AN ABSTRACT OF THE THESIS OF

Dustin L. McGuire for the degree of Master of Science in Animal Sciences presented on August 29, 2013
Title: Influence of Supplement Composition on Utilization of Low-Quality Cool-Season Forages by Beef Cattle

Abstract approved:

David W. Bohnert                                           Reinaldo F. Cooke

Two studies were conducted to evaluate the influence of supplement composition on intake and digestibility of a low-quality (< 6% CP), cool-season forage, as well as cow performance. Treatments included a non-supplemented control (CON), corn (approximately 8% CP), corn and urea (LU = corn + 0.09 mg/kg BW urea, approximately 27% CP; HU = corn + 0.17 mg/kg BW urea, approximately 43% CP) and a positive control of SBM (approximately 51% CP). In Experiment 1, 5 ruminally cannulated Angus x Hereford steers (560 ± 79 kg of BW) were used in an incomplete 5 x 4 Latin square with four 28-d periods to compare the effects of urea addition to a corn-based supplement on forage intake, digestibility and ruminal fermentation characteristics. Forage intake and digestibility were not influence by supplementation (P > 0.10); however, intake was greater for SBM than HU (P = 0.01). Ruminal NH₃-N increased with supplementation (P < 0.01), increased linearly with urea inclusion (P < 0.01) and was greater for HU than SBM (P < 0.01). However, ruminal NH₃-N for non-supplemented steers was 1.61 mM, within the range believed to support optimal growth of rumen microbes in vivo, suggesting that ruminally available-N was not limiting forage utilization. Total volatile fatty acid concentration was not influenced by supplement composition (P > 0.10). In Experiment 2,
80 late gestation (approximately 190 d pregnant) Angus x Hereford cows (507 ± 10 kg) were stratified by age, BCS, and BW and randomly allotted to the treatments described in Experiment 1 (20 pens; 4 cows/pen; 4 pens/treatment). Cow BW and BCS change were improved with supplementation (P < 0.01) and with increasing urea inclusion (P < 0.01), but did not differ between the HU and SBM treatments (P > 0.10). Cow BUN (P = 0.05), glucose and NEFA were not influenced by supplementation (P > 0.10); supplementation increased IGF-I (P < 0.01) and tended to increase insulin (P = 0.07). Blood variable concentrations did not differ between HU and SBM. These results suggest that a starch-based energy supplement fed at less than 0.5% of BW in conjunction with urea is an acceptable management alternative to supplementation with natural protein for ruminants consuming low-quality, cool-season forages.
Influence of Supplement Composition on Utilization of Low-Quality, Cool-Season Forages by Beef Cattle

by

Dustin L. McGuire

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______________________________
Co-Major Professor, representing Animal Sciences

______________________________
Co-Major Professor, representing Animal Sciences

______________________________
Head of the Department of Animal and Rangeland Sciences

______________________________
Dean of the Graduate School

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Dustin L. McGuire, Author
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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Literature Review</td>
</tr>
<tr>
<td>1.1</td>
<td>Introduction</td>
</tr>
<tr>
<td>1.2</td>
<td>Forage Quality</td>
</tr>
<tr>
<td>1.3</td>
<td>Cool-Season vs. Warm-Season Forages</td>
</tr>
<tr>
<td>1.4</td>
<td>Seasonal Variation of Forage Quality on Oregon Rangelands</td>
</tr>
<tr>
<td>1.5</td>
<td>Low-Quality Forage Supplementation</td>
</tr>
<tr>
<td>1.6</td>
<td>Protein Supplementation of Low-Quality Forages</td>
</tr>
<tr>
<td>1.7</td>
<td>Energy Supplementation of Low-Quality Forages</td>
</tr>
<tr>
<td>1.8</td>
<td>Energy Metabolism and Reproduction</td>
</tr>
<tr>
<td>1.9</td>
<td>Endocrine Control of Reproduction</td>
</tr>
<tr>
<td>1.10</td>
<td>Glucose</td>
</tr>
<tr>
<td>1.11</td>
<td>Insulin</td>
</tr>
<tr>
<td>1.12</td>
<td>Insulin-Like Growth Factor-I (IGF-I)</td>
</tr>
<tr>
<td>1.13</td>
<td>Non-Esterified Fatty Acids (NEFA)</td>
</tr>
<tr>
<td>1.14</td>
<td>Conclusion</td>
</tr>
<tr>
<td>2</td>
<td>Influence of Supplement Composition on Utilization of Low-Quality Cool-Season Forages by Beef Cattle</td>
</tr>
<tr>
<td>2.1</td>
<td>Introduction</td>
</tr>
<tr>
<td>2.2</td>
<td>Materials and Methods</td>
</tr>
<tr>
<td>2.3</td>
<td>Results and Discussion</td>
</tr>
<tr>
<td>2.4</td>
<td>Conclusion</td>
</tr>
<tr>
<td>3</td>
<td>Literature Cited</td>
</tr>
</tbody>
</table>
4 Appendix .......................................................................................................................... 68

4.1 Abbreviations ............................................................................................................. 69
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ingredient and nutrient content of fine fescue straw and supplements</td>
</tr>
<tr>
<td>2</td>
<td>Effects of supplement composition on intake and diet digestibility in steers consuming low-quality, cool-season forage</td>
</tr>
<tr>
<td>3</td>
<td>Effects of supplement composition on ruminal fill and fermentation characteristics in steers consuming low-quality, cool-season forage</td>
</tr>
<tr>
<td>4</td>
<td>Effects of supplement composition on blood characteristics in steers consuming low-quality, cool-season forage</td>
</tr>
<tr>
<td>5</td>
<td>Effects of supplement composition on cow performance and calf birth weight</td>
</tr>
<tr>
<td>6</td>
<td>Effects of supplement composition on blood characteristics in cows consuming low-quality, cool-season forage</td>
</tr>
</tbody>
</table>

# Figures

<table>
<thead>
<tr>
<th>Figures</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Effects of supplement composition on steer ruminal ammonia-N</td>
</tr>
</tbody>
</table>
LITERATURE REVIEW

Introduction

In the Pacific Northwest, many producers feed low-quality roughages to their cattle during the winter feeding period, corresponding to late gestation for spring calving cows. These low-quality forages often require some form of supplementation, normally protein and/or energy, to maximize forage utilization by ruminants (DelCurto et al., 1990a,b; Köster et al., 1996). Protein supplementation of ruminants consuming low-quality (< 6% CP) warm-season forage has been shown to increase forage intake and digestibility (DelCurto et al., 1990b). Recent research suggests that these findings may not hold true for cool-season, low-quality forages. Bohnert et al. (2011) reported that forage intake response to supplemental protein may depend on forage type, with cool-season forage intake not normally altered with protein supplementation, most likely due to greater forage digestibility and intake without supplementation compared with comparable quality warm-season forages. Also, energy supplementation above 0.5 and 0.8% of BW (Bowman and Sanson, 1996; Garcés-Yépez et al., 1997) for starch and fiber sources, respectively, has been shown to decrease forage intake and utilization by substituting supplement for forage (DelCurto et al., 1990a). Similarly, forage intake is decreased when the fat concentration is greater than 3% of diet DM (Hess et al., 2008).

The primary goal of a beef cow-calf operation is to annually have each cow produce a calf. However, this often does not occur, as reproductive disorders prevent cows from becoming pregnant, costing beef producers between $441 and $502 million dollars per year (Bellows et al., 2002). Yet, improved reproduction may cost more than
the income it returns. Thus, successful producers may not always have the highest conception rate in their cowherd, but rather manage best for economic profitability (DelCurto et al., 2000). Therefore, it is important to develop nutritional systems that enhance reproduction without negatively impacting net return.

Proper nutrition, specifically energy intake, is the most essential factor influencing reproductive function in cattle (Hess et al., 2005). Energy intake has a large influence on the postpartum interval, and as a result, the reproductive efficiency of beef cattle. Postpartum intervals greater than 83 d will result in cows that do not calve within a 365 d period from their last calf (Hess et al., 2005). Additionally, hormones and metabolites associated with energy metabolism are important regulators of reproduction. For example, circulating NEFA regulate reproduction by negatively influencing the synthesis and release of gonadotropins. Conversely, insulin and glucose can stimulate the release of GnRH from the hypothalamus, leading to a subsequent release of gonadotropins from the pituitary (Arias et al., 1992).

Little research utilizing low-quality cool-season forages has evaluated cow performance and forage intake and utilization in response to energy supplements of varying protein concentrations. Therefore, our objective was to evaluate the effects of energy and protein supplementation strategies on nutrient intake and utilization, metabolic hormones, and performance of ruminants consuming low-quality cool-season forage.
**Forage Quality**

Many characteristics are important in determining forage quality. In an article discussing the regulation of forage intake, Mertens (1994) suggests that intake is the most important factor for determining forage quality, since animal performance is dependent on the intake of digestible and metabolizable nutrients. He reports that differences in feed intake are responsible for 60 to 90% of the variation in digestible DM and/or DE among animals and feeds compared to only 10 to 40% for differences in digestibility. This fact is especially apparent when low-quality diets are fed because feed intake is limited by gastrointestinal tract capacity, thus limiting the intake of digestible nutrients (Mertens, 1994).

Also, because forage digestibility influences feed intake, it is an important factor to consider when determining forage quality. Forage digestibility is heavily influenced by the carbohydrate content, specifically structural carbohydrate content, of the forage. Carbohydrates can be divided into non-structural carbohydrates and structural carbohydrates. Non-structural carbohydrates are almost completely utilized by ruminants. They are degraded to simple sugars, which are rapidly fermented to yield VFA and ultimately serve as a primary substrate for energy metabolism (Morrison, 1979; Mertens, 1994). Conversely, structural carbohydrates are significantly less digestible, and thus slow digesta flow and decrease intake (Mertens, 1994; Buxton, 1996).

While it is important to remember that many forage characteristics influence quality, CP levels are highly correlated with many other factors, such as digestibility and
concentrations of other nutrients (Ganskopp and Bohnert, 2001). Due to this correlation, CP level can be used as the primary indicator of forage quality for beef cattle (Sullivan 1962).

**Cool-Season vs. Warm-Season Forages**

Forages can be grouped into cool- and warm-season types, which are distinguished by physiological and biochemical differences. One difference is their respective photosynthetic pathways. The first organic product during carbon fixation in cool-season (C3) grasses is the 3-C 3-phosphoglycerate, whereas the first product in warm-season (C4) forages is the 4-C oxaloacetate (Lambers et al., 1998). These forage types also differ in anatomy. Cool-season grasses have a high proportion of mesophyll, which is generally considered to be the most rapidly degraded plant tissue (Wilson, 1993). Additionally, the loosely arranged mesophyll of cool-season plants allows for quicker bacterial penetration and thus, faster digestion, than does the tightly packed mesophyll of warm-season plants (Akin, 1989; Wilson, 1993). In addition, the bundle sheath cells of cool-season plants are thin-walled and more readily digested than those found in warm-season plants (Wilson, 1993).

Cool-season forages are generally assumed to be of greater nutritional quality than warm-season forages (Galyean and Goetsch, 1993; Barbehenn et al., 2004), which is attributed to higher levels of protein and non-structural carbohydrates, as well as lower fiber content (Barbehenn and Bernays, 1992). Increased fiber content and slower
digestion of the cell wall in warm-season forages results in slower digesta flow and consequently, decreased intake by ruminants (Galyean and Goetsch, 1993).

