# EFFECTS OF PLANT SAPONINS ON FORAGE DIGESTION AND PERFORMANCE OF GRAZING BEEF CATTLE

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# CHAPTER I

# INTRODUCTION

Micro-Aid® has been shown to improve microbial efficiency and fiber digestibility in dairy diets, as well as feed conversion and microbial efficiency in feedlot diets. In the U.S., approximately 30 million beef cows graze pasture year around, while roughly 20 million growing cattle graze for some period of time after being weaned and before entering the breeding herd or entering the feedlot for finishing. Limited data suggests that Micro-Aid® improves performance of grazing cattle while improving nitrogen utilization and reducing methane and urea emissions. Further study is necessary to determine if the inclusion of Micro-Aid® in supplements for forage-fed cattle is a cost effective practice. Addition of Micro-Aid® to forage-fed cattle supplements could result in improved forage utilization, improved performance of forage-fed cattle or reduced amount of protein supplement required. It is estimated that on an annual basis, the approximate 50 million head of grazing cattle in the U.S. are supplemented for an average of 120 days. Therefore, at the inclusion rate of 1 gram of Micro-Aid® per head per day during the supplementation period alone would result in a total market potential of 6,000 metric tons of Micro-Aid® annually.

Micro-Aid® improved feed to gain an average of 4.7% in fifteen independent studies of cattle at three different locations. In vitro work suggests that at least a portion

of this response can be attributed to improved microbial efficiency when cattle are supplemented with Micro-Aid®.

Similarly, addition of Micro-Aid® to a dairy ration improved in vitro digestibility of ADF, microbial growth and microbial efficiency. In the Southern Great Plains, standing forage supplies adequate nutrients to maintain beef cows and (or) allow for weight gain in cows and growing cattle three to six months of the year. Said another way, forage is of low quality in six to nine months when supplemental protein and (or) energy are not required. Previous work suggests that Micro-Aid® and other *Yucca Schidigera*-based surfactants have defaunating properties (Hristov et al., 2004). For example, inclusion of *Yucca Schidigera* resulted in a reduction in rumen protozoa and an increase in rumen bacteria and fungi (Hristov et al., 1999). Therefore, in high roughage diets the defaunating effects of Micro-Aid® could potentially increase fiber digestion and improve microbial nitrogen supply to the small intestine. Addition of Micro-Aid® to forage-fed cattle supplements could result in improved forage utilization, improved performance of forage-fed cattle or reduced amount of protein supplement required. Therefore, the purpose of this dissertation was to determine if Micro-Aid® can improve forage utilization and ultimately impact animal performance of growing cattle and spring-calving cows consuming a forage based diet.

# CHAPTER II

## REVIEW OF LITERATURE

### *Background of Saponins*

Micro-Aid® is an all-natural, dry or liquid feed additive for use in animal feeds. It is manufactured from a purified extract of the *Yucca schidigera* plant that grows in the Southwest United States and Mexico. Micro-Aid® is thought to have surfactant like properties because it contains saponins. By definition, saponins are glucosides that occur in plants and are characterized by the property of producing a soapy lather (Merriam-Webster, 2010). Saponins are either triterpenoids or steroids in nature and have a dydrophobic aglycone, more commonly named sarsapogenin (Figure 1), attached to a sugar (Wina et al., 2005). Steroidal saponins from *Yucca schidigera* and *Quillaja saponaria* plants are the most commonly used commercial saponins. Yucca extract has a concentration of 4.4% steroidal saponins (Wina et al., 2005). The actual role of steroidal saponins in nature is not known, but has been suggested that they may inhibit mold (antimicrobial) and protect plants from insects (Francis et al., 2002) or even provide a source of monosaccharides for the plant (Barr et al., 1998). The draw to saponin technology can be attributed to their known lytic action on erythrocyte membranes. This action is believed to be due to their affinity to membrane sterols, particularly cholesterol (Glauert et al., 1962). When treated with saponins, cell membranes from human erythrocytes developed pores 40-50 Å in diameter (Seeman et al., 1973). The interaction

between saponins and membrane lipids seems to be complicated, but it is thought that yucca saponins are effective at suppressing rumen protozoa by reacting with cholesterol in the protozoal cell membrane, causing it to lyse (Cheeke, 2000). Saponins form micelles with sterols when the sapogenin (the hydrophobic portion) lipophilically binds with the hydrophobic sterol nucleus (Oakenfull and Sidhu, 1989). These characteristics allow saponins to bind to cholesterol membranes of protozoa and lyse them. A reduction in protozoa should increase the rate of fermentation in high roughage diets via the subsequent increase in bacterial and fungal populations.

***Saponins for the feed industry***

The primary saponin-containing plants that have potential use as feed additives for ruminants are listed in Table 1. The plant itself is not typically used as a feedstuff, rather the extracted saponins from the plant. The process for obtaining yucca extract starts with the harvest of the trunk of the plant "yucca logs" which are macerated and then either ground or pressed to produce yucca powder or juice, respectively (Cheeke, 2000). Some commercial uses for saponins include surfactants for mining and ore separation, emulsions for photographic films and in cosmetics (Cheeke, 2000). However, these extracts are currently being used as dietary feed additives for livestock, primarily for the control of ammonia and odor (Cheeke, 2000). It is thought that the effects of saponins on nitrogen metabolism are caused by the non-butanol-extractable fractions which contains primarily carbohydrates (Cheeke, 2000), but in ruminants it is thought that yucca extracts reduce rumen ammonia concentrations as a consequence of suppressing rumen protozoa concentrations (Hristov et al., 1999). This potential use as a defaunating agent has led to the investigation of saponins as

feed additives over the past few decades (Goetsch and Owens, 1985; Wilson et al., 1998; Hristov et al., 2004). The consequences from defaunating the rumen include but are not limited to, decreased bacterial proteolysis, improved nitrogen conservation, decreased methanogenesis, and a shift in VFA production toward propionate which all aid in the improvement in overall animal efficiency. These benefits to animal efficiency may be a domino effect or they might be independent results of steroidal saponins.

***Saponins Role on Rumen Fermentation***

First, the data shows sufficient support that there is a reduction in rumen protozoa with the inclusion of saponins in ruminant diets (Valdez et al., 1986; Lu and Jorgenson, 1987; Wallace et al., 1994; Klita et al., 1996; Hristov et al., 1999). This reduction in rumen protozoa may have several positive associative effects including improved nitrogen metabolism efficiency, a reduction in methane emissions, shift's in bacterial and fungal populations and a potential increase in bacterial protein flow to the lower gastrointestinal tract (Wallace et al., 1994). The addition of 0.1% Yucca schidigera to rumen fluid inhibited the motion of the cilia of entodiniomorphs and the contraction of holotrichs, while decreasing the rate of breakdown of [$^{14}$C] leucine-labeled *Selenomonas ruminantium* (Wallace et al., 1994). This impairment can eventually lead to protozoal cell lysis and consequently provide a competitive advantage for some bacteria (i.e., *S. ruminantium*) and fungi as well as reduce intrarumen nitrogen cycling and potentially improve microbial synthesis.

One of the primary benefits from the use of saponins and a potential result of rumen defaunation is the impact on nitrogen metabolism. It has been well documented that an improvement in nitrogen retention can be expected as a response to rumen defaunation (Williams and Coleman, 1997). Summarized data from Wina et al. (2005) showed that

ammonia was decreased in 50% of the reviewed studies while protozoa concentrations were decreased in 70% of the studies (Table 2). This discrepancy may be due to method of determination of one concentration or the other or there may not be a linear relationship between reduced protozoa concentrations and reduced ammonia. It may also be due to the independent ability of yucca saponins to bind ammonia, reducing the rumen ammonia concentrations or the subsequent reduction in rumen proteolysis (Wallace et al., 1994). It has also been suggested that when rumen ammonia concentrations are high, yucca extract can bind ammonia-N and release it when concentrations are low (Hussain and Cheeke, 1995). Makkar et al. (1999) also demonstrated this rumen nitrogen conservation when urea-supplemented straw was fed.

Another method of action on nitrogen metabolism in ruminants is via blood and milk urea concentrations. There are some instances where no effect has been seen on blood urea nitrogen (Hristov et al., 1999; Wilson et al. 1998), but more often it has been cited to reduce blood urea nitrogen (Hussain and Cheeke, 1995; Hussain et al., 1996; Killeen et al., 1998a; Killeen et al., 1998b). Wilson et al. (1998) also found that it had no effect on milk urea nitrogen in lactating dairy cows. It seems pretty evident that saponins interact with protein metabolism in the ruminant, but diet or stage of production may be of great importance to the overall impact.

Another positive associative effect of rumen defaunation is the potential to reduce methanogens associated with protozoa. Methane is a hydrogen sink in the rumen and is not only a loss of carbon and hydrogen, but it is harmful to the environment. Therefore, a reduction in methanogensis would be beneficial to the whole system of beef cattle production. Nevertheless, when Yucca extract was included in a high roughage diet or to a mixed diet of hay and barley grain there was no effect on methane production in a rumen simulation system (Sliwinski et al., 2002). Other studies have conversely demonstrated a

reduction in methane production (Santoso et al., 2004; Hu et al., 2005). Sanotoso et al. (2004) also calculated a reduction in energy losses through methane as a percent of gross energy when *Yucca schidigera* was included in the diet. These results may be independent of rumen defaunation and rather a direct inhibition of methane producing bacteria.

A source of ruminal ammonia is from the proteolysis of bacterial protein by protozoal ingestion. Not only are bacteria engulfing protozoa decreased by saponins, but sterols are absent on bacterial membranes (Cheeke, 2000), and therefore should be able to proliferate in the presence of saponins. Wallace et al. (1994) observed an increase in *Prevotella ruminicola* growth, no affect on *Selenomonas ruminantium*, suppressed growth of *Streptococcus bovis*, and complete inhibition of *Butyrivibrio fibrisolvens* when *Yucca schidigera* was introduced to the medium at 1%. *Prevotella ruminicola* is a gram-negative bacteria and it is suggested that yucca extract is more potent to gram-positive bacteria (Wang et al., 2000), which could lead to a subsequent increase in gram-negative bacteria. Antibiotics such as monensin also decreases gram-positive bacteria, leading to an increase in propionate production, a decrease in passage rate and an improvement in overall efficiency (Perry et al., 1976). Wang et al. (2000) also reported a suppressing effect on *Streptococcus bovis*, but they also saw a substantial reduction in cellulytic bacteria and fungi. Effects on certain types of bacteria may be saponin or product specific and may be dependent on diet (i.e., high fiber vs. high concentrate).

Potential for increases in fungal populations may also be due to the decrease in protozoa concentrations (Hsu et al., 1991), and fungi are an important component of fiber digestion in the rumen. Francis et al. (2002) observed that *Sapindus rarak* actually decreased fungal RNA concentration in rumen liquor in an in vitro fermentation experiment. However,

Francis et al. (2002) also presented data from Diaz et al. (1993) demonstrating increased fungal populations in sheep fed 25-30 g of *Sapindus saponaria* for 30 days. There are inconsistent results pertaining to saponins effects on fungal populations and there is little knowledge as to which fungi are important to ruminant digestion.

It is thought that a decrease in protozoa should decrease microbial engulfment, increasing microbial protein synthesis and bacterial protein flow to the small intestine. Again, results have been varied. Lu and Jorgensen (1987) reported a decrease in microbial protein concentration while Goetsch and Owens (1985) reported a contradictory improvement in efficiency of protein synthesis by 36%. Hu et al. (2005) also showed an improvement in microbial protein (mg/mL) when tea saponins were included in vitro at 6 and 8 milligrams. With respect to Lu and Jorgensen (1987), even if there is a decrease in microbial protein concentration there may not be a decrease in microbial protein flow to the small intestine because bacterial nitrogen is more easily washed out of the rumen than protozoal nitrogen (Abe et al., 1981)

In addition, shifts in volatile fatty acid profiles have been reported in the presence of saponins. In this case there is typically a shift toward propionate production, resulting in a decrease in the acetate to propionate ratio (Hristov et al., 1999) which is consistent with a decrease in protozoa numbers (Williams and Coleman, 1992). This shift is seen when ionophores are fed (Perry et al., 1976). In most cases a shift in propionate leads to a shift in rumen pH. However, this has not consistently been reported with the use of saponins.

Hristov et al. (1999) observed a numerical decrease in pH from the control diet when yucca extract was added at 20 and 60 g/day (6.28 vs. 6.18 and 6.19), whereas others have observed no differences in pH with yucca extract (Valdez et al., 1986; Wilson et al., 1998).

Conversely, there have also been reports of increased pH values (Hussain and Cheeke, 1995; Zinn et al., 1999). Regardless of pH changes, data suggests that an increase in propionate can be expected when saponins are added to the rumen.

### *Fate of Saponins in the Rumen*

As for degradation of saponins in the ruminant, most are merely degraded in the rumen to derivatives of the sarsapogenin and are poorly absorbed (Oakenfull and Sidhu, 1989). Wang et al. (2000) recorded a decrease in Yucca saponins when added to the pure culture of *Fibrobacter succinogenes*. It is thought that because some bacteria can fully degrade dietary saponins that rumen adaptation could occur and that the results obtained from the administration of saponins may be transient. Therefore it has been suggested that feeding saponins intermittently might be more effective and alleviate bacterial adaptation (Cheeke, 2000). Thalib and others (1995) actually observed that a feeding frequency of 3 days per week was an effective means of supplying saponins with successful suppression of protozoa and ammonia concentrations. However, the appropriate feeding regimen and complete metabolism of saponins in the ruminant needs to be further investigated.

### *Saponins on Intake and Digestibility*

Most reports suggest that the inclusion of saponins in the diet has no effect on dry matter intake (Valdez et al., 1986; Wilson et al., 1998; Hristov et al., 1999). Again, there have been inconsistent results with regard to digestibility. It has been shown that defaunation resulted in a decrease in apparent organic matter, nitrogen, neutral detergent fiber and acid detergent fiber digestion (Koenig et al., 2000). However, Santoso et al. (2004) saw no effect on apparent organic matter, nitrogen, neutral detergent fiber and acid detergent fiber digestibility in sheep fed

orchardgrass silage and concentrates, and Hristov et al. (1999) saw no differences in in situ dry matter disappearance, or total tract digestibility when fed to heifers. Conversely, Goetsch and Owens (1985) saw an improvement in apparent total tract organic matter digestibility. Hristov et al. (2004) did report an increase in the immediately soluble dry matter portion of corn, enabling it to have an increase in in situ rumen dry matter degradability when a yucca extract product was fed. As for passage rate, there have been no reports that saponins have any effect on particulate passage rate (Goetsch and Owens, 1985; Hristov et al., 2004).

Nitrogen conservation might be improved due to consequences previously mentioned. However, when evaluating nitrogen digestibility and retention there have been mixed results reported. Hristov et al. (1999) reported an increase in urinary nitrogen excretion of 5%, resulting in a slight decrease in nitrogen retention when yucca saponin was included in the diet at 60 g/day. As for nitrogen digestion, Lu and Jorgensen (1987) observed a decrease in digestibility when high levels of alfalfa saponins were provided in a forage-based diet. Similar findings have been reported for a 35% reduction in apparent nitrogen digestibility (Valdez et al., 1986).

### *Saponins on Animal Performance*

There have been very few studies evaluating animal performance in the presence of yucca extracts. In a study with dairy cows, Wilson et al. (1998) reported a tendency for reduced crude protein and true protein in the milk ($P = 0.13$ and 0.07, respectively), but no difference in daily milk yield. Coinciding data from Valdez and others (1986) also showed no impact on body weight change, milk yield or composition of the milk. However, Valdez et al. (1986) suggested that there was a consistent improvement in milk production by 1 to 3% with the inclusion of sarsaponin. Mader and Brumm (1987) evaluated the replacement of soybean meal with urea in a steer supplement and observed an improvement in rate of body weight gains from steers consuming the urea containing supplement with saponin as compared to urea only as the source of degradable protein. More research needs to be conducted to evaluate animal performance when providing dietary plant saponins.

### *Protein Supplementation*

The potential for saponins to benefit grazing cattle is due to the fact that from late summer until early spring, tall-grass prairie forage is poor in quality and rumen ammonia is first limiting, reducing forage intake and digestibility (McCollum and Horn, 1990). Therefore it is essential to provide a supplement with enough crude protein to alleviate this rumen nitrogen deficit. McCollum and Horn (1990) outlined numerous studies that unveiled the increase in performance by supplementing low levels of protein when cattle graze low quality forage. The "Oklahoma Gold" program developed at Oklahoma State University was established on the basis that providing 0.45 kg of a high protein supplement (38 to 40% crude protein) three times/wk will improve performance of grazing steers by 0.20 kg/d (Lalman and Gill, 2010). Additionally, in the Southern Great Plains it is a common practice to supply either a 40% crude protein cube three times/wk during the winter months to spring-calving cows and a 30% cube to fall-calving cows four times/wk due to their increased energy demand. These protein cubes are typically formulated with cottonseed meal and wheat middlings as the primary ingredients. The crude protein in cottonseed meal is moderate to high in rumen degradability (57 to 78%; NRC, 1996; Winterholler et al., 2009) and serves as a good source of nitrogen for rumen bacteria.

