5	NS (P>0.1)	-
Trial 6	NS (P>0.1)	-
Trial 7	Ladak 65 > AC Grazeland (P< 0.1) (averaged across fertilizer status)	N-Fertilized > Non fertilized (P< 0.05) (averaged across variety)
Trial 8	NS (P>0.1)	NS (P>0.1)
Trial 9	AC Grazeland > Ladak 65 (P< 0.05) (averaged across fertilizer status)	N-Fertilized > Non fertilized (P< 0.05) (averaged across variety)
Trial 10	NS (P>0.1)	NS (P>0.1)

In Situ Tests

Comparison of DMD Between Ladak 65 and AC Grazeland

In 1999, whole plant forage samples were used for two *in situ* DMD trials. Nylon bags were filled with randomly selected forage from a larger main sample. Data were extremely variable and erratic. No significant differences were detected (data not shown). Random mixtures of leaves and stems were difficult to achieve. Stems would have a lower initial rate of digestion than the leaves. Also, an estimate for percent DM was obtained by weighing forage material from each variety by maturity combination, then drying and re-weighing it. Leaf dry matter would undoubtedly be higher and stem tissue lower in dry matter than the sample average. Variance between samples was very high and the data were not used.

Fresh, Frozen and Dry Samples

In 1999, a 4-h *in situ* DMD test was conducted with samples of fresh, frozen or dried samples of AC Grazeland or Ladak 65 alfalfa. Equivalent samples of both pre-bud and mature alfalfa (Fig. 26, 27) were prepared in nylon bags and simultaneously tested in five cannulated cows. In the pre-bud fresh group, AC Grazeland had significantly ($P < 0.05$) less digestion at 4-h than Ladak 65. However, values of pre-bud alfalfa averaged across treatments showed no significant differences ($P > 0.1$) in 4-h digestion between varieties.

Initial rate of digestion of fresh forage in both mature and pre-bud alfalfa was different than either frozen or dried forage. These differences between sample storage

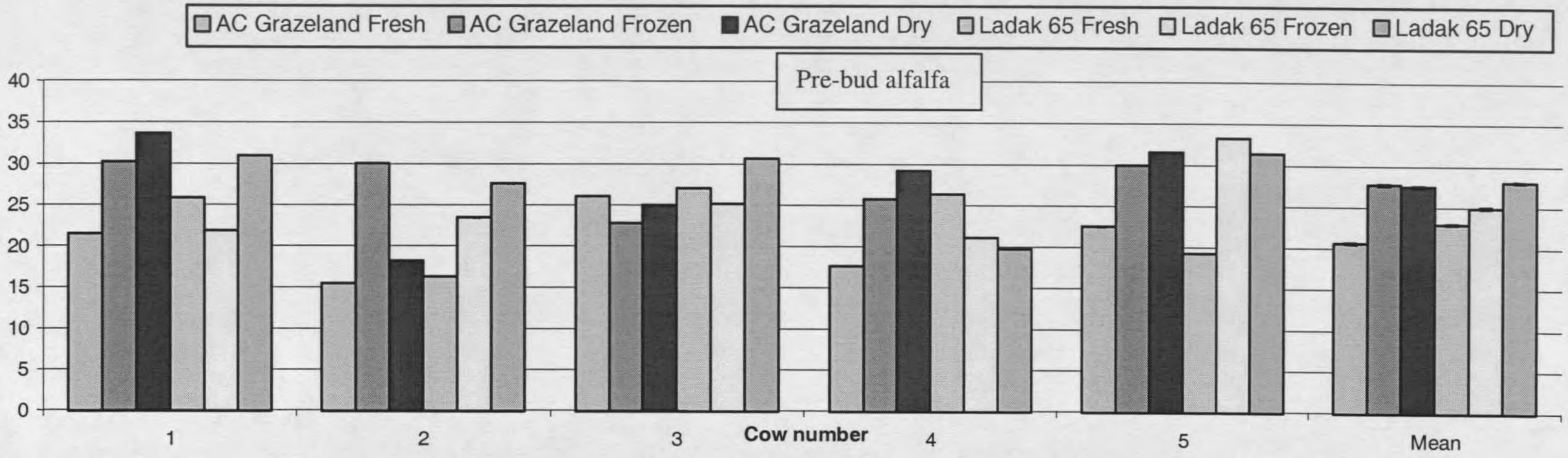

Figure 26. *In sacco* 4-h forage disappearance of fresh, frozen or dried mature Ladak 65 and AC Grazeland alfalfa in five mature, cannulated beef cows. Bars denote 95% confidence interval. Means based on multiple measurements from five replications of each treatment.

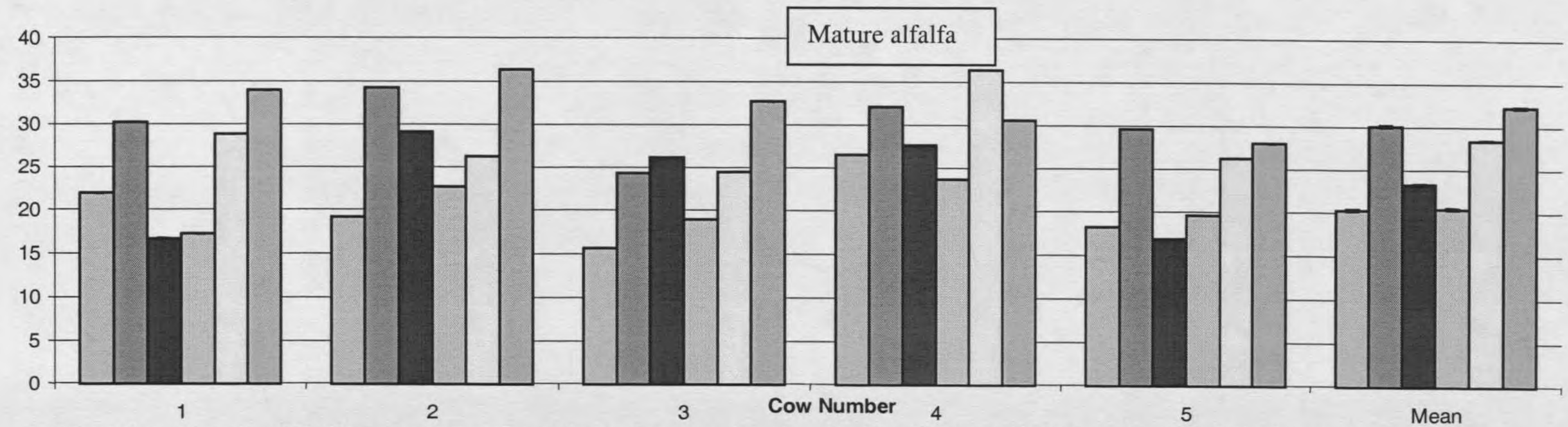

Figure 27. *In sacco* 4-h forage disappearance of fresh, frozen or dried pre-bud Ladak 65 and AC Grazeland alfalfa in five mature, cannulated beef cows. Bars denote 95% confidence interval. Means based on multiple measurements from five replications of each treatment.

techniques suggested that IRD trials using dry or frozen samples might yield inaccurate results. Frozen samples had a higher 4-h digestion than fresh forage in all cases (P< 0.05). This effect in pasture could explain the bloat storms that occurred with frosts on 10 June, 1999 (Fig. 13) and 10 September, 1999 (Fig. 19).

In situ DM and N disappearance of Ladak 65 and AC Grazeland with and without N fertilizer

In 2000, leaves of AC Grazeland and Ladak 65 alfalfa were digested *in situ* in three mature cannulated beef cows, and sampled at 0, 2, 4, 6, and 24 h. AC Grazeland with N had significantly higher DMD at 2-h than any other group ($P < 0.05$) [Table 6]. Fertilized Ladak 65 had a significantly greater DMD than any other group at 6-h ($P < 0.05$) and greater DMD than AC Grazeland without N at 24 h ($P < 0.1$). The 2-h results for AC Grazeland suggest that AC Grazeland has a lower initial DMD than Ladak 65 and may explain the trend toward lower bloat than Ladak 65.

Crude protein (TN x 6.25) was initially higher ($P < 0.05$) in Ladak 65 whether fertilized or unfertilized (Table 7). Crude protein levels remained fairly stable in digested fresh forage over 24 h, and may not reflect large conversions of plant protein to microbial proteins or other bound forms of N. Crude protein levels have been implicated in bloat. However, during this trial, the digestion of N from fresh forage CP did not appear rapid enough to explain the immediacy and severity of bloat.

Table 6. Effect of N fertilization on *in situ* disappearance of dry matter in fresh leaves (g kg^{-1}) of AC Grazeland (AGC) and Ladak 65 in 2000. Values based on three replications (cows) of each treatment.

	WITHOUT N		WITH N		LSD		N FERTILIZATION STATUS (F) ACROSS VARIETIES			VARIETIES (V) ACROSS FERT STATUS			VxF
TIME	LADAK 65	ACG	LADAK 65	ACG	P=0.05	P=0.1	WITHOUT N	WITH N	P <	LADAK 65	ACG	P <	P <
0 h	0.0	0.0	20.0	0.0	NS	NS	0.0	0.0	NS	0.0	0.0	NS	NS
2 h	70.0	230.0	100.0	400.0	90.0	60.0	150.0	70.0	NS	9.0	140.0	NS	0.05
4 h	250.0	320.0	250.0	200.0	NS	NS	280.0	220.0	NS	250.0	260.0	NS	NS
6 h	330.0	300.0	390.0	290.0	90.0	60.0	310.0	340.	NS	360.0	290.0	1.0	NS
24 h	900.0	860.0	920.0	890.0	70.0	50.0	880.0	900.0	NS	910.0	880.0	1.0	NS

Table 7. Crude protein concentrations (g kg^{-1}) of *in situ* digested leaf samples taken from cannulated beef cows over 24 hours. Values based on three replications (cows) of each treatment.

	WITHOUT N		WITH N		LSD		N FERTILIZATION STATUS (F) ACROSS VARIETIES			VARIETIES (V) ACROSS FERT STATUS			VxF
TIME	LADAK 65	ACG	LADAK 65	ACG	P=0.05	P=0.1	WITHOUT N	WITH N	P <	LADAK 65	ACG	P <	P <
0 h	368.4	341.6	370.2	337.0	3.4	2.3	355.0	353.6	NS	369.3	339.3	0.1	NS
2 h	368.3	344.1	363.0	299.6	9.8	6.6	356.2	331.3	NS	365.6	321.8	NS	NS
4 h	365.6	362.0	362.0	335.6	NS	NS	362.0	348.8	NS	363.8	330.0	NS	NS
6 h	356.3	342.2	357.4	351.6	3.5	2.3	349.2	354.5	NS	356.8	346.9	NS	NS
24 h	383.1	260.6	327.3	299.1	16.7	11.2	321.9	313.2	NS	355.2	279.8	NS	NS

Concentration of *in situ* NO_3 was initially higher in Ladak 65 than AC Grazeland when averaged across fertilizer status (Table 8). Ladak 65 with N fertilization had significantly higher NO_3 levels than other treatments throughout most of the digestion trial. Pre-digested fertilized alfalfa contained higher levels of NO_3 than alfalfa without fertilizer ($P < 0.1$). No concurrent bloat data were available during the period of this *in situ* trial, but initial NO_3 disappearance was very rapid. The levels of NO_3 present in the fertilized alfalfa prior to digestion in this study were high enough to potentially lead to NO_3 toxicity symptoms of pregnant livestock (Crawford et al., 1966). Rapid release of NO_3 and other soluble forms of N can likely cause increased risk of nitrate toxicity or bloat.