**Seasonal Variation of Forage Quality on Oregon’s Rangelands**

Oregon rangeland is typically characterized by arid and semi-arid environments, high-elevations, short-growing seasons and shallow, poor-quality soils (DelCurto et al., 2000; Ganskopp and Bohnert, 2001). These characteristics lead to limited forage production and long periods of nutrient deficiencies. High-elevation rangelands are also typified by high climate variation between years and seasons (DelCurto et al., 2000). Ganskopp and Bohnert (2001) analyzed the nutrient composition of seven of the most common cool-season grasses in the Great Basin for April through November. Looking at the years 1992 and 1993 and adopting a 7.5% CP level as adequate, they found a great disparity in the length of time when adequate CP levels were available. In 1992, the various forages provided adequate CP at different times within the growing season, resulting in forage with adequate CP being available for about 185 d; whereas an abundance of soil moisture in the spring of 1993 resulted in all grasses maturing quickly and herbage was CP deficient by late July, thereby yielding a total of 83 d with adequate CP (Ganskopp and Bohnert, 2001). This creates a need for supplementation programs designed to address these types of management challenges.

**Low-Quality Forage Supplementation**

As previously mentioned, limited forage availability and significant variation in forage quality leaves much of Oregon's cow-calf industry dependent on low-quality,
cool-season forages for a significant portion of the year. These forages are often supplemented, typically with protein and/or energy, to maximize utilization based on previous work with low-quality, warm-season forages (DelCurto et al., 1990a,b; Koster et al., 1996). However, warm-season forages are a minor constituent of beef cattle diets in Oregon.

**Protein Supplementation of Low-Quality Forages**

*Cow Performance.* Research on the effects of protein supplementation of low-quality forages on cow performance typically focuses on changes in BW and BCS. DelCurto et al. (1990b) supplied isocaloric supplements of increasing CP (13, 25, and 39%) at 0.5% of BW to cows grazing dormant tallgrass prairie and noted linear decreases in BW and BCS loss with increasing levels of supplemental protein. Additionally, Bohnert et al. (2002b) reported that pre- and postcalving cow BW and BCS change were more positive with protein supplementation for cows consuming low-quality (5% CP) meadow hay. Also, Mathis et al. (1999) designed a study to identify the optimal level of supplemental soybean meal (SBM) for cows consuming low-quality prairie forage (3% CP). They offered supplemental SBM (54% CP) at 0.08, 0.12, 0.16, 0.20, 0.24, 0.28, 0.32, 0.40 and 0.48% BW/d for 69 d during the winter grazing period. Cow BW and BCS loss were decreased with increasing levels of supplemental SBM until reaching a plateau at 0.32% BW. Each 0.1% BW decrease in supplement below the aforementioned plateau resulted in a 0.5 unit decrease in BCS.
**Forage Intake.** Protein supplementation of low-quality (<~6% CP) warm season forages has been shown to increase forage intake, thus increasing overall energy intake (McCollum and Gaylean, 1985; DelCurto et al., 1990b; Köster et al., 1996). McCollum and Gaylean (1985) increased intake of low-quality prairie hay (6% CP) in steers by 27% with supplementation of cottonseed meal (CSM; 38% CP). Köster et al. (1996) ruminally infused increasing levels of soluble protein (sodium caseinate; 90% CP) in cows consuming low-quality prairie hay (2% CP). Forage OMI was increased in comparison to an unsupplemented control for cows supplemented with up to 720g/d of soluble protein.

To evaluate the effects of protein concentration on intake of low-quality, warm-season forage, DelCurto et al. (1990a) provided isocaloric supplements with increasing levels of CP (12, 28, and 41%). Forage intake as a percent of BW was increased with protein supplementation and responded in a quadratic fashion. Supplementation at the high and moderated levels resulted in steers consuming 60 and 42% more forage, respectively, than those supplemented at the low level.

**Digestibility.** Increases in forage intake with protein supplementation are likely a result of improved forage digestibility (McCollum and Gaylean, 1985; DelCurto et al., 1990a). McCollum and Gaylean (1985) reported IVDMD of prairie hay was increased with protein supplementation. Similarly, DelCurto et al. (1990a) noted that total tract DMD increased with protein supplementation. Mathis et al. (1999) showed increased total tract OM and NDF digestibility with increasing levels of CP compared to an
unsupplemented control, reaching a plateau when supplemental SBM reached 0.33% BW. In contrast, Mathis et al. (2000) noted that total tract digestion of OM and NDF were not affected by protein supplementation of steers consuming forage of 6 to 8% CP. However, digestibility was increased with supplementation when forage quality decreased to 4% CP (Mathis et al., 2000). These findings suggest that increases in digestibility of low-quality forages with protein supplementation are largely the result of improved N availability for rumen microorganisms.

*Rumen Degradable Protein (RDP).* Protein, specifically RDP, is generally considered to be the first limiting nutrient for ruminants consuming low-quality forage. The ability of rumen microorganisms to grow and reproduce is largely dependent on RDP availability (Köster et al., 1996; Bohnert et al., 2002a). Consequently, when RDP is deficient, microbial growth is limited resulting in depressed fiber digestion. As a result, forage intake is decreased by increased ruminal fill and decreased passage rates (McCollum and Gaylean 1985; Köster et al., 1996). Thus, supplements that result in adequate RDP to cattle fed low-quality forages typically increase forage intake and digestibility (Köster et al., 1996). The idea that protein supplements should consist primarily of RDP is further supported by studies investigating the effects of RUP on intake and digestibility of low-quality forage. Sletmoen-Olson et al. (2000) reported that supplemental RUP had little effect on cow performance and forage utilization when RDP is adequate to support normal rumen function. However, RUP supplementation can be beneficial when RDP is limiting. Rumen-undegradable protein can be absorbed from the small intestines as NH₃, free amino acids or peptides, which can be directly used by the
animal. The absorbed N can also enter the N recycling process and be deaminated to urea N by the liver and ultimately used as a source of RDP (Bohnert et al., 2002a,b,c).

**Ruminal Fermentation.** Maximum growth of rumen microbes in vivo has been shown to occur when ruminal NH$_3$ is between 1.18 and 2.94 mM (Satter and Slyter, 1974; Slyter et al., 1979). Supplementation of CP to ruminants consuming low-quality forages routinely increases ruminal NH$_3$ concentration (Horney et al., 1996; Köster et al., 1996; Mathis et al., 1999). Consequently, protein supplementation when ruminal NH$_3$ concentration limits microbial growth helps to increase fiber digestion and forage intake.

Fiber digestion by cellulolytic microorganisms is also dependent on branch chain VFA (BCVFA) production resulting from the deamination of branch chain amino acids by non-cellulotic bacteria (Yokoyama and Johnson, 1988). These BCVFA are used as the carbon skeletons for the synthesis of amino acids, as well as for synthesis of long-chain fatty acids typically incorporated into the bacterial cell membranes. Protein supplements have increased total VFA concentration (Hannah et al., 1991; Köster et al., 1996; Mathis et al., 1999; 2000) and the proportion of BCVFA (Mathis et al., 1999; 2000). Conversely, others have noted no increase in total and/or BCVFA concentration with CP supplementation (McCollum and Gaylean, 1985; Bodine et al., 2000), possibly due to increased rumen fluid dilution rate and liquid volume as a result of CP supplementation (Bohnert et al., 2002c).

Rumen microbes also require S for microbial growth (Hume and Bird, 1970), as well as for the synthesis of S-amino acids (methionine and cystine) for microbial protein
synthesis (Durand and Komisarczuk, 1988; Leng, 1990). Microbial growth is believed to be optimized at an N:S ratio of 10:1 (Hume and Bird, 1970; NRC, 1996). Furthermore, feed digestibility has been reported to be decreased when dietary sulfur is inadequate (Rumsey, 1978; Leng, 1990).

Cool- vs Warm-Season Forages. While many studies suggest that protein supplementation increases intake and digestibility of low-quality, warm-season forages, recent research suggests that these findings may not hold true for cool-season, low-quality forages. Forage intake has not been reported to increase in most studies with protein supplementation of low-quality, cool-season forages (Mathis et al., 2000; Bohnert et al., 2002a,b; Currier et al., 2004a,b). Cool-season forages are reported to have a greater proportion of CP as RDP compared with warm-season forages (Bohnert et al., 2011; 2013). This greater RDP concentration likely results in increased in ruminally available-N capable of supporting improved microbial growth. Additionally, Bohnert et al. (2011) suggest that the difference in forage intake response to supplemental protein between cool- and warm-season forages is likely due to the greater digestibility and intake of cool-season forages without supplementation compared with warm-season forages of comparable quality.

Forage DMI without supplementation is typically observed to be greater than 1.7% of BW in studies utilizing low-quality cool-season forages, resulting in no increase in forage intake with protein supplementation (Mathis et al., 2000, Currier et al., 2004a,b, Bohnert et al., 2011). Conversely, intake of low-quality warm-season forages is often
less than 1% of BW without supplementation, allowing for a greater potential increase in response to protein supplementation (DelCurto et al., 1990b; Köster et al., 1996; Mathis et al., 1999).

Differences in intake response to protein supplementation between cool- and warm-season forages may be associated with NDF intake. Research suggests that intake is maximized when NDF intake is approximately 12.5 g∙kg of BW\(^{-1}\)·d\(^{-1}\) (Mertens 1985, 1994). In agreement with this, Bohnert et al. (2011) reported an NDF intake below 12.5 g∙kg of BW\(^{-1}\)·d\(^{-1}\) (10.8 g∙kg of BW\(^{-1}\)·d\(^{-1}\)) for an unsupplemented warm-season forage and an increase to 16.0 g∙kg of BW\(^{-1}\)·d\(^{-1}\) with protein supplementation (an almost 50% increase in forage DM intake). Conversely, the same study reported an NDF intake greater than 12.5 g∙kg of BW\(^{-1}\)·d\(^{-1}\) for an unsupplemented cool-season forage and a minimal increase (less than 7%) in forage intake in response to protein supplementation (Bohnert et al., 2011). Similar results with cool-season forages have been seen in other studies (Galloway et al., 1991; Mathis et al., 2000) in which NDF intake was above 12.5 g∙kg of BW\(^{-1}\)·d\(^{-1}\) without supplementation and no increase in forage intake was seen with protein supplementation. In contrast, studies with warm season forages (DelCurto et al., 1990a,b; Köster et al., 1996; Bandyk et al., 2001) showed NDF intakes without supplementation of 6.4, 5.1, and 8.2 g∙kg of BW\(^{-1}\)·d\(^{-1}\) respectively and 14.3, 11.3 and 13.3 g∙kg of BW\(^{-1}\)·d\(^{-1}\) with supplementation. Consequently, increases in forage intake were noted for each of these studies.
Another factor possibly influencing the intake response to protein supplementation of cool- and warm-season forages may be OM intake. In a review on the effects of supplementation on voluntary forage intake, Moore et al. (1999) suggested that forage intake should not be expected to increase if forage OM intake is greater than 17.5 g·kg of BW\(^{-1} \cdot \text{d}^{-1}\) without supplementation. Studies by Mathis et al. (2000) and Bohnert et al. (2002a,b; 2011) noted forage OM intake greater than 17.5 g·kg of BW\(^{-1} \cdot \text{d}^{-1}\) for unsupplemented cool-season forages and indicated no increase in forage intake with supplementation. Additionally, findings by Mathis et al. (2000) suggest a similar relationship in steers consuming warm-season forages. When OM intake was greater than 17.5 g·kg of BW\(^{-1} \cdot \text{d}^{-1}\), protein supplementation resulted in no increase in forage intake in steers consuming a warm-season grass hay (Mathis et al., 2000). Most studies on supplementation of low-quality, warm-season forages in which OM intake is less than 17.5 g·kg of BW\(^{-1} \cdot \text{d}^{-1}\) without supplementation report an increase in both OM intake and forage intake with supplementation (Lintzenich et al., 1995; Köster et al., 1996; Bohnert et al., 2011). Nevertheless, studies by Horney et al. (1996) and Currier et al. (2004a,b) saw no increase in forage OM intake or forage intake with protein supplementation, despite forage OM intake less than 17.5 g·kg of BW\(^{-1} \cdot \text{d}^{-1}\) without supplementation.