### *Urea Supplementation*

Alternative options to alleviate rumen ammonia deficiency in grazing cattle diets are to include non-protein nitrogen sources, which have the potential to decrease feed costs. Farmer et al (2004) concluded that incorporating urea up to 30% of rumen degradable protein

did not have a negative effect on performance of dry-pregnant cows when utilized in an alternate day feeding system, but did decrease the acetate to propionate ratio compared to no dietary urea. They observed supplement refusals when urea was included at 45% of supplemental rumen degradable protein (30% CP; 188 g/d of urea). Farmer et al. (2004) went on to conclude that when feeding gestating cows on alternate days it would be safe, without supplement refusal, to include urea at a level equivalent to 3.1% of the diet or 22% of crude protein in a 40% crude protein supplement delivered at 119 g urea/feeding. In concurring literature, Koster et al (2002) supplied 60% of the supplemental rumen degradable protein via urea (103 g/d). Similarly, Koster et al (1997) supplied 100% of supplemental rumen degradable protein through urea (132 g/d) and Forero et al. (1980) supplied 62.5% of the supplemental crude protein with urea (108 g/d) and saw no palatability or toxicity issues. Therefore it seems reasonable to conclude that urea could be included at levels near 100 g/d regardless of its percentage of the crude protein or rumen degradable protein. However, there is little information on maximum urea levels for lactating cows. In most fall-calving systems feed is delivered four times per week at a common rate of 2.06 kg/day or 3.61 kg/feeding. This has to be considered when including urea as a substitute for a natural protein. In a fall-calving system an inclusion rate of 2.6% urea (101 g/feeding) or urea included at 25% of crude protein or 37% of rumen degradable protein should result in no supplement refusals. When substituting a natural protein source with a slow-release urea product, palatability issues have not been an issue. Owens et al. (1980) determined from observed ammonia levels that toxicity would not occur until urea intake reached 900 g/day.

High inclusion rates of urea in supplements for cattle consuming a forage based diet have decreased total organic matter intake, decreasing performance as compared to natural

protein supplements. Performance losses when substituting natural protein with urea (Rush and Totusek, 1975) may be due to a metabolizable protein deficiency (NRC, 1996) or an increase in removal of urea from the kidneys and excretion in the urine. As previously mentioned, data suggests that saponins may have the potential to conserve this nitrogen and improve performance (Mader and Brumm, 1987).

Correction of rumen ammonia deficiency could be arguably the most important mechanism, yet the most controversial in relation to animal performance. It is the classic example of the chicken and the egg. The primary reason for the controversy is due to the fact that supplemental protein typically increases intake and digestibility, but in some instances digestibility is increased without a subsequent increase in dry matter intake. This has been the focus of several studies, primarily due to the fact that non-protein nitrogen (i.e., urea) has increased microbial synthesis similar to plant protein, but has been less effective at improving dry matter digestibility (Rush et al., 1976; Kropp et al., 1977). If rumen ammonia deficiency was the sole mechanism of improving performance a source of non-protein nitrogen should prove to be as effective as plant proteins. The fact that non-protein nitrogen is not as effective as plant protein has established the foundation for further mechanisms. Plant protein has been shown to be more effective at improving digestion of dry matter and organic matter due to bypass of some of the feed protein to the small intestine (Kropp et al., 1977).

***Protein Supplementation on Animal Performance***

Increased non-ammonia nitrogen flow to the small intestine from microbial cell protein or un-degraded plant proteins could increase performance. When protein has been

supplied to cattle consuming low-quality forage the non-ammonia nitrogen flow to the small intestine has been consistently improved. This is thought to be the reason for increased intake. Egan and Moir (1965) showed that infusion of urea or casein into the small intestine increased intake, however infusion with casein into the small intestine did not increase organic matter digestibility. This demonstrated that amino acid supply to the small intestine may increase intake independent of rumen ammonia supply, but that digestibility is influenced by rumen ammonia. This increases microbial production and efficiency (Bandyk et al., 2001; Wickersham et al., 2008). The following two (a and b) mechanisms serve as potential reasons for the performance responses observed from grazing cattle provided supplemental protein.

a. Provide amino acids to the small intestine for tissue metabolism (McCollum and Horn, 1990). It is well understood that the small intestine utilizes several amino acids for metabolism. Supplying undegraded plant proteins to the small intestine may provide amino acids not sufficiently supplied by microbial cell protein. This process may also provide glucogenic precursors to tissues in the form of amino acids to help oxidize acetate (Hannah, 1991). This will enhance the use of metabolizable energy supplied to the animal and aid in the catabolism of the volatile fatty acid, acetate, which prevails in a high roughage diet.

b. Balance amino acid requirements in the rumen and at the tissue level (McCollum and Horn, 1990). It has been demonstrated that microbial efficiency can be improved by supplementing individual amino acids (Clark and Petersen, 1985).

Improvements in dry matter intake may also be the driving force behind performance enhancements when supplemental protein is provided. Typically, physical fill in high roughage diets is the limiting factor influencing intake. This is due to tension receptors

located in the reticulum. When small amounts of protein are supplied to cattle consuming low quality forage the dry matter digestibility is improved and dry matter intake is also improved (Mathis et al., 1999). However, it has also been demonstrated that intake can be increased with duodenal infusion of casein (Egan and Moir, 1965) without influencing digestibility. This provides evidence that more than one mechanism exists to improve intake of low quality forage and ultimately improved performance.

***Conclusions***

In conclusion, protein supplementation is a must in the Southern Great Plains and is unquestionably beneficial to cattle grazing low quality forage. However, in recent years the cost of production has increased markedly and one of the primary reasons is supplemental feed costs. A current enterprise budget from Oklahoma State Cooperative Extension suggests that a conservative estimate for yearly production of spring-calving cows is nearly $500.00/cow/year. Over 50% of these costs can be attributed to feed costs (hay, pasture and supplement) and nearly $50/cow can be directly attributed to protein supplementation costs (Lalman, 2010). These cost estimates are more likely exceeded in most cow-calf operations in the Southern Great Plains. Therefore, any potential feed additives that can be easily incorporated into protein supplements already being provided and improve nutrient efficiency will decrease feed costs and increase profits. This is why products such as Micro-Aid® have the potential to be an effective means of improving performance of grazing cattle provided supplemental protein.

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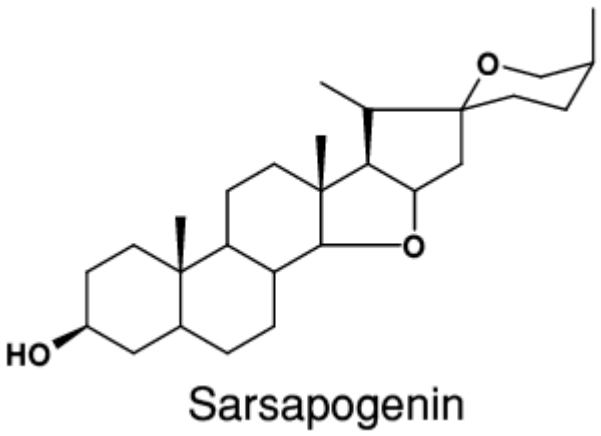


Figure 1. Spiro-furostanol saponin

Table 1. Saponin-containing plants used for feed additives in ruminant diets

| Family and Species | Plant Part | Saponin or Sapogenin Name |
|---|---|---|
| | | Rosaceae |
| *Quillaja saponaria* | bark | Quillaic acid |
| | | Agavaceae |
| *Yucca Schidigera* | trunk, root | Sarsapogenin, gloriogenin, markogenin |
| | | Sapindaceae |
| *Sapindus saponaria* | fruit | Hederagenin (aglycone) |
| *Sapindus rarak* | fruit | Hederagenin (aglycone), mukorozi-saponin |
| | | Theaceae |
| *Camellia sinensis* | seed, leaf | Theasaponin, camelliasaponins |

Adapted from Wina et al., 2005

Table 2. Effects of commercial *Yucca schidigera* extract products on protozoa, ammonia and propionate concentrations in rumen contents in vitro and in vivo

| | | | | Effect On, % | | |
|---|---|---|---|---|---|---|
| | Method | Dosage | Substrate/feed | Protozoa | Ammonia | Propionate |
| *Yucca Schidigera* (commercial) | In vitro | 1 & 10 mg/mL | No substrate | -22 and -100(A) | 0 and -6 | ND |
| | In vitro | 1-100 mg/kg DM | Grass silage & hay | No effect | No effect | No effect |
| | In vivo (cows) | 125 mg/kg diet | Hay-corn-cottonseed | ND | No effect | No effect |
| | In vitro | 200 mg/L | Hay | ND | No effect | No effect |
| | In vivo (sheep) | 5-30 g/d | Soybean hull:alfalfa:corn:oat | -18[a] | ND | ND |
| *Yucca Schidigera* (commercial) sarsaponin | In vivo (heifers) | 20-60 g/d | Alfalfa hay:barley grain (39:61) | -20 to -32 | No effect | +17 to +18 |
| | In vitro | 1-4 mg/mL | Casein | No effect | -5 to -27 | No effect |
| | In vitro | 1.2-3.2 g/L | Potato starch | -6 to -30 | -20 to -50 | +11 to +29 |
| | In vitro | 1.2-3.2 g/L | Corn starch | -13 to -32 | -20 to -39 | +5 to +14 |
| | In vitro | 1.2-3.2 g/L | Hay:concentrate (6:4) | -18 to -43 | -18 to -38 | +10 to +37 |
| | In vitro | 33-77 mg/kg | Corn:alfalfa:soybean meal | -8 to -19 | No effect | ND |
| | In vivo (cows) | 77 mg/kg | Concentrate:sorghum silage (55:45) | ND | No effect | +17 to +18 |
| | In vivo (sheep) | 0.01 & 0.25% | Hay-barley | No effect | -56 to -59 | No effect |

A = protozoal activity measured by $^{15}N$ released from labeled bacteria
ND = no data
[a]Effect was significant at the highest level
Adapted from Wina et al., 2005

# CHAPTER III

# EFFECT OF MICRO-AID® ON HAY INTAKE AND UTILIZATION BY BEEF CATTLE

ABSTRACT

Sixteen ruminally cannulated crossbred steers (BW = 529 ± 45 kg) were used to evaluate in situ DM and NDF degradation characteristics of low quality prairie hay (Exp. 1), blood urea nitrogen (BUN) and rumen fermentation parameters (Exp. 2) in steers provided a protein supplement with or without Micro-Aid® (MA; plant derived saponin). Steers were allowed ad libitum access to chopped prairie hay (4.9% CP and 73.8% NDF) and randomly assigned to one of four treatments. Supplements included: 1) no supplement (C), 2) cottonseed meal/wheat midds supplement with no MA (PC; 40% CP); 3) MA added to PC to supply 1 g·steer$^{-1}$·d$^{-1}$ (MA1); 4) MA added to PC to supply 2 g·steer$^{-1}$·d$^{-1}$(MA2). Steers were individually supplemented 0.92 kg (DM basis) once daily at 0800 along with a vitamin and mineral mix to ensure requirements were met. Orthogonal contrasts were used to determine the effects of protein supplementation, addition of MA and level of MA inclusion. In Exp. 1, in situ forage samples were incubated for a 96 h period. Protein supplementation increased DMI, Kp, and ruminal digestibility of DM and NDF ($P < 0.01$), but there was no effect on rumen N degradability. Dry matter intake was not affected by the inclusion of MA in either experiment. The addition of MA decreased Kp compared to PC (2.78 and 2.27%/h, respectively; $P = 0.02$), resulting in an increase in rumen NDF and DM digestibility. However, there was no influence of MA on apparent total tract digestibility in Exp. 2. Rumen protozoa concentrations were suppressed with MA inclusion while lactate concentrations were increased ($P < 0.01$). There was no impact on BUN, rumen ammonia or pH for MA as compared to PC diets. Total concentrations of VFA and independent acetate and propionate concentrations were increased due to supplementation. These concentrations were not different from MA steers, but MA1 did increase total VFA and acetate, propionate and

butyrate concentrations compared to MA2. Providing low quantities of a protein supplement to steers consuming low-quality prairie hay improved forage DMI and apparent disappearance of DM, NDF, ADF and N along with an improvement in nitrogen balance. Including MA in protein supplements increased rumen DM and NDF digestibility of forage and reduced protozoa. Therefore, MA may potentially increase the supply of bacterial protein to the small intestine and reduce methane production. However, more research is needed to validate this and to evaluate its impact on animal performance.

## INTRODUCTION

It is a common practice to supply additional protein to cattle consuming low quality forage. This practice is a result of a deficiency in rumen ammonia-N and is thought to be effective when the CP of forage is less than 7% (DM basis; McCollum and Horn, 1990). In the Southern Great Plains, it has been reported that standing native range pastures reach this minimum CP content in late July (McMurphy et al., 2009). Supplying additional protein to cattle has resulted in improvements in intake and digestibility (McCollum and Horn, 1990). Rising costs of supplemental protein and environmental policy encouraging the reduction of greenhouse gases in animal production have led to research to improve supplementation efficiency.

Micro-Aid® is an all-natural, dry or liquid feed additive for use in animal feeds. It is manufactured from a purified extract of the *Yucca schidigera* plant that grows in the Southwest United States and Mexico and contains saponins. Saponins are either triterpenoids or steroids in nature and have a dydrophobic aglycone, more commonly named sarsapogenin,

attached to a sugar (Wina et al., 2005). The interest in steroidal saponin technology can be attributed to their known lytic action on rumen protozoa. This action is believed to be due to their affinity to membrane sterols, particularly cholesterol (Glauert et al., 1962). The consequences from defaunating the rumen include, but are not limited to decreased bacterial proteolysis, improved nitrogen conservation, decreased methanogenesis, and a shift in VFA production toward propionate which all improve in overall animal efficiency. These benefits to animal efficiency may be a direct effect from reduced protozoa concentrations or mere functions of the yucca extract itself. The objectives of this study were to determine if the addition of MA had a positive impact on digestion in steers consuming low-quality prairie hay and its influence on rumen fermentation and nitrogen metabolism.

## MATERIALS AND METHODS

***Animals.*** This experiment was conducted at the Nutrition and Physiology Barn located on campus at Oklahoma State University in accordance with an approved Oklahoma State University Animal Care and Use Committee protocol. Sixteen ruminally cannulated crossbred steers (BW = 529 ± 45 kg) were housed individually in slatted-floor pens (2.4 x 4.6 m) and allowed ad libitum access to chopped prairie hay (5 cm; 4.6% CP, 56.0% TDN, 75.7%, NDF, 4.2% ADIA; DM basis). Hay was harvested in late July, 2008 from an old world bluestem (*Bothriochloa ischaemum*) hay meadow. Steers were randomly assigned to one of four supplement treatments: 1) no supplement (C; negative control), 2) cottonseed meal/wheat midds-based supplement with no Micro-Aid® (PC; positive control, 40% CP, DM basis), 3) Micro-Aid® added to PC to supply 1 $g \cdot steer^{-1} \cdot d^{-1}$ (MA1), 4) Micro-Aid® added to PC to supply 2 $g \cdot steer^{-1} \cdot d^{-1}$

(MA2). Steers were provided 0.92 kg (DM basis) once daily of supplement in order to meet but not exceed rumen degradable protein requirements (RDP; NRC, 1996). A vitamin and mineral mix was provided in feed pans with supplement daily to all steers. Steers had continuous access to fresh water and diets were fed at 0800 for 10 d prior to initiation of the study to allow for ruminal adaptation.

***Experiment 1: In Situ Digestibility***

Characteristics of forage in situ degradation were evaluated in steers consuming the experimental diet (4 steers per treatment) and using standardization techniques presented by Vanzant et al. (1998). Dacron bags (Ankom Technology, Macedon, NY; 10 × 20 cm, 53 ± 15 $\mu$m pore size) were labeled with waterproof permanent marker and bag weight was recorded. All forage samples were ground in a Wiley mill (Model-4, Thomas Scientific, Sweedesboro, NJ) to pass a 2-mm screen prior to incubation. Forage samples were weighed into Dacron bags (5 g; as-fed) and heat sealed in duplicate for each incubation time point. Prior to ruminal insertion, bags were soaked in tepid water (39$^{o}$C) for 20 min to remove water soluble fractions and reduce wetting lag time. All bags (except 0 h) were inserted into the ventral rumen, under the ruminal mat, in a mesh laundry bag in reverse order at 0730 on d 0; 0730 on d 1; 0730 and 1930 on d 2; 0730, 1530, 1930, and 2330 on d 3; and 0130, 0330, 0530 on d 4. These times of insertion correspond to incubation times of 2, 4, 6, 8, 12, 16, 24, 36, 48, 72 and 96 h. At 0730 on d 4, all bags were removed from the rumen and 0-h bags were soaked in tepid water for 20 min. All bags were rinsed with 39$^{o}$C water to remove particles adhering to the outside of the bags and then washed, by steer, in a washing machine (Model LSR7233EQO, Whirlpool, Benton Harbor, MI) on delicate setting 10 times for 1-min rinse and 2-min spin cycles. Following rinsing, bags were

oven dried at 50°C for 72 h. Dry sample bags were allowed to equilibrate with atmospheric conditions for 60 min to room temperature before being weighed. Samples from each incubation time were composited and subsamples from each composite were analyzed for NDF using an ANKOM200 Fiber Analyzer (ANKOM Technology, 2005), and nitrogen (N) content using a Leco TruSpec CN analyzer (Leco Corporation, St. Joseph, MI).

Total N, NDF and DM were segmented into three fractions (A, B and C) based on susceptibility to ruminal degradation. The A fraction was considered to be immediately soluble while the C fraction was deemed unavailable to rumen degradation and the B fraction was the portion that was degraded at a measurable rate (Coblentz et al., 2002). Nonlinear regression was used to determine degradation kinetics of the percentage of DM and NDF remaining on incubation time, using the PROC NLIN procedure of SAS (SAS Inst. Inc., Cary, NC). Data were fitted to the nonlinear regression model described by Mertens and Loften (1980). The A and B fractions, lag time and the fractional rate constant ($K_d$) were determined directly from the nonlinear model. The C fraction was determined experimentally and equals the residual in the 96-h bags. The effective rumen degradability (RD) was calculated according to Ørskov and McDonald (1979) using the equation:

$$\text{Extent} = A + [(B \times k_d)/( k_d + k_p)]$$

where $k_d$ = rate of degradation of B fraction and $k_p$ = rate of particulate passage from the rumen as described below. .