Forage Quality Constituents of Pasture During Grazing

Crude Protein Concentration

Samples from the top 15 cm of the plant in Trial 4, 1999

Samples of AC Grazeland and Ladak 65 alfalfa were taken from the upper 15 cm on days when animals were introduced to fresh forage. Crude protein concentration of Ladak 65 was higher than AC Grazeland on one day ($P < 0.05$) of the 1999 season (Fig. 28). Across the 16 days, Ladak 65 had ($P < 0.05$) higher CP concentration than AC Grazeland. During this period, AC Grazeland was slightly more mature than Ladak 65, however, no statistical difference of maturity between varieties was detected.

Table 8. Nitrate concentration (g kg^{-1}) in digested fresh forage *in situ* leaf samples taken from cannulated beef cows over 24 hours. Values based on three replications (cows) of each treatment.

	Without N		With N		LSD		N Fertilization status (F) Across Varieties			Varieties (V) Across Fert Status			VxF
TIME	Ladak 65	ACG	Ladak 65	ACG	P=0.05	P=0.1	Without N	With N	P <	Ladak 65	ACG	P <	P <
0 h	2.4	2.1	5.0	2.7	2.2	1.5	2.2	3.9	0.5	3.7	2.4	0.1	NS
2 h	2.6	2.1	3.4	2.1	NS	1.3	2.4	2.8	NS	3.0	2.1	NS	NS
4 h	1.6	1.6	2.3	2.7	NS	1.0	1.6	2.5	1.0	1.9	2.2	NS	NS
6 h	1.2	1.3	2.0	1.8	NS	0.8	1.2	1.9	1.0	1.6	1.5	NS	NS
24 h	0.0	0.3	0.0	0.2	NS	NS	0.2	0.1	NS	0.0	0.3	NS	NS

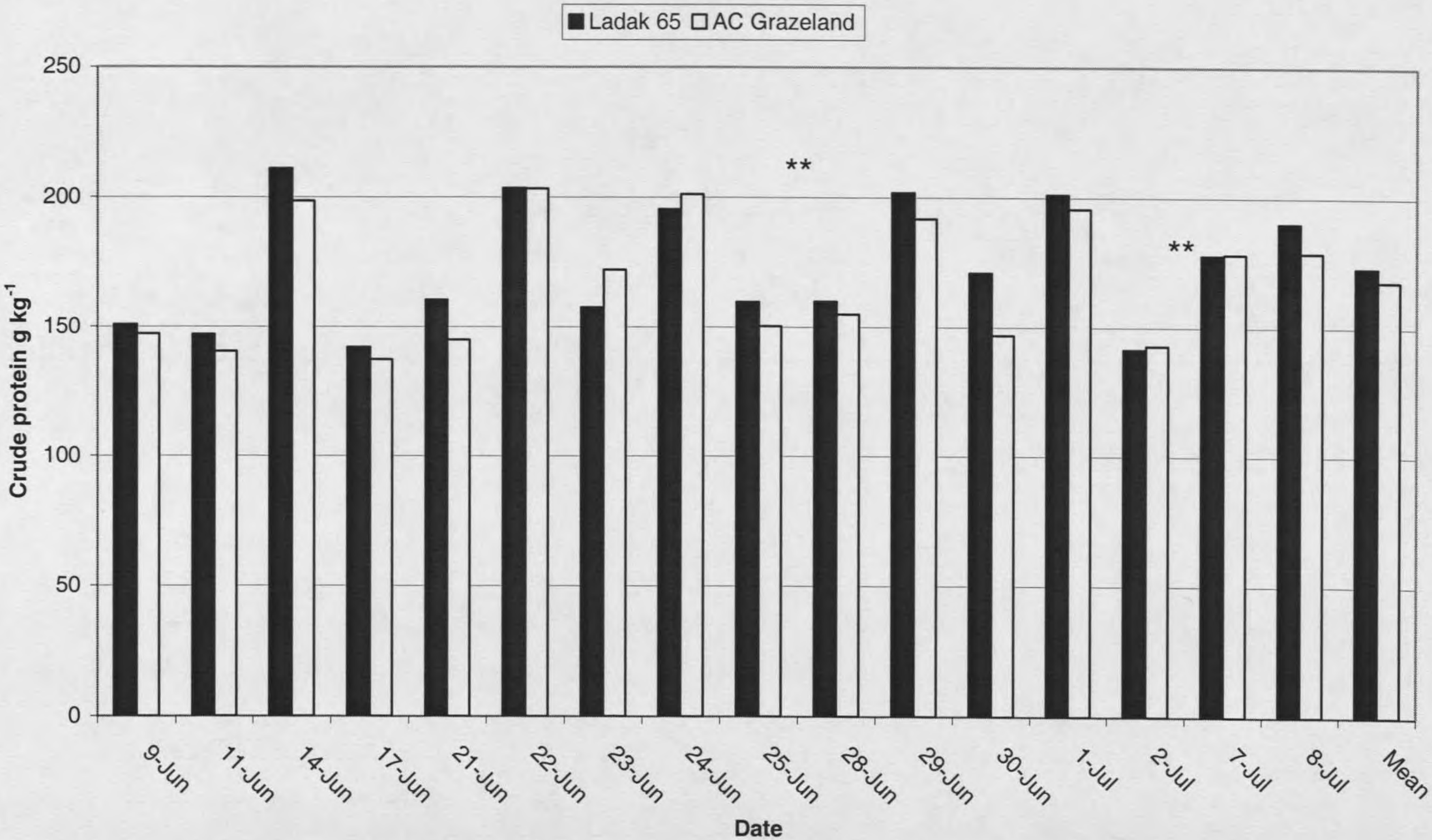

Figure 28. Crude protein concentrations of the top 15 cm of plants of Ladak 65 and AC Grazeland alfalfa during trial 4, 1999. * or ** denote significant differences at P<0.1 and 0.05, respectively. Values based on four replications of each variety.

Considering the importance of maturity and protein on bloat incidence and severity, these factors could play a role in the differences in mean BSI scores of the two varieties. Across this period, the correlation coefficient between BSI and CP was $r=0.4$, ($P < 0.05$).

Pre and post-grazed samples, Trial 4, 1999

Whole plant samples were cut at ground level from a 0.3 m^2 quadrat, from the previous day's grazed cells and from fresh forage. Over the 23-day period average CP of pre-grazed alfalfa declined from 232 to 173 g kg^{-1}. There were five of nine days with significant differences ($P < 0.1$) in CP content between pre- and post-grazed samples (Table 9). Means of both varieties before grazing were higher than samples after grazing. This is likely due to grazed upper leaves and stems containing higher levels of CP than lower stems, with sheep grazing the plant components higher in CP. Averaged across grazing status, only one day had significant differences between varieties ($P < 0.05$). Again, CP levels in Ladak 65 were slightly higher than those of AC Grazeland.

Samples from the top 15 cm of the alfalfa plant during Trial 9, sheep in 2000

There were significant differences among treatments for CP concentration on several days (Table 10). Across the 12 days sampled, there were no significant differences between treatments. The CP levels from this trial were higher than those of Trial 4, likely due to sampling upper 15 cm vs. whole plant. Interestingly, spring N fertilization (175 kg ha^{-1}) did not significantly increase CP levels in 2000. In Trial 9, there were seven of 16 days with significant differences among treatments for bloat severity (Table 3). However, levels of CP did not appear to explain these differences.

Table 9. Crude protein concentrations (g kg^{-1}) of AC Grazeland (ACG) and Ladak 65 before and after grazing, Trial 4, 1999. Values based on two replications of each treatment.

	PRE-GRAZE		POST-GRAZE		LSD		GRAZING (G) STATUS ACROSS VARIETIES			VARIETY (V) ACROSS GRAZING STATUS			VxG
DATE	LADAK 65	ACG	LADAK 65	ACG	P=0.05	P=0.1	PRE-GRAZE	POST-GRAZE	P <	LADAK 65	ACG	P <	P <
9 June	243.5	221.3	219.4	152.8	NS	NS	232.4	186.1	NS	231.4	187.0	NS	NS
11 June	241.5	227.2	252.7	185.8	NS	NS	234.3	219.3	NS	247.1	206.5	NS	NS
17 June	227.2	220.5	172.0	154.1	NS	NS	223.9	163.0	.05	199.6	187.3	NS	NS
21 June	214.6	211.3	146.8	145.4	45	30	213.0	146.1	.05	180.7	178.3	NS	NS
23 June	193.7	200.0	126.3	135.5	41	27	196.8	130.9	.05	160	167.7	NS	NS
25 June	192.8	178.1	138.2	138.9	52	35	185.4	138.5	.05	165.5	158.5	NS	NS
28 June	188.3	171.4	147.3	129.2	16	11	179.9	138.2	.05	167.8	150.3	.05	NS
30 June	211.4	160.2	151.7	143.2	NS	NS	185.8	147.5	NS	181.6	151.7	NS	NS
2 July	156.0	189.9	126.4	120.9	45	30	172.9	123.6	NS	141.2	155.4	NS	NS
Mean	206.6	195.72	162.2	144.3	29.5	19.77	201.14	153.21	.05	184.36	169.99	0.1	NS

Table 10. Crude protein concentrations (g kg^{-1}) of AC Grazeland (ACG) and Ladak 65 with or without N fertilizer during Trial 9, 2000. Values based on two replications of each treatment.

	Without N		With N		LSD		Fertilization Status (F) Across Varieties			Variety (V) Across Fertilization Status			VxF
DATE	Ladak 65	ACG	Ladak 65	ACG	P=0.05	P=0.1	Without N	With N	P <	Ladak 65	ACG	P <	P <
1 June	320.9	303.9	314.9	302.1	NS	NS	312.4	308.5	NS	317.9	303.0	NS	NS
2 June	311.4	314.1	334.6	314.3	6.3	4.2	312.7	324.4	NS	323.0	314.2	NS	0.05
5 June	305.5	306.0	293.2	299.3	NS	NS	305.8	296.2	NS	299.4	302.6	NS	NS
6 June	312.1	286.6	304.4	282.6	34.4	23.1	299.3	293.5	NS	308.3	284.6	0.05	NS
7 June	301.9	303.1	313.3	292.9	24.0	16.1	302.5	303.1	NS	307.6	298.0	NS	NS
8 June	267.2	278.2	292.5	279.7	23.4	15.7	272.7	286.1	0.1	279.9	278.9	0.1	NS
9 June	276.5	254.3	265.6	278.6	NS	NS	265.4	272.1	NS	271.1	266.5	0.1	NS
13 June	257.5	277.2	273.3	252.2	NS	NS	267.3	262.8	NS	265.4	264.7	NS	NS
15 June	286.7	256.9	279.0	293.9	NS	NS	271.8	286.4	NS	282.8	275.4	NS	NS
16 June	268.3	284.1	279.7	264.1	NS	20.0	276.2	271.9	NS	274.0	274.1	0.1	NS
18 June	284.4	254.0	249.5	256.1	NS	50.3	251.2	252.8	NS	248.9	255.0	NS	NS
Mean	286.9	283.5	290.9	283.2	NS	NS	285.2	287.1	NS	288.9	283.3	NS	NS

Samples from the top 15 cm of the alfalfa plant during Trial 10, cattle in 2000

Crude protein concentrations were significantly different among treatments on most days (Table 11). In contrast with Trial 9, the late summer growth responded to spring application of N (175 kg ha^{-1}). Across the six sample dates, mean levels of CP were highest for fertilized Ladak 65. Without fertilization, Ladak 65 had higher levels of CP than AC Grazeland. Mean levels of CP were significantly different between varieties ($P < 0.05$) across N levels but not between fertilizer levels across varieties. During this portion of the trial the varieties had visibly different growth. While both varieties were managed the same, Ladak 65 recovered better. Grazing data were only collected in paired cells in which growth was approximately equal. However, if grazing had been conducted in all cells rather than in paired cells with similar growth, differences in plant components would likely exist. Differences in CP did not appear to fully explain differences in mean BSI during Trial 10.