*Physical Form of Protein Supplements.* Protein can be supplemented in numerous forms, such as oilseed meals, NPN, legumes and various byproducts. The most widely used sources of supplemental protein are derivatives of oilseed byproducts, such as SBM. These protein sources offer energy densities similar to those of cereal grains, while also providing high concentrations of protein (NRC, 1996). Much of the research indicating
an increase in intake and digestibility of low-quality forages in response to protein supplementation has utilized SBM as the supplemental protein source (DelCurto et al., 1990a,b,c; Mathis et al., 1999) However, the high cost of these feeds has led to increased efforts to use cheaper alternatives, such as NPN.

Nutritionists have known since the late 1800's that ruminants are capable of converting NPN to protein (Helmer and Bartley, 1971). The most widely used source of NPN is urea, which offers a high N content and a low cost per unit N. However, urea is hydrolyzed to NH$_3$ within the rumen at a rate exceeding the capability of rumen microbes to assimilate NH$_3$ into microbial protein (Chalupa, 1968; Helmer and Bartley, 1971; Bartley et al., 1976). Ruminal pH increases in response to this rapid release of ammonia, stimulating increased absorption of NH$_3$ from the rumen into the portal blood. Excess blood NH$_3$ is removed predominantly by conversion to urea in the liver, although ammonia may also be excreted in the urine as ammonia salts or used to form glutamine via transamination (Essig et al., 1988).

Urea toxicity occurs when the ammonia-urea conversion capacity of the liver is exceed, resulting in increased blood NH$_3$ levels (Chalupa, 1968; Essig et al., 1988). Consequently, it is suggested that urea intake not exceed 0.3 g/kg BW (Helmer and Bartley, 1971). Other sources of NPN, such as biuret, can be used to help avoid NH$_3$ toxicity by offering slower ruminal release of NH$_3$ (Owens and Zinn, 1988); however, a higher cost per unit N hinders their use when compared to urea, limiting industry adoption of biuret and other slow release compounds.
Numerous studies have evaluated the usefulness of NPN as a supplemental protein source. Currier et al. (2004a,b,c) reported that supplementation of low-quality forage with urea or biuret to ruminants had no adverse effects on forage intake, nutrient digestibility, site of digestion, or microbial efficiency when compared with non-supplemented controls. These findings suggest that ruminants can efficiently use NPN as a source of supplemental nitrogen in place of expensive natural protein supplements. This agrees with earlier research by Köster et al. (1997; 2002) that indicated urea can be substituted for supplemental protein without negatively impacting diet intake, digestibility or palatability. However, Köster et al. (2002) suggest that maximal performance occurs with urea inclusion levels of less than 40% of RDP.

Legumes can also be effectively used as a source of supplemental protein. Many legumes, such as soybeans, peas and lentils, are typically produced for human consumption, with cull seeds fed to livestock (Kellems and Church, 2009). Forage legumes (i.e. alfalfa hay) are often used as a source of high protein forage and can be used for supplemental protein as effectively as the more common oilseed meals (DelCurto et al., 1990c).

Aside from oilseed meals, many other byproducts can be used as a source of supplemental protein. Byproducts of the milling (i.e. corn gluten meal, corn gluten feed), distillery (i.e. distillers' grain) and brewery (i.e. brewers' grain) industries generally offer over 20% CP (Kellems and Church, 2009). Animal byproducts typically offer greater
than 80% CP and include blood meal, fishmeal and feathermeal (Kellems and Church, 2009), among others.

**Energy Supplementation of Low-Quality Forages**

Energy supplements can be divided into starch-based, fiber-based and fat based. The effect of physical form of energy supplements is discussed further in the section titled *Physical Form of Energy Supplements*. In general, high levels of energy supplementation typically result in decreased intake and digestibility of low-quality forages (Chase and Hibberd, 1987; Sanson et al., 1990). Chase and Hibberd (1987) noted a decrease in hay and fiber digestibility as supplemental corn increased. However, the cubic response seen for NDF, ADF and hay OM digestibility suggests that small quantities of supplemental corn may not have a large negative impact. Despite increased total OM digestibility, total digestible OMI was not increased with high levels of supplemental corn. The lack of differences in digestible OMI, along with a similar total VFA concentration suggests that cattle fed large quantities of supplemental energy substitute the supplemental energy source for forage which may result in a similar energy balance to non-supplemented controls (Chase and Hibberd, 1987). The lower substitution rate often seen with low levels of supplemental energy, routinely results in an increase in digestible OMI and an improvement in energy status of the animal (Chase and Hibberd, 1987). Other researchers have also noted a decrease in forage digestibility at high levels of supplemental energy, with little to no impact at low levels. (Sanson and Clanton, 1989; Sanson et al., 1990).
Corn and other starch-based supplements offered at greater than 0.5% BW (Bowman and Sanson, 1996; Garcés-Yépez et al., 1997) with inadequate RDP have long been shown to decrease forage intake and digestibility (Chase and Hibberd, 1987; DelCurto et al., 1990a). Decreased ruminal pH is often believed to be a cause of decreased digestibility and intake of forages in cattle supplemented with starch based energy supplements. Lower ruminal pH often shifts the rumen microbes from a cellulolytic population towards an amylolytic population, resulting in impaired digestion of forages (Caton and Dhuyvetter, 1997). However, in a review on the requirements and responses of grazing cattle to energy supplementation, Caton and Dhuyvetter (1997) report that ruminal pH response to starch supplementation is inconsistent, with some studies indicating a decrease in forage intake despite no changes in pH. These findings suggest that the decreased intake and digestibility of forages associated with starch supplementation cannot be entirely attributed to a decline in ruminal pH.

Decreases in ruminal NH$_3$ concentration with energy supplementation may also cause the decrease in forage digestibility. Chase and Hibberd (1987) noted a linear decrease in ruminal NH$_3$ as supplemental corn increased. Additionally, energy supplementation resulted in ruminal NH$_3$ levels dropping below those previously recommended for maximal microbial growth and activity (Chase and Hibberd 1987). DelCurto et al. (1990a) also noted decreases in ruminal NH$_3$ with increasing levels of energy supplementation. Because low ruminal NH$_3$ concentrations appear to limit forage utilization, supplementation of additional RDP may offset the negative impacts of energy supplementation.
As previously mentioned, low levels of supplemental energy typically have little effect on intake and digestibility of low-quality forages when fed along with adequate supplemental protein (DelCurto et. al., 1990a; 2000). This is in agreement with Bodine et al. (2001), who indicated that the addition of RDP can lessen the negative effects associated with energy supplementation of low-quality forages. However, results from studies investigating the effects of high-protein, high-energy supplements have varied. DelCurto et al. (1990a) reported no effect of increased energy supplementation at high levels of protein in one study, but observed decreased forage intake with additional energy supplementation in another study utilizing the same forage and supplementation treatments. Chase and Hibberd (1987) also noted variable responses to increased supplemental energy.

As a result of decreased forage intake, energy supplementation of low-quality forages can often have little impact on beef cattle performance when forage is readily available (DelCurto et al., 2000; Sanson et al., 1990). Sanson et al. (1990) reported that cows grazing range estimated to contain roughly 5% CP lost more weight when supplemented with ear corn than with ear corn and a protein supplement. Additionally, both treatments resulted in greater weight loss than cows receiving a protein supplement alone (Sanson et al., 1990).

While data suggest that energy supplementation of beef cows consuming low-quality forage often has little effect on cow BW and BCS (Caton and Dhuyvetter, 1997; DelCurto et al., 2000), many studies report improved reproductive efficiency. While
discussing nutritional strategies to improve reproductive performance, Bohnert and Cooke (2011) suggest that energy supplements contain high-starch ingredients to promote propionate synthesis. Propionate synthesis is closely related to circulating levels of glucose, insulin and IGF-I (Randel, 1990); which are vital for optimal reproductive performance (Wettemann et al., 2003).

**Physical Form of Energy Supplements.** While much of the research on energy supplementation has utilized high-starch supplements, many researchers have evaluated the impacts of degradable fiber, as well as fats on forage intake and utilization. Studies investigating the effects of highly degradable fiber as an energy supplement have produced different responses than those seen with starch supplementation. In their aforementioned review, Caton and Dhyuvetter (1997) reported that studies utilizing highly degradable fiber supplements (such as soybean hulls, wheat middlings, beet pulp, and corn gluten feed) have resulted in minimal decreases in forage intake when compared to starch-based supplements. Additionally, Highfill et al. (1987) reported improved fiber digestion with high fiber energy supplements when compared with highly soluble carbohydrate supplements. In general, fibrous energy sources can be offered at higher levels than starch based energy supplements (0.8 compared with 0.5% of BW, respectively) without negatively effecting forage intake and digestibility (Bowman and Sanson, 1996; Garcés-Yépez et al., 1997).

Supplemental fat may also be used to increase the energy density of the diet, as fat yields more energy when metabolized than other nutrients (DM basis). However,
supplemental fat should be limited to 2 to 3% of diet DM (Hess et al., 2008) in order to avoid negatively impacting forage intake and digestibility. Supplementation programs designed to improve reproductive performance often aim to increase energy balance. However, the enhancement of reproductive performance associated with supplemental fat may not be entirely due to an improvement in energy balance. Cows fed calcium salts of fatty acids have been shown to have decreased plasma estradiol, increased LH, and improved follicular growth when compared to an isocaloric control (Hightshoe et al., 1991). Also, Lopes et al. (2009; 2011) suggested that Ca salts of PUFA can have beneficial impacts on reproduction, independently from the effects of energy supplementation. Cows receiving PUFA supplements during the expected time of luteolysis (around d 16 after ovulation) had greater pregnancy rates to timed AI than those receiving isocaloric, isonitrogenous, and isolipidic supplements of SFA, likely due to decreased pregnancy loss around d 16 of pregnancy (Lopes et al., 2011). The positive effects of PUFA supplementation are likely due to modulation of PGF$_{2\alpha}$ synthesis and increased circulating levels of progesterone (P4; Lopes et al., 2009; 2011).

**Energy Metabolism and Reproduction**

Houghton et al. (1990) demonstrated that PPI is greater in cows calving with a BCS <6 (on the standard 1 to 9 scale; Wagner et al., 1988). However, first-service conception rate and overall pregnancy rate were shown to be considerably higher in thin cows with increasing BCS than in fat cows. Similarly, cows with adequate energy status have been shown to have a decreased PPI and increased pregnancy rate when compared to cows
with inadequate energy status (Ciccioli et al., 2003; Hess et al., 2005). Ciccioli et al. (2003) reported that increased nutrient intake, as well as a corresponding increasing in cow BW and BCS, was positively correlated with increased blood concentrations of glucose, insulin and IGF-I, suggesting that the positive impact of energy on reproductive function may be partially due to the effects of energy intake and reserves on plasma hormones and metabolites (glucose, insulin, and IGF-1; Wettemann et al., 2003) and their influence on endocrine control of reproduction.