After *in situ* procedures were completed steers were allowed an additional 10-d adaptation period in which hay intake was measured during the final 5 d. Following measurement of intake, four consecutive days were used to ascertain passage rate by procedures described by Coblentz et al. (1999). Briefly, manual evacuation of ruminal contents of each of

four steers (one treatment replication/d) was conducted before feeding (0 h) and at four h post-feeding. Total ruminal contents were weighed, mixed, subsampled in triplicate, and returned to the rumen. Ruminal subsamples were dried at 50°C for 96 h. Hay and orts samples were collected throughout the study, composited by steer and dried at 50°C in a forced air oven for 48 h. All dried samples were ground with a Wiley mill (Model-4, Thomas Scientific, Sweedesboro, NJ) to pass through a 2-mm screen. Acid detergent insoluble ash (ADIA) contents in prairie hay, orts, and ruminal contents were determined by ashing ADF residues in a muffle furnace at 500°C for 8 h. Fractional passage rate of ADIA ($k_p$) was determined by dividing the mean ADIA intake (grams per h) by the mean (from the 0- and 4-h ruminal evacuations) ruminal mass of ADIA (Waldo et al., 1972). The hourly intake of ADIA for each steer was calculated by dividing total daily intake of ADIA by 24 h.

***Statistical Analysis.*** Degradation characteristics were analyzed using the PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) and the Satterthwaite approximation for degrees of freedom. The model included supplement treatment as the independent variable. Orthogonal contrasts were tested for: 1) C vs. others, 2) PC vs. MA1 + MA2, 3) MA1 vs. MA2. Alpha level was set at 0.05.

### *Experiment 2: Metabolism Study*

The same sixteen ruminally-cannulated crossbred beef steers from Exp. 1 remained on their treatments and were moved to individual metabolism stanchions and used to evaluate rumen fermentation, blood urea nitrogen (BUN) and urine and fecal excretion. Once in place, steers were adapted to the metabolism stanchions for 7 d before 5 d of forage intake measurement and sample collection (d 8 through 13). Steers had unlimited access to fresh

water and were offered prairie hay at 130% of the average voluntary intake measured during the 7-d adaptation. Supplement and mineral was provided using the same methods as described in Exp. 1.

Forage and supplement samples were collected from d 8 through 12, while orts, urine and fecal samples were collected on d 9 through 13. Urine was kept in an environment with a pH < 3 between sampling periods by using 6 *N* HCl in the urine containers (Farmer et al., 2004). Urine was weighed and sampled every 24 h unless sample collection container was over half full at 12 h and then subsamples were collected and weights were determined at that time. Specific gravity was determined and subsamples were collected and frozen (-20°C) for later analysis of urinary-N (2400 Kjeltec, FOSS Analytical, Slangerupgade, Denmark). Fecal output was weighed every 24 h and immediately placed in the drying oven (50°C) for DM determination.

Supplement, hay, orts and fecal samples were dried at 50ºC and ground in a Wiley mill (Model-4, Thomas Scientific, Sweedesboro, NJ) to pass a 2-mm screen before analysis. After grinding, supplement, hay, orts and fecal samples were composited within steer across all days for the experiment. All composite samples were analyzed for NDF, ADF, and N. Forage and supplement NDF and ADF content were determined using an ANKOM200 Fiber Analyzer (ANKOM Technology, 2005). Nitrogen was determined using a Leco TruSpec CN Analyzer (Leco Corporation, St. Joseph, MI).

At 0800 on d 16, 0-h blood and rumen samples were collected. Blood samples were collected with a BD vacutainer (BD, Franklin Lakes, NJ), immediately placed on ice and allowed to coagulate before serum harvest. Serum was harvested via centrifugation at 1,500 x *g* for 20 min and stored (-20°C) for later evaluation of BUN concentration (mg/dL) according

to manufacturers guidelines (TECO Diagnostics, Anaheim, CA, USA) using 96 well plates and a spectrophotometer (Multiskan Ascent, MTX Labsystems Inc., Vienna, VA, USA; filter 595). After blood collection, 0-h rumen fluid was hand collected from the ventral rumen. Following h zero, rumen and blood samples were collected at 3, 6, 9, 12, 15, 18, 21, and 24 h post-feeding. Rumen fluid pH was immediately determined using a portable pH meter. Two whole rumen content samples were collected. Sample one was strained through four layers of cheesecloth and 100 mL of strained rumen fluid was acidified with 10 mL of 0.1 N HCl for analysis of VFA (mM), ammonia-nitrogen (RAN; mg/dL) and lactate (mM). Sample two (50 mL) was mixed with 50 mL of 50% formalin (1:2 dilution) for determination of protozoa concentrations.

Rumen samples stored for RAN analysis were thawed and analyzed using a phenol-hypochlorite assay adapted from Broderick and Kang (1980) and modified by Galyean (1997), using a spectrophotometer (Multiskan Ascent, MTX Labsystems Inc., Vienna, VA, USA; filter 630). Samples for VFA analysis were centrifuged at 3,800 x *g* for 10 min. Rumen fluid was then removed from the centrifuge and 1 mL of supernatant was filtered through a 0.45 $\mu$m filter into 1.5 mL microcentrifuge tubes. At this time 250 $\mu$L of 25% (w/v) metaphosphoric acid solution was added to the supernatant. Tubes were vortexed, allowed to stand in ice water for 30 min, and then centrifuged at 15,000 x *g* for 15 min. Supernatant was loaded into gas chromatography (GC) vials at 900 $\mu$L with 100 $\mu$L 2-ethyl butyric acid as the internal standard and concentrations were determined by gas chromatography and injected onto a ZB-FFAP capillary column (30m x 0.53mm x 1 $\mu$m; no. 7HK-G009-22, Phenomenex Inc., Torrance, CA) with helium carrier gas at 620 kPa and a flow rate of 8.0 mL/min. Injector and detector temperatures were 250 and 280°C. Lactate was determined

colorimetrically according to Pennington and Sutherland (1956) and modified by Schatz (1990) using a spectrophotometer (Multiskan Spectrum, Thermo Scientific, Waltham, MA; filter 560).

To determine protozoa concentrations, two drops of Brilliant Green dye were added to 1 ml of the mixed rumen sample and then allowed to stand overnight. Nine milliliters of a 30:70 (v/v) glycerol solution was added to each sample, giving it a final dilution of 1:20. One milliliters of the 1:20 dilution was pipetted into a Sedgewick-Rafter chamber and protozoa were counted under 10 x magnification as described by Dehority (1984).

***Statistical Analysis.*** Intake, digestibility, microbial synthesis and nitrogen retention data measurements were analyzed using PROC MIXED MODEL procedures of SAS and the Satterthwaite approximation for degrees of freedom. Model terms included supplement treatment, time and treatment x time as independent variables. Blood urea nitrogen, and rumen fermentation parameters were analyzed using time as a repeated measure factor, and an autoregressive(period 1) covariance structure was used to model within steer variability. Orthogonal contrasts were tested for: 1) C vs. others, 2) PC vs. MA1 + MA2, 3) MA1 vs. MA2. Alpha level was set at 0.05.

## RESULTS AND DISCUSSION

***Experiment 1. Intake and Passage Rate.*** Intakes and passage rate parameters for Exp. 1 are shown in Table 2. Hay intakes were lower than those reported for late gestating cows in an environment similar to production settings, consuming similar low-quality prairie hay (1.9 kg·100kg of $BW^{-1}\cdot d^{-1}$ BW; Johnson et al., 2003; Banta et al., 2008; Winterholler, 2009a), but similar to those observed with cannulated steers in a similar confinement setting

(Guthrie and Wagner, 1988). Protein supplementation increased hay DMI ($P < 0.01$) by 80%. Protein supplementation of steers consuming low-quality prairie hay has consistently resulted in greater forage DMI (Guthrie and Wagner, 1988; Mathis et al., 1999; Bodine et al., 2001). This result is a consequence of a deficit in rumen ammonia being corrected with a supplement high in RDP (McCollum and Horn, 1990). However, MA inclusion did not impact hay DMI as compared to PC. The lack of response to DMI with the inclusion of MA is consistent with other reported data when saponins were included in the diet (Valdez et al., 1986; Wilson et al, 1998; Hristov et al., 1999).

Particulate passage rate calculated from ADIA concentrations at 0 and 4-h post-feeding was greater for supplemented steers than for C steers. In correlation with increased forage DMI, it should be expected that rate of particulate passage from the rumen would also increase. This has been confirmed with data from protein supplemented cattle consuming low-quality prairie hay (McCollum and Galyean, 1985; Guthrie and Wagner, 1988; Olson et al., 1999). Olson and others (1999) also reported similar rates of passage using ADIA as a marker for non-supplemented steers (1.83%/h) and for steers provided 0.32 kg of RDP (2.78%/h), similar to this study (approx. 0.29 kg RDP; Winterholler et al., 2009b). Most reports suggest that saponins have no effect on particulate passage rate (Hristov et al., 2004; Goetsch and Owens, 1985). However, these data suggest that the inclusion of MA negatively impacts particulate passage rate by 22.5% compared to PC ($P = 0.02$), and this coincides with Goodall and Matsushima (1980). This decrease in particulate passage rate is not understood with these data. However, this effect may be a result in an increase in lactic acid concentration in the rumen from an increased rate of fermentation. Another potential explanation might be a change in rumen fluid viscosity from the foam-forming characteristics

of saponins found in yucca extracts (Cheeke, 2000). It has been shown that foam formation is negatively correlated with particulate passage rate (Okine et al., 1988).

***In situ Digestibility.*** In situ digestibility kinetics are presented in Table 3. Protein supplementation increased the B and C fraction, $k_d$, and rumen digestibility of DM ($P < 0.01$), with a tendency to increase the immediately soluble fraction ($P = 0.08$). Similarly, McCollum and Galyean (1985) observed an improvement in in vitro DM disappearance with inoculums from protein supplemented steers as compared to non-supplemented steers. Protein supplementation did not affect time to the onset of fermentation of DM ($P > 0.10$). The inclusion of MA increased the proportion of the A fraction, $k_d$ and rumen degradability with a tendency for decreased lag time for DM as compared to PC ($P < 0.05$). MA increased in situ rumen DM digestibility by 7 units compared to PC, which can be attributed to the increase in rumen retention time.

The kinetics for the NDF portion was similar to DM, with the following exceptions. There were no differences among treatments in the A fraction and there was a tendency for decreased lag time ($P = 0.09$) for supplemented compared to non-supplemented steers. MA increased rate of degradation (4.15 vs. 3.33%/h for MA vs. PC; $P = 0.02$). This increase in $K_d$ for DM and NDF may be a consequence of reduced rumen protozoa. Veira (1986) suggested that faunated ruminants have slowed fermentation rates as compared to defaunated animals. This is evident by reduced lactic acid production in faunated animals.

Protein supplementation increased the potentially degradable nitrogen fraction, resulting in a trend for a reduction in the insoluble portion when expressed as a percent of total nitrogen ($P = 0.08$). The discrete lag time for nitrogen was reduced when steers were supplemented with protein and further reduced for steers consuming MA. The degradable

nitrogen portion is relatively low compared to published values (Vanzant et al., 1996), but with warm season grasses the low degradability is thought to be due to the nitrogen association with bundle-sheath cells (Mullahey et al., 1992).

***Experiment 2. Intake and Digestibility.*** Results for intake and digestibility parameters from Exp. 2 are in Table 4. There was no significant difference in hay DMI for supplemented steers as compared to C, nor did the inclusion of MA affect DMI. The lack of response to protein supplementation contradicts Exp. 1, but can be explained by the combination of compressed treatment means and the nearly two fold difference in standard errors compared to Exp. 1.

Total tract apparent digestibility of DM, NDF, ADF and CP was increased due to protein supplementation as anticipated (Guthrie and Wagner, 1988; Beaty et al., 1994; Mathis et al., 1999), with no change due to dietary MA inclusion. Contradictory to rumen digestibility improvements from MA inclusion for DM and NDF in Exp. 1, there was no difference in apparent total tract digestibility for MA as compared to PC ($P > 0.10$). This discrepancy might be due to compensatory hind gut digestibility for PC steers. Goetsch and Owens (1985) suggested that particles passing from the rumen the slowest must pass through the hindgut the fastest. Sultan and Loerch (1992) observed an eight unit improvement in apparent ruminal NDF digestion when lambs were supplied a high protein supplement, but actually observed a 1.3 unit decrease in apparent total tract digestibility. They also recorded a three unit increase in ruminal DM digestibility when total tract apparent DM digestibility was again 1.3 units lower than the low protein supplemented lambs. Brink and Steele (1985) also demonstrated that postruminal digestion was inversely related to ruminal digestion of OM and that postruminal digestion increases as OM supply increases.

Protein supplementation also improved nitrogen balance in steers consuming low-quality prairie hay. Even though nitrogen intake and fecal excretion of nitrogen was increased and urinary nitrogen was numerically greater ($P = 0.07$) for supplemented steers there was still a substantial improvement in retained nitrogen. This suggests that rumen microbes were successfully able to incorporate feed protein into microbial protein and further validates the need for additional protein in diets of cattle consuming low-quality forage. Nitrogen excretion or retention was not different for MA-fed steers as compared to PC-fed steers. Hristov et al. (1999) reported an increase in urinary nitrogen excretion of 5%, resulting in a reduction in nitrogen retention when yucca saponin was included in the diet at 60 g/d.

***Rumen Fermentation and Blood Urea Nitrogen.*** There were no treatment by time interactions observed for BUN or any of the fermentation parameters; therefore, main effect means for treatment over time are presented in Table 5.

Protein supplementation increased BUN in steers by over 50%, with no additional effect from MA. This increase in BUN has been observed when providing a cottonseed-meal based supplement to forage fed lambs (Caton et al., 1988). As seen here, some reports show no affect on BUN when ruminants are supplemented with saponins (Wilson et al., 1998; Hristov et al., 1999), but more often it has been cited to reduce BUN (Hussain and Cheeke, 1995; Hussain et al., 1996; Killeen et al., 1998).

As compared to C steers, supplemental protein decreased rumen pH over time. Nevertheless, there was not a difference in pH between PC and MA supplemented steers. Hristov et al. (1999) observed no decrease in pH from the control diet when yucca extract was added at 20 and 60 g/d (6.28 vs. 6.18 and 6.19). Similarly, others have observed no

differences in pH with yucca extract supplementation (Valdez et al., 1986; Wilson et al., 1998).

Rumen ammonia-nitrogen concentrations were greater for supplemented steers, but MA inclusion did not impact RAN. It has been suggested that yucca saponins have nitrogen binding potential and a subsequent ability to release it when concentrations are low (Hussain and Cheeke, 1995). However, this response was not present in cattle consuming ad libitum low-quality forage.

Protozoa concentrations were the lowest for the C steers. This was expected as protozoa have an amino acid requirement for proliferation and their excretion of ammonia-nitrogen is utilized by rumen bacteria as a substrate for synthesis of bacterial protein (Dehority, 2003). Therefore, when rumen degradable protein is deficient, microbial populations will be reduced (Dehority, 2003). Positive control steers had the greatest concentration of protozoa with MA supplemented steers being intermediate and less than PC steers ($P = 0.01$). Data demonstrate consistent results on the suppression of protozoa populations when exposed to yucca extract (Valdez et al, 1986; Wallace et al., 1994; Hristov et al., 1999). The interaction between saponins and membrane lipids is complicated, but it is thought that yucca saponins are effective at suppressing rumen protozoa by reacting with cholesterol in the protozoal cell membrane, causing it to lyse (Cheeke, 2000).

Rumen lactate concentrations were minimal, as expected by diet, but there was an increase in lactate concentrations for MA steers as compared to PC and C (0.15 vs. 0.10 mM; $P = 0.05$). This may be due to the reduced protozoa populations. Protozoa have been known to be mediators in lactic acid production by engulfing some of the lactic acid producing bacteria (Veira, 1986).

Protein supplementation increased total VFA concentrations and independent concentrations of acetate, propionate, butyrate, and valerate ($P < 0.05$) with no effect on isobutyrate, and isovalerate. However, the acetate to propionate ratio was the lowest for C steers. This is a consequence of having the lowest total VFA concentrations. There were no differences in total and individual VFA or acetate to propionate ratios for PC as compared to MA. Still, there was a decrease in acetate, propionate and total VFAs for MA2 versus MA1 steers. When including dietary saponins, others have reported a shift toward propionate production, resulting in a decrease in the acetate to propionate ratio (Hristov et al., 1999), which is consistent with a decrease in protozoa numbers (Williams and Coleman, 1992). A decrease in acetate to propionate was not seen in this case, but MA1 did increase concentrations of propionate by 8.3% ($P = 0.17$) when compared to PC.

## IMPLICATIONS

Additional plant protein supplied to cattle consuming low-quality prairie hay improved intake and digestibility as well as nitrogen balance. The inclusion of Micro-Aid® in the protein supplement improved rumen DM and NDF degradability via a decrease in rumen particulate passage rate, but was not successful at improving apparent total tract digestibility. This improvement in rumen digestion may have the potential to increase the efficiency of nutrient use for subsequent improvements in animal efficiency. In addition, Micro-Aid® successfully suppressed protozoa after continuous administration, over 20 d, suggesting that complete adaptation to saponins by protozoa was not observed. However, yucca saponins have demonstrated toxic effects to bacteria such as *Streptococcus bovis*, lactic acid producing bacteria, and it has been suggested that bacteria can adapt to saponins (Cheeke, 2000). This

in combination with an increase in fermentation rate may explain the increase in lactic acid production from MA supplemented steers. Therefore it would be beneficial to examine different feeding regimens of Micro-Aid® and its effect on digestion. The reduction in protozoa has also been shown to increase microbial efficiency (Veira, 1986), which would be beneficial to lactating cows that have a greater metabolizable protein requirement than cannulated steers or even gestating cows. More research is needed to determine the effects of Micro-Aid® on animal performance.

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**Table 1: Supplement composition and amount of nutrients supplied daily**

| | Supplement[1] | | | |
|---|---|---|---|---|
| Item (DM basis) | C | PC | MA1 | MA2 |
| | % of DM | | | |
| Cottonseed meal | - | 75.5 | 75.5 | 75.5 |
| Wheat middlings | - | 19.5 | 19.5 | 19.5 |
| Cane Molasses | - | 5.0 | 5.0 | 5.0 |
| Micro-Aid® | - | - | 0.1 | 0.2 |
| | Nutrient supplied | | | |
| DM, kg/d | - | 0.92 | 0.92 | 0.92 |
| CP, kg/d | - | 0.37 | 0.38 | 0.38 |
| TDN, kg/d | - | 0.64 | 0.65 | 0.67 |
| Crude fat, kg/d | - | 0.02 | 0.03 | 0.03 |

[1]Supplements included: 1) no supplementation (C); 2) 0.92 kg/d of a 40% CP cottonseed meal and wheat middlings-based supplement (PC); 3) PC plus 1 g/d of Micro-Aid® (MA1); and 4) PC plus 2 g/d of Micro-Aid® (MA2).