Nitrate Concentration

Nitrate concentrations were determined by wet chemistry for the dried forage samples collected during grazing. The NIRS equations were unreliable for accurate prediction of NO_3 levels in alfalfa samples. Initially, samples were taken between 0800 and 1200 for daily values and would not account for the rapid or diurnal variation in NO_3 concentrations. Because most forage samples were not taken concurrent with bloat events, most were not analyzed or used in correlations with bloat. Samples taken after

Table 11. Crude protein concentrations (g kg^{-1}) of AC Grazeland (ACG) and Ladak 65 with or without N fertilizer during Trial 10, 2000. Values based on two replications of each treatment.

	Without N		With N		LSD		Fertilization Status (F) Across Varieties			Variety (V) Across Fertilization Status			VxF
DATE	Ladak 65	ACG	Ladak 65	ACG	P=0.05	P=0.1	Without N	With N	P <	Ladak 65	ACG	P <	P <
9 Sept	282.3	270.7	294.1	314.5	11.5	7.7	276.5	304.3	0.05	288.2	292.6	0.05	NS
10 Sept	283.0	272.4	284.0	305.7	10.0	6.7	277.7	294.9	NS	283.5	289.0	NS	NS
11 Sept	282.9	268.2	282.9	295.2	NS	19.2	275.5	289.0	NS	282.9	281.7	NS	NS
12 Sept	275.3	245.8	208.9	299.9	74.3	49.8	260.5	254.4	NS	242.1	272.8	0.05	NS
15 Sept	267.8	253.8	258.3	271.9	NS	NS	260.8	265.1	NS	263.0	262.9	NS	NS
Mean	278.3	262.1	265.6	297.4	9.0	6.0	270.2	281.5	NS	271.9	279.8	0.05	NS

1 September 1999 were taken concurrent with daily initiation of grazing and were utilized in statistical comparisons to bloat.

Alfalfa NO_3 concentration declined from approximately 2.0 (9 June) to 0.3 g kg^{-1} on 8 July (Fig. 29). However, no significant differences were found between varieties for NO_3 concentration on any of the 18 days, or across dates ($P > 0.1$). Sample numbers were low and variance was high, likely accounting for the lack of significance between varieties.

During the pre- and post-frost grazing study (Trial 7), forage samples were taken from the top 15 cm of plants from cells of Ladak 65 and AC Grazeland with and without N fertilization, concurrent with bloat data. Fertilized Ladak 65 and AC Grazeland had higher levels of NO_3 ($P < 0.05$) than unfertilized alfalfa when averaged over 3 days pre-frost (Table 12). These values correspond to the mean BSI scores for the same period (Table 13), as well as for the 0-h samples from the 2000 *in situ* trial (Table 6). These results suggest that NO_3 could play an important role in bloat.

A major limitation to pasture bloat studies is sampling protocol for SPN or NPN compounds. If NO_3 or other soluble components of NPN play an important role in bloat, these could easily have been overlooked. Improper storage conditions will change NPN to other forms before it can be accurately measured (Howarth et al., 1977). Any sample in which chemical activity has not been immediately halted, (for example, conventional drying or freezing) or is allowed to re-initiate is problematic. A low ratio of soluble to insoluble protein is a characteristic of samples that have been frozen and thawed more

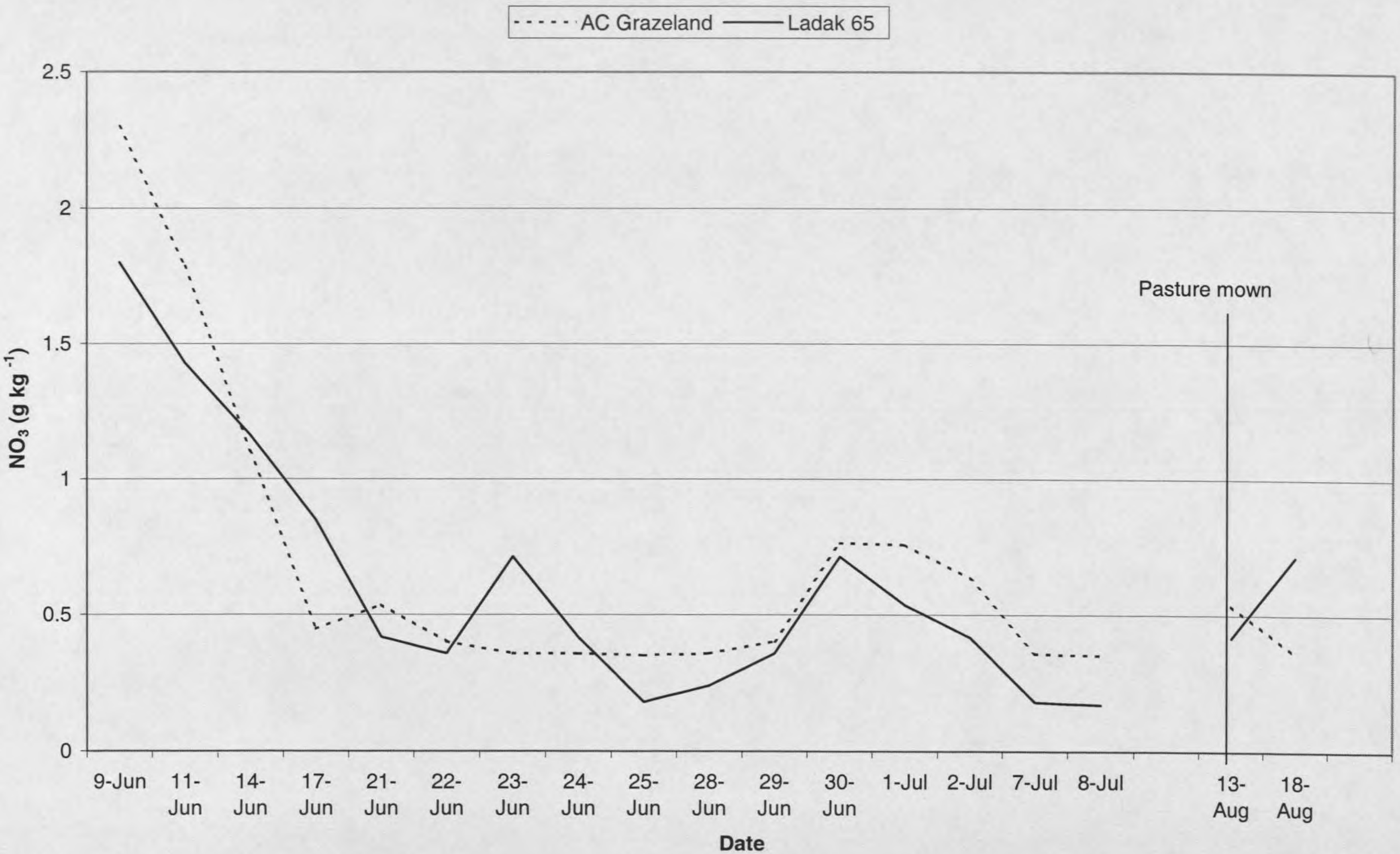

Figure 29. Nitrate (NO_3) concentrations of unfertilized AC Grazeland and Ladak 65 alfalfa during Trials 4 and 5, 1999. Values based on four replications of each variety.

Table 12. Nitrate concentrations in samples (g kg^{-1}) of AC Grazeland (ACG) and Ladak 65 with or without N fertilizer during Trial 7, 1999. Values based on two replications of each treatment.

BSI	Without N		With N		LSD		N Fertilization status (F) Across Varieties			Varieties (V) Across Fert Status			VxF
Mean	Ladak 65	ACG	Ladak 65	ACG	P=0.05	P=0.1	Without N	With N	P <	Ladak 65	ACG	P <	P <
Pre-frost	3.1	3.5	8.1	6.0	0.5	3.0	3.3	7.1	NS	5.6	3.3	NS	0.05
Post-frost	3.1	3.6	7.2	6.6	2.5	1.7	3.4	6.9	0.05	5.1	5.1	NS	NS

Table 13. Mean BSI (BSI, 1=no bloat, 6=dead) of 24 mixed purebred ewes grazing Ladak 65 or AC Grazeland (ACG) with or without N fertilizer during Trial 7, 1999. Values based on two replications of each treatment.

BSI	Without N		With N		LSD		N Fertilization status (F) Across Varieties			Varieties (V) Across Fert Status			VxF
Mean	Ladak 65	ACG	Ladak 65	ACG	P=0.05	P=0.1	Without N	With N	P <	Ladak 65	ACG	P <	P <
Pre-frost	1.0	1.0	3.9	2.9	1.5	1.0	1.0	3.4	0.05	2.4	2.0	0.1	NS
Post-frost	1.6	1.7	3.7	2.8	1.0	0.8	1.6	3.2	0.05	2.6	2.3	0.1	NS

than once (Howarth et al., 1977). In many bloat studies, the forage samples were frozen. Active nitrate reductase in the cytoplasm may continue to convert NO_3 to NH_4, and incorporate it into amino acids, thus, changing the form of N from soluble non-protein N (SNPN) to SP. Howarth et al. (1977) freeze-dried alfalfa samples prior to analysis and found a significant correlation between soluble NPN and bloat. The SPN average over three years of trial was not significantly correlated to bloat, however SNPN had a significant, but low, correlation to bloat ($r=0.28$, $P < 0.05$) [Howarth et al. 1977).

Another reason NPN may have been overlooked is because NPN in alfalfa can contain a large percentage of NO_3. Alfalfa has very high N_2 fixation capacity and it has not been commonly recognized as an NO_3-accumulating plant. However, many studies have confirmed accumulation of NO_3 by alfalfa (Smith and Sund, 1965; Lee and Smith, 1972; Shertz and Miller, 1972; Nelson, 1984; Puschner, 2000). Alfalfa utilizes NO_3 by taking it into the root, transporting it to the leaves, reducing it to NH_4, and incorporating it into amino acids. Nitrate is reduced to nitrite (NO_2) by the enzyme nitrate reductase (NR) present in the cytoplasm. Nitrate reductase has a high turnover rate and is dependent on favorable conditions to function. During adverse conditions, NR synthesis of many plants is reduced and NO_3 accumulates. Drought, cloudy weather (Buck et al., 1976; Edwards and McCoy, 1980), reduction of light intensity (George et al., 1971; Cantliffe, 1972; Hicks and Peterson, 1976; Strizke et al., 1976; Gomm, 1979c;), and levels of N fertilizer that exceed the rate of crop utilization (Edwards and McCoy, 1980; Nelson, 1984) can cause NO_3 to accumulate. If NO_3 plays a role in the initiation of bloat these climatic conditions could in turn lead to bloat, explaining the contradictory anecdotal evidence concerning weather conditions and bloat.