**Endocrine Control of Reproduction**

It has long been recognized that a prompt resumption of estrus is essential for optimal reproductive efficiency. This resumption and maintenance of reproductive ability are mediated by the hypothalomo-hypophyseal-ovarain axis, as outlined by Hess et al. (2005) in a review on the nutritional control of reproduction in cattle. Hypothalamic GnRH is released into the hypophyseal portal blood system, via neurosecretory neurons. The GnRH is subsequently transported to the anterior pituitary gland, where it stimulates the synthesis and secretion of the gonadotropins, FSH and LH (Hess et al., 2005).

Early follicular growth, up until follicle deviation, is stimulated by FSH (Ginther et al., 1996; Hess et al., 2005). Tonic secretion of GnRH stimulates hypophyseal LH secretion, which stimulates maturation of the dominant follicle. Once estradiol production by ovarian follicles reaches threshold levels, a GnRH surge and subsequent LH surge lead to ovulation (Ginther et al., 2001; Hess et al., 2005).
Following ovulation, P4 produced by the corpus luteum suppresses GnRH release, as well as the subsequent gonadotropin synthesis. Consequently, regression of the corpus luteum must occur for ovulation to be repeated (Hess et al., 2005). Furthermore, as explained by Mann and Lamming (1999) in their review on the influence of P4 during early pregnancy, P4 is essential for the establishment and maintenance of pregnancy. Looper et al. (2003) reported normal estrous cycles for 81% of cows with the transient increase in P4 compared to 36% in cows without, suggesting that P4 may prepare the reproductive tract for the resumption of estrus and rebreeding. Similarly, Werth et al. (1996) reported that the percentage of cows conceiving at their first estrus post-parturition was greater in cows in which a transient increase in progesterone prior to estrus was detected (76%) than in those without the transient increase (41%).

**Glucose**

Blood glucose concentrations have been show to be positively associated with feed intake and rate of BW gain in beef cattle (Vizcarra et al., 1998; Hersom et al., 2004). In ruminants, fermentation of carbohydrates by rumen microbes predominately yields VFAs, with little glucose being absorbed from the gastro-intestinal tract (Fahey and Berger, 1988). Although many factors affect glucose absorption, Young (1977) suggests that less than 10% of glucose requirements are absorbed directly from the digestive tract of ruminants.
Therefore, forage-fed cattle are dependent on liver gluconeogenesis to meet glucose requirements (Young, 1977; Huntington, 1997). The rate of gluconeogenesis is largely dependent on the amount of precursors available, and therefore is increased with increased feed intake (Fahey and Berger, 1988; Vizcarra et al., 1998; Hersom et al., 2004).

Glucose is the primary energy source for the central nervous system and, as a result, reductions in glucose availability may lead to a decline in the synthesis and release of GnRH, as well as the subsequent release of gonadotropins (Hess et al., 2005). The impairment of GnRH secretion with low blood glucose, along with the return to normal when glucose levels rise, suggests that the hypothalamus may detect low blood glucose in a threshold-dependent manner. (Hess et al., 2005). Additionally, the positive effects of glucose on the reproductive system may be associated with improving energy status and concentrations of blood metabolites and hormones (Randel, 1990; Hess, 2005).

**Insulin**

Insulin secretion is primarily stimulated by high blood glucose concentration, after which it acts to increase anabolic processes, acting predominately on the liver, muscle and adipose tissue. Elevated insulin levels prompts rapid glucose uptake in fat and muscle cells, where it is converted to fatty acids and glycogen, respectively (Nusse and Whitehead, 2001). In the liver, insulin acts to promote glycogenesis (Nussey and Whitehead, 2001).
There is increasing evidence that circulating insulin concentrations are decreased by dietary restriction and negative energy balance (Diskin et al., 2003; Webb et al., 2004). Insulin, along with glucose, has been shown to influence GnRH release from the hypothalamus (Hess et al., 2005) and play a role in the regulation of ovarian responsiveness to gonadotropins (Diskin et al., 2003). Insulin has also been shown to modulate circulating levels of progesterone (Lopes et al., 2009; Vieira et al., 2010), a hormone required for pregnancy recognition and maintenance, as well estrous resumption (Graham and Clark, 1997; Looper et al., 2003). However, recent research indicate these effects are dependent on circulating glucose concentrations (Cooke et al., 2012). Research from Gong et al. (2002) indicate that diets formulated to increase circulatory insulin concentrations during early lactation in dairy cattle can hasten the first ovulation postpartum. In a review on the control of follicular growth, Webb et al. (2004) reported that insulin infusion in beef heifers increases dominant follicle diameter, as well as ovulation rate in energy deprived heifers, suggesting that management practices designed to increase circulating insulin concentrations may help to improve reproductive performance.

**Insulin-Like Growth Factor-I (IGF-I)**

The majority of blood IGF-I is bound to one of six IGFBPs (Beattie et al., 2006) which act to transport, protect from degradation, and regulate the action of IGF-I by enhancing or blocking activity in target cells (Le Roith et al., 2001). Insulin-like growth factor-I synthesis is regulated primarily by GH (McGuire et al., 1992; Cooke et al.,
However, IGF-I concentrations have been shown to be negatively associated with GH levels (Elleberger et al., 1989), suggesting that other mechanisms must be involved in the synthesis of IGF-I. For example, hepatic synthesis of IGF-I is improved by the increased receptiveness of hepatic GH receptors in response to insulin (McGuire et al., 1995; Molento et al., 2002). Consequently, insulin concentrations are typically positively associated with IGF-I concentrations in cattle (Keisler and Lucy, 1996; Webb et al., 2004; Cooke et al., 2007).

Research suggests that IGF-I is positively associated with feed intake and BW (Bossis et al., 2000; Rausch et al., 2002) and increases in response to protein supplementation (Perry et al. 2002; Sullivan et al., 2009). Furthermore, Lents et al. (2013) reported that reductions in circulating IGF-I concentrations resulting from feed restriction were accompanied by reduced LH pulse frequency, decreased dominant follicle size and the absence of a preovulatory LH surge, resulting in no ovulation for 44 to 70% of heifers. Blood concentrations of IGF-I have been shown to be greater in mature beef cows that resumed estrous cycles within 20 weeks postpartum than in cows that remained in anestrous (Roberts et al., 1997). In addition, increased IGF-I concentration postweaning appears to be genetically associated with increased conception and calving rates (Zang et al., 2013). The effects of IGF-I on cattle reproduction appear to be the result of autocrine, paracrine and endocrine mechanisms. In their review of the relationship between nutrition and reproduction in cattle, Wettemann et al. (2003) suggest that IGF-I may modulate GnRH and gonadotropin secretion due to the presence of IGF-I receptors in the hypothalamus and pituitary. These findings are in agreement with the
results compiled by Diskin et al. (2003), which indicated that IGF-I alters hypothalamic and pituitary functions. Receptors for IGF-I have also been detected in ovarian cells, such as granulosa, thecal, and luteal cells (Spicer and Echternkamp, 1995). Additionally, Echternkamp et al. (2004) noted an increase in blood IGF-I and enhanced follicular development in cows selected for increased ovulation and twinning rates compared with control animals despite similar gonadotropin secretion and ovarian steroid production. These results suggest that IGF-I may increase ovarian sensitivity to gonadotropins and consequently increase fertility in cattle.

**Non-Esterified Fatty Acids (NEFA)**

Inadequate nutrition is often associated with elevated levels of NEFA, caused by fat tissue mobilization (Brown et al., 2012). After entering the blood stream, NEFA are taken up by the liver and other tissues. Partial oxidation in the liver can result in ketosis, while triglyceride formation can lead to fatty liver (de Vries and Veerkamp, 2000; Brown et al., 2012). These conditions can contribute to delayed ovulation, estrus and pregnancy (Jorritsma et al., 2000; Brown et al., 2012).

Studies evaluating the effects of elevated NEFA concentrations on the gonadotropins have been inconsistent. Some studies suggest that LH secretion is not influenced by NEFA concentrations (Estienne et al., 1990; DiCostanzo et al., 1999). However, recent research by Lents et al. (2013) suggests that nutrient restriction results in elevated NEFA concentrations and a concurrent decrease in LH pulse frequency. Similarly, in a review on the manipulation of reproduction with supplementation,
Dhuyvetter and Caton (1996) suggest a correlation between LH pulse frequency and plasma NEFA concentrations.

Additionally, increased NEFA concentrations have been shown to be correlated with insulin resistance in dairy cows (Oikawa and Oetzel, 2006). In a study designed to test the relationship between insulin response and changes associated with fasting, Oikawa and Oetzel (2006) reported that decreased insulin response was associated with increased plasma NEFA concentration. To further study the relationship between plasma NEFA concentrations and insulin response, Pires et al. (2007) utilized nicotinic acid as an antilipolytic agent in order to induce different plasma NEFA concentrations. The resulting low NEFA levels resulted in enhanced glucose clearance, despite low insulin levels, suggesting an increased responsiveness to endogenous insulin (Pires et al., 2007).

**Conclusion**

Research has shown that supplement composition can have significant impacts on forage intake and digestibility, as well as cow performance and reproductive efficiency. It has long been understood that protein supplementation increases forage intake and digestibility of low-quality forages. Conversely, high levels of energy supplements routinely decrease intake and digestibility of forage. Still, energy supplementation can positively impact reproductive performance by influencing plasma hormones and metabolites; however, much of this work has been done with low-quality, warm-season forages. Few studies have utilized low-quality, cool-season forages to compare the effects of energy supplements with varying protein concentrations on cow performance.
and forage intake and utilization. Therefore, the current research was designed to evaluate the influence of supplement composition on intake and digestibility of low-quality cool-season forages, as well as cow performance.
Influence of Supplement Composition on Utilization of Low-Quality, Cool-Season Forages by Beef Cattle

Low-quality forages are a vital part of beef cattle diets; however, forage utilization is typically limited without supplementation (DelCurto et al., 1990a,b; Köster et al., 1996), leading to reduced BW and BCS (DelCurton et al., 1990b; Bohnert et al., 2002b). This impaired nutritional status and animal performance often leads to reduced reproductive efficiency (Wiltbank et al., 1962; Bellows and Short, 1978; Hess et al., 2005) when compared with an adequate nutritional state. Consequently, many studies have tried to optimize low-quality forage utilization while maintaining animal performance. Protein supplementation typically increases intake and digestibility of low-quality, warm-season forages (DelCurto et al., 1990a,b; Köster et al., 1996); whereas, starch-based supplementation at greater than 0.5% of BW typically decrease forage utilization (Bowman and Sanson, 1996; Garcés-Yépez et al., 1997).

Studies with low-quality, cool-season forages suggest that intake is not increased by protein supplementation (Mathis et al., 2000; Bohnert et al., 2002b). Cool-season forages have a greater proportion of CP as RDP than warm-season forages (Bohnert et al., 2011), suggesting that ruminal NH₃-N may not limit intake and digestibility to the same extent as with warm-season forages. As a result, protein supplementation likely does not have the same positive impact on energy balance as seen with warm-season forages. Therefore, we hypothesize that energy supplementation will be more beneficial than protein supplementation for ruminants consuming low-quality, cool-season forages.
Little data is available on the effects of supplementing low-quality, cool-season forages with energy-dense supplements containing varying protein concentrations on cow performance and forage utilization. Therefore, the objective of these experiments was to evaluate the influence of supplement composition on intake and digestibility of low-quality cool-season forages, as well as cow performance.

**Materials and Methods**

All experimental procedures used in this study were approved by the Oregon State University Institutional Animal Care and Use Committee.