*All steers received 57 g of a complete vitamin and mineral premix (DM basis; 28.6% NaCl, 12.8% Ca, 8.8% P, 1.2% Mg, 1044 ppm Cu, 12.33 ppm Se, 3116.8 ppm Zn, 409,000,000 IU Vitamin A and 455,000 IU Vitamin E.

**Table 2. Effect of supplement on feed intake and passage rate in steers consuming low quality prairie hay in Experiment 1**

| | Treatments[1] | | | | | *P*-Value[2] | | |
|---|---|---|---|---|---|---|---|---|
| Item | C | PC | MA1 | MA2 | SEM | $C_1$[3] | $C_2$[3] | $C_3$[3] |
| No. | 4 | 4 | 4 | 4 | | | | |
| Initial BW, kg | 523 | 510 | 526 | 556 | 23 | 0.78 | 0.29 | 0.38 |
| Intake | | | | | | | | |
| Hay, kg·100kg of $BW^{-1}$·$d^{-1}$ | 0.78 | 1.50 | 1.44 | 1.27 | 0.15 | <0.01 | 0.40 | 0.38 |
| DM, kg·100kg of $BW^{-1}$·$d^{-1}$ | 0.78 | 1.70 | 1.63 | 1.46 | 0.15 | <0.01 | 0.36 | 0.35 |
| Rumen Contents | | | | | | | | |
| Fill, kg (DM basis) | 7.89 | 9.37 | 10.56 | 10.7 | 0.77 | 0.02 | 0.15 | 0.88 |
| ADIA, % | 4.67 | 5.01 | 5.19 | 5.24 | 0.00 | <0.01 | <0.01 | <0.01 |
| Passage rate, %/h | 1.82 | 2.78 | 2.38 | 2.15 | 0.18 | 0.01 | 0.02 | 0.31 |

[1]Treatements included: 1) no supplementation (C); 2) 0.92 kg/d of a 40% CP cottonseed meal and wheat middlings-based supplement (PC); 3) PC plus 1 g/d of Micro-Aid® (MA1); and 4) PC plus 2 g/d of Micro-Aid® (MA2).
[2]Probability of a greater F-statistic.
[3]$C_1$ = C vs. others; $C_2$ = PC vs. MA1 + MA2; $C_3$ = MA1 vs. MA2.

**Table 3. Effect of supplement on in situ digestibility kinetics of low-quality prairie hay in Experiment 1**

| | Treatments[1] | | | | | *P*-Value[2] | | |
|---|---|---|---|---|---|---|---|---|
| Item | C | PC | MA1 | MA2 | SEM | $C_1$[3] | $C_2$[3] | $C_3$[3] |
| No. | 4 | 4 | 4 | 4 | | | | |
| DM | | | | | | | | |
| A, % | 12.94 | 13.03 | 14.39 | 14.15 | 0.43 | 0.08 | 0.02 | 0.66 |
| B, % | 43.90 | 55.28 | 56.48 | 54.51 | 1.20 | <0.01 | 0.87 | 0.21 |
| C, % | 43.17 | 31.69 | 29.13 | 31.34 | 1.27 | <0.01 | 0.30 | 0.18 |
| Lag, h | 9.90 | 7.02 | 9.41 | 8.63 | 1.18 | 0.26 | 0.14 | 0.60 |
| $k_d$, %/h | 2.05 | 3.20 | 3.90 | 3.97 | 0.28 | <0.01 | 0.02 | 0.84 |
| RD, % | 35.66 | 42.44 | 49.44 | 49.42 | 1.59 | <0.01 | <0.01 | 0.99 |
| NDF | | | | | | | | |
| A, % | 2.09 | 2.42 | 3.36 | 3.86 | 0.73 | 0.20 | 0.15 | 0.58 |
| B, % | 52.62 | 65.41 | 67.50 | 64.42 | 1.25 | <0.01 | 0.69 | 0.07 |
| C, % | 45.29 | 32.18 | 29.15 | 31.71 | 1.58 | <0.01 | 0.32 | 0.21 |
| Lag, h | 11.51 | 6.88 | 7.86 | 8.37 | 1.85 | 0.09 | 0.54 | 0.83 |
| $k_d$, %/h | 2.09 | 3.33 | 4.19 | 4.11 | 0.29 | <0.01 | 0.02 | 0.84 |
| RD, % | 29.59 | 37.90 | 46.22 | 46.10 | 2.19 | <0.01 | <0.01 | 0.96 |
| Nitrogen | | | | | | | | |
| A, % | 21.01 | 19.9 | 17.73 | 19.56 | 1.70 | 0.33 | 0.50 | 0.40 |
| B, % | 27.80 | 33.08 | 39.24 | 31.97 | 2.38 | 0.02 | 0.34 | 0.03 |
| C, % | 51.19 | 47.03 | 43.04 | 48.47 | 2.35 | 0.08 | 0.62 | 0.09 |
| Lag, h | 38.59 | 34.07 | 25.42 | 23.63 | 3.88 | 0.03 | 0.04 | 0.71 |
| $k_d$, %/h | 3.17 | 3.88 | 3.95 | 3.38 | 0.32 | 0.15 | 0.55 | 0.17 |
| RD, % | 38.34 | 38.92 | 42.11 | 39.10 | 2.08 | 0.48 | 0.46 | 0.26 |

A = Immediately soluble fraction, B = Fraction degraded at a measurable rate, C = Fraction unavailable to rumen degradation, Lag = Time lapse until fermentation begins, $k_d$ = Rate of degradation, RD = Rumen degradability.

[1]Treatements included: 1) no supplementation (C); 2) 0.92 kg/d of a 40% CP cottonseed meal and wheat middlings-based supplement (PC); 3) PC plus 1 g/d of Micro-Aid® (MA1); and 4) PC plus 2 g/d of Micro-Aid® (MA2).

[2]Probability of a greater F-statistic.

[3]Orthogonal contrasts: $C_1$ = C vs. PC + MA1 + MA2; $C_2$ = PC vs. MA1 + MA2; $C_3$ = MA1 vs. MA2.

**Table 4. Effect of supplement on DMI, total tract apparent digestibility, and nitrogen balance in steers consuming low quality prairie hay in Experiment 2**

| | Treatments[1] | | | | | *P*-Value[2] | | |
|---|---|---|---|---|---|---|---|---|
| Item | C | PC | MA1 | MA2 | SEM[4] | $C_1$[3] | $C_2$[3] | $C_3$[3] |
| No. | 3 | 3 | 4 | 4 | | | | |
| Initial BW, kg | 423 | 538 | 550 | 577 | 27 | 0.32 | 0.45 | 0.48 |
| Hay intake, g/kg BW | 0.88 | 1.22 | 1.25 | 1.08 | 0.27 | 0.33 | 0.85 | 0.60 |
| DMI, g/kg BW | 0.88 | 1.39 | 1.42 | 1.24 | 0.27 | 0.15 | 0.83 | 0.59 |
| Apparent Digestibility, % | | | | | | | | |
| DM, % | 43.62 | 59.46 | 59.33 | 60.70 | 4.23 | <0.01 | 0.90 | 0.80 |
| NDF, % | 47.50 | 62.50 | 64.67 | 64.44 | 4.34 | <0.01 | 0.66 | 0.97 |
| ADF, % | 44.10 | 59.86 | 61.56 | 62.07 | 4.69 | <0.01 | 0.70 | 0.93 |
| CP, % | 41.66 | 61.15 | 54.56 | 59.78 | 4.18 | <0.01 | 0.39 | 0.33 |
| Nitrogen Balance | | | | | | | | |
| Intake, g/d | 38.7 | 114.3 | 118.3 | 113.9 | 12.3 | <0.01 | 0.90 | 0.78 |
| Fecal, g/d | 22.6 | 45.8 | 54.5 | 46.7 | 8.7 | 0.02 | 0.61 | 0.48 |
| Urine, g/d | 15.1 | 24.1 | 25.0 | 31.8 | 5.2 | 0.07 | 0.45 | 0.31 |
| Retained, g/d | 1.0 | 44.5 | 38.8 | 35.4 | 8.4 | <0.01 | 0.42 | 0.75 |
| N retained/N intake, % | 2.0 | 39.1 | 32.4 | 30.2 | 6.3 | <0.01 | 0.27 | 0.77 |

[1]Treatements included: 1) no supplementation (C); 2) 0.92 kg/d of a 40% CP cottonseed meal and wheat middlings-based supplement (PC); 3) PC plus 1 g/d of Micro-Aid® (MA1); and 4) PC plus 2 g/d of Micro-Aid® (MA2).
[2]Probability of a greater F-statistic.
[3]Orthogonal contrasts: $C_1$ = C vs. PC + MA1 + MA2; $C_2$ = PC vs. MA1 + MA2; $C_3$ = MA1 vs. MA2.
[4]Most conservative SEM, n = 3.

**Table 5. Effect of supplement on rumen fermentation, blood urea nitrogen and protozoa counts in steers consuming low quality prairie hay in Experiment 2**

| | Treatments[1] | | | | | *P*-Value[2] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Item | C | PC | MA1 | MA2 | SEM[3] | Trt[4] | Trt x T[5] | $C_1$[6] | $C_2$[6] | $C_3$[6] |
| No. | 3 | 3 | 4 | 4 | | | | | | |
| BUN[7], mg/dL | 1.80 | 2.67 | 2.98 | 2.88 | 0.21 | <0.01 | 0.36 | <0.01 | 0.26 | 0.72 |
| Rumen | | | | | | | | | | |
| pH | 7.05 | 6.50 | 6.47 | 6.32 | 0.16 | 0.03 | 0.57 | <0.01 | 0.55 | 0.47 |
| RAN[8], mM | 0.12 | 0.57 | 0.61 | 0.77 | 0.18 | 0.11 | 0.66 | 0.02 | 0.57 | 0.49 |
| Lactate, mM | 0.09 | 0.10 | 0.16 | 0.14 | 0.02 | 0.07 | 0.55 | 0.09 | 0.05 | 0.69 |
| Protozoa, $10^3$/mL | 5.48 | 12.45 | 10.54 | 10.15 | 0.73 | <0.01 | 0.63 | <0.01 | 0.01 | 0.69 |
| VFA, mM | | | | | | | | | | |
| Total | 55.99 | 75.83 | 83.51 | 73.66 | 3.79 | <0.01 | 0.29 | <0.01 | 0.49 | 0.04 |
| Acetate | 37.62 | 54.16 | 60.45 | 51.80 | 3.01 | <0.01 | 0.29 | <0.01 | 0.54 | 0.03 |
| Propionate | 8.63 | 10.62 | 11.51 | 10.21 | 0.52 | <0.01 | 0.29 | <0.01 | 0.66 | 0.05 |
| Butyrate | 5.41 | 6.40 | 6.79 | 6.60 | 0.34 | 0.03 | 0.15 | <0.01 | 0.43 | 0.65 |
| Valerate | 1.45 | 1.63 | 1.67 | 1.66 | 0.04 | <0.01 | 0.75 | <0.01 | 0.47 | 0.84 |
| Isobutyrate | 1.63 | 1.65 | 1.67 | 1.83 | 0.12 | 0.57 | 0.83 | 0.51 | 0.47 | 0.31 |
| Isovalerate | 1.27 | 1.36 | 1.42 | 1.57 | 0.12 | 0.33 | 0.89 | 0.21 | 0.30 | 0.36 |
| Acetate:Propionate | 4.32 | 5.10 | 5.24 | 5.06 | 0.10 | <0.01 | 0.61 | <0.01 | 0.64 | 0.16 |

[1]Treatements included: 1) no supplementation (C); 2) 0.92 kg/d of a 40% CP cottonseed meal and wheat middlings-based supplement (PC); 3) PC plus 1 g/d of Micro-Aid® (MA1); and 4) PC plus 2 g/d of Micro-Aid® (MA2).
[2]Probability of a greater F-statistic.
[3]Most conservative SEM, n = 3.
[4]Trt = Treatment.
[5]T = Time
[6]Orthogonal contrasts: $C_1$ = C vs. PC + MA1 + MA2; $C_2$ = PC vs. MA1 + MA2; $C_3$ = MA1 vs. MA2.
[7]Blood urea nitrogen.
[8]Rumen ammonia nitrogen

# CHAPTER IV

# EFFECTS OF INCLUDING MICRO-AID® IN A PROTEIN SUPPLEMENT ON PERFORMANCE OF GROWING STEERS AND SPRING-CALVING COWS

# ABSTRACT

Three experiments were conducted to evaluate the use of Micro-Aid® on the performance of stocker steers and spring-calving cows. In Exp. 1, fall-born crossbred (n = 68; 289 ± 34 kg initial BW) steers were randomly allotted to one of four supplement treatments (DM basis): 1) 0.15 kg·steer$^{-1}$·d$^{-1}$ soybean hulls (C; control), 2) 0.41 kg·steer$^{-1}$·d$^{-1}$ of a cottonseed meal/wheat midds-based supplement with no Micro-Aid® (PC; positive control, 40% CP), 3) Micro-Aid® added to PC to supply 1 g·steer$^{-1}$·d$^{-1}$ (MA1), and 4) Micro-Aid® added to PC to supply 2 g·steer$^{-1}$·d$^{-1}$ (MA2). Exp. 2 and 3 utilized Angus and Angus x Hereford beef cows (n = 93) stratified by age and body weight and randomly assigned to one of two supplement treatments (DM basis), 1) a cottonseed meal/wheat midds-based supplement with no Micro-Aid® (PC; 30% CP) and 2) Micro-Aid® added to PC to supply 1 g·cow$^{-1}$·d$^{-1}$ (MA) provided at 1.91 kg/d, 3d/wk during gestation and 0.82 kg/d, 4 d/wk during lactation. All cattle were individually fed in either a 32 animal stall barn (Exp. 1 and 2) or an individual pen (Exp. 3). Exp. 2 evaluated the use of MA on the performance of spring-calving cows, while Exp. 3 examined the use of MA on forage intake and digestibility, milk yield and milk composition during early- and late-lactation. In Exp. 1 protein supplementation improved ADG as compared to C, but providing MA to stocker steers had no impact on total BW gain or ADG ($P > 0.10$). Cow BW and BCS was increased during gestation for MA cows, but to a lesser extent than PC ($P = 0.03$), but a numerically lower change for MA cows after calving resulted in similar BW and BCS at the end of supplementation. At this time, calves from cows fed MA were 5 kg heavier ($P < 0.01$), but calf BW was similar at weaning. Including MA had no effect on milk yield, milk composition, hay DMI, or digestibility at either stage of production. These data are

interpreted to indicate that MA may be effective at improving calf performance when forage quality is low and therefore may be more applicable to a fall-calving system. Also, all experiments provided supplements 3 or 4 d/wk which may affect the ability of MA to sustain a stable rumen environment and diminish its impact on performance. Therefore, the feeding regimen of MA needs to be further evaluated.

## INTRODUCTION

In the U.S., approximately 30 million beef cows graze pasture year around, while roughly 20 million growing cattle graze for some period of time after being weaned and before entering the breeding herd or the feedlot for finishing. In the Southern Great Plains, standing forage supplies adequate nutrients to maintain beef cows and (or) allow for weight gain in cows and growing cattle three to six months of the year when supplemental protein and (or) energy are not required. McCollum and Horn (1990) outlined numerous studies that unveiled the increase in performance by supplementing low levels of protein when cattle graze low quality forage. The "Oklahoma Gold" program developed at Oklahoma State University was established on the basis that providing 0.45 kg of a high protein supplement (38 to 40% CP) three times/wk will improve performance of steers grazing late summer pasture by 0.20 kg/d (Lalman and Gill, 2010). Additionally, it is a common practice to supply a high CP supplement three times/wk during the winter months to spring-calving cows. These are typically oilseed-meal based supplements because cottonseed meal is moderate to high in rumen degradability (57 to 78%; NRC, 1996, and Winterholler, 2009a) and serve as a good source of nitrogen to stimulate rumen bacterial growth.

Micro-Aid® is an all-natural, dry or liquid feed additive manufactured from a purified extract of the *Yucca schidigera* plant. Micro-Aid® and other *Yucca Schidigera*-based saponins have defaunating properties (Hristov et al., 2004). For example, inclusion of *Yucca Schidigera* resulted in a reduction in rumen protozoa and an increase in rumen bacteria and fungi (Hristov et al., 1999). Therefore, in high roughage diets the defaunating effects of Micro-Aid® could potentially increase fiber digestion and improve microbial N supply to the small intestine. Addition of Micro-Aid® to forage-fed cattle supplements could result in improved forage utilization, improved performance of forage-fed cattle or reduced amount of protein supplement required. Therefore, the objectives of these studies were to investigate the effects of MA inclusion in protein supplements for stocker steers and spring-calving cows.

## MATERIALS AND METHODS

These experiments were conducted in accordance with an approved Oklahoma State University Animal Care and Use Committee protocol.

### *Experiment 1: Stocker Steer Performance*

***Animals.*** This experiment was conducted at the OSU Range Cow Research Center, North Range Unit located approximately 16 km west of Stillwater, OK. Fall-born crossbred (n = 68; 289 ± 34 kg initial BW) steers weaned in early July were implanted with Component TE-G® (29 mg tylosin tartrate, 40 mg trenbolone acetate (TBA), 8 mg estradiol USP; Ivy Animal Health, Overland Park, KS) and used to determine the effects of MA inclusion on stocker steer performance during late summer.