Many of the conditions that cause NO_3 accumulation are similar to those associated with bloat (Table 14).

Nitrate may be a readily available component of soluble N, which is needed for stable froth. However, it is also possible that a product of NO_3 conversion, or another component of NPN play a separate role, accounting for the variation and fluctuation in bloat incidence and severity. For example, a difference in composition of ruminal gas or activity of microbes caused by excess NO_3 might trigger bloat.

Pre- and post-grazed samples

There was extreme variation in NO_3 concentration of pre- and post-grazed samples (Table 15) and the 2000 samples of the top 15 cm (Table 18). Further, the NO_3 levels differ among whole plant and samples of the top 15 cm. This disparity probably exists for two reasons. First, pre- and post-grazed samples were from the whole plant while grab samples were from the top 15 cm. Stems and lower leaves contain higher levels of NO_3 normally (Wright and Davidson, 1964) and because pre- and post-grazed samples contain stems and lower leaves, some disparity would be expected. Secondly, pre and post-grazed samples were dried while grab samples were frozen. Frozen samples have typically yielded different results than dried samples (Howarth, 1977).

Table 14. Factors associated with bloat hazard or NO_3 in forage crops.

FACTOR	BLOAT CITATION	NO_3 CITATION
Alfalfa as a species	Fay et al., 1980,1981; Howarth 1982 Howarth 1991; Majak et al., 1995	Shertz and Miller, 1972; Smith and Sund, 1965; Lee and Smith, 1984; Nelson, 1984; Cherney, 1994.
Immature alfalfa	Hall et al., 1994b Howarth 1991; Majak et al., 1995	Wright and Davidson 1964; Smith and Sund, 1965
Alfalfa in pasture mix	Guyer, 1983	Smith and Sund, 1964
Alfalfa as the sole forage	Fay et al., 1980,1981; Howarth 1982 Guyer, 1983; Howarth 1991; Majak et al., 1995	Smith and Sund, 1964
Immediately post-frost	Hall et al., 1991; Hall and Majak, 1991	Buck et al., 1976; Edwards and McCoy, 1980
Cloudiness	Walgenbach et al., 1981; Walgenbach and Marten, 1981	Stritzke et al., 1976
Early A.M.	Majak and Hall, 1993; Hall and Majak,1995	Stritzke et al., 1976
Drought	Walgenbach et al., 1981; Walgenbach and Marten, 1981	Buck et al., 1976; Edwards and McCoy, 1980
Immature ryegrass	Garry, 1990	Nelson, 1984
Maize	Leek, 1982	Nelson, 1984
Trifolum	Fay et al., 1980, 1981; Howarth, 1982	Smith and Sund, 1965
FACTOR	**LOW BLOAT**	**LOW NO_3**
Mature alfalfa	Howarth 1991; Majak et al., 1995	Wright and Davidson, 1964; Smith and Sund, 1965
Birdsfoot trefoil	Fay et al., 1980, 1981; Howarth, 1982	Smith and Sund, 1964

Table 15. Variation in NO_3 concentration (g kg^{-1}) of whole plant samples of AC Grazeland and Ladak 65 alfalfa before and after grazing, during Trial 4. Values based on 18 replications (days).

	PRE GRAZE		POST GRAZE	
VARIETY	**LADAK 65**	**AC GRAZELAND**	**LADAK 65**	**AC GRAZELAND**
Mean, 18d	0.453	0.693	0.856	0.734
Standard Deviation	0.464	0.582	0.642	0.302

During 2000, NO_3 levels were high at the beginning of the growing season and tapered off as the plants matured (Fig. 29). Across the period, mean NO_3 levels for whole plant samples were 5 g kg^{-1} with fertilized Ladak 65, 5.3 g kg^{-1} for non-fertilized Ladak 65, 5.5 g kg^{-1} for fertilized AC Grazeland and 3.00 g kg^{-1} for non-fertilized AC Grazeland. However, due to high variability there were no statistical differences among treatments (Table 16). Despite high variation in NO_3 concentrations, these data indicate that alfalfa can accumulate toxic levels of NO_3 under field conditions. It was not possible to conclusively determine a role of NO_3 in alfalfa pasture bloat. However, based on the potentially high NO_3 levels and rapid release of NO_3 *in situ*, roles of NO_3 and other SPN fractions deserves further research attention. Particular attention should be paid to proper sample collection and storage protocols.

Table 16. Variation for whole plant NO_3 concentration (g kg^{-1}) of AC Grazeland and Ladak 65 alfalfa samples in Trial 9. Values based on 16 replications (days).

	LADAK 65		AC GRAZELAND	
VARIETY	WITH N FERTILIZATION	WTHOUT N FERTILIZATION	WITH N FERTILIZATION	WTHOUT N FERTILIZATION
Mean	2.42	2.82	2.64	1.34
Standard Deviation	5.02	5.26	5.51	3.07

Acid Detergent Fiber

Samples taken from the top 15 cm, Trial 4, 1999

Samples were collected to compare ADF levels between AC Grazeland and Ladak 65. The ADF concentrations of upper 15 cm of forage were measured by NIRS for Trial 4 (Fig. 30). Acid detergent fiber levels increased as the season progressed, similar to the results of Howarth (1991). Across all sample dates, AC Grazeland had significantly higher ADF levels ($P < 0.05$) than Ladak 65 (Fig. 31). Overall, Ladak 65 had higher digestible dry matter than AC Grazeland ($P< 0.05$). This suggests that the portion of the alfalfa plant readily grazed on AC Grazeland is less digestible.

Pre and post-graze (whole plant), Trial 4, 1999

Plants were cut at ground level from $0.3m^2$ quadrats to determine livestock diet as well as to compare ADF between varieties and grazing status. The ADF of pre- and post-

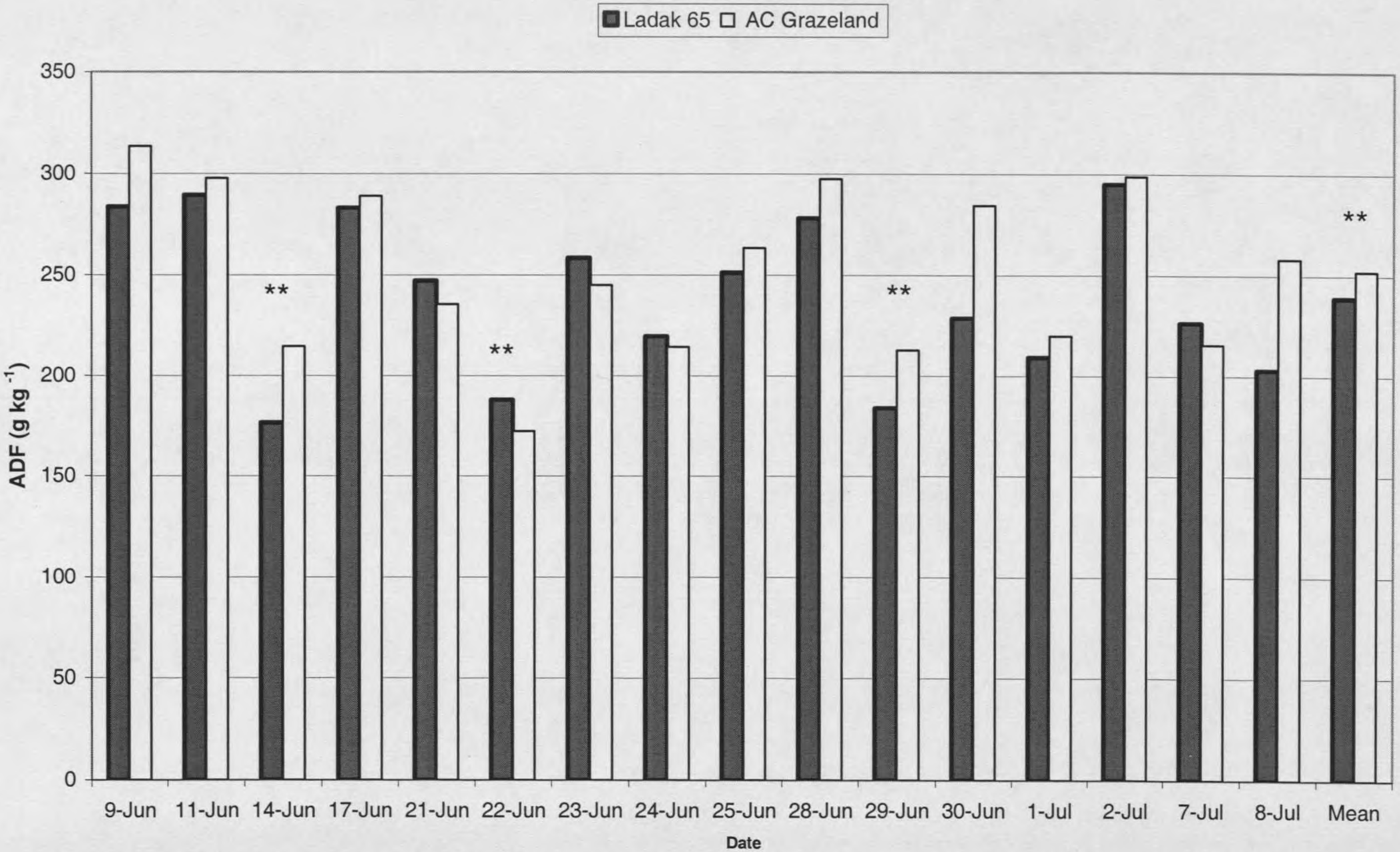

Figure 30. Mean acid detergent fiber (ADF) concentration of samples of AC Grazeland and Ladak 65 alfalfa taken from the top 15 cm of the plant during Trial 4, 1999. * or ** denote significant differences at P<0.1 and 0.05, respectively. Values based on four replications of each variety.

grazed (whole plant) samples of AC Grazeland and Ladak 65 was measured during Trial 4. The level of ADF was significantly different among treatments on six of the nine sample dates (Table 17). These data contrasted with the results taken from the top 15 cm of the plant, in which ADF levels of AC Grazeland were significantly higher, suggesting that the sheep selected similar diets despite potential plant differences. Post-grazing samples of Ladak 65 were higher in ADF than AC Grazeland ($P < 0.05$). This suggests that sheep consumed more ADF in AC Grazeland than Ladak 65. These differences in digestibility correspond to the mean BSI of Trial 4, and suggest that higher ADF levels in AC Grazeland could play a role in bloat reduction.

Trial 9, with or without N Fertilizer, 2000

Levels of ADF during this portion of the trial were highest for fertilized AC Grazeland (Table 18). Overall, N increased ADF concentration in AC Grazeland, and across fertilizer levels, AC Grazeland had significantly higher ($P < 0.1$) ADF than Ladak 65.