**Experiment 1. Influence of Supplement Composition on Forage Intake and Digestibility in Steers**

Five ruminally cannulated Angus x Hereford steers (560 ± 79 kg of BW) were used in an incomplete 5 x 4 Latin square (Cochran and Cox, 1957) and housed in individual pens (4 x 8 m) within an enclosed barn with continuous lighting. Treatments consisted of a non-supplemented, negative control (CON), 3 high energy corn-based supplements with low, moderate and high levels of protein (Corn = 1.26 g/kg BW Corn, approximately 8 % crude protein, CP; LU = Corn + 0.09 g/kg BW urea, approximately 27% CP; HU = Corn + 0.17 g/kg BW urea, approximately 43% CP) and a positive control (1.26 g/kg BW SBM, approximately 51% CP, Table 1). All supplements were formulated to be provide similar caloric intakes and the SBM treatment was formulated to provide approximately 100% of the estimated RDP requirement assuming a microbial efficiency of 10% (NRC, 1996; Model 1). In addition, the HU supplement was formulated to be isonitrogenous to the
SBM supplement; however, a lower than anticipated CP concentration in the corn resulted in the HU supplement having a lower CP concentration than the SBM supplement. The LU supplement was designed to have a CP concentration halfway between that of the Corn and HU supplements. Supplement ingredient and nutrient compositions are outlined in Table 1. Supplements and a mineral-salt mix (Cattleman’s Choice; Performix Nutrition Systems, Nampa, ID) containing 14% Ca, 10% P, 16% NaCl, 1.5% Mg, 6000 mg/kg Zn, 3200 mg/kg Cu, 65 mg/kg I, 900 mg/kg Mn, 140 mg/kg Se, 136 IU/g of vitamin A, 13 IU/g of vitamin D3, and 0.05 IU/g of vitamin E were placed directly into the rumen via ruminal cannula at 0700 h daily. Steers had continuous access to fresh water and chopped (4- to 8-cm) fine fescue grass seed straw (4.7% CP; DM basis). Straw was provided at 0710 h daily at 120% of the previous 5 d average intake; previous day feed refusals were determined prior to supplementation. Additionally, steers were administered an intramuscular injection of vitamins A and D at trial onset to safeguard against deficiency (500,000 and 75,000 IU of vitamins A and D, respectively; Vitamin A and D, Vedco, St. Joseph, MO).

The 4 experimental periods were 28 d each with 20 d of diet adaptation and 8 d of sampling. At least 3 d were allowed between periods when steers were removed from pens and placed in a common pen with continuous access to water and low-quality, fine fescue grass seed straw. Forage intake was measured d 21 through d 26. Treatment effects on ruminal DM and indigestible acid detergent fiber (IADF) were determined on d 21 by manually removing the contents of the reticulorumen from each steer 4 h after feeding. Total ruminal contents were weighed, mixed by hand and subsampled in triplicate (approximately 400 g per triplicate). The remaining ruminal contents were
immediately replaced into the animal. Ruminal samples were weighed, dried in a forced-air oven (55°C; 96 h), reweighed for DM, ground to pass a 1-mm screen in a Wiley mill (Model 4; Arthur H. Thomas, Philadelphia, PA) and composited within steer and period. Straw, corn and SBM were collected on d 21 through 26 and orts were collected on an equal-weight basis (5% as-fed) on d 22 through 27. Feed and orts samples were dried at 55°C for 48 h. On d 23 to 28 fecal grab samples were collected every 12 h with a 2 h advancement each day to allow for sampling on each even hour of a 24-h day. Fecal samples were dried at 55°C for 96 h. Dried samples of feed, orts and feces were ground as previously described. Feed samples were composited by period, whereas orts and feces were composited by steer within period.

Ground samples of feed, orts and feces were analyzed for DM and OM (AOAC, 1990) and N (Leco Tru Mac CN, Leco Corp., St. Josph, MI). Straw, orts and feces were analyzed for NDF (Robertson and Van Soest, 1981) and ADF (Goering and Van Soest, 1970) using procedures modified for use in an Ankom 200 Fiber Analyzer (Ankom Technology Corp.); NDF and ADF of corn and SBM were determined by a commercial laboratory (Dairy One; Ithaca, NY). Feed, orts, feces and rumen particulate samples were analyzed for IADF using procedures described by Bohnert et al. (2002c). The passage of IADF was determined by dividing IADF intake by the quantity of IADF in the rumen 4 h post-feeding using techniques described by Van Soest (1982). In addition, diet digestibility was determined by using IADF fecal concentration in conjunction with nutrient concentration of forage and supplements (Merchen, 1988).
Blood samples were collected into commercial blood collection tubes containing 0.1 mL of a 15% EDTA solution (Tyco Healthcare Group, Mansfield, MA) via coccygeal venipuncture 4 h after feeding on d 23 through d 28. Samples were immediately placed on ice for transport to the lab, centrifuged (2,500 x g for 30 min; 4°C) and plasma harvested and stored (-80°C).

Steers were intra-ruminally pulse-dosed with 5 g of Co-EDTA in a 150-mL aqueous solution (Uden et al., 1980) on d 28. The Co marker was administered throughout the rumen by injection through a stainless steel probe with a perforated tip. Approximately 100 mL of ruminal fluid was collected by suction strainer (Raun and Burroughs, 1962) immediately before dosing and at 1, 3, 6, 9, 12, 18, and 24 h after dosing. Ruminal fluid pH was measured immediately after collection (Orion SA 520, American Instrument Exchange Inc., Haverhill, MA). Twenty milliliters of ruminal fluid was stored (−20°C) for later analysis of Co concentration and 5 mL was acidified with 1 mL of 25% (wt/vol) meta-phosphoric acid and stored (−20°C) for subsequent analysis of VFA and NH3-N. Frozen (−20°C) ruminal samples were prepared for analysis by thawing, centrifuging (15,000 × g for 10 min at room temperature for VFA and NH3-N, and 2,000 × g for 20 min at room temperature for Co), and collecting the supernatant. Cobalt was analyzed by atomic absorption using an air-acetylene flame (Model 351 AA/AE Spectrophotometer, Instrumentation Laboratory Inc., Lexington, MA). Ruminal liquid volume and liquid dilution rate were estimated by regressing the natural logarithm of Co concentration against sampling time as described by Warner and Stacy (1968). Volatile fatty acids were analyzed as described by Harmon et al. (1985), and NH3-N was
analyzed using a modification (sodium salicylate substituted for phenol) of the procedure described by Broderick and Kang (1980) using an absorbance microplate reader (VersaMAX Microplate Reader, Molecular Devices; Sunnyvale, CA).

**Experiment 2. Influence of Supplement Composition on Cow Performance**

Eighty late gestation (approximately 190 d pregnant) Angus x Hereford cows (507 ± 10 kg BW) were stratified into 4 blocks by age, BCS (standard 1 to 9 scale; Wagner, 1988) and BW. Cows were then randomly assigned within block to 1 of 5 treatments. Cows were then sorted by treatment, within block, and randomly allotted to 1 of 20 pens (4 cows/pen; 4 pens/treatment). The same treatments as described in Exp. 1 were used. Water and a mineral-salt mix was available free choice (same composition as previously described; Cattleman’s Choice; Performix Nutrition Systems, Nampa, ID). Cows were provided ad libitum access to low-quality (5.0% CP; DM basis) fine fescue grass seed straw. Also, the supplements provided to cows are provided in Table 1. The quantity of SBM supplement provided was calculated to meet 100% of the estimated RDP requirement (NRC, 1996; Model 1), while the Corn, LU and HU supplements were provided in amounts estimated to be isocaloric with the SBM treatment. Corn was offered at 816.5g DM hd⁻¹ d⁻¹, with 59 and 115.2g DM hd⁻¹ d⁻¹ of urea added for the LU and HU treatments, respectively; SBM was offered at 816.5g DM hd⁻¹ d⁻¹. Supplemental CP was approximately 8, 27, 43, and 51% of DM for the corn, LU, HU and SBM treatments, respectively.

Straw, corn, and SBM samples were collected weekly and analyzed for CP, OM, NDF and ADF, as described in Exp. 1. Cow BW and BCS were measured every 14 d until
calving and within 24 h post-calving. Calf BW was also obtained within 24 h post-calving. Blood samples were collected into 2 commercial 10-mL blood collection tubes (1 containing 0.1 mL of a 15% EDTA solution for plasma harvest, Tyco Healthcare Group, Mansfield, MA; and 1 vacutainer for serum harvest, Becton Dickinson, Franklin Lakes, NJ) via jugular venipuncture at trial onset, d 49 and within 24 h post-calving. Samples were immediately placed on ice for transport to the lab. Plasma samples were centrifuged (2,500 x g for 30 min; 4°C) and plasma harvested and stored (-80°C). Serum samples were refrigerated overnight, centrifuged (2,500 x g for 30 min; 4°C) and serum harvested and stored (-80°C).

**Blood Analysis**

Plasma glucose and BUN concentrations were determined using a quantitative colorimetric kit (catalog numbers G7521 B7551, respectively; Pointe Scientific, Inc., Canton, MI). Concentration of IGF-I was determined using a human-specific commercial kit (SG100; R&D Systems, Inc., Minneapolis, MN) as previously described and validated for bovine samples (Moriel et al., 2012). Serum NEFA concentration was determined using a commercial kit (HR Series NEFA - 2; Wako Pure Chemical Industries Ltd. USA, Richmond, VA) with modifications described by Pescara et al. (2010). Insulin concentration was determined using a commercially available radioimmunoassay Coat-A-Count kit (Siemens Healthcare Diagnostics, Los Angeles, CA). For Exp. 1, intra- and interassay CV were, respectively, 4.75 and 1.81% for IGF-I, 4.08 and 8.40% for BUN 3.70 and 7.88% for glucose; all insulin samples were analyzed
in a single run and the intra-assay CV was 2.60%. The intra- and interassay CV for Exp. 2 were 8.52 and 8.30% for IGF-I, 6.40 and 3.82% for BUN, 0.89 and 2.36 for glucose, 8.52 and 8.30% for insulin and 5.94 and 4.27% for NEFA.

Statistical Analysis

Exp. 1. Intake and digestibility data were analyzed as a 5 x 4 incomplete Latin square with the MIXED procedure of SAS. The model included period and treatment and steer was used as the random variable. Contrasts used to partition specific treatment effects consisted of: 1) supplemented vs non-supplemented; 2) linear effect of urea; 3) quadratic effect of urea; and 4) HU vs SBM.

Ruminal pH, NH\textsubscript{3}-N and VFA data were analyzed using the REPEATED statement with the MIXED procedure of SAS. The model included period, treatment, hour and treatment x hour. Steer was used as the RANDOM statement to specify variation and steer( period) was used as the subject. The specific term for the repeated statement was hour. Autoregression (AR1) was determined to be the most appropriate covariance structure based on the Akaike information criterion. The same contrasts as previously noted were used to partition specific treatment effects.

Blood samples were analyzed using the REPEATED statement with the MIXED procedure of SAS. The model included period, treatment, day and treatment x day. Steer was used as the random variable and steer( period) was used as the subject. The specific term for the repeated statement was day. Autoregression (AR1) was determined to be the most appropriate covariance structure based on the Akaike information criterion. The same
contrasts as previously noted were used to partition specific treatment effects. If no
treatment x time interactions were detected (P > 0.05), overall treatment means were
compared.