July 28, 2009, steers were weighed after removal from feed and water for 16 h and then stratified by weight and randomly assigned to one of four supplement treatments in a completely randomized design. Steers were randomly allocated so that BW was equal across treatments at the initiation of the experiment and then individually identified with a colored ear-tag indicative of treatment. Supplement treatments (DM basis) included: 1) 0.15 kg·steer$^{-1}$·d$^{-1}$ soybean hulls (C; control), 2) 0.41 kg·steer$^{-1}$·d$^{-1}$ of a cottonseed meal and wheat midds-based supplement with no Micro-Aid® (PC; positive control, 40% CP), 3) Micro-Aid® added to PC to supply 1 g·steer$^{-1}$·d$^{-1}$ (MA1), and 4) Micro-Aid® added to PC to supply 2 g·steer$^{-1}$·d$^{-1}$ (MA2). Three fewer steers were allocated to C because there is ample research demonstrating a low standard error for capturing substantial improvements in ADG of stocker steers provided protein during late summer (Lalman, 2008). The low quantity of soybean hulls provided to the C-fed steers as means to prevent grazing time and animal handling differences due to supplementation procedures. Steers were individually fed in a 32 animal stall barn (Winterholler et al., 2009b) on Monday, Wednesday and Friday each week. The amount supplied at each feeding was determined by multiplying daily feeding rates by 7 and dividing by 3. With the exception of C, supplements were formulated to be isonitrogenous (Table 1) and fed as 1.27-cm diameter pellets for an 85 d period. Throughout the experiment, steers had ad libitum access to water and a free choice mineral supplement (28.6% NaCl, 12.8% Ca, 8.8% P, 1.2% Mg, 1,044 mg/kg Cu, 12 mg/kg Se, 3,117 mg/kg Zn, 409,000,000 IU Vitamin A and 455,000 IU Vitamin E; DM basis). Shrunk weights (16 h) were obtained every 28 d until the conclusion of the experiment (October 21, 2009), resulting in a 85 d experimental period.

Steers were managed as a single herd and rotated biweekly through three pastures (34.5 ± 9.9 ha; mean ± SD) consisting primarily of big bluestem (*Andropogon gerardii*), little bluestem (*Schizachyrium scoparium*) and Indiangrass (*Sorghastrum nutans*). Hand plucked

forage samples were collected in triplicate along with random duplicate 0.61 $m^2$ quadrat ground-clipped samples to determine forage quality and standing forage mass, respectively, at the introduction to each pasture throughout the supplementation period. Initial forage mass calculations were 12.0 ± 0.41, 14.3 ± 3.4 and 7.0 ± 1.6 animal unit months (AUM) for pastures 1, 2 and 3, respectively and each were grazed for 3.3, 2.3 and 1.6 AUM's, respectively.

For hand plucked and quadrat samples, forage DM (oven drying at 50°C until no further weight loss) was determined immediately following collection and after drying, samples were ground through a Wiley Mill grinder (Model-4, Thomas Scientific, Sweedesboro, NJ) using a 2-mm screen and stored for future analysis. Forage samples were analyzed for neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Ankom Tech Corp, Fairport, NY), Ash (combusted 6-h in a muffle furnace at 500°C), and CP (% N x 6.25; TruSpec CN, LECO Corporation, St. Joseph, MI 49085).

***Statistical Analysis.*** Animal performance was analyzed using PROC MIXED of SAS (SAS Inst. Inc., Cary, NC) and the Satterthwaite approximation for degrees of freedom. Steer was considered the experimental unit because supplements were individually fed. The model included supplement treatment as a fixed effect. Orthogonal contrasts were tested for: 1) C vs. others, 2) PC vs. MA1 + MA2, 3) MA1 vs. MA2. Alpha level was set at 0.05. One steer from the MA1 treatment was removed due to supplement refusal and morbidity.

### *Experiment 2: Spring-Calving Cow Performance*

***Animals.*** This experiment was also conducted at the OSU Range Cow Research Center. Angus and Angus x Hereford beef cows (n = 93; 549 ± 77 kg initial BW ± SD; 5.4 ±

0.5 initial BCS ± SD; Wagner et al., 1988) were stratified by age and BW and randomly assigned to one of two supplement treatments so that age and BW were similar across treatments.

Supplements (DM basis) included: 1) 0.82 kg/d during gestation and 1.55 kg/d during lactation of a cottonseed meal/wheat middling-based supplement (PC; 30% CP) and 2) PC plus the inclusion of 1 g/d of Micro-Aid® (MA). Supplements were formulated to be isonitrogenous (Table 2) and individually supplied as a 0.64-cm pellet in feed pans in a 32 animal stall barn (Winterholler et al., 2007). During gestation, supplements were delivered 3 d each week at the rate calculated by dividing weekly amounts (daily supplement x 7) by 3. However, due to increased nutrient requirements during lactation, supplement amounts and feeding frequency were increased. After calving, feed was delivered on Monday, Wednesday, Friday, and Saturdays at 0700. Supplements were balanced to be at or near RDP requirements with respect to MP requirements. This was determined by using level one model calculations of NRC (1996) for a 590 kg beef cow consuming low-quality prairie hay at 1.8 and 2.5 kg/100 kg of BW for a gestating and lactating cow, respectively. Supplement RDP values from Winterholler et al. (2009) were used in combination with an estimated microbial efficiency of 9 percent. Supplements were also formulated to meet P, Ca, and vitamin A requirements (NRC, 1996). Supplementation began on January 15, 2010, prior to the initiation of calving (average calving day = April 3, 2010) and continued until May 3, 2010, at which time eight cows had not yet calved.

This experiment did not utilize a non-supplemented, negative control treatment because lack of protein supplementation during winter months consistently results in

significant losses in BW and BCS when cows graze dormant native range (Clanton and Zimmerman, 1970; Schauer et al., 2005; Steele et al., 2007).

During gestation, cows were managed as a contemporary group in a single pasture (30 ha). After parturition, cow/calf pairs were moved to a nearby pasture (11 ha) and managed as a single contemporary group due to a modified feeding regimen. Regardless of pasture, cows had limited access to stockpiled native range forage and ad libitum access to tall-grass prairie hay (4.8% CP, 56.0% TDN, 74.1%, NDF, 4.4% ADIA; DM basis) and a mineral supplement (28.6% NaCl, 12.8% Ca, 8.8% P, 1.2% Mg, 1,044 mg/kg Cu, 12 mg/kg Se, 3,117 mg/kg Zn, 409,000,000 IU Vitamin A and 455,000 IU Vitamin E; DM basis). Supplement and hay samples were collected and composited for determination of DM (oven drying at 50°C until no further weight loss), NDF, ADF (Ankom Tech Corp, Fairport, NY), ash (combusted 6-h in a muffle furnace at 500°C), and CP (% N x 6.25; TruSpec CN, LECO Corporation, St. Joseph, MI).

Individual cow BW and BCS were determined at the beginning of the treatment period (January 15, 2010), prior to calving, immediately after calving, at supplementation termination (May 3, 2010), prior to breeding (June 4, 2010), and at weaning (September 16, 2010). All weights were recorded after 16-h withdrawal from feed and water. Body condition scores (1 = emaciated, 9 = obese; Wagner et al., 1988) were determined by the same two independent evaluators throughout the experiment. Calf birth weight was determined within 24-h of parturition and then recorded at the end of the supplementation period and again at weaning. With the exception of four potential herd sires (2 per treatment group), bulls were banded at the time of vaccination (June 4, 2010).

The percentage of cows cycling at the start of the breeding season was determined by quantifying progesterone concentration (Vizcarra et al., 1997) in plasma samples. Blood samples were obtained via coccygeal venipuncture on d -9 and d 0 of the breeding season in 10-mL vacutainer tubes containing EDTA (BD, Franklin Lakes, NJ). Plasma was harvested within 4 h (2500 x *g* for 25 min at 4˚C) and stored at -20˚C until progesterone was quantified with a solid phase RIA (Coat-A-Count progesterone kit, Diagnostic Products Corp., Los Angeles, CA). Cows with one or more plasma samples containing ≥ 1.0 ng/mL progesterone were considered to be cycling (i.e., exhibiting luteal activity).

On d 0 of the breeding season all cows received an intramuscular injection of 5 mL of $PGF_{2\alpha}$ (Lutalyse, Pfizer Inc.) and fitted with an Estrus Alert® patch to aid in heat detection. Once standing estrus was observed or after the color on the patch was completely exposed cows were artificially inseminated the following morning or evening. A second injection of $PGF_{2\alpha}$ (Lutalyse, Pfizer Inc.) was administered on d 10 of the breeding season to those cows that did not exhibit signs of estrus after the first injection. Cows that were artificially inseminated were moved to another pasture 10 d post insemination and exposed to a bull for natural service. First service artificial insemination continued until 75% of the cows had been serviced and then cows were exposed to bulls for an accumulative breeding season of 63 d. On October 28, 2010 blood samples were collected via coccygeal venipuncture in 10-mL vacutainer tubes (BD, Franklin Lakes, NJ), allowed to coagulate and then serum was aspirated for detection of pregnancy by quantifying Pregnancy-Specific Protein B at a Biopryn® certified commercial laboratory (Circle H Laboratories, Dalhart, TX).

***Statistical Analysis.*** Cow was considered the experimental unit because supplements were individually fed. All non-categorical data was analyzed using PROC MIXED of SAS

(SAS Inst. Inc., Cary, NC) and the Satterthwaite approximation for degrees of freedom. The model included supplement treatment as a fixed effect. Calf performance was analyzed using calf age as a co-variable. Least squares means were separated and reported using pair-wise t-tests with the PDIFF option in the LS means statement ($\alpha = 0.05$) when the *P*–value for the was $\leq 0.05$. Categorical data for reproduction parameters were analyzed using FREQ procedures in SAS (SAS Inc., Cary, NC) and Chi Square calculations to separate mean percent differences. The tables included supplement treatment by cycling and supplement treatment by pregnancy results.

***Experiment 3: Milk Production, Intake and Digestibility***

***Animals.*** This experiment was also conducted at the OSU Range Cow Research Center to evaluate supplement treatments on milk yield, milk composition, hay intake and apparent digestibility. During early lactation, cows were blocked by days in milk (DIM; 29 ± 9 d) in a randomized complete block design. The milking procedure took place on 3 d and included 5, 9 and 9 cows per treatment for block 1, 2, and 3, respectively for a total of 23 cows per supplement treatment described in Exp. 2. Cows from blocks 2 and 3 were reevaluated during late lactation (195 ± 9 DIM) and also used to determine hay intake and digestibility during each stage of production. Experiments 2 and 3 were conducted concurrently.

For determination of milk yield and composition during early lactation, calves were separated from the cows at 1600 h on the day prior to milking. The pairs were reunited at 2300 h and calves were allowed to nurse their dams ad libitum, but for < 45 min. After nursing, cows and calves were again separated until milking was completed. Machine

milking began at 0800 h the following morning and was completed by 1200 h. Hay and water was provided to cows ad lib during this period.

Prior to milking, a 1.0 mL injection of oxytocin (20 USP units/mL, intramuscularly; Phoenix Pharmaceutical Inc., St. Joseph, MO) was given to each cow to facilitate milk ejection. A portable milking machine was used to individually milk the cows until milk cessation, at which time the milking apparatus was removed and each teat was hand-stripped to ensure complete emptying of each quarter. Hand-stripped milk was mixed thoroughly with mechanically obtained milk, weighed and subsampled (50 mL). The subsample was preserved in 2-bromo-2-nitropropane-1,3-diol and shipped to the Heart of America DHIA (Manhattan, KS) for analysis of milk urea N, protein, butterfat, lactose, and solids not fat. Milk weights were used to calculate 24-h milk yield using this equation: P = (MW/MIN) * 1,440, where P = 24-h milk yield, MW = weight of milk obtained from milking procedure described above, MIN = minutes from calf-separation to termination of milking procedure, and 1,440 = minutes in 24-h period.

Cows from blocks 2 and 3 were used to determine hay intake and digestibility prior to milk yield determination. Cows were housed in 18 individual outdoor 3.7 x 9.1-m pens, so that they were exposed to the same environmental conditions as their herd mates in Exp. 2. When 9 cows from each treatment were 18 ± 9 DIM, they were gathered and removed from feed and water for 16-h before shrunk BW and BCS was determined. Cows (n = 18) and their calves were then randomly allotted to 1 of 18 pens where they had ad libitum access to the same tall-grass prairie hay fed in Exp. 2 and subjected to the same supplement feeding regimen as Exp. 2 (Monday, Wednesday, Friday and Saturday mornings). This process was repeated a second time for a total of 18 cows per treatment. During late lactation these same

cow/calf pairs were gathered at 175 ± 9 DIM and given ad libitum access to tall-grass prairie hay round bales (4.4% CP, 55.0% TDN, 69.4% NDF, 3.2% ADIA; DM basis) in a dry-lot for 10 d prior to determination of intake and diet digestibility. Square bales from the same hay harvest were used for the collection period. In early lactation, cows and calves (pre-ruminants) were allowed to be together for the entire period. However, during late lactation calves were only reunited with their dams and allowed to nurse at 0700 and 1900 h for < 20 min to ensure that hay intake was not affected by intake of the calf. Therefore, calves were held in a single dry-lot pen and provided ad libitum access to hay and water during the interim periods.

Two 10-d periods were used to evaluate intake and digestibility. The cows were adapted to the pens and hay feeders for 5 d and data were collected over the next 5 d. Following adaptation, hay supplied was 120% of the average intake from d 0 to 5 and actual intake was measured from d 6 through 10. Fecal samples were collected twice daily during these time at 0800 and 1700 h to predict fecal output from acid detergent insoluble ash (ADIA) concentration. Supplement, hay, ort and fecal samples were dried at 50°C and ground in a Wiley mill (Model-4, Thomas Scientific, Sweedesboro, NJ) to pass a 2-mm screen before analysis. Supplement and hay samples were composited by period while ort and fecal samples were composited by cow within each period. All composite samples were analyzed for NDF and ADF (Ankom Tech Corp, Fairport, NY), ash (combusted 6-h in a muffle furnace at 500°C), CP (% N x 6.25; TruSpec CN, LECO Corporation, St. Joseph, MI) and ADIA (combustion of ADF residues 6-h in a muffle furnace at 500°C).

Apparent DM, OM, NDF, ADF and CP digestibility were calculated for each cow. In addition, digestible DMI (DMI, kg/100 kg of BW x DM digestibility) and digestible OM intake were calculated for each cow.

***Statistical Analysis.*** Cow was considered the experimental unit and milk yield, milk composition, hay intake and digestibility measurements were analyzed as a randomized complete block design using PROC MIXED procedures of SAS (SAS Inst. Inc., Cary, NC) and the Satterthwaite approximation for degrees of freedom. The model included supplement treatment and supplement treatment X stage of production as fixed effects and calf age and period as random variables. Least square means were separated and reported using pair-wise t-tests with the PDIFF option in the LS means statement ($\alpha = 0.05$) when the *P*–value was $\leq$ 0.05.

## RESULTS AND DISCUSSION

### *Experiment 1*

***Forage quality.*** Forage approximate analyses for hand plucked samples over time are shown in Table 3. Forage CP concentration declined from wk 1 to wk 11 throughout the experimental period. These values also fall in the suggested forage CP range (< 7%) that requires supplemental protein (McCollum and Horn, 1990).

***Steer performance.*** Animal performance results are presented in Table 4. There were no differences in initial BW, but there was an improvement in final BW for steers supplemented with protein ($P < 0.01$). This improvement was also seen in BW gain and rate of BW gain over the entire supplementation period for supplemented steers as compared to C

(0.94 vs. 0.81 kg/d; $P < 0.01$). This improvement in ADG is slightly less than summarized data by Lalman (2008) who suggested an improvement of 0.17 ± 0.04 kg/day (7 studies). As a result, the amount of supplement provided per additional kg of BW gain (3.50 kg; as-is basis) was slightly lower than those data reported by Lalman (2008) (2.80 kg; as-is basis). These improvements in performance were not affected by the inclusion of MA ($P = 0.22$). Even though not analyzed, supplement conversion was the lowest for MA2 steers (3.03, 3.24, and 5.04 kg of supplement per kg of additional BW gain for PC, MA1 and MA2, respectively). Without forage DMI it is difficult to understand this trend, but when evaluating forage DMI in cannulated steers there was a numerical decrease in hay DMI for MA2 as compared to PC steers while MA1 DMI was nearly identical to PC (McMurphy et al., unpublished data). These data also resulted in similar total tract apparent DM digestibilities for PC, MA1 and MA2 and the cumulative effect may reduce performance of MA2 steers over a period of time because of reduced digestible energy intake. Additionally, data obtained from McMurphy et al. (unpublished data) were from steers supplemented on a daily basis. These steers were fed at a prorated amount for delivery three times per week. This feeding regimen actually supplied 2.3 g MA per feeding ((1 g/d x 7 d) / 3 d), which may not allow for a direct comparison. Data evaluating feeding intervals for MA to grazing cattle is unavailable, however Thalib et al. (1995) did deem saponins from *Sapindus rarak* successful at suppressing protozoa when feed at 3 d intervals. This needs to be further investigated to determine the appropriate quantity of MA or if it would be more appropriate to add MA to a mineral or liquid supplement in order to maintain a daily supply of MA for stocker steers.

***Experiment 2***

***Cow BW and BCS.*** Supplementation with a source of plant protein high in RDP has shown to be an effective means of mediating BW and BCS during winter months when cows are consuming low-quality forage (Clanton and Zimmerman, 1970; Schauer et al., 2005; Steele et al., 2007). It has also been shown that there is a point of diminishing returns for protein supplementation (Mathis et al., 1999). Data for cow BW, BW change, BCS and BCS change are reported in Table 5. There were no differences in BW or BCS at the initiation of the experiment, the end of the supplementation period or at weaning for PC and MA treated cows. At the end of the supplementation period BW was reduced for both treatments, but there was a slight recovery from this time until weaning (12 and 19 kg for PC and MA, respectively). This improvement in BW during the summer grazing period was not as substantial as those observed by Banta et al. (2008), but were similar to data from Winterholler et al. (2009b). During late gestation BW was improved to a greater extent for PC as compared to MA ($P = 0.03$), while there was not an improvement in BCS change ($P = 0.15$). This prepartum difference was eliminated after parturition. This was not the case in the studies by Winterholler et al. (2009b) and Steele et al. (2007), but similar to those changes reported by Schaurer et al. (2005) where supplementation improved BCS by 0.44 units during late gestation as compared to non-supplemented cows. Therefore it is concluded that protein supplementation with and without MA was effective at improving BW and BCS during late gestation. After calving, regardless of supplement treatment BCS was reduced until time of weaning (-0.45). This reduction in BCS after parturition is typical of spring-calving cows consuming low-quality hay even when energy supplied by the supplement is slightly increased (Banta et al., 2006; Winterholler et al., 2009b). Other researchers have

been unsuccessful at capturing a difference in BW change with the addition of dietary saponins (Valdez et al., 1986).