Trial 10, with or without N Fertilizer, 2000

During this portion of the trial, levels of ADF were significantly lower for fertilized AC Grazeland than for non-fertilized AC Grazeland (Table 19). These results contrast with those of Trial 9, but are similar to the late summer CP response to N application (175 kg ha^{-1}). In September 2000, N-fertilized Ladak 65 had faster regrowth, lower CP levels (Table 11) and higher ADF levels (Table 19) than N-fertilized AC Grazeland. However, bloat incidence and severity of cattle were not different ($P > 0.1$,

Table 17. Acid detergent fiber concentrations (g kg^{-1}) of AC Grazeland (ACG) and Ladak 65 (whole plant) before and after grazing by sheep in Trial 4, 1999. Values based on two replications of each treatment.

	Pre-Graze		Post-Graze		LSD		Grazing Status (G) Across Varieties			Variety (V) Across Grazing Status			VxG
DATE	Ladak 65	ACG	Ladak 65	ACG	P=0.05	P=0.1	Pre-Graze	Post-Graze	P <	Ladak 65	ACG	P <	P <
9 June	276.8	312.5	324.3	450.2	131.0	88.0	294.6	387.2	NS	300.5	381.3	NS	NS
11 June	285.7	312.4	301.3	358.4	NS	NS	299.1	329.9	NS	293.5	335.4	NS	NS
17 June	283.0	287.4	353.2	378.2	42.0	28.0	285.2	365.7	NS	318.1	332.8	NS	NS
21 June	305.5	313.5	377.9	397.8	32.0	21.0	309.5	387.9	.05	341.7	355.7	NS	NS
23 June	319.7	336.3	409.9	404.6	40.0	27.0	328.0	407.2	.05	364.8	370.5	NS	NS
25 June	329.8	331.8	398.6	398.1	77.0	52.0	330.8	398.4	.05	364.2	364.9	NS	NS
28 June	343.6	363.0	390.3	414.3	60.0	40.0	353.3	402.3	.05	366.9	388.7	NS	NS
30 June	294.4	383.4	387.1	418.0	NS	NS	338.9	402.5	NS	340.8	400.7	NS	NS
2 July	372.2	351.8	414.6	393.6	NS	NS	362.0	404.1	NS	393.4	372.7	NS	NS
Mean	339.3	336.7	390.2	379.6	45.0	30.0	338.0	384.9	NS	364.8	358.2	NS	NS

Table 18. Acid detergent fiber concentrations (g kg^{-1}) of the upper 15 cm of forage of AC Grazeland (ACG) and Ladak 65 with or without N fertilization during Trial 9, 2000. Values based on two replications of each treatment.

	WITHOUT N		WITH N		LSD		N FERTILIZER STATUS (F) ACROSS VARIETIES			VARIETY (V) ACROSS N FERTILIZER STATUS			VxF
DATE	LADAK 65	ACG	LADAK 65	ACG	P=0.05	P=0.1	WITHOUT N	WITH N	P <	LADAK 65	ACG	P <	P <
1 June	145.6	170.9	161.7	190.9	35.2	23.6	158.2	176.3	0.1	153.6	180.9	NS	NS
2 June	160.1	172.6	133.8	147.3	56.1	37.6	166.3	140.5	NS	146.9	159.9	NS	NS
5 June	159.0	148.3	200.2	184.0	86.7	58.1	153.6	192.1	NS	179.6	166.1	NS	NS
6 June	136.5	199.8	152.4	192.6	47.8	32.0	168.1	172.5	NS	144.4	196.2	NS	NS
7 June	172.1	162.5	150.7	183.9	66.0	44.2	167.3	167.3	NS	161.4	173.2	NS	NS
8 June	208.5	214.0	164.3	180.1	59.8	40.1	211.2	172.2	0.1	186.4	197.0	NS	NS
9 June	195.1	215.2	196.1	195.5	100.5	67.4	205.1	195.8	NS	195.6	205.3	NS	NS
13 June	239.0	206.7	210.6	250.2	50.2	33.6	222.9	230.4	NS	224.8	228.4	0.05	0.05
15 June	208.4	238.3	216.5	204.8	90.5	60.6	223.3	210.6	NS	212.5	221.5	NS	NS
16 June	193.5	190.2	195.3	254.5	27.0	18.1	191.8	224.9	0.05	194.4	222.3	0.05	0.05
18 June	242.2	262.4	232.0	241.3	56.8	38.1	252.3	236.7	NS	237.1	251.8	NS	NS
Mean	187.3	198.2	183.0	202.3	NS	18.7	192.8	192.7	NS	185.2	200.2	NS	.05

Table 19. Acid detergent fiber concentrations (g kg^{-1}) of the upper 15 cm of forage of AC Grazeland (ACG) and Ladak 65 with or without N fertilization during Trial 10, 2000. Values based on two replications of each treatment.

	Without N		With N		LSD		N Fertilizer status (F) Across Varieties			Variety (V) Across N Fertilizer Status			VxF
DATE	Ladak 65	ACG	Ladak 65	ACG	P=0.05	P=0.1	Without N	With N	P <	Ladak 65	ACG	P <	P <
9 Sept	178.8	199.0	168.0	143.3	15.8	10.6	188.9	155.6	NS	173.4	171.1	0.05	NS
10 Sept	184.5	205.1	178.0	151.7	14.7	9.8	194.8	164.8	NS	181.2	178.4	NS	NS
11 Sept	187.6	211.3	191.8	153.9	33.7	22.5	199.4	172.8	0.05	189.7	182.6	0.05	NS
12 Sept	197.7	243.8	304.8	161.7	144.3	96.7	220.7	233.2	NS	251.2	202.7	0.05	NS
15 Sept	207.8	244.3	269.5	163.3	80.1	53.7	226.1	216.4	NS	238.7	203.8	NS	NS
Mean	191.2	220.7	222.4	154.8	41.5	27.8	206.0	188.6	NS	206.8	187.7	NS	.05

Table 4). These data reflect the interactions among the alfalfa varieties with growth conditions and fertility levels.

Neutral Detergent Fiber

Samples taken from the top 15 cm of the alfalfa plant, Trial 4, 1999

As with ADF, NDF levels in the upper portions of the plant maintained a constant level throughout the summer as plants matured (Fig. 30, 31). NDF levels were higher in AC Grazeland on two days ($P < 0.1$ and $P < 0.05$) and overall ($P < 0.05$). Across all dates, mean NDF of AC Grazeland was 305 g kg^{-1} and Ladak 65 was 285 g kg^{-1} NDF ($P < 0.05$).

Pre- and post-graze, whole plant, Trial 4, 1999

Mean levels of NDF during June were highest for post-grazed AC Grazeland samples (Table 20). Pre-grazed AC Grazeland had lower levels than Ladak 65 ($P < 0.05$), suggesting that NDF consumption by sheep was lower for AC Grazeland than for Ladak 65. Data from the upper 15 cm of the same area during the same time frame have different fiber levels than these data. Higher NDF levels in the stems could account for the differences between sample types in fiber levels.

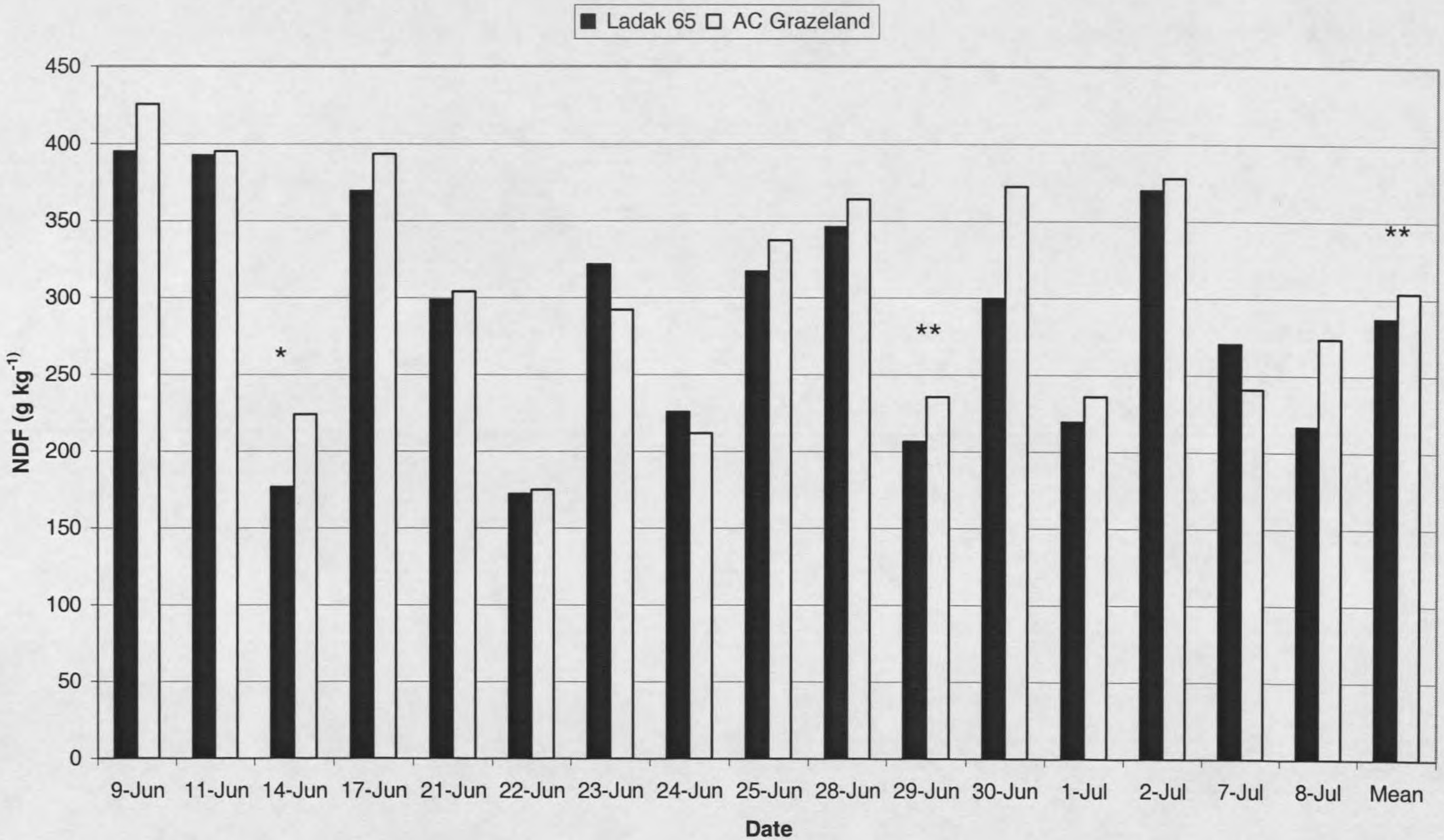

Figure 31. Mean NDF concentration of samples of AC Grazeland and Ladak 65 alfalfa taken from the top 15 cm of the plant during Trial 4, 1999. * or ** denote significant differences at P<0.1 and 0.05, respectively. Values based on four replications of each variety.

Table 20. Neutral detergent fiber concentrations (g kg^{-1}) of AC Grazeland (ACG) and Ladak 65 (whole plant) before and after grazing during Trial 4, 1999. Values based on two replications of each treatment.