**Exp. 2.** Cow performance data was analyzed as a randomized block design using
the MIXED procedure of SAS. The model included block, treatment and treatment x
block. Blood samples were analyzed using the REPEATED statement with the MIXED
procedure of SAS. Model included block, treatment, day and all resulting interactions.
Values were adjusted covariatełty to values obtained at trial onset. Cow(pen) and
pen(treatment) were used as the repeated variables, the subject was cow(pen) and
appropriate covariate structure was determined by the Akaike information criterion; AR1
was used for insulin and NEFA and compound symmetry (CS) was used for BUN, glucose
and IGF-I. The same contrasts as previously described were used to partition specific
treatment effects. If no treatment x time interactions were detected (P > 0.05), treatment
means were compared.

Results and Discussion

**Exp. 1 Forage intake, digestibility and ruminal fermentation characteristics in steers**

*Intake and Digestibility.* Protein supplementation has long been shown to increase
low-quality forage intake and digestibility in ruminants (DelCurto et al., 1990a,b; Köster et
al., 1996); however, much of this work has utilized warm-season forages. Contrary to
work done with low-quality, warm-season forages, intake of low-quality, cool-season
forages is typically not increased with protein supplementation (Mathis et al., 2000;
Bohnert et al., 2002a,b). Bohnert et al. (2011) suggest that this could be due to greater overall intake of cool-season forages when compared with warm-season forages, with forage DM intake typically exceeding 1.7% of BW for cool-season forages (Mathis et al., 2000; Bohnert et al., 2011) and often below 1.0% of BW for warm season forages (Köster et al., 1996; Mathis et al., 1999). In the present study, forage DMI exceeded 2.0% of BW for all treatments (Table 2). Also, cool-season forages have been shown to have a greater concentration of RDP, NSC and WSC when compared with warm-season forages with similar CP (Barbehenn and Bernays, 1992; Bohnert et al., 2011; 2013). Bohnert et al. (2011) noted approximately 28% greater RDP for cool-season forages compared with warm-season forages. Further research by Bohnert et al. (2013) indicated that cool-season forages have greater soluble and degradable protein fractions than warm-season forages. The higher proportion of CP as RDP, as well as greater NSC and WSC concentrations, in cool-season forages could explain the greater intake and digestibility seen when compared with warm-season forages, as well as the lack of a CP supplementation effect.

Grass seed straw DM and OM intake, as well as NDF and ADF intake were not increased with supplementation (P > 0.10) but were greater for steers receiving SBM than for HU steers (P = 0.01; Table 2). Previous research suggests that forage intake may not be affected by the supplements provided, with low energy levels (DelCurto et al., 1990a; Bowman and Sanson, 1996; Garcés-Yépez et al., 1997) not influencing intake, and protein supplementation generally not altering intake of cool-season forages (Mathis et al., 2000; Bohnert et al., 2011). Additionally, straw DMI and NDF intake tended to respond in a quadratic fashion (P = 0.10) to urea supplementation, with LU stimulating greater intake
than corn and HU. This is in agreement with previous research suggesting energy based supplements with adequate RDP may result in improved forage intake when compared with energy supplements alone (Bodine et al., 2001).

Energy supplementation of ruminants consuming low-quality forages typically decreases forage intake and digestibility (Chase and Hibberd, 1987; Sanson et al., 1990). However, starch-based energy supplements offered at below 0.5% of BW typically have no negative impact on forage intake when fed along with adequate protein (DelCurto et al., 1990a; Bowman and Sanson, 1996; Garcés-Yépez et al., 1997). In the present study, corn was provided at approximately 0.1% of BW and, therefore, was not expected to negatively impact forage utilization.

The lack of a supplementation effect on forage intake is further supported by OM and NDF intake, as well as ruminal NH₃-N concentration. Moore et al. (1999) suggest that forage intake should not be expected to increase when forage OM intake exceeds 17.5 (g · kg BW)/d. Previous research (Mertens 1985, 1994) suggests that intake is maximized when NDF intake is approximately 12.5 (g · kg BW)/d. In the present study, non-supplemented forage OM and NDF intake were approximately 19.4 and 16.0 (g · kg BW)/d, respectively. Consequently forage intake was not expected to increase in response to increasing levels of protein supplementation. Furthermore, ruminal NH₃-N concentration in non-supplemented steers was 1.61 mM, which is within the range typically believed to support optimal growth of rumen microbes in vivo (1.18 to 2.94 mM; Slyter et al., 1979).
Straw DM and OM intake were greater for SBM steers than for HU steers. Although statistically significant, only a slight numerical increase was seen, with intake being approximately 0.25% BW greater for the SBM steers. A possible explanation for this could be the greater N and MP intake for steers supplemented with SBM than for those supplemented with HU.

Natural proteins, such as SBM, contain RUP, which serves as a source of MP in addition to that obtained from microbial protein. Past research has noted increased MP can have a direct stimulatory effect on low-quality forage intake (Egan, 1977). As designed, N intake increased with supplementation and increased linearly with increasing urea supplementation. However, a lower than expected corn CP concentration resulted in greater N intake with SBM supplementation than with HU.

Due to intake of supplemental DM, total DM and OM intake were increased with supplementation (P = 0.02; Table 2). Additionally, differences in supplement DMI were due to the addition of urea to a common amount of grain supplement. Total DM and OM intake were greater for SBM steers than for their HU counterparts (P < 0.01). This is in contrast with previous work (Ammerman et al., 1972; Swingle et al., 1977; Köster et al., 1997) suggesting that urea- and natural protein-based supplements stimulate similar DMI. However, SBM steers in the present study had greater forage DMI, possibly due to differences in N and MP intake, resulting in an increase in total DMI.

Nitrogen intake increased with supplementation (P < 0.01) and increased linearly with increasing urea (P < 0.01; Table 2). However, as noted earlier, the corn used in the
study had a lower concentration of CP than expected resulting in a greater N intake for SBM supplemented steers than HU steers (P < 0.01). Also, fecal N was greater with supplementation (P = 0.02) and for SBM compared with HU (P = 0.01). Fecal N has been shown to increase with energy and/or protein supplementation, possibly due to increased hindgut fermentation (Bodine and Purvis, 2003)

Apparent total tract DM and OM digestibility were increased with supplementation (P = 0.05 and P = 0.03, respectively; Table 2). Apparent digestibility has been reported to increase with protein supplementation (Horney et al., 1996; Bohnert et al., 2002a,b) and energy supplementation (DelCurto et al., 1990a; Caton and Dhuyvetter, 1997), due to the greater digestibility of the supplement when compared to the forages. We noted no differences for SBM vs HU or for urea inclusion (P > 0.10). Similarly, Ammerman et al. (1972) reported no difference in OM digestibility between ruminants supplemented with natural protein or a NPN source. Total tract NDF and ADF digestibility showed no differences due to treatments (P > 0.10; Table 2). This agrees with previous work that suggests ruminal fiber digestibility is not influenced by protein supplementation (Litzenich et al., 1995; Bohnert et al., 2002b; Currier et al., 2004b). Furthermore, low-levels of energy supplementation typically do not alter fiber digestibility ( Bowman and Sanson, 1996; Garcés-Yépez et al., 1997). Total tract N digestibility increased with supplementation (P < 0.01; Table 2) and increased linearly with increasing urea inclusion (P < 0.01), likely due to greater N digestibility of the supplements.
**Ruminal Fermentation.** A treatment x time interaction (\(P < 0.01\)) was noted for ruminal NH\(_3\)-N; however, due to the nature of the interaction we concluded that discussing treatment means while providing the time x treatment figure would facilitate data interpretation and discussion (Table 3; Figure 1). Ruminal NH\(_3\)-N increased with supplementation (\(P < 0.01\)), increased linearly with urea inclusion (\(P < 0.01\)) and was greater for HU supplemented steers compared with SBM steers (\(P < 0.01\)). Non-supplemented steers had a ruminal NH\(_3\)-N concentration of 1.61 mM, which is within the range of 1.18 to 2.94 mM believed to support optimal growth of rumen microbes in vivo (Slyter et al., 1979). Consequently, we can assume that NH\(_3\)-N was not limiting ruminal fermentation in non-supplemented controls.

Past research has consistently shown increased ruminal NH\(_3\)-N with increasing protein supplementation (Köster et al., 1996; Mathis et al., 1999; Bohnert et al., 2002c), which was also noted in the current study. Additionally, RDP supplementation has been shown to have greater increases in ruminal NH\(_3\)-N when compared with RUP supplementation (Bandyk et al., 2001; Bohnert et al., 2002c). This helps explain the greater ruminal NH\(_3\)-N noted for HU when compared with SBM, as the N in urea consists entirely of RDP.

Ruminal pH tended to decrease with supplementation (\(P = 0.08\); Table 3) and was lower for HU steers than SBM steers (\(P = 0.01\)). However, ruminal pH remained above 6.4 for all treatments and sampling times (data not shown). This is well within the range typically considered to support growth of cellulolytic bacteria and fiber digestion,
assuming other nutrients are available in adequate amounts (Yokoyama and Johnson, 1988). This further supports the lack of a supplementation effect on ruminal fiber digestibility, as rumen microbe growth should not have been effected.

No treatment effects were seen on total VFA concentration or the molar proportions of propionate or butyrate (P > 0.05; Table 3). Additionally, the acetate:propionate ratio did not differ between treatments (P > 0.10), suggesting similar energy efficiencies of ruminal fermentation. Nevertheless, the molar proportion of acetate was greater for HU steers than for SBM steers (P = 0.01) while steers supplemented with SBM had greater molar proportions of the branch chain VFA isobutyrate, isovalerate and valerate (P ≤ 0.01). This was expected, as branch-chain VFA are formed by the fermentation of branch-chain amino acids present in natural proteins, such as SBM (Leng, 1973). Supplemented steers had greater molar proportions of isovalerate than non-supplemented steers (P < 0.01). Isobutyrate tended to decrease linearly with increasing urea inclusion (P = 0.06).

No differences were noted for IADF intake, fill, passage rate or outflow (P > 0.10; Table 3) for supplemented steers compared to non-supplemented controls. Additionally, no effects were noted for urea inclusion (P > 0.10). This is in agreement with previous research, in that rumen particulate dynamics typically are not influenced by protein supplementation (Bohnert et al., 2002c; Currier et al., 2004c). However, IADF intake and outflow were greater with SBM than with HU (P = 0.01), likely due to the greater forage, and therefore IADF, intake noted for SBM supplements when compared with HU supplements. Nonetheless, IADF fill did not differ between HU and SBM steers,
supporting the assumption that in ruminants offered low-quality forages feed intake matches the capacity of the gastrointestinal tract to accommodate digesta (Mertens, 1994).

**Blood Variables.** As noted by Knaus et al. (2002), plasma insulin and glucose concentrations were not influenced by increased dietary CP (P > 0.10; Table 4) despite increases in DMI. In cattle, circulating glucose concentrations are dependent gluconeogenesis in the liver (Young, 1977). The rate of hepatic gluconeogenesis is influenced by the availability of glucose precursors, primarily propionate derived from ruminal fermentation (Huntington, 1997; Vieira et al., 2010). In the current study, ruminal propionate, as well as total VFA concentration, was not influence by treatment, suggesting similar rates of glucose production. Similarly, McGuire et al. (1992a) noted no difference in plasma glucose concentrations with protein supplementation; however, the authors did report increased plasma insulin. Steer BUN was increased with supplementation (P < 0.01; Table 4) agreeing with past research showing increased BUN in response to protein supplementation of low quality forages (Krehbiel et al., 1998; Ferrell et al., 1999; Bohnert et al., 2002a) and is directly correlated to N intake (Harmeyer and Martens, 1980).