***Calf Performance.*** There was no difference in age (31 ± 17 d) or birth weight (39 ± 0.75 kg; mean BW ± SEM) of calves at the conclusion of the supplementation period (Table 6). At the end of the supplementation period calves from MA cows were 5 kg heavier than those from the PC cows. This improvement in BW may be due to a slight improvement of milk yield by MA cows during early lactation (0.19 kg/d; Table 8). Furr and Nelson (1964) reported strong correlations between dam milk yield and calf ADG (0.75 to 0.91) that were significant in six of nine groups of cattle. But similar to this study, improved calf BW at the end of supplementation did not necessarily improve weaning BW (Furr and Nelson, 1964). Marston et al. (1992) also reported a good correlation between total milk yield and adjusted weaning BW equating to an increase in 0.014 kg of ADG per kg of additional milk. This would not explain the 5 kg increase in calf BW. However this correlation is for total milk yield and not for early lactation. To fully understand this improvement in calf performance a greater number of cows and more milk yield measurements per cow during early lactation may be needed.

***Cow Reproductive Performance.*** There was no affect on luteal activity, or pregnancy rate at weaning for PC and MA treated cows, respectively (Table 7). There were also no differences in BW, BCS at the beginning of the breeding season. Therefore, these data suggest that MA has no dramatic effect on reproductive performance.

***Experiment 3***

***Milk Yield and Composition.*** There were no interactions ($P > 0.05$) for stage of production and treatment, so simple means for early and late lactation on milk yield and composition are presented in Table 8. There were no effects of MA on milk yield or milk constituents as compared to PC other than a trend for reduced solids not fat concentrations during early lactation for MA cows ($P = 0.08$). This was not observed for cows in late lactation. Findings from Valdez et al. (1986) also showed no difference in milk yield or composition of the milk when sarsaponins were included in the diet. It is thought that MA plays an important role in nitrogen metabolism, but in accord with these findings Wilson et al. (1998) also observed saponins had no effect on milk urea nitrogen in lactating dairy cows. As expected, milk production was lower during late lactation with higher concentrations of butterfat, protein and lactose as compared to early lactation.

***Intake and Digestibility.*** Hay DMI and digestibility of DM, NDF, ADF and CP for early and late lactation are reported in Table 9. There were no differences in forage DMI or any of the digestibility parameters for early and late lactation. This is in contrast to Goetsch and Owens (1995) who observed an improvement in total tract OM digestibility, but confirms results observed in cannulated steers fed MA (McMurphy et al., unpublished data). McMurphy et al. (unpublished data) did find an improvement in rumen digestibility of DM and NDF, but PC steers appeared to exhibit compensatory hind gut digestibility.

Hay DMI and apparent total tract digestibility of DM and OM during early lactation are comparable to those reported by Winterholler et al. (2009b) using similar techniques at the OSU Range Cow Research Center, North Range Unit. There was a 25% decrease in hay DMI (kg·100kg of $BW^{-1}\cdot d^{-1}$) and an increase in digestibility of DM and OM by more than 10

units for cows in late lactation as compared to those in early lactation. Interestingly, this resulted in similar digestible DMI and OM intake for each stage of production. Although to a lesser extent, Johnson et al. (2003) also observed a slight decrease in forage DMI and an increase in apparent total tract OM digestibility for multiparous cows in late gestation. These cows also consumed similar quantities of digestible OM regardless of stage of production. Ovenell et al. (1991) did not see a change in particulate passage rate or DM digestibility when comparing non-lactating and lactating cows, but no data could be found comparing the passage rate of particulate matter for cows at different stages of lactation consuming the same diet.

The improvement in digestibility could also be due to the internal marker used (i.e. ADIA) to determine apparent digestibility. This concentration was lower in the prairie hay during late lactation compared with hay used in early lactation (4.4 vs. 3.2% ADIA for early and late lactation, respectively). This might be attributed to the timing in which the hay was utilized during late lactation. Typically hay from the previous year's harvest is supplied to cows, but this hay was cut in July, 2010 and used for determination of intake in October, 2010. No data are available on the concentration of ADIA overtime in stored hay and this impact on its use as an internal marker.

## IMPLICATIONS

The inclusion of MA did not impact performance of stocker steers or beef cows during the supplementation period. However, MA did improve calf performance during the supplementation period. This advantage was lost due to compensatory gain after

supplementation ceased, but could prove to be of great importance to a fall-calving cow herd consuming low-quality forage for its entire lactation period (assuming a 205 d weaning practice).

Also, the feeding regimen needs to be evaluated as a potential role in animal performance. This study intermittently supplied MA as opposed to a continuous supply. This may negatively impact the microflora populations intermittently and never sustain a stable environment. It would also be beneficial to evaluate the use of MA in combination with NPN. Mader and Brumm (1987) evaluated the replacement of soybean meal with urea in a steer supplement and observed an improvement in rate of body weight gains from steers consuming the urea supplement with saponin as compared to urea only as the source of degradable protein. With the rising costs of feed protein, this may be an economical option to upgrade the use of NPN in grazing cow diets. More research needs to be conducted to determine the impact of daily provision of MA and its interaction with diet on performance of grazing animals.

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**Table 1: Supplement composition and amount of nutrients supplied daily for stocker steers grazing late summer native range (Exp. 1)**

| | Supplement[1] | | | |
|---|---|---|---|---|
| Item (DM basis) | C | PC | MA1 | MA2 |
| | % of DM | | | |
| Cottonseed meal | - | 75.5 | 75.5 | 75.5 |
| Wheat middlings | - | 19.5 | 19.5 | 19.5 |
| Soybean Hulls | 100.0 | - | - | - |
| Cane Molasses | - | 5.0 | 5.0 | 5.0 |
| Micro-Aid® | - | - | 0.2 | 0.4 |
| | Nutrient supplied | | | |
| DM, kg/d | 0.13 | 0.40 | 0.40 | 0.40 |
| CP, kg/d | 0.02 | 0.16 | 0.16 | 0.16 |
| TDN, kg/d | 0.08 | 0.29 | 0.30 | 0.29 |
| Crude fat, kg/d | 0.01 | 0.01 | 0.01 | 0.01 |

[1]Supplements included (DM basis) 1) 0.15 kg/d of soybean hulls (C); 2) 0.40 kg/d of a 40% CP cottonseed meal and wheat middlings-based supplement (PC); 3) PC plus 1 g/d of Micro-Aid® (MA1); and 4) PC plus 2 g/d of Micro-Aid® (MA2).

*All steers had ad libitum access to a complete vitamin and mineral premix (28.6% NaCl, 12.8% Ca, 8.8% P, 1.2% Mg, 1,044 mg/kg Cu, 12 mg/kg Se, 3,117 mg/kg Zn, 409,000,000 IU Vitamin A and 455,000 IU Vitamin E; DM basis).

**Table 2: Supplement composition and amount of nutrients supplied daily during gestation and lactation (Exp. 2 and 3)**

| | Supplement[1] | |
|---|---|---|
| Item (DM basis) | PC | MA |
| | % of DM | |
| Cottonseed meal | 46.8 | 46.8 |
| Wheat middlings | 42.0 | 42.0 |
| Micro-Aid® | - | 0.1 |
| Cane Molasses | 5.0 | 5.0 |
| Dicalcium Phosphate | 5.2 | 5.2 |
| Salt | 0.8 | 0.7 |
| Vitamin-A, 30,000 IU[2] | 0.2 | 0.2 |
| | Nutrient supplied, gestation | |
| DM, kg/d | 0.82 | 0.82 |
| CP, kg/d | 0.25 | 0.25 |
| TDN, kg/d | 0.65 | 0.57 |
| | Nutrient supplied, lactation | |
| DM, kg/d | 1.55 | 1.55 |
| CP, kg/d | 0.47 | 0.47 |
| TDN, kg/d | 1.22 | 1.07 |

[1]Supplements (DM basis) included 1) 1.91 kg delivered 3d/wk during gestation (0.82 kg/d) and 2.71 kg delivered 4d/wk (1.55 kg/d) during lactation of a 30% CP cottonseed meal and wheat middling-based supplement (PC); and 2) PC plus the inclusion of 1 g/d of Micro-Aid® (MA).

[2]Provided 60,037 IU of Vitamin A per kg of diet DM.

**Table 3. Composition of tallgrass native range during summer supplementation from late July until early October, 2009 (Exp. 1)**

| | Week | | | | | |
|---|---|---|---|---|---|---|
| Chemical Component, %DM | 1 | 3 | 5 | 7 | 9 | 11 |
| DM | 50.5 | 45.0 | 40.5 | 42.9 | 50.0 | 83.2 |
| CP | 6.9 | 6.2 | 5.7 | 5.9 | 5.1 | 4.3 |
| NDF | 76.6 | 74.1 | 74.7 | 76.3 | 76.3 | 74.9 |
| ADF | 43.2 | 43.4 | 44.8 | 46.1 | 45.3 | 44.3 |
| OM | 93.3 | 91.7 | 90.5 | 93.3 | 92.9 | 90.4 |

**Table 4. Effect of supplement on performance of steers grazing native range in late summer and fall in Exp. 1**

| | Treatments[1] | | | | | *P*- Value[2] | | |
|---|---|---|---|---|---|---|---|---|
| Item | C | PC | MA1 | MA2 | SEM[3] | $C_1$[4] | $C_2$[4] | $C_3$[4] |
| No. | 15 | 18 | 17 | 18 | | | | |
| BW, kg | | | | | | | | |
| Initial | 288 | 290 | 293 | 289 | 8.8 | 0.81 | 0.87 | 0.71 |
| Final | 357 | 371 | 374 | 365 | 9.4 | <0.01 | 0.22 | 0.21 |
| Gain | 69 | 82 | 80 | 76 | 2.5 | <0.01 | 0.22 | 0.21 |
| ADG, kg | | | | | | | | |
| d 0 - 28 | 0.96 | 1.00 | 1.00 | 0.94 | 0.05 | 0.74 | 0.66 | 0.39 |
| d 28 - 56 | 0.79 | 1.00 | 0.98 | 0.98 | 0.07 | 0.02 | 0.86 | 0.97 |
| d 56 - 85 | 0.67 | 0.89 | 0.85 | 0.76 | 0.07 | 0.06 | 0.34 | 0.35 |
| Total | 0.81 | 0.96 | 0.95 | 0.90 | 0.03 | <0.01 | 0.23 | 0.20 |

[1]Treatements included 1) no supplementation (C); 2) 0.40 kg/d of a 40% CP cottonseed meal and wheat middlings-based supplement (PC); 3) PC plus 1 g/d of Micro-Aid® (MA1); and 4) PC plus 2 g/d of Micro-Aid® (MA2).
[2]Probability of a greater F-statistic.
[3]Most conservative SEM, n = 15.
[4]$C_1$ = C vs. others; $C_2$ = PC vs. MA1 + MA2; $C_3$ = MA1 vs. MA2.

**Table 5: Effect of winter supplement on cow BW and BCS in Exp. 2**

| | Supplement[1] | | | |
|---|---|---|---|---|
| Item | PC | MA | SEM[2] | *P*-value[3] |
| No. | 46 | 47 | | |
| Supplementation period, d | 107 | 107 | | |
| Age, yrs | 5.5 | 5.6 | 0.38 | 0.89 |
| Initial BW (1/15/10), kg | 543 | 554 | 11.28 | 0.46 |
| BW change before calving[4], kg | 14 | 8 | 2.14 | 0.03 |
| BW change after calving[5], kg | -17 | -16 | 3.62 | 0.90 |
| BW change 107-d[6], kg | -50 | -56 | 5.25 | 0.42 |
| BW at end of Supplementation (5/3/10), kg | 492 | 498 | 10.36 | 0.70 |
| BW change 244-d[7], kg | -38 | -39 | 4.53 | 0.91 |
| BW at Weaning (9/16/10), kg | 504 | 517 | 9.79 | 0.37 |
| | | | | |
| Initial BCS (1/15/10) | 5.20 | 5.27 | 0.08 | 0.54 |
| BCS change before calving[4] | 0.36 | 0.24 | 0.05 | 0.15 |
| BCS change after calving[5] | -0.68 | -0.21 | 0.41 | 0.42 |
| BCS change 107-d[6] | -0.32 | -0.33 | 0.07 | 0.87 |
| BCS at end of Supplementation (5/3/10) | 4.88 | 4.94 | 0.07 | 0.59 |
| BCS change 244-d[7] | -0.54 | -0.46 | 0.11 | 0.59 |
| BCS at Weaning (9/16/10) | 4.65 | 4.80 | 0.14 | 0.46 |

[1]Supplements (DM basis) included 1) 0.82 kg/d during gestation and 1.55 kg/d during lactation of a 30% CP cottonseed meal and wheat middling-based supplement (PC); and 2) PC plus the inclusion of 1 g/d of Micro-Aid® (MA).
[2]Most conservative SEM, n= 47.
[3]Probability of a greater F-statistic.
[4]Precalving measurements obtained prior to calving.
[5]Change postcalving to end of supplementation period.
[6]Change over supplementation period (January 15, 2010, to May 3, 2010).
[7]Change from beginning of supplementation to weaning (January15, 2010, to September 16, 2010).

**Table 6: Effect of winter supplement for spring-calving cows on calf performance in Exp. 2**

| | Supplement[1] | | | |
|---|---|---|---|---|
| Item | PC | MA | SEM[2] | *P*-value[3] |
| No. | 46 | 47 | | |
| Birth Weight, kg | 39 | 39 | 0.75 | 0.70 |
| May 3 BW (age = 31 d, SD = 17 d), kg | 66 | 71 | 1.44 | <0.01 |
| Weaning BW (age = 165 d, SD = 18 d), kg | 189 | 193 | 3.53 | 0.46 |

[1]Supplements (DM basis) included 1) 0.91kg/d during gestation and 1.73 kg/d during lactation of a 30% CP cottonseed meal and wheat middling-based supplement (PC); and 2) PC plus the inclusion of 1 g/d of Micro-Aid® (MA).
[2]Most conservative SEM, n= 46.
[3]Probability of a greater F-statistic.

**Table 7: Effect of winter supplement on cow reproductive performance in Exp. 2**

| | Supplement[1] | | | |
|---|---|---|---|---|
| Item | PC | MA | SEM[2] | *P*-value[3] |
| No. | 46 | 47 | | |
| Supplementation period, d | 107 | 107 | | |
| Prebreeding BW (6/4/2010), kg | 471 | 484 | 10.11 | 0.38 |
| Prebreeding BCS (6/4/2010) | 4.4 | 4.6 | 0.14 | 0.20 |
| Luteal Activity[4], % | 60.9 | 55.3 | 0.29 | 0.59 |
| Pregnancy rate at weaning, % | 76.1 | 80.9 | 0.31 | 0.58 |
| Calving to start of breeding season, d | 60 | 61 | 2.67 | 0.91 |

[1]Supplements (DM basis) included 1) 0.91kg/d during gestation and 1.73 kg/d during lactation of a 30% CP cottonseed meal and wheat middling-based supplement (PC); and 2) PC plus the inclusion of 1 g/d of Micro-Aid® (MA).
[2]Most conservative SEM, n= 46.
[3]Probability of a greater F-statistic.
[4]Percentage of cows exhibiting ovarian luteal activity at the beginning of the breeding season.
[5]No. for AI conception rate = for PC and MA treatments, respectively.

**Table 8: Effect of winter supplement on beef cow milk production and composition during early and late lactation in Exp. 3**

| Item | Supplement[1] | | SEM | *P*-value[2] |
|---|---|---|---|---|
| | PC | MA | | |
| | Early Lactation | | | |
| No. | 23 | 23 | | |
| Butterfat, % | 2.66 | 2.62 | 0.22 | 0.86 |
| Protein, % | 2.85 | 2.79 | 0.05 | 0.34 |
| Lactose, % | 5.21 | 5.15 | 0.03 | 0.21 |
| Solids not fat, % | 9.03 | 8.89 | 0.05 | 0.08 |
| Milk urea N, mg/dl | 2.47 | 2.68 | 0.62 | 0.29 |
| Milk yield, kg/d[3] | 7.96 | 8.15 | 0.58 | 0.72 |
| | Late Lactation | | | |
| No. | 18 | 18 | | |
| Butterfat, % | 3.30 | 3.28 | 0.07 | 0.80 |
| Protein, % | 4.86 | 4.84 | 0.05 | 0.84 |
| Lactose, % | 9.05 | 9.01 | 0.07 | 0.69 |
| Solids not fat, % | 2.87 | 2.77 | 0.18 | 0.71 |
| Milk urea N, mg/dl | 1.91 | 1.76 | 0.21 | 0.21 |
| Milk yield, kg/d[3] | 4.93 | 4.32 | 0.66 | 0.21 |

1Supplements (DM basis) included 1) 1.55 kg/d during lactation of a 30% CP cottonseed meal and wheat middling-based supplement (PC); and 2) PC plus the inclusion of 1 g/d of Micro-Aid® (MA).
2Probability of a greater F-statistic.
3Calculated 24-h milk production from machine milking.