	PRE-GRAZE		POST-GRAZE		LSD		GRAZING STATUS (G) ACROSS VARIETIES			VARIETY (V) ACROSS GRAZING STATUS			VxG
DATE	LADAK 65	ACG	LADAK 65	ACG	P=0.05	P=0.1	PRE-GRAZE	POST-GRAZE	P <	LADAK 65	ACG	P <	P <
9 June	380.6	430.2	439.2	604.1	153.0	102	405.4	521.6	.05	409.9	517.1	.05	NS
11 June	409.4	409.8	398.9	462.6	NS	NS	409.6	430.7	NS	401	436.2	NS	NS
17 June	369.5	389.6	485.8	503.4	NS	NS	379.5	494.6	.05	427.7	446.5	NS	NS
21 June	414.5	421.2	397.5	541.3	NS	NS	417.8	469.4	NS	406.0	481.2	NS	NS
23 June	437.4	453.5	555.6	564.0	76.0	51.0	445.5	559.8	.05	496.5	508.7	NS	NS
25 June	453.4	455.3	542.7	548.9	109.0	73.0	454.4	545.8	.05	498.1	502.1	NS	NS
28 June	474.7	530.5	533.0	610.8	56.0	37.0	502.6	571.9	.05	503.9	570.6	.05	NS
30 June	390.0	563.2	528.7	558.2	NS	NS	476.6	543.5	NS	459.4	560.7	0.1	NS
2 July	512.6	479.3	571.4	578.4	42.0	28.0	496.0	574.9	.05	542	528.9	NS	NS
Mean	467.7	458.3	526.7	557.8	53.0	36.0	463.3	542.2	.05	497.2	508.0	NS	NS

Trial 9, with or without N fertilizer, 2000

Levels of NDF during June 2000 were highest for fertilized AC Grazeland alfalfa (Table 21). Overall, AC Grazeland had 8% higher levels of NDF than Ladak 65 across fertilizer levels. These results are similar to the results from 1999, however, they do not fully explain differences in mean BSI in Trial 9.

Trial 10, with or without N fertilizer, 2000

During this trial, only fertilized Ladak 65 had significantly higher levels of NDF than each of the other sample groups (Table 22). This is in contrast to Trial 4 and Trial 9. The differences in these trials may have been due to the response of AC Grazeland to insufficient irrigation.

Correlation of Bloat Incidence with Bloat and Plant Factors

Thirty paired data groups were used to calculate correlation between bloat and plant factors. Correlation to percent bloat (Table 23) showed CP as the factor most highly correlated to bloat. A model (M1) was developed predicting bloat with CP. Residuals from this model showed NO_3 having the highest correlation to residuals from M1. A second model (M2) was developed predicting bloat with CP and NO_3. P values for both factors were less than 0.1. The overall model had a multiple R^2 of 0.26 and P-value less than 0.05. Residuals from M2 were used to develop three factor models.

Table 21. Neutral detergent fiber concentrations (g kg^{-1}) of the upper 15 cm of forage of AC Grazeland (ACG) and Ladak 65 with or without N fertilization during Trial 9, 2000. Values two replications of each treatment.

	Without N		With N		LSD		N Fertilizer status (F) Across Varieties			Variety (V) Across N Fertilizer Status			VxF
DATE	Ladak 65	ACG	Ladak 65	ACG	P=0.05	P=0.1	Without N	With N	P <	Ladak 65	ACG	P <	P <
1 June	197.2	233.6	222.1	255.6	53.2	35.7	215.4	238.8	NS	209.7	244.6	0.1	NS
2 June	220.6	235.3	109.5	204.0	45.7	30.6	227.9	199.7	0.1	208.0	219.6	NS	NS
5 June	193.4	184.5	222.8	220.4	89.0	59.6	188.9	221.6	NS	208.1	202.4	NS	NS
6 June	176.0	236.0	192.4	258.3	131.3	27.9	206.0	225.3	NS	184.2	247.1	0.1	NS
7 June	215.4	217.1	195.2	259.2	71.0	47.6	216.2	227.2	NS	205.3	238.1	NS	NS
8 June	252.2	254.8	216.3	235.9	34.2	22.9	253.5	226.1	0.05	234.2	245.4	NS	NS
9 June	236.9	265.8	251.9	253.1	135.8	91.0	251.4	252.5	NS	244.4	259.4	NS	NS
13 June	296.7	258.2	265.5	319.8	112.8	75.6	277.5	262.6	NS	281.1	289.0	NS	NS
15 June	261.2	301.2	270.9	249.3	97.7	65.4	281.2	260.1	NS	266.0	275.2	NS	NS
16 June	254.1	243.8	249.1	316.1	1.34	9.0	248.9	282.6	0.05	251.6	280.0	0.05	0.05
18 June	301.3	340.9	299.8	296.9	160.5	107.5	321.1	298.3	NS	300.5	318.9	NS	NS
Mean	236.8	251.9	234.7	260.7	42.6	28.6	244.4	247.7	NS	235.7	256.3	0.1	NS

Table 22. Neutral detergent fiber concentrations (g kg^{-1}) of the upper 15 cm of forage of AC Grazeland (ACG) and Ladak 65 with or without N fertilization during Trial 10, 2000. Values based on two replications of each treatment.

	Without N		With N		LSD		N Fertilizer status (F) Across Varieties			Variety (V) Across N Fertilizer Status			VxF
DATE	Ladak 65	ACG	Ladak 65	ACG	P=0.05	P=0.1	Without N	With N	P <	Ladak 65	ACG	P <	P <
9 Sept	219.0	240.7	216.2	183.3	19.3	13.0	229.8	199.9	0.05	217.6	212.1	0.05	NS
10 Sept	241.4	267.8	260.7	244.3	NS	NS	254.6	252.5	NS	251.0	256.0	NS	NS
11 Sept	288.4	347.5	304.1	289.1	NS	NS	317.9	296.6	NS	296.3	318.3	NS	NS
12 Sept	248.6	220.6	372.0	256.6	NS	NS	234.6	314.3	NS	310.3	238.6	NS	NS
15 Sept	249.6	247.3	320.4	248.7	NS	87.3	248.4	284.6	NS	285.0	248.0	NS	NS
Mean	249.4	264.8	294.7	244.4	NS	38.9	257.1	269.5	NS	272.0	254.6	NS	0.1

Table 23. Correlation (r) of plant factors with percent bloat. Values based on 30 samples from Trial 9.

FACTORS	r	P <
Bloat Severity	0.60	0.05
Variety	0.25	0.05
N Fertilization	0.27	0.05
CP	0.41	0.05
ADF	-0.31	0.05
NDF	-0.34	0.05
NO_3	0.29	0.05

However, P-values for additional factors were all greater than 0.1. Model M2 suggests that 26 percent of the variability in bloat incidence is due to CP and NO_3. Model M1 had a multiple R^2 of 0.17 suggesting that CP accounts for 17 percent of the variability in bloat incidence and that NO_3 accounts for 9 percent. Variety had the lowest correlation to bloat of all factors measured. However, this result might be substantially different if the varieties used in this study were extremely different.

Correlation to bloat severity was 0.07, -0.10, -0.11, 0.17, 0.12 and 0.23 for variety, fertilizer level, CP, ADF, NDF and NO_3, respectively (Table 24). These results did not yield adequate linear models. However, they are often in contrast both in degree and direction. This suggests that incidence and severity may be caused by different factors.

Table 24. Correlation (r) of plant factors with bloat severity. Values based on 30 samples from Trial 9.

FACTORS	r	P <
Bloat Severity	0.60	0.05
Variety	0.07	0.05
N Fertilization	-0.10	0.05
CP	-0.11	0.05
ADF	0.17	0.05
NDF	0.12	0.05
NO_3	0.23	0.05

CHAPTER 5

CONCLUSIONS

Both incidence and severity of bloat in livestock grazing AC Grazeland appear to be slightly less than that of Ladak 65. Mean BSI across all trials were 1.60 for Ladak 65 vs. 1.53 for AC Grazeland. These results are similar to several Canadian evaluations. However, plant maturity, environment and animal susceptibility all appear to play a greater role than cultivar in both bloat incidence and severity.

The 4-h DMD of fresh samples of AC Grazeland was significantly lower than that of Ladak 65 ($P < 0.1$), but the differences were never more than two percent. This difference is far below the hypothesized 50% reduction in IRD needed to eliminate bloat. Ladak 65 is a hardy dryland variety, and may have characteristics that cause it to be a previously unrecognized LIRD variety also.

At the beginning of the growing season, maturity and growth of both varieties were similar. However, AC Grazeland required more water to continue growth than Ladak 65 and went dormant earlier in the fall.

On half of the 10 grazing trials, bloat severity on AC Grazeland pasture with sheep and cattle was less than that of Ladak 65. In one trial, sheep bloat severity on Ladak 65 was lower than that of AC Grazeland. In four trials there were no significant differences between varieties. While no single factor causes bloat, 4-h DMD, frost, fiber, and N fractions appear to play a role in initiation and severity of bloat.

AC Grazeland does appear to have less bloat tendency than Ladak 65. However, bloat storms tended to occur simultaneously on both varieties apparently stimulated by frost or high levels of N. During these storms, AC Grazeland was not bloat-safe. AC Grazeland does not reduce bloat consistently or to the degree necessary to work as the sole bloat management tool in production.

In these trials, NO_3 appeared to be related to bloat initiation. Nitrogen fertilization increased CP and NO_3 concentrations of alfalfa forage, and generally corresponded to increased bloat. In *in situ* digestion studies, N-fertilized alfalfa had a high NO_3 level which was rapidly digested. Pasture NO_3 levels associated with increased bloat incidence and severity were also potentially toxic. It appears that NO_3 has been overlooked as a potential component of SN involved with alfalfa pasture bloat. However, due to difficulties in obtaining simultaneous bloat and appropriate samples for SN and NO_3, this finding is inconclusive. Certainly the role of NO_3 in bloat requires more study.

REFERENCES CITED

Association of Official Analytical Chemists International. 2000. Official methods of analysis. Vol. 1. 17th ed. AOAC. Washington, DC.

Bailey, C. B. and C. C. Balch. 1961. Saliva secretion and its relation to feeding in cattle. 1. The composition and rate of secretion of parotid saliva in a small steer. Br. J. Nutr. 15: 371-382.

Bartley, E. E. 1976. Bovine saliva. Production and function. In: Buffers in Ruminant Physiology and Metabolism (M.W. Weinberg and A.L. Sheffner, Eds.), Church Dwight, New York. pp. 61-77.

Berg, B. P., R. M. Tait, T. A. McAllister, W. Majak, S., Achary and K.-J. Cheng. 1996. Bloat in cattle grazing an alfalfa cultivar selected for reduced digestibility and low bloat incidence. Proceedings of the Forage and Grassland Conference, (M. J. Williams, Ed.)Vancouver, B. C. 13-15 June. American Forage and Grassland Council, Georgetown, TX. pp. 111-115.

Berg, B. P., B. Coulman, B. Coplen, J. Hall, W. Majak, T. A. McAllister, D. McAriney, R. M. Tait, and K.-J. Cheng. 1997. An alfalfa cultivar selected for low initial rate of digestion reduces bloat in grazing steers. p. 73 In: Proceedings of the 2nd National Alfalfa Grazing Conference (J. Johnson, ed.) Omaha, NE, 16 Aug, Certified Alfalfa Seed Council, Davis, CA.

Berg, B. P., W. Majak, T. A. McAllister, J. W. Hall, D. McCartney, B. E. Coulman, B. P. Goplen, S. N. Acharya Jr., M. Tait and K.-J. Cheng. 1999. Bloat in cattle grazing alfalfa cultivars selected for a low initial rate of digestion: A review. Can J. Plant Sci. 20: 494-502.

