EFFECT OF PROTEIN SUPPLEMENT SOURCES ON INTAKE AND DIGESTION OF STEERS FED LOW-QUALITY FORAGE

A Thesis

by

COURTNEY CHANEL STEFAN

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Approved by:

Chair of Committee,	Tryon A. Wickersham
Committee Members,	Jason E. Sawyer
	Larry Redmon
	Steve Riechman
Head of Department,	H. Russell Cross

May 2013

Major Subject: Animal Science

ABSTRACT

Potential protein supplements to grazing cattle were evaluated in two projects. In the first project, Karanja seedcake samples were prepared and ruminally incubated using the in situ method to measure nutrient disappearance in cattle consuming low-quality forage. Organic matter and CP were fractionated by degradability into A, B, and C fractions. Organic matter fraction A degradability ranged from 59.6 to 89% ($P < 0.05$) for all the karanja seedcake samples. Fraction A of CP ranged from 61.6 to 96.2% degradability for all the samples ($P < 0.05$). Karanja seedcake samples were observed to contain highly degradable nutrients and our results indicate karanja seedcake may possibly be utilized in grazing cattle protein supplements, consequently increasing economic sustainability of biofuel production. In the second experiment, four non-protein nitrogen supplements were ruminally infused in steers consuming low-quality forage. Supplements included a 40% CP mineral mix (40MM), 60% CP mineral mix (60MM), 25% CP liquid (25L), or 35% CP liquid (35L). Protein provision stimulated forage OM intake and total OM intake for both liquid supplements. Forage OM intake tended to be greater for the liquid supplements; 25L ($P = 0.06$) and 35L ($P = 0.08$), then control. Total OM intake significantly increased ($P < 0.01$) for both liquid supplements, when compared to the control treatment. Total digestible OM intake was greater ($P < 0.01$) for 25L (3.4 kg/d) and 35L (3.36 kg/d) than control (2.94 kg/d). Forage and total OM intake were not significantly affected by 40MM or 60MM treatments. Total tract digestions (OM and NDF) were not observed to be significantly different ($P \geq 0.11$) between supplements and control. Ruminal ammonia was greater ($P < 0.01$) for all

supplements then control and total ruminal VFA concentrations increased from 84.7 to 98.7 mM for control versus supplemented. Supplementation with liquids had a significant effect on intake. Our results indicate of