**Table 9: Effect of supplement treatment during early and late lactation on hay intake and apparent total tract digestibility of dietary components (DM basis) Exp. 3**

| | Supplement[1] | | | |
|---|---|---|---|---|
| Item | PC | MA | SEM | *P*-value[2] |
| No. | 18 | 18 | | |
| | Early Lactation | | | |
| Initial BW, kg | 490 | 499 | | |
| Hay intake, kg·100kg of $BW^{-1}\cdot d^{-1}$ | 2.33 | 2.39 | 0.11 | 0.50 |
| OM intake, kg·100kg of $BW^{-1}\cdot d^{-1}$ | 2.45 | 2.51 | 0.12 | 0.56 |
| Fecal output, kg·100kg of $BW^{-1}\cdot d^{-1}$ | 1.25 | 1.27 | 0.04 | 0.74 |
| Digestible, DMI kg·100kg of $BW^{-1}\cdot d^{-1}$ | 1.47 | 1.50 | 0.11 | 0.60 |
| Digestible OM intake, kg·100kg of $BW^{-1}\cdot d^{-1}$ | 1.26 | 1.29 | 0.11 | 0.57 |
| DM digestibility, % | 54.84 | 55.18 | 1.86 | 0.74 |
| OM digestibility, % | 58.07 | 57.60 | 2.02 | 0.67 |
| NDF digestibility, % | 55.25 | 55.94 | 2.17 | 0.64 |
| ADF digestibility, % | 48.82 | 49.15 | 3.83 | 0.84 |
| CP digestibility, % | 46.25 | 48.84 | 1.56 | 0.25 |
| | Late Lactation | | | |
| Initial BW, kg | 539 | 546 | | |
| Hay intake, kg·100kg of $BW^{-1}\cdot d^{-1}$ | 1.92 | 1.85 | 0.14 | 0.39 |
| OM intake, kg·100kg of $BW^{-1}\cdot d^{-1}$ | 2.09 | 2.03 | 0.14 | 0.45 |
| Fecal output, kg·100kg of $BW^{-1}\cdot d^{-1}$ | 0.78 | 0.76 | 0.03 | 0.55 |
| Digestible, DMI kg·100kg of $BW^{-1}\cdot d^{-1}$ | 1.50 | 1.47 | 0.12 | 0.67 |
| Digestible OM intake, kg·100kg of $BW^{-1}\cdot d^{-1}$ | 1.45 | 1.42 | 0.12 | 0.70 |
| DM digestibility, % | 66.60 | 66.86 | 1.22 | 0.86 |
| OM digestibility, % | 69.11 | 69.48 | 1.15 | 0.81 |
| NDF digestibility, % | 67.79 | 67.97 | 1.36 | 0.91 |
| ADF digestibility, % | 64.84 | 65.55 | 2.24 | 0.68 |
| CP digestibility, % | 59.92 | 60.17 | 1.00 | 0.86 |

[1]Supplements (DM basis) included 1) 1.55 kg/d during lactation of a 30% CP cottonseed meal and wheat middling-based supplement (PC); and 2) PC plus the inclusion of 1 g/d of Micro-Aid® (MA).
[2]Probability of a greater F-statistic.

# APPENDICES

## Oklahoma State University
## Institutional Animal Care and Use Committee (IACUC)

Protocol Expires: 6/4/2012

Date : Friday, June 05, 2009 Animal Care and Use Protocol (ACUP) No : AG0910

Proposal Title: Effects of Micro-Aid on Forage Utilization and Production of Forage-Fed Beef Cattle

Principal Investigator:

David Lalman
Animal Science
201 Ani Sci
Campus

Reviewed and Processed as: Special Review

Approval Status Recommended by Reviewer(s) : Approved

Approved for a total of 16 cattle (Bos Taurus) for the next three years.

Signatures : Charlotte L. Ownby

Charlotte Ownby, IACUC Chair

Friday, June 05, 2009
Date

cc: Department Head, Animal Science
Director, Animal Resources

**Approvals are valid for three calendar years, after which time a request for renewal must be submitted. Any modifications to the research project, course, or testing procedure must be submitted for review and approval by the IACUC, prior to initiating any changes. Modifications do not affect the original approval period. Approved projects are subject to monitoring by the IACUC. OSU is a USDA registered research facility and maintains an Animal Welfare Assurance document with the Public Health Service Office of Laboratory Animal Welfare, Assurance number AA3722-01.**

**Effects of Implant Type and Protein Source on Growth of Steers Grazing Summer Pasture**[1,2]

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[2]The authors would like to thank Elanco Animal Health for partial funding of this experiment.

**Abstract**

A split-plot design was used to investigate the effects of implant type and protein source on performance of steers grazing summer pasture. Crossbred steers (n = 196 each year) 216 ± 24 kg and 208 ± 23 kg (initial BW ± SD) for 2008 and 2009, respectively, were ranked by weight and randomly assigned to implant and supplement treatments. Supplement treatments were no supplement (control), cottonseed meal-based supplement (CSM; 33% CP), and dried distillers grains-based supplement (DDGS; 33% CP). Implant treatments were control (no implant), Ralgro® (R) and Component TE-G® (TEG). Steers were grazed for 126 days starting in early June. Supplementation was initiated in late July when supplements were group fed, within pasture, three times each wk at a rate of 0.95 kg/steer. Protein supplementation increased BW and ADG by 12 and 0.16 kg, respectively ($P < 0.05$). Rate of BW gain was also improved by DDGS (0.05 kg; $P < 0.05$) as compared with CSM, resulting in 2.67 vs. 3.78 kg of supplement per kg of additional ADG for DDGS and CSM, respectively. Implantation increased final BW ($P = 0.02$) and improved ADG 8.1% ($P < 0.05$) during the first ~95 days, regardless of implant type. However, TEG increased ADG (0.08 kg; $P < 0.05$) during the final ~31 d of the grazing season as compared with control and R. DDGS is an effective protein source for cattle grazing Old World Bluestem or tall-grass native range in late summer and TEG improved cattle performance compared to R when grazing season was longer than 100 days.

**Key Words:** implants, protein supplementation, grazing steers

## Introduction

Stocker cattle are a major component of the beef industry in the southern Great Plains. Within this industry, there are several technologies available to operators to improve efficiency and increase profits. Some of these management strategies include, but are not limited to, implants, protein supplementation, and the inclusion of ionophores in mineral or feed supplements. Implants are one of the most profitable technologies available. Ralgro®, an estrogenic implant (zeranol), is frequently used in the stocker industry. Kuhl (1997) reported that body weight gains of 12 kg were seen when Ralgro® was used, but that it is only efficacious for approximately 100 days. However, Component TE-G®, a trenbolone acetate/estradiol implant for grazing cattle, has a suggested payout period of 120 days and might be more applicable to season-long grazing systems in the Southern Great Plains. These season-long grazing systems also benefit from protein supplementation during late summer, when grasses are reaching maturity and quality is diminishing. During this period of time forage intake and digestibility are hindered due to a deficiency in rumen ammonia-N (McCollum and Horn, 1990). The "Oklahoma Gold" program developed at Oklahoma State University was established on the basis that providing 0.45 kg/day of a high protein supplement with an ionophore (38-40% CP) during late summer will improve performance of grazing steers by 0.20 kg/d. This program was established using oilseed meals as a base commodity. In recent years these oilseed meals have increased in price relative to alternative protein sources such as dried distillers grains with solubles. On average, cottonseed meal has been $0.07 higher on a cost per unit of crude protein during the past five years (USDA, 2010), deeming it a viable substitute for cottonseed meal.

Accordingly, the hypothesis is that Component TE-G® will have a longer payout period and that dried distillers grains with solubles can be an effective replacement for cottonseed meal as a protein source for summer stocker steers grazing warm season grasses. Therefore, the objectives of this study were to evaluate the effects of implant type and protein source on performance of steers grazing summer pasture.

## Materials and Methods

**Study site, Vegetation and Stocking Rate.** This study was conducted during 2 consecutive years at the Oklahoma State University Crosstimbers-Bluestem Stocker Range, 11 km southwest of Stillwater from late Spring until early Fall. Each year, 12 pastures (106 ha) consisting primarily of introduced Old World Bluestem (**OWB;** *Bothriochloa ischaemum*) and 3 tallgrass native range pastures (**NR**; 97 ha) consisting primarily of big bluestem (*Andropogon gerardii*), little bluestem (*Schizachyrium scoparium*) and Indiangrass (*Sorghastrum nutans*) were used to evaluate steer performance. In May of each year Nitrogen fertilizer was applied at a rate of 90 kg/ha to the OWB pastures.

Introduced OWB pastures represented a more homogeneous grazing site in contrast to the NR pastures. Accordingly, it has been suggested that an improvement in forage available for use is increased by 25% for pastures containing introduced forages as opposed to NR pastures (Redfearn et al., 2008). Therefore, stocking rates were adjusted accordingly. Each year, OWB pastures (8.80 ± 2.22 ha) were grazed at a stocking density of 311 kg/ha resulting in 1.56 ± 0.13 steers/ha (165 steers). This stocking rate was a conservative estimate to ensure forage availability was not limiting during the grazing period based from previous research at

the Crosstimbers-Bluestem Stocker Range (Ackerman et al., 2001). Native pastures (32 ± 14 ha) were also lightly stocked at a rate of 0.38 ± 0.06 steers/ha for yr 1 (35 steers) and 0.45 ± 0.07 steers/ha for yr 2 (42 steers). Prior to green-up in yr 2, previous yr litter was removed by an unintentional range fire, so stocking density was increased in an effort to try and control year to year variation in forage availability and quality. Multiple sources of water were present, including free flowing streams, ponds and improved water sources so that livestock had ad libitum access to water.

Hand plucked forage samples, from each pasture, were collected in triplicate, every other week throughout the supplementation phase of the study to determine forage quality (Table 1). Dry matter (oven drying at 55°C) was determined immediately following collection and after drying, samples were ground through a Wiley Mill grinder using a 2 mm screen and stored for future proximate analysis.

**Animals.** All experimental protocols were approved by the Oklahoma State University Animal Care and Use Committee. In both years steers arrived in late spring. In yr 1, crossbred stocker steers (n = 200) consisting of primarily Bos-Indicus breeds arrived from Arizona and in yr 2, crossbred stocker steers (n = 207), consisting of primarily British breeds with modest Bos-Indicus influence, arrived from Hawaii, via California, on two separate shipment dates. Upon arrival, cattle were dewormed with Ivermax® according to label directions (5 mg ivermectin/ml; American Livestock Supply, Inc.), individually weighed and identified with a treatment tag. Steers were provided a brief acclimation period at which time therapeutic treatments were administered whenever necessary for morbidity. In yr 2, due to a late arrival, load 2 was metaphylactically treated with Micotil® (7 ml/hd; Elanco Animal Health). In two years, only 2 steers were excluded due to mortality.

Crossbred steers (n = 196 each year) 216 ± 24 kg and 208 ± 23 kg (BW ± SD) for 2008 and 2009, respectively were stratified by arrival weight and randomly allotted to one of three implant treatments. Treatment groups were then randomly assigned to 1 of 15 pastures. Steers were assigned to treatments so that initial weight was uniform across all three implant treatments and across all 15 pastures. Each year, 196 steers were used to evaluate performance while the extra steers were equally dispersed across NR pastures and included in stocking rate calculations. On day 0, steers were removed from access to feed and water overnight (14 h) and on d 1 (May 29, 2008 and June 6, 2009) steers were weighed, palpated for implant presence, tagged by treatment and implanted according to treatment. There was no treatment by year interaction for initial BW ($P = 0.97$).

Pastures were randomly assigned to one of three supplement treatments (Table 2): 1) no supplement (control); 2) cottonseed meal-based supplement (**CSM**; 33% CP); and 3) dried distillers grains with solubles-based supplement (**DDGS**; 33% CP). Supplements were formulated to provide 125 mg·steer$^{-1}$·day$^{-1}$ of monensin. Supplements were group fed 3 times/wk and delivered as 0.48 cm pellets in bunks at a rate of 0.95 kg/steer (DM basis). Beginning in late July, supplements were fed for 70 and 84 d for yr 1 and 2, respectively. Implant treatments consisted of: 1) Control (no implant); 2) Ralgro® (**R**; 36 mg zeranol; Schering-Plough Animal Health Corp., Union, NJ 07083); and 3) Component TE-G®, with Tylan (**TEG**; 40 mg trenbolone acetate (**TBA**), 8 mg estradiol USP, 29 mg tylosin tartrate; Ivy Animal Health, Overaland Park, KS 66214). All implants were administered on d 1 (same technician each year), in the middle third of the ear using the standard implanting device for the respective product. Prior to implantation, the ear and the implant-gun needle were disinfected and after implantation, each ear was palpated to verify proper implant

placement. Implant sites were evaluated via palpation of the ears at ~day 95 and day 126 and scored as follows: 1 = implant present, normal; 2 = implant present, abnormal; 3 = no implant present, normal; 4 = no implant present, abnormal.

Cattle were maintained in treatment groups for a grazing period of 126 days and individual shrunk BW were obtained at the beginning of the supplementation period (~day 49), the mid-point (~day 95), and the conclusion of the experiment (day 126). Cattle were observed regularly throughout the study for morbidity.

**Lab Analysis.** Forage and supplement samples were analyzed for lab DM (oven drying at 105°C), NDF and ADF (Ankom Tech Corp, Fairport, NY), Ash (combusted 6-h in a muffle furnace at 500°C), CP (% N x 6.25; Truspec-CN LECO Corporation, St. Joseph, MI 49085) and RDP (Krishnamoorthy et al., 1983). Supplement samples were also sent to an independent laboratory (Dairy One; Ithica, NY) and subjected to analysis of neutral detergent insoluble nitrogen (**NDIN**), ADIN, ether extract (**EE**), and lignin. Additionally, IVDMD was determined using the DAISY$^{II}$ incubator (Ankom Tech Corp, Fairport, NY). These data were used to calculate TDN according to Weiss et al. (1992).

**Statistical Analysis.** Effects of type of implant and protein source on growth performance of steers were analyzed as a split-plot design using MIXED procedures of SAS (SAS Inc., Cary, NC) with $\alpha = 0.05$. Whole-plot was supplement treatment (pasture = experimental unit) and sub-plot was implant treatment (steer = experimental unit). Random variables included source, pasture and source*pasture type*supplement within pasture. Source of cattle was used to control year and multiple shipment dates in year 2. While pasture type was used to manage influences of forage type and quality. Orthogonal contrasts were used to determine implant, implant type, supplement and supplement type effects on

performance. Effects of implant, supplement, and the interaction on ear score were analyzed using GENMOD. Finding no effects on ear score due to supplement or its interaction with implant, differences due to implant were determined using FREQ procedures in SAS (SAS Inc., Cary, NC) and Chi Square calculations to separate mean percent differences. The tables included implant by ear score at day ~95 and day 126.

## Results and Discussion

The implant by supplement interaction for BW or ADG was not significant ($P > 0.05$), nor was protein source by forage type ($P > 0.05$) and therefore main effect means are presented (Table 3 and 4).

**Supplementation.** Protein supplementation increased BW and ADG by 12 and 0.16 kg, respectively ($P < 0.05$). This increase in BW gain from small amounts of a monensin containing protein supplement during summer grazing is consistent with summarized data (Lalman, 2008). This summary reports a rate of BW gain of 0.17 ± 0.04 kg/day (7 studies). This additional gain validates the adequacy of forage available for animal growth and that rumen ammonia-N is a limiting factor affecting energy intake and its utilization (McCollum and Horn, 1990). When comparing sources of protein, rate of BW gain was improved by DDGS (0.05 kg; $P < 0.05$) as compared with CSM. This resulted in a supplement conversion of 2.67 vs. 3.78 kg of supplement per kg of additional ADG for DDGS and CSM, respectively. When compared with summarized data (Lalman, 2008), observed supplement conversion for CSM is below average (2.8 ± 0.08), but within one standard deviation of the mean, whereas supplement conversion for DDGS is above average. This amount of protein

(0.14 kg·steer$^{-1}$·day$^{-1}$) is the minimum allowance suggested by Lalman (2008) for an expected increase in growth performance and could explain the decrease in supplement conversion of CSM. Another potential reason may be the inadequacy of digestible energy from CSM (69.2 vs. 86.3% TDN) to support equivalent microbial growth and protein utilization as DDGS. However, energy provided by forage may actually be less for DDGS supplemented steers than steers provided CSM due to differences in forage DMI. It has been shown that supplementing DDGS to weaned calves consuming low quality forage has a negative influence on hay DMI of 0.32 kg for every 1 kg of DDGS supplemented (Winterholler et al., 2009a). In the current study, DDGS were supplied at 0.64 kg 3 times/wk resulting in an estimated potential decrease in forage DMI of 0.20 kg each time supplemented. Moreover, Morris et al. (2006) showed that when supplementing DDGS to summer stocker steers, forage DMI decreased linearly with increasing levels of DDGS, but ADG also increased linearly. This suggests that the energy provided by DDGS can overcome the potential loss of energy intake from a small decrease in forage intake.

The energy supplied by DDGS is in the form of fat (9.95%) and MacDonald et al. (2006) demonstrated that the inclusion of oil at the same ether extract concentration as DDGS did not increase ADG similarly. Therefore, it was suggested that DDGS may potentially fulfill a deficiency of metabolizable protein (**MP**; MacDonald et al., 2006). It has been suggested by McCollum and Horn (1990) that providing escape protein to cattle consuming low quality forages may potentially reduce the amount of protein needed. Winterholler et al. (2009b) demonstrated that CP of CSM is 73.5% ruminally degradable in cattle consuming low quality roughage, resulting in minimal escape protein. Whereas, tabular values from NRC (2000) show that DDGS is only 45.1% ruminally degradable. However,

when urea was added to DDGS in supplements deficient in rumen degradable protein there was no increase in performance of heifers consuming grass hay (Stalker et al., 2004). Therefore, this study may provide evidence that combining a protein source high in rumen undegradable protein with a highly rumen degradable plant protein source can improve ADG in summer stocker steers.