Branine, M. E., and M. L. Galyean. 1990. Influence of grain and monensin supplementation on ruminal fermentation, intake, digesta kinetics and incidence and severity of frothy bloat in steers grazing winter wheat pasture. J. Anim. Sci. 68: 1139-1150.

Bryant, M. P., I. M. Robinson, and I. L. Lindahl. 1961. A note on the flora and fauna in the rumen of steers fed a feedlot bloat-provoking ration and the effect of penicillin. Appl. Microbial. 9: 511-515.

Buck, W. B., 1976. Clinical and Diagnostic veterinary toxicology. Kendall/Hunt Publishing Co., Dubuque, Iowa. (G. A. Van Gelder ed.)

Bush, L. and H. Burton. 1994. Intrinsic chemical factors in forage quality. In: Forage quality, evaluation and utilization. (G. C. Fahey, M. Collins, D. R. Mertens, and L. E. Mosereds, eds.) Madison, WI 364-405. American Society of Agronomy, 1994. pp. 77-83, 109-117

Cantliffe, D. J. 1972. Nitrate accumulation in spinach cultivars and plant introductions. Can. J. Plant Sci. 53: 365-367.

Carruthers, V. R., A. M. Bryant and F. R. M. Cockrem. 1988. Quantity of digesta in the reticulo-rumen of cows differing in susceptibility to bloat. N. Z. J. Agric. Research. 31: 111-119

Cheng, K.-J., T. A. McAllister, J. D. Popp, A. N. Hristov, Z. Mir and H. T. Shin. 1998. A review of bloat in feedlot cattle. J. Anim Sci. 76: 299-308.

Cheng, K.-J. and J. W. Costerton. 1987. The electron microscopy of bacteria involved in the digestion of forage material by cattle. An Rep Okla Agric Exp Stn. Stillwater: The Station. July 1987 121: 114-125.

Cheng, K.-J. and J. W. Costerton. 1975. Ultrastructure of cell envelopes of bacteria of the bovine rumen. Appl. Microbiol. 29: 841-849.

Cheng, K.-J., R. Hironaka, G. A. Jones, T. Nicas and J. W. Costerton. 1976. Frothy feedlot bloat in cattle: production of extracellular polysaccharides and development of viscosity in cultures of Streptococcus bovis. Can J. Microbiol. 22: 450-459.

Cherney, D. J. R., J. H. Cherney, and A.N. Pell. 1994. Inorganic nitrogen supply effects on alfalfa forage quality. J. Dairy Sci. 77: 230-236.

Clarke, R. T. J. 1966. Bloat in cattle. Preliminary observations with the antiprotozoal agent, I, Z-dimethyl-5-Nitromidazole for the prevention of bloat. N. Z. J. Agric. Research. 9: 437-443.

Clarke, R. T. J., and R. E. Hungate. 1971. Bloat in cattle. 35. Microbiologial activities in the reticulo-rumens of cows differently susceptible to legume bloat. N. Z. J. Agr. Res. 14 108-121.

Clarke, R.T. J. and C. S. W. Reid. 1969. Bloat in cattle. The effect of dimetridazole on the rumen ciliate protezoa of dry and lactating cows. N. Z. J. Agric. Research. 12: 437-445.

Clarke, R. T. J. and C. S. W. Reid. 1970. Legume bloat.n In: Physiology of Digestion and Metabolism and the Ruminant. (A. T. Philipson, ed.) Oriel Press. pp. 599-602

Clarke, R. T. J., and C. S. W. Reid. 1973. Foamy bloat of cattle. A review. J. Dairy Sci. 57: 753-781.

Clarke, R. T. J., W. T. Jones, and C. S. W. Reid. 1974. Bloat in cattle. N. Z. J. Agric. Res. 17: 411-415.

Cockrem, F. R. M., 1975. Physiological genetics as a basis for the study of susceptibility to bloat in cows. Proc. N. Z. Soc. Anim. Prod. 35: 21-28.

Cockrem, F. R. M., J. T. McIntosh, and R. McLaren. 1983. Selection for and against susceptibility to bloat in dairy cows- a review. Proc. N. Z. Soc. Anim. Prod. 43: 101-106.

Cockrem, F. R. M., J. T. McIntosh, R. McLaren, and C. A. Morris. 1987. The relationship between volume of rumen contents and genetic susceptibility to pasture bloat in cattle. Br. Soc. Anim. Prod. 45: 43-47.

Coulman, B., B. Goplen, W. Majak, T. McAllister, K.-J. Cheng, B. Berg, J. Hall, D. McCanterey and S. Acharya. 1999. A review of the development of a bloat-reduced alfalfa cultivar. Can. J. Plant Sci. 80: 487-491.

Crawford, R.R., W.K. Kennedy, and K.L. Davison. 1966. Factors influencing toxicity of forages that contain nitrate when fed to cattle. Cornell Vet. 56: 1-17.

Edwards W. C. and C. P. McCoy. 1980. Nitrate poisoning in Oklahoma cattle. Vet. Med. Small. Anim. Clin. March 1980: 457-458.

Fay, J. P., K.-J. Cheng, M. R. Hanna, R. E. Howarth and J. W. Costerton. 1980. In vitro digestion of bloat-safe and bloat-causing legumes by ruminal microorganisms: gas and foam production. J. Dairy Sci: 63: 1273-1281.

Fay, J. P., K. J. Cheney, M. R. Hanna, R. E. Howarth and J. W. Costerton. 1981. A scanning electron microscope study of the invasion of leaves of a bloat-safe or bloat-causing legume by rumen microorganisms. Can. J. Microbiol 27: 390-399.

Garry, F. 1990. Managing bloat in cattle. Vet. Med. 85: 643-650.

George, J. R. C. L. Rhykerd, and C. H. Noller. 1971. Effect of light intensity, temperature, nitrogen, and stage of growth on nitrate accumulation and dry matter production of a sorghum-sudangrass hybrid. Agron. J. 63: 413-415.

Gomm, F. B., 1979. Alfalfa for hay and pasture in southeastern Oregon. Special Report 5-31. Ag. Exp. Station, Oreg. S. Univ, Corvallis

Gomm, F. B., 1979c. Herbage yield and nitrate concentration in meadow plants as affected by environmental variables. J. Range Manage. 35: 359-364.

Goplen, B. P., R. E. Howarth and G.L. Lees. 1992. Selection of alfalfa for a lower initial rate of digestion and corresponding changes in epidermal and mesophyll cell wall thickness. Can. J. Plant Sci. 73: 111-122.

Gutierrez, J., R. E. Davis. I. L. Lindahl, and E. J. Warwick. 1959. Bacterial changes in the rumen during the onset of feedlot bloat of cattle and characteristics of Peptostreptococcus osdetii n. sp. J. Anim. Sci. 7: 16-22.

Guyer, P. Q. and A Hogg, 1980. Bloat prevention and treatment. In Cattlemans Library. Great Plains State Publication, GPE, 1701.

Hall, J. W. and W. Majak. 1983. Plant and animal factors in legume bloat. In Natural Toxicants in feeds and poisonous plants. pp 253-257 (P. R. Cheeke and L. R. Shull eds.) Proteins and Amino Acids. Avi Publishing, Inc. Westport, CT.

Hall, J. W., and W. Majak. 1991. Relationship of weather and plant factors to alfalfa bloat in autumn. Can. J. Anim. Sci. 71: 861-866.

Hall, J. W., and W. Majak. 1992. Rapid screening of feed supplements for the prevention of legume bloat. Can. J. Anim. Sci. 72: 613.

Hall, J. W., W. Majak, A. L. Van Ryswyk, R. E. Howarth and C. M. Kalnin. 1988. The relationship of rumen cations and soluble protein with predisposition of cattle to alfalfa bloat. Can. J. Anim. Sci. 68: 431-437.

Hall, J. W. and J. Majak. 1995. Effect of time of grazing or cutting and feeding on the incidence of alfalfa bloat in cattle. Can J. Anim Sci. 75: 271-273.

Hall, J. W., W. Majak, D. G. Stout, K.-J. Cheng, B. P. Goplen, and R. E. Howarth. 1994a. Bloat in cattle fed alfalfa selected for a low initial rate of digestion. Can. J. Anim. Sci. 74: 451-456.

Hall, J. W., W. Majak, R. J. Williams and R. E. Howarth. 1984. Effect of daily weather conditions on bloat in cattle fed fresh alfalfa. Can. J. Anim. Sci. 64: 943-950.

Hall, J. W., I. Walker and W. Majak. 1994b. Evaluation of supplements for the prevention of alfalfa bloat. Can Vet J. 35: 702-705.

Hicks, D. R. and R. H. Pederson. 1976. Defoliation and nitrogen fertilizer effects on nitrate nitrogen profiles in maize. Agron. J. 68: 476-478.

Howarth, R. E. 1975. A review of bloat in cattle. Can. Vet. J. 16: 281-294.

Howarth, R. E., R. K. Chaplin, K.-J. Cheng, B. P. Goplen, J. W. Hall, R. Hironaka, W. Majak and R. M. Radostits. 1991. Bloat in cattle. Agriculture Canada Publ. 1858/E. Ottowa, Ontario, Canada pp. 1-34.

Howarth, R. E., B. P. Goplen, S. A. Brandt and K.-J. Cheng. 1982. Disruption of leaf tissues by rumen microorganisms: An approach to breeding bloat-safe forage legumes. Crop Sci: 22: 564-568.

Howarth, R. E., B. P. Goplen, J. P. Fay and K.-J. Cheng. 1979. Digestion of bloat-causing and bloat-safe legumes. Annales de Recherches Veterinaires. 10: 332-334.

Howarth, R. E., B. P. Goplen, A. C. Fesser and S. A. Brandt. 1978. A possible role for leaf cell rupture in legume pasture bloat. Crop Sci. 18: 129-133.

Howarth, R. E., W. Majak, D. E. Waldern, S. A. Brandt, A. C. Fesser, B. P. Goplen, and D. T. Spurr. 1977. Relationship between ruminant bloat and the chemical composition of alfalfa herbage. I. Nitrogen and protein fractions. Can J. Anim. Sci. 57: 345-357.

Johns, A. T. 1954. Bloat in cattle on red clover. 1. N. Z. J. Sci. Tech. 36A: 289-293.

Jones, W. T. and J. W. Lyttleton. 1969. Bloat in Cattle XXIX. The foaming properties of clover proteins. N. Z. J. Agric. Res. 12: 31-46.

Jones, W. T. and J. W. Lyttleton. 1972. Bloat in Cattle XXXIX. Detection of protein precipitants (flavolans) in legumes. N. Z. J. Agric. Res. 16: 441-446.

Katz, M. P., T. G. Nagaraja, and L. R. Fina. 1986. Ruminal changes in monensin- and lasolocid-fed cattle grazing bloat-provocative alfalfa pasture. J. Anim. Sci. 63: 1246-1257.

Kopecny J. and Wallace R. J. 1982. Cellular location and some properties of proteolytic enzymes of rumen bacterial. Appl. Environ. Microbiol. 43: 1026-1033.

Kudo, H., K.-J. Cheng, M. R. Hanna, R. E. Howarth, B. P. Goplen and J. W. Costerton. 1985. Ruminal digestion of alfalfa strain selected for slow and fast initial rates of digestion. Can. J. Anim. Sci. 65: 157-161.