Urea, like many NPN sources, is quickly hydrolyzed to NH$_3$-N in the rumen, which is then utilized or absorbed across the rumen wall and transported to the liver. Blood NH$_3$-N is rapidly converted to urea by the liver (Van Soest, 1982). While a portion of N from SBM is converted to NH$_3$-N in the rumen, a lower, delayed peak in ruminal NH$_3$-N is typically observed when compared to rapidly degradable and soluble NPN sources (Owens and Zinn, 1988). Therefore, increased ruminal NH$_3$-N, and consequently a increased
BUN, should be expected with urea when compared with SBM supplementation. In the current study, BUN increased linearly with increasing levels of supplemental urea (P < 0.01) and was greater for HU steers than SBM steers (P < 0.01).

Protein supplementation has increased plasma IGF-I in beef cattle (Perry et al., 2002; Sullivan et al., 2009). Our data supports this as plasma IGF-I concentration increased with supplementation (P = 0.02; Table 4) and responded to urea inclusion in a quadratic fashion (P = 0.03). Furthermore, IGF-I has been shown to increase with greater DMI (Bossis et al., 2000; Rausch et al., 2002), suggesting that our increase in IGF-I with supplementation may have been due to greater energy and DM intake resulting from supplementation.

Exp.2 Cow Performance

Protein supplementation of beef cows consuming low-quality forage typically improves weight and BCS change compared with not providing a supplement (Bohnert et al., 2002a; Currier et al. 2004a). Pre-calving (within 14 d of calving) BW and BCS change were improved with supplementation (P < 0.01; Table 5) and increased linearly with increasing urea supplementation (P < 0.01). Likewise, post-calving BW and BCS change were increased with supplementation (P < 0.01) and increased linearly with greater urea inclusion (P < 0.01). Similarly, Sanson et al. (1990) reported that cows receiving a corn and protein supplement had improved BW change compared with cows receiving only corn during the winter pre-calving period.
Past research suggests that urea can be included at approximately 3% of supplement DM in a high protein (30 to 40% CP) supplement without negatively influencing cow performance (Köster et al., 2002; Farmer et al., 2004). In contrast, supplements in the current study included urea at 6.7 and 12.4% of DM for LU and HU, respectively, and cows showed a linear increase in performance characteristics in response to increased urea inclusion. Although results from Exp. 1 suggests that DMI may have differed between HU and SBM treatments, no differences were noted in pre- or post-calving BW and BCS change for HU cows compared to SBM cows (P > 0.10).

Calf birth weight increased linearly (P = 0.04) with increasing urea. The effects of protein supplementation on calf birth weight have been inconsistent. Stalker et al. (2007) noted an increase in calf birth weight with protein supplementation. Similarly, other studies (DelCurto et al. 1990b; Larson et al., 2009) have noted an tendency for calf birth weight to increase with protein supplementation. Conversely, other studies have noted no differences between calves born to cows offered a protein supplement and non-supplemented controls (Bohnert et al., 2002a; Martin et al. 2007). However, it has long been understood that an improvement in dam nutritional status can lead to increased calf birth weight (Bellows and Short, 1978). This, in combination with the improved cow performance noted with increasing urea in the current study, suggests that the increase in calf birth weight may be the result of an improvement in overall nutritional status, as opposed to a direct result of increased protein supplementation.
Plasma IGF-I concentrations increased with supplementation ($P < 0.01$; Table 6) and responded in a quadratic fashion to increasing urea inclusion ($P = 0.05$), with IGF-I concentrations appearing to plateau at when supplemental protein reached the level corresponding to the LU supplement. This supports previous work with pregnant beef cattle that reported increased IGF-I concentrations in response to increases in dietary protein (Perry et al., 2002; Sullivan et al., 2009). Despite differences in animal performance between treatments, no treatment effects were detected for plasma glucose or serum NEFA concentrations ($P > 0.10$; Table 6). This result was unexpected, as NEFA concentrations are typically elevated with inadequate nutritional status, caused by fat tissue mobilization (Brown et al., 2012). However, Vieira et al. (2010) also noted no difference in serum NEFA concentrations between different nutritional statuses. As previously noted, other studies (McGuire et al., 1992a; Knaus et al., 2002) reported no difference in plasma glucose concentration with protein supplementation. Additionally, McGuire et al. (1992a) reported an increase in plasma insulin, while in the current study plasma insulin tended ($P = 0.07$) to increased with supplementation (Table 6). Also, insulin concentration has been positively associated with intake and rate of BW gain (Vizcarra et al., 1998; Bossis et al., 2000) which both increased with supplementation in the current study. Plasma BUN tended to increase linearly ($P = 0.08$) with increasing urea concentration of the supplement. As previously noted, BUN (Krehbiel et al., 1998; Ferrel et al., 1999; Bohnert et al., 2002a) has been shown to increase with increasing protein supplementation. In Exp. 1, HU steers had greater plasma BUN concentration than SBM steers; however, no differences were detected in Exp. 2, which can likely be explained by the timing of blood collection. Blood
samples were collected 4 hr after supplementation in Exp. 1 and immediately prior to supplementation in Exp. 2. This would allow for greater conversion of the natural proteins in SBM to NH$_3$-N in the rumen, and a subsequent increase in plasma BUN. No treatment effects were detected for other blood variables between HU and SBM supplemented cows (P > 0.10).

**Conclusion**

The results of these experiments suggest that intake of low-quality, cool-season forage was not limited by ruminally available-N. However, the improvement in animal performance with supplementation indicates that both energy and protein were limiting performance. The addition of supplemental energy necessitated the addition of RDP to optimize forage utilization and performance, resulting in similar performance between animals supplemented with natural protein and those receiving an energy dense supplement with added urea. As a result, a starch-based energy supplement, along with a source of NPN, appears to be an acceptable management alternative to sources of natural protein for ruminants consuming low-quality, cool-season forage.
### Tables

Table 1. Ingredient and nutrient content of fine fescue straw and supplements

<table>
<thead>
<tr>
<th>Supplement Composition, % DM</th>
<th>Treatment&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Exp. 1</th>
<th>Exp. 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>- 100.0 93.3 87.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Urea</td>
<td>- - 6.7 12.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SBM</td>
<td>- - - 100.0</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nutrient Composition, % DM</th>
<th>Exp. 1</th>
<th>Exp. 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP, %DM</td>
<td>8.3</td>
<td>4.7</td>
</tr>
<tr>
<td>TDN, %DM&lt;sup&gt;b&lt;/sup&gt;</td>
<td>87.5</td>
<td>48.2</td>
</tr>
<tr>
<td>NE&lt;sub&gt;m&lt;/sub&gt;, Mcal/kg&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.19</td>
<td>0.90</td>
</tr>
<tr>
<td>NE&lt;sub&gt;g&lt;/sub&gt;, Mcal/kg&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.51</td>
<td>0.36</td>
</tr>
<tr>
<td>OM, %DM</td>
<td>98.5</td>
<td>75.2</td>
</tr>
<tr>
<td>ADF, %DM</td>
<td>10.4</td>
<td>77.5</td>
</tr>
</tbody>
</table>

<sup>a</sup> Con = control; Corn = corn; LU = low urea; HU = high urea; SBM = soybean meal.

<sup>b</sup> Calculated with the following equation (Bath and Marble, 1989): TDN = 88.9 - (0.779 * ADF)

<sup>c</sup> Calculated with the following equations (NRC, 1996):<br>
NE<sub>m</sub> = 1.37ME - 0.138ME<sup>2</sup> + 0.0105ME<sup>3</sup> - 1.12;<br>
NE<sub>g</sub> = 1.42ME - 0.174ME<sup>2</sup> + 0.0122ME<sup>3</sup> - 1.65, given that ME = 0.82 × DE, and 1 kg of TDN = 4.4 Mcal of DE.
Table 2. Effects of supplement composition on intake and diet digestibility in steers consuming low-quality, cool-season forage (Exp. 1)

<table>
<thead>
<tr>
<th>Treatmenta</th>
<th>Con</th>
<th>Corn</th>
<th>LU</th>
<th>HU</th>
<th>SBM</th>
<th>SEMb</th>
<th>Con vs Supp</th>
<th>L Urea</th>
<th>Q Urea</th>
<th>HU vs SBM</th>
</tr>
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<tbody>
<tr>
<td>DMI, g/kg of BW</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Forage</td>
<td>21.5</td>
<td>20.8</td>
<td>21.7</td>
<td>20.5</td>
<td>23.0</td>
<td>0.71</td>
<td>0.87</td>
<td>0.64</td>
<td>0.10</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Supplement</td>
<td>0.00</td>
<td>1.26</td>
<td>1.35</td>
<td>1.43</td>
<td>1.27</td>
<td></td>
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<tr>
<td>Total</td>
<td>21.5</td>
<td>22.1</td>
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<td>22.0</td>
<td>24.3</td>
<td>0.71</td>
<td>0.02</td>
<td>0.85</td>
<td>0.10</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>OMI, g/kg of BW</td>
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<td></td>
<td></td>
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<tr>
<td>Forage</td>
<td>19.4</td>
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<td>18.6</td>
<td>20.8</td>
<td>0.63</td>
<td>0.88</td>
<td>0.72</td>
<td>0.10</td>
<td>&lt;0.01</td>
</tr>
<tr>
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<td>1.24</td>
<td>1.24</td>
<td>1.17</td>
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</tr>
<tr>
<td>Total</td>
<td>19.4</td>
<td>20.0</td>
<td>20.8</td>
<td>19.8</td>
<td>22.0</td>
<td>0.63</td>
<td>0.01</td>
<td>0.72</td>
<td>0.10</td>
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<tr>
<td>N Intake, g/kg BW</td>
<td>0.165</td>
<td>0.181</td>
<td>0.229</td>
<td>0.258</td>
<td>0.280</td>
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<tr>
<td>NDF Intake, g/kg BW</td>
<td>16.0</td>
<td>15.7</td>
<td>16.4</td>
<td>15.5</td>
<td>17.3</td>
<td>0.53</td>
<td>0.61</td>
<td>0.68</td>
<td>0.10</td>
<td>&lt;0.01</td>
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<tr>
<td>ADF Intake, g/kg BW</td>
<td>9.8</td>
<td>9.5</td>
<td>9.9</td>
<td>9.4</td>
<td>10.6</td>
<td>0.32</td>
<td>0.62</td>
<td>0.79</td>
<td>0.12</td>
<td>&lt;0.01</td>
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<tr>
<td>Apparent total tract digestibility, %</td>
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<tr>
<td>DM</td>
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<td>N</td>
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<td>43.2</td>
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<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.79</td>
<td>0.84</td>
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</table>

a Con = control; Corn = corn; LU = low urea; HU = high urea; SBM = soybean meal.

b n = 5

c Con vs Supp = control vs supplemented treatments; L urea = linear effect of urea; Q urea = quadratic effect of urea; HU vs SBM = high urea vs soybean meal.
Table 3. Effects of supplement composition on ruminal fill and fermentation characteristics in steers consuming low-quality, cool-season forage (Exp. 1)