**Implantation.** Final BW was increased when steers were implanted with TEG as compared with control ($P < 0.05$). Change in BW was increased due to implantation by 5 and 11 kg for R and TEG, respectively ($P < 0.05$). This gain in BW from R is less than the average improvement of 12 kg reported by Kuhl (1997). However, there is no published data reporting an average increase in BW gain from TEG during the stocker phase on growing steers. Implantation increased ADG 7.0% (0.86 vs.. 0.92 kg/d; $P < 0.05$) during the entire grazing period (126 days). This improvement is slightly lower than the range suggested by Reuter et al. (2008) of 0.08 to 0.12 kg ADG. Differences in type of cattle and year of forage production may cause these differences. Furthermore, type of implant influenced ADG ($P < 0.05$). When compared to R, TEG positively influenced rate of BW gain by 0.04 kg for the entire grazing period. But more importantly, ADG was increased by 0.08 kg during the final ~31 d, while R was similar to control (0.66 and 0.67 kg/d, respectively). This may be due to a slower payout rate of TEG as suggested by ear palpation results presented in Table 5. There was an increased presence of implants in cattle administered TEG at ~95 and 126 days ($\chi^2 < 0.01$) as compared with R implanted steers. There was a lower presence of implants at day 126 than at day ~95 for TEG (76 vs. 34% present) suggesting that the payout period may continue past day 126 for some steers. In contrast, the lack of R implants, upon palpation, at day ~95 explains why there was a difference in ADG between implant type during the final

~31 days of the grazing season. This decrease in payout rate is potentially due to the implant carrier. The carrier utilized in TEG is cholesterol as compared with the carrier lactose for R (personal communication with Dr. Robert Botts, Elanco Animal Health). Implants with a lactose matrix (carrier) have been deemed "short-acting" vs. "long-acting" implants with a cholesterol carrier (Istasse et al., 1988).

Implanting site defects occurred at a rate of 5.3 and 10% at day ~95 for R and TEG, respectively. However, the final palpation data demonstrates a very low detection rate of defects which is not different than non-implanted steers ($P > 0.05$). Anderson and Botts (2002) reported that implant site defects in feedlot cattle range from 6 to 10%.

**Economics.** To evaluate the potential economic return from the use of these management strategies, a simple anaylsis was conducted using average feeder cattle prices from 1999 to 2009 (Cattlefax, 2010) and average commodity prices from 2004 to 2009 (USDA, 2010). The value of gain was calculated using the average initial price for a 204 kg steer on June 1 and the average final price for a 318 kg steer on October 15 (i.e. final value – initial value / weight change). The calculated value of gain during this period was $1.36 per kilogram of body weight gain. Feed costs were calculated using the price per kilogram of CSM, DDGS and wheat middlings in each supplement. Figure 1 displays the cost of gain for supplement types and implant types relative to this cost of gain. It is evident that supplementation and implantation are cost effective means of improving production and profitability.

## Implications

Utilizing dried distillers grains with solubles as the primary ingredient of a monensin containing protein supplement in combination with cottonseed meal can increase growth and improve supplement conversion of steers grazing summer warm-season grasses. In addition, Ralgro® and Component TE-G® implants cost $1.12 and $1.34, respectively (Valley Vet Supply) and return 5 and 11 kg to calves grazing summer warm-season grasses for 126 days. This results in a cost of gain of $0.22 and $0.12 per kilogram of body weight gain for Ralgro® and Component TE-G®, respectively. These low costs for improvements in rate of body weight gain are complimentary to those captured by providing small amounts of a monensin containing protein supplement.

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Table 1. Composition of old world bluestem and tallgrass native range forages during summer supplementation from late July until early October during 2008 and 2009

| | | | | | | | *P*-Value[3] | | |
|---|---|---|---|---|---|---|---|---|---|
| | Wk2 | Wk4 | Wk6 | Wk8 | Wk10 | Wk12 | Ptype[4] | Wk | Ptype* Wk |
| Chemical Component, %DM | | | | | | | | | |
| DM | | | | | | | | | |
| OWB | 42.6$^{a1}$ | 38.9$^{b}$ | 39.2$^{b}$ | 41.7$^{a}$ | 48.3$^{c}$ | 46.0$^{c}$ | 0.02 | 0.01 | 0.09 |
| NR | 48.4$^{a2}$ | 43.3$^{b}$ | 40.0$^{c}$ | 43.0$^{bc}$ | 47.9$^{a}$ | 47.8$^{ab}$ | | | |
| OM | | | | | | | | | |
| OWB | 94.5$^{a}$ | 94.5$^{a}$ | 94.5$^{a}$ | 94.3$^{ab1}$ | 94.5$^{a1}$ | 93.7$^{c}$ | 0.01 | 0.01 | 0.01 |
| NR | 94.1$^{a}$ | 94.6$^{ab}$ | 94.7$^{b2}$ | 94.9$^{b2}$ | 95.4$^{c2}$ | 94.1$^{a}$ | | | |
| RDP | | | | | | | | | |
| OWB | 55.1[1] | 57.4[1] | 58.3 | 57.8 | 56.3 | 51.8 | 0.01 | 0.13 | 0.38 |
| NR | 41.1[2] | 45.2[2] | 51.2 | 54.5 | 53.0 | 47.0 | | | |
| CP | | | | | | | | | |
| OWB | 8.2$^{b1}$ | 10.1$^{c1}$ | 8.7$^{bc1}$ | 9.2$^{bc1}$ | 7.7$^{ab1}$ | 6.8$^{a1}$ | 0.01 | 0.01 | 0.70 |
| NR | 5.2[2] | 6.3[2] | 5.4[2] | 5.5[2] | 5.0[2] | 4.5[2] | | | |
| NDF | | | | | | | | | |
| OWB | 73.2$^{a1}$ | 73.3$^{a}$ | 74.3$^{b}$ | 74.4$^{b}$ | 75.2$^{c}$ | 77.7$^{d1}$ | 0.32 | 0.01 | 0.01 |
| NR | 71.5$^{a2}$ | 72.2$^{a}$ | 74.9$^{b}$ | 75.2$^{b}$ | 76.5$^{c}$ | 75.2$^{bc2}$ | | | |
| ADF | | | | | | | | | |
| OWB | 38.4$^{a}$ | 38.9$^{a}$ | 40.0$^{b1}$ | 40.5$^{b}$ | 40.8$^{b1}$ | 44.7$^{c}$ | 0.03 | 0.01 | 0.10 |
| NR | 39.4$^{a}$ | 39.6$^{a}$ | 42.6$^{b2}$ | 42.3$^{b}$ | 43.9$^{b2}$ | 46.4$^{c}$ | | | |

[a,b,c,d] Means within a row lacking a common superscript differ ($P < 0.05$)
[1,2] Means within a column lacking a common superscript differ ($P < 0.05$)
[3] Probability of a greater F-statistic
[4] Ptype = forage type of pastures (OWB or NR)

Table 2. Ingredients and chemical composition of cottonseed meal (CSM) and dried distillers grains with solubles (DDGS) supplements

| | Supplement | |
|---|---|---|
| Ingredients, %DM | CSM | DDGS |
| Cottonseed Meal (44% CP) | 55.70 | 31.49 |
| Dried Distillers Grains w/ Solubles | - | 61.11 |
| Wheat Middlings | 37.74 | - |
| Cane Molasses (pellet binder) | 4.18 | 4.23 |
| Limestone | 2.22 | 1.86 |
| Dical | - | 1.15 |
| Rumensin 80 | 0.16 | 0.16 |

| | Year | | Year | |
|---|---|---|---|---|
| Chemical Composition, %DM | 2008 | 2009 | 2008 | 2009 |
| CP | 31.80 | 33.90 | 34.30 | 34.60 |
| RDP[a] | 75.04 | 76.51 | 50.64 | 52.89 |
| Fat | 3.20 | 3.90 | 9.90 | 10.00 |
| TDN[b] | 69.40 | 69.00 | 87.00 | 85.60 |
| Ca | 1.08 | 1.44 | 0.94 | 1.04 |
| P | 1.04 | 1.16 | 1.12 | 1.25 |
| S | 0.34 | 0.45 | 0.62 | 0.76 |

[a]Rumen Degradable Protein – determined using *streptomyces griseus* procedure (Krishnamoorthy et al., 1983)
[b]Calculated using a multiple-component model including CP, lignin, ash, ether extract, ADIN, NDIN, NDF, IVNDFD (Weiss, 1992)

Table 3. Effects of protein source on performance of steers grazing summer warm-season grass pastures during 2008 and 2009

| | Treatments[1] | | | | *P*-value[2] | | |
|---|---|---|---|---|---|---|---|
| Item | Control | CSM | DDGS | SEM | Trt[3] | $C_1$[4] | $C_2$[4] |
| Steers, No. | 131 | 130 | 131 | | | | |
| BW, kg | | | | | | | |
| Initial | 252 | 252 | 254 | 4.59 | 0.83 | 0.85 | 0.56 |
| Final | 317[a] | 325[b] | 331[b] | 2.61 | 0.01 | 0.01 | 0.12 |
| BW Gain, kg | 64[a] | 74[b] | 78[c] | 3.61 | 0.01 | 0.01 | 0.02 |
| ADG, kg | | | | | | | |
| D ~49 to ~95 | 0.95[a] | 1.10[b] | 1.14[b] | 0.08 | 0.01 | 0.01 | 0.25 |
| D ~95 to 126 | 0.61[a] | 0.72[b] | 0.73[b] | 0.11 | 0.04 | 0.01 | 0.83 |
| Overall | 0.81[a] | 0.93[b] | 0.98[c] | 0.01 | 0.01 | 0.01 | 0.02 |

[1] Treatments include; 1) no supplement (control); 2) cottonseed meal based supplement (**CSM**; 33% CP); and 3) dried distillers grains with solubles based supplement (**DDGS**; 33% CP).
[2]Probability of a greater F-statistic
[3]Trt = Treatment
[4] Orthoganol contrasts: $C_1$ = no supplement vs. Supplementation; $C_2$ = Cottonseed meal (CSM) vs. Dried distillers grains with solubles (DDGS)
[a,,b,c] Means within the same row with the same letter are not statistically different at a 0.05 level of significance

Table 4. Effects of the type of single dose, moderate term implant on performance of steers grazing summer warm-season grass pastures during 2008 and 2009

| | Treatments[1] | | | | *P*-value[2] | | |
|---|---|---|---|---|---|---|---|
| Item | Control | R | TEG | SEM | Trt[3] | $C_1$[4] | $C_2$[4] |
| Steers, No. | 130 | 132 | 130 | | | | |
| BW, kg | | | | | | | |
| Initial | 211 | 211 | 210 | 3.14 | 0.97 | 0.98 | 0.81 |
| Final | 319[a] | 325[ab] | 329[b] | 2.62 | 0.02 | 0.01 | 0.17 |
| BW Gain, kg | 108[a] | 113[b] | 119[c] | 3.01 | 0.01 | 0.01 | 0.01 |
| ADG, kg | | | | | | | |
| D 0 to ~95 | 0.93[a] | 0.99[b] | 1.02[b] | 0.07 | 0.01 | 0.01 | 0.13 |
| D ~95 to 126 | 0.67[a] | 0.66[a] | 0.74[b] | 0.12 | 0.02 | 0.34 | 0.01 |
| Overall | 0.86[a] | 0.90[b] | 0.94[c] | 0.02 | 0.01 | 0.01 | 0.01 |

[1] Treatments include; 1) Control (no implant); 2) Ralgro® (**R**; 36 mg zeranol; Schering-Plough Animal Health Corp., Union, NJ 07083); and 3) Component TE-G®, with Tylan (**TEG**; 40 mg trenbolone acetate (**TBA**), 8 mg estradiol USP, 29 mg tylosin tartrate; Ivy Animal Health, Overaland Park, KS 66214).
[2] Probability of a greater F-statistic
[3] Trt = Treatment
[4] Orthogonal contrasts: $C_1$ = Control (no implant) vs. Implant; $C_2$ = Ralgro® vs. Component TE-G®
[a,b,c] Means within the same row with the same letter are not statistically different at a 0.05 level of significance

Table 5. Ear palpation score ~95 d and 126 d post implantation with Ralgro® (R) or Component TE-G® (TEG)

| | Treatments[1] | | | *P*-value[2] |
|---|---|---|---|---|
| Item | Control | R | TEG | Implant |
| No. | 130 | 132 | 130 | |
| Ear Score[4] | | | | |
| D ~95 | | | | |
| Abnormality[5] present | | | | |
| Percent present | 0.0%[a] | 5.3%[b] | 10.0%[c] | 0.01 |
| Number/Total | 0/130 | 7/132 | 13/130 | |
| Palpable Implant | | | | |
| Percent present | 0.0%[a] | 3.8%[b] | 76.2%[c] | <0.01 |
| Number/Total | 0/130 | 5/132 | 99/130 | |
| D 126 | | | | |
| Abnormality[5] present | | | | |
| Percent present | 0.0% | 3.9% | 3.1% | 0.53 |
| Number/Total | 0/130 | 5/132 | 4/130 | |
| Palpable Implant | | | | |
| Percent present | 0.0%[a] | 0.8%[b] | 34.4%[c] | 0.03 |
| Number/Total | 0/130 | 1/132 | 44/130 | |

[1] Treatments include; 1) Control (no implant); 2) Ralgro® (**R**; 36 mg zeranol; Schering-Plough Animal Health Corp., Union, NJ 07083); and 3) Component TE-G®, with Tylan (**TEG**; 40 mg trenbolone acetate (**TBA**), 8 mg estradiol USP, 29 mg tylosin tartrate; Ivy Animal Health, Overaland Park, KS 66214).

[2] Probability of chi square

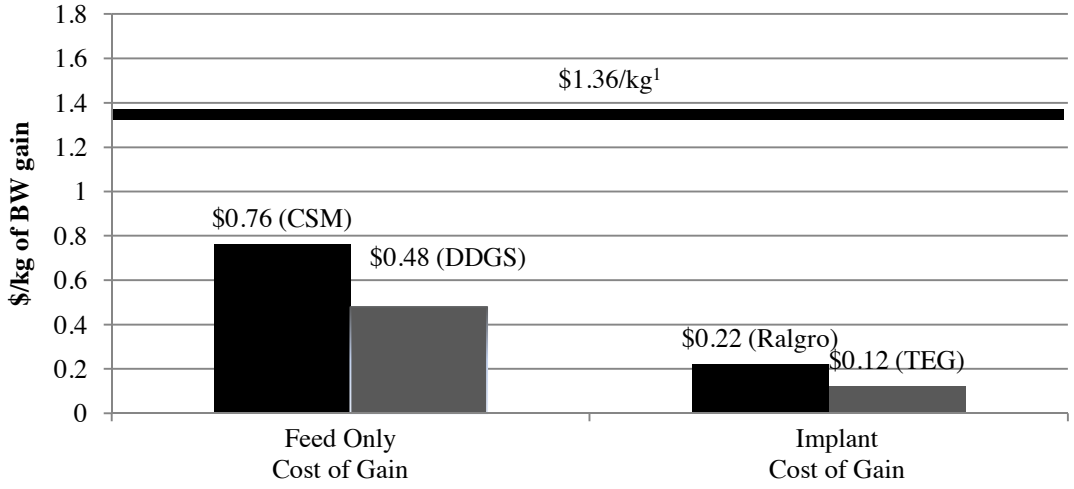
[3] Trt = Implant treatment

[4] 1 = implant present, normal; 2 = implant present, abnormal; 3 = no implant present, normal; 4 = no implant present, abnormal

[5] Abnormality = any blemish found on the ear at the location of implant administration

[a,,b,c] Means within the same row with the same letter are not statistically different at a 0.05 level of significance

**Figure 1. Economic evaluation of implanting and supplementing summer stocker steers**




[1] Average value of gain from 1999 to 2009 during the summer grazing season (Cattle Fax, 2010)

VITA

Casey Paul McMurphy

Candidate for the Degree of

Doctor of Philosophy

Thesis: EFFECTS OF PLANT SAPONINS ON FORAGE DIGESTION AND PERFORMANCE OF GRAZING BEEF CATTLE

Major Field: Animal Nutrition

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Completed the requirements for the Doctor of Philosophy in Animal Nutrition at Oklahoma State University, Stillwater, Oklahoma in May, 2011.

Completed the requirements for the Master of Science in Animal Science at The University of Arizona, Tucson, Arizona in 2007.

Completed the requirements for the Bachelor of Science in Agriculture Business at Oklahoma State University, Stillwater, Oklahoma in 2004.

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- Lecturer/Judging Coach, The University of Arizona, August 2004 – 2007
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- Member of the American Angus and Red Angus Associations
- Member of Oklahoma Red Angus Association

Name: Casey McMurphy Date of Degree: May, 2011

Institution: Oklahoma State University Location: Stillwater, Oklahoma

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The inclusion of Micro-Aid® (MA) in protein supplements improved rumen DM and NDF degradability via a decrease in rumen particulate passage rate, but was not successful at improving apparent total tract digestibility. This improvement in rumen digestion may have the potential to increase the supply of nutrients to the small intestine for subsequent improvements in animal performance. Micro-Aid® successfully suppressed protozoa after continuous administration, over 20 days, suggesting that complete adaptation to saponins by protozoa was not observed. Also, the reduction in protozoa has been shown to increase microbial efficiency (Veira, 1986) which would be beneficial to lactating cows that have a greater metabolizable protein requirement than cannulated steers or even gestating cows. The inclusion of MA did not impact performance of stocker steers or beef cows during the supplementation period. However, MA did improve calf performance when supplementing cows during early lactation. This advantage was lost due to compensatory gain after supplementation ceased, but could prove to be of great importance to a fall-calving cow herd, consuming low-quality forage for its entire lactation period. There was a non-significant increase in milk yield of 2.4% during early lactation, similar to other findings. Feeding frequency of MA needs to be evaluated as a potential role in animal performance. These performance studies intermittently supplied MA as opposed to a continuous supply. This may negatively impact the microflora populations intermittently and never sustain a stable environment. It would also be beneficial to evaluate the use of MA in combination with non-protein nitrogen. With the rising costs of feed protein, this may be an economical option to upgrade the use of non-protein nitrogen in grazing cow diets. More research needs to be conducted to determine the impact of daily provision of MA and its interaction with diet on performance of grazing animals.

ADVISER'S APPROVAL: Dr. David Lalman