Lee, C. and D. Smith. 1972. Influence of nitrogen fertilizer on stands, yields of herbage and protein, and nitrogenous fraction of field-grown alfalfa. Agron. J. 64: 527-530.

Leek, B. F. 1983. Clinical diseases of the rumen: A physiologist's view. Vet. Record 113: 10-14.

Lees, G. L., R. E. Howarth, B. P. Goplen and A. C. Fesser. 1981. Mechanical disruption of leaf tissues and cells in some bloat-causing and bloat-safe forage legumes. Crop Sci 21: 444-448.

Lees, G.L., R.E. Howarth and B.P. Goplen. 1982. Morphological characteristics of leaves from some legumes forages: relation to digestion and mechanical strength. Can J. Bot. 60: 2126-2132.

Lees, G.L. 1984. Cutical and cell wall thickness: relation to mechanical strength of whole leaves and isolated cells from some forage legumes. Crop Sci. 24: 1077-1081.

Lindahl, I.L., R.E. Davis and R.T. Tertell. 1957. Production of bloat and other symptoms in sheep by alfalfa saponin administration. In: Alfalfa saponins. USDA Tech. Bull. 1161, Agric. Res. Serv., Washington, DC.

MacAdams J. W., J. Hill, and R. E. Whitesides. 1995. Frost-induced movement of potassium to the apoplast may increase bloat in ruminants grazing alfalfa. Crop Sci. 35: 1133-11362.

MacAdams J. W., and R. E. Whitesides. 1996. Growth at low temperatures increases alfalfa leaf cell constituents related to pasture bloat. Crop Sci. 36: 378-382.

Majak, W., J. W. Hall, L. M. Rode and C. M. Kalnin. 1986. Rumen clearance rates in relation to the occurrence of alfalfa bloat in cattle. J. Dairy Sci. 69: 1560-1567.

Majak, W. and J. W. Hall. 1990. Sodium and potassium concentrations in ruminal contents after feeding bloat-inducing alfalfa to cattle. Can. J. Anim. Sci. 70: 235-241.

Majak, W. and J. W. Hall, 1993. Effect of time of feeding on the incidence of alfalfa bloat in cattle. Proc. 22nd conf. On rumen function. Chicago, Il. (Abstr)

Majak, W., J. W. Hall, and W. P. McCaughey. 1995. Pasture management strategies for reducing the risk of legume bloat in cattle. J. Anim. Sci. 73: 1493-1498.

Majak, W., R. E. Howarth, A. C. Resser, B. P. Goplen, and M. W. Pedersen. 1980. Relationships between ruminant bloat and the composition of alfalfa herbage; aII. Saponins. Can J. Anim. Sci. 60: 699-708.

Majak, W., R. E. Howarth, K.-J. Cheng and J. W. Hall. 1983. Rumen conditions that predispose cattle to pasture bloat. J. Dairy Sci. 66: 1683-1688.

Majak. W., R. E. Howarth and P. Narasimhalu. 1985. Chlorophyll and protein levels in bovine rumen fluid in relation to alfalfa pasture bloat. Can. J. Ani . Sci. 65: 147-156.

Mangan, J. R., 1959. Bloat in cattle XI. Foaming properties of proteins, saponins, and rumen liquor. N. Z. J. Agr. Res. 2: 47-61.

McArthur, J. M. and J. E. Miltmore, 1969. Bloat investigations, the pH of rumen contents and its role in legume bloat. Can J. Anim. Sci: 49: 59-67.

McIntosh, J. T. and F. R. M. Cockrem. 1977. The genetics of susceptibility to bloat in cattle. II. Preliminary results from saliva samples from cows of high and low susceptibility. N.Z. J. Agric. Res 20: 263-268.

McMahon L. R., T. A. McAllister, B. P. Berg, W. Majak, S.M. Acharya, J. D. Popp, B. E. Coulman, Y. Wang and K.-J. Cheng. 1999. A review of the effects of forage condensed tannins on ruminal fermentation and bloat in grazing cattle. Can. J. Plant Sci. 20: 471-485.

Mendel, V. E., and J. M. Boda. 1961. Physiological studies of the rumen with emphasis on the animal factors associated with bloat. J. Dairy Sci. 44: 1881-98.

Miller, K. P., and E. C. Frederick,. 1966. Relationship between feedlot bloat and daily gain with dairy steers. J. Anim. Sci. 25: 1254.

Miltimore, J. E., J. M. McArthur, B. P. Goplen, W. Majak and R. E. Howarth. 1974. Variability of fraction I protein and heritabiliy estimates for fraction I protein and total phenolic constituents in alfalfa. Agron. J. 66: 384-386.

Miltimore, J. E., J. M McArthur and J. L. Mason. 1970. Bloat investigations. The threshold Fraction I (18s) protein concentrations for bloat and relationships between bloat and lipid, tannin, Ca, Mg, Ni and Zn concentrations in alfalfa. Can. J. Anim. Sci. 50: 61-68.

Mishra, B. D., E. E. Bartley, C. R. Fina, and M. P. Bryant. 1968. Bloat in cattle. XIV. Mucinolytic activity of several anaerobic rumen bacteria. J. Anim. Sci. 27: 1651-1656.

Montana Agricultural Statistics. 2000. MT Dept. Ag. and USDA. National Agriculture Statistics Service.

Moate, P. J., T. Clarke, L. H. Davis and R. H. Laby. 1997. Rumen gases and bloat in grazing dairy cows. J. Ag Sci. 129: 459-469.

Morris, C. A., N. G. Cullen, and H. G. Geertsema. 1997. Genetic studies of bloat susceptibility in cattle. Proc. N.Z. Soc. Anim. Prod. 57: 19-21.

Nelson, D. W. 1984. Effect of nitrogen excess on quality of food and fiber pp. 643-661. In: Nitrogen in Crop Production. Purdue University, West Lafayette, IN.

Okine, E. K., G. W. Mathison and R. T. Hardin. 1989. Relations between passage rates of rumen fluid and particulate matter and foam production in rumen contents of cattle fed on different diets ad lib. Br. J. Nutr. 61: 387-395.

Puschner, B. 2000. Anti-nutritional factors in alfalfa hay. In: Proceedings, 2000 National Alfalfa Symposium, (10-12) Dec. 2000; Las Vegas, NV.

Rajan, G. H., C. A. Morris, V. R. Carruthers, R. J. Wilkins and T. T. Wheeler. 1996. The relative abundance of a salivary protein, bSP30, is correlated with susceptibility to bloat in cattle herds selected for high or low bloat susceptibility. Anim. Genet. 27: 407-414.

Shertz, D. L. and D. A. Miller. 1972. Nitrate N accumulation in soil profile under alfalfa. Agron. J. 64: 660-664.

Shirley, R. L. 1986. Nitrogen and energy, nutrition of ruminants. In Animal Feeding and Nutrition. (Tony J. Cunha, ed.) Academic Press Inc., Harcourt, Brace, Jovanovich, Orlando. pp. 26-57.

Smith, D. and J. M. Sund. 1965. Influence of stage of growth and soil nitrogen on nitrate content of herbage of alfalfa, red clover, ladino, clover, trefoil, and bromegrass. J. Agr. Food Chem. 13: 81-84.

Stritzke, J. R., L. I. Croy and W. E. McMurtry, 1976. Effect of shade and fertility on NO_3,-N accumulation, carbohydrate content, and dry matter production of tall fescue. Agron. J. 68: 387-389.

Turner, M. A. 1981. Dietary potassium-sodium imbalance as a factor in the aetiology of bloat of primary ruminal tympany in dairy cows. Vet. Res. Commun. 5: 159-164.

Undersander, D.J., D.R. Mertens, and N. Thiex. 1993. pp 83-108. Forage Analysis Procedure.

Walgenbach, R. P. and G. C. Marten. 1980. Release of soluble protein and nitrogen in alfalfa. III. Influence of shading. Crop Sci. 21: 859-862.

Walgenbach, R. P., G. C. Marten and G. R. Blake. 1981. Release of soluble protein and nitrogen in alfalfa. I. Influence of growth temperature and soil moisture. Crop Sci. 21: 843-849.

Wheeler, T. T., B. J. Haigh, R. J. Wilkins, J. Y. McCracken and C. A. Morris. 1998. A candidate gene marker for susceptibility in cattle? Proc. N.Z. Soc. Anim. Prod. 58: 10-12.

Wright, M. J. and K. L. Davison. 1964. Nitrate accumulation in crops and nitrate poisoning in animals. Adv. Agron. 16: 197-248.

APPENDIX A

NIRS SAMPLE ANALYSIS

A NIRSystems inc. Model 4500 Spectrophotometer (NIRSystems, Inc. Silver Spring, MD, 301-680-9600) was used to scan all plant samples. The scans were entered into the database of the ISI Infra-red software named "Infratec 2" (NIRSystems, Inc. Silver Spring, MD 301-680-9600). A calibration equation was developed using the "center" and "Select" programs in the software, which isolated a range of scans from a group of samples that had been selected from all samples throughout the research. These samples were analyzed for TN, ADF, NDF and $N0_3$, at Midwest Labs, Omaha, NE. Midwest Labs uses wet chemistry methods following the National Forage Testing Association recommended methods. The $N0_3$ analyses were also conducted at the MSU Animal Nutrition Laboratory using the AOAC official method of $N0_3$ analysis (AOAC Official Methods of Analysis, 2000). Results from the laboratory were entered into the software database. The laboratory data were entered into the "Calibration" program of the software, and a calibration equation for each different portion of our research was developed. The standard error of calibration (SEC) for each constituent was used in the NIRS quality control calculations to establish error limits (Undersander et al., 1993) [Table 25]. The scans were analyzed using the "Predict" program. The "Predict" program used the calibration equation to predict CP, ADF, NDF, $N0_3$, and bloat. Validation of the calibration equation was accomplished by selection for a validation set. The validation set represented alfalfa samples taken at different times and with different methods throughout the trial. These samples were analyzed for CP, ADF, NDF and $N0_3$, at Midwest Labs, Omaha, NE. The samples were scanned by the spectrophotometer and entered into the database. The scans were analyzed using the "Predict" program in the software providing predicted results. The predicted results were compared to the laboratory results using the "Statistical" program in the software. The standard error of prediction corrected for bias (SEPC) was produced for each constituent. The NIRS provided excellent prediction ($R^2 > 0.97$, $P < 0.01$) of CP, ADF, and NDF of the alfalfa in pasture studies.

Table 25. Calibration and validation statistics for CP, ADF and NDF concentrations (g kg $^{-1}$) by NIRS.

	Mean	N	Standard Error	Standard Error Confidence Limit	Bias	Bias Confidence Limit	R^2
CP							
Calibration	226.1	14	8.43	--	--	--	0.95[1]
Validation	226.2	14	8.74	18.8	0.0	08.7	0.98[2]
ADF							
Calibration	291.9	14	13.18				0.97
Validation	291.9	14	13.46	24.3	0.0	11.2	0.99
NDF							
Calibration	356.0	14	15.89				0.97
Validation	356.0	14	16.2	40.4	0.0	18.6	0.97

[1] Multiple correlation coefficients squared for equation development.
[2] Correlation coefficient squared between predicted and measured constituents.