<table>
<thead>
<tr>
<th></th>
<th>Treatmenta</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>SEMb</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Con</td>
<td>Corn</td>
<td>LU</td>
<td>HU</td>
<td>SBM</td>
<td></td>
<td>Con</td>
<td>L</td>
<td>Q</td>
<td>HU vs</td>
<td></td>
</tr>
<tr>
<td>NH₃-N, mM</td>
<td>1.61</td>
<td>1.50</td>
<td>3.20</td>
<td>4.72</td>
<td>2.96</td>
<td>0.21</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.69</td>
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</tr>
<tr>
<td>pH</td>
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<td>6.81</td>
<td>6.76</td>
<td>6.88</td>
<td>0.04</td>
<td>0.08</td>
<td>0.38</td>
<td>0.61</td>
<td>0.01</td>
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<tr>
<td>Total VFA, mM</td>
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<td>136.4</td>
<td>134.2</td>
<td>135.5</td>
<td>128.0</td>
<td>0.73</td>
<td>0.91</td>
<td>0.93</td>
<td>0.84</td>
<td>0.44</td>
<td></td>
</tr>
<tr>
<td>VFA, mol/100 mol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetate</td>
<td>63.85</td>
<td>63.41</td>
<td>63.22</td>
<td>63.66</td>
<td>61.07</td>
<td>0.67</td>
<td>0.19</td>
<td>0.80</td>
<td>0.71</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Propionate</td>
<td>18.06</td>
<td>17.51</td>
<td>18.07</td>
<td>17.99</td>
<td>18.11</td>
<td>0.38</td>
<td>0.63</td>
<td>0.22</td>
<td>0.33</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td>Isobutyrate</td>
<td>1.88</td>
<td>1.85</td>
<td>1.72</td>
<td>1.64</td>
<td>2.38</td>
<td>0.09</td>
<td>0.86</td>
<td>0.06</td>
<td>0.81</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Butyrate</td>
<td>10.86</td>
<td>11.79</td>
<td>11.35</td>
<td>11.48</td>
<td>10.91</td>
<td>0.31</td>
<td>0.15</td>
<td>0.48</td>
<td>0.47</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>Isovalerate</td>
<td>1.92</td>
<td>2.08</td>
<td>2.25</td>
<td>1.93</td>
<td>3.54</td>
<td>0.20</td>
<td>&lt;0.01</td>
<td>0.51</td>
<td>0.21</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Valerate</td>
<td>3.35</td>
<td>3.22</td>
<td>3.52</td>
<td>3.40</td>
<td>3.94</td>
<td>0.14</td>
<td>0.28</td>
<td>0.36</td>
<td>0.23</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Acetate:propionate</td>
<td>3.56</td>
<td>3.66</td>
<td>3.52</td>
<td>3.58</td>
<td>3.39</td>
<td>0.11</td>
<td>0.84</td>
<td>0.54</td>
<td>0.39</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>Ruminal IADF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IADF intake, g/kg of BW</td>
<td>4.74</td>
<td>4.58</td>
<td>4.79</td>
<td>4.60</td>
<td>5.06</td>
<td>0.15</td>
<td>0.89</td>
<td>0.88</td>
<td>0.14</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Fill, g/kg of BW</td>
<td>8.86</td>
<td>9.15</td>
<td>8.36</td>
<td>8.79</td>
<td>8.38</td>
<td>0.40</td>
<td>0.64</td>
<td>0.48</td>
<td>0.19</td>
<td>0.44</td>
<td></td>
</tr>
<tr>
<td>Passage rate, % /h</td>
<td>2.23</td>
<td>2.10</td>
<td>2.39</td>
<td>2.28</td>
<td>2.57</td>
<td>0.17</td>
<td>0.45</td>
<td>0.34</td>
<td>0.22</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>Outflow (g·kg of BW⁻¹)/h</td>
<td>0.20</td>
<td>0.19</td>
<td>0.20</td>
<td>0.19</td>
<td>0.21</td>
<td>0.006</td>
<td>0.89</td>
<td>0.88</td>
<td>0.14</td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>

a Con = control; Corn = corn; LU = low urea; HU = high urea; SBM = soybean meal.

b n = 5

c Con vs Supp = control vs supplemented treatments; L urea = linear effect of urea; Q urea = quadratic effect of urea; HU vs SBM = high urea vs soybean meal.
Table 4. Effects of supplement composition on blood characteristics in steers consuming low-quality, cool-season forage (Exp. 1)

<table>
<thead>
<tr>
<th></th>
<th>Treatmenta</th>
<th></th>
<th></th>
<th></th>
<th>SEMb</th>
<th>Con vs Supp</th>
<th>L Urea</th>
<th>Q Urea</th>
<th>HU vs SBM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin, ng/mL</td>
<td>1.51</td>
<td>1.61</td>
<td>1.40</td>
<td>1.65</td>
<td>1.46</td>
<td>0.330</td>
<td>0.95</td>
<td>0.91</td>
<td>0.42</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>53.8</td>
<td>54.1</td>
<td>55.2</td>
<td>54.7</td>
<td>55.2</td>
<td>1.58</td>
<td>0.54</td>
<td>0.75</td>
<td>0.66</td>
</tr>
<tr>
<td>IGF-I, ng/mL</td>
<td>139</td>
<td>131</td>
<td>167</td>
<td>161</td>
<td>176</td>
<td>14.4</td>
<td>0.02</td>
<td>0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>BUN, mg/dL</td>
<td>10.2</td>
<td>10.2</td>
<td>17.4</td>
<td>22.8</td>
<td>18.5</td>
<td>0.94</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.35</td>
</tr>
</tbody>
</table>

a Con = control; Corn = corn; LU = low urea; HU = high urea; SBM = soybean meal.

b n = 5

c Con vs Supp = control vs supplemented treatments; L urea = linear effect of urea; Q urea = quadratic effect of urea; HU vs SBM = high urea vs soybean meal.
<table>
<thead>
<tr>
<th></th>
<th>Treatment(^a)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Con</td>
<td>Corn</td>
<td>LU</td>
<td>HU</td>
<td>SBM</td>
</tr>
<tr>
<td>Initial Wt., kg</td>
<td>516</td>
<td>518</td>
<td>505</td>
<td>509</td>
<td>497</td>
</tr>
<tr>
<td>Initial BCS</td>
<td>4.76</td>
<td>4.75</td>
<td>4.82</td>
<td>4.62</td>
<td>4.79</td>
</tr>
<tr>
<td>Weight change, kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Precalving</td>
<td>18.9</td>
<td>21.0</td>
<td>50.6</td>
<td>66.6</td>
<td>70.7</td>
</tr>
<tr>
<td>Postcalving</td>
<td>-36.0</td>
<td>-29.6</td>
<td>5.2</td>
<td>15.6</td>
<td>17.3</td>
</tr>
<tr>
<td>BCS change</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Precalving</td>
<td>-0.49</td>
<td>-0.32</td>
<td>0.05</td>
<td>0.12</td>
<td>0.26</td>
</tr>
<tr>
<td>Postcalving</td>
<td>-0.63</td>
<td>-0.57</td>
<td>-0.22</td>
<td>-0.05</td>
<td>0.15</td>
</tr>
<tr>
<td>Days to Calving</td>
<td>94.8</td>
<td>93.9</td>
<td>96.1</td>
<td>99.1</td>
<td>98.8</td>
</tr>
<tr>
<td>Calf Birth Wt., kg</td>
<td>37.7</td>
<td>34.1</td>
<td>37.8</td>
<td>38.3</td>
<td>40.3</td>
</tr>
</tbody>
</table>

\(^a\) Con = control; Corn = corn; LU = low urea; HU = high urea; SBM = soybean meal.

\(^b\) n = 4

\(^c\) Con vs Supp = control vs supplemented treatments; L urea = linear effect of urea; Q urea = quadratic effect of urea; HU vs SBM = high urea vs soybean meal.
Table 6. Effects of supplement composition on blood characteristics in cows consuming low-quality, cool-season foragea (Exp. 2)

<table>
<thead>
<tr>
<th></th>
<th>Treatmentb</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Con</td>
<td>Corn</td>
<td>LU</td>
<td>HU</td>
<td>SBM</td>
<td>SEMc</td>
<td>Con</td>
<td>L</td>
<td>Q</td>
<td>HU vs</td>
</tr>
<tr>
<td>Insulin, ng/mL</td>
<td>0.80</td>
<td>0.89</td>
<td>1.20</td>
<td>1.08</td>
<td>1.22</td>
<td>0.149</td>
<td>0.07</td>
<td>0.36</td>
<td>0.23</td>
<td>0.50</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>56.6</td>
<td>62.7</td>
<td>60.3</td>
<td>63.1</td>
<td>61.0</td>
<td>5.06</td>
<td>0.45</td>
<td>0.96</td>
<td>0.71</td>
<td>0.79</td>
</tr>
<tr>
<td>IGF-I, ng/mL</td>
<td>25.0</td>
<td>26.6</td>
<td>39.0</td>
<td>39.4</td>
<td>41.7</td>
<td>2.52</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.05</td>
<td>0.51</td>
</tr>
<tr>
<td>BUN, mg/dL</td>
<td>9.32</td>
<td>7.63</td>
<td>12.72</td>
<td>14.88</td>
<td>18.41</td>
<td>1.150</td>
<td>0.13</td>
<td>0.08</td>
<td>0.43</td>
<td>0.21</td>
</tr>
<tr>
<td>NEFA, mEq/L</td>
<td>0.48</td>
<td>0.47</td>
<td>0.53</td>
<td>0.58</td>
<td>0.48</td>
<td>0.116</td>
<td>0.82</td>
<td>0.59</td>
<td>0.95</td>
<td>0.62</td>
</tr>
</tbody>
</table>

a Evaluated for blood samples collected on d 49 and within 24 h of calving. Least square means adjusted covariately to values obtained on d 0.

b Con = control; Corn = corn; LU = low urea; HU = high urea; SBM = soybean meal.

c n = 4

d Con vs Supp = control vs supplement treatments; L urea = linear effect of urea; Q urea = quadratic effect of urea; HU vs SBM = high urea vs soybean meal.
Figure 1. Effects of supplement composition on steer ruminal ammonia-N (Exp. 1). Columns from left to right for each treatment represent 0, 1, 3, 6, 9, 12, 18 and 24 h after feeding, respectively. Treatments were Con = control; Corn = corn; LU = low urea; HU = high urea; SBM = soybean meal. Treatment x time interactions were (P <0.001) and SEM is 0.50.
Literature Cited


APPENDIX
Abbreviations

ADF - Acid Detergent Fiber
AI - Artificial Insemination
BCS - Body Condition Score
BCVFA - Branch Chain Volatile Fatty Acid
BUN - Plasma Urea Nitrogen
BW - Body Weight
C3 - Cool-Season Plant
C4 - Warm-Season Plant
CON - Control Treatment
CP - Crude Protein
CSM - Cotton Seed Meal
d - Day
DE - Digestible Energy
DM - Dry Matter
DMD - Dry Matter Digestibility
DMI - Dry Matter Intake
FSH - Follicle Stimulating Hormone
GH - Growth Hormone
GnRH - Gonadotropin Releasing Hormone
h - hour
hd - head
HU - High Urea Treatment
IADF - Indigestible Acid Detergent Fiber

IGF - Insulin-Like Growth Factor

IGFBP - Insulin-Like Growth Factor Binding Protein

IVDMD - In vitro Dry Matter Disappearance

LH - Luteinizing Hormone

LU - Low Urea Treatment

MP - Metabolizable Protein

NDF - Neutral Detergent Fiber

NEFA - Non-Esterified Fatty Acid

NH3 - Ammonia

NH3-N Ammonia Nitrogen

NPN - Non-Protein Nitrogen

OM - Organic Matter

OMI - Organic Matter Intake

P4 - Progesterone

PPI - Postpartum Interval

PUFA - Polyunsaturated Fatty Acids

RDP - Rumen Degradable Protein

RUP - Rumen Undegradable Protein

SBM - Soybean Meal

SFA - Saturated Fatty Acids

VFA - Volatile Fatty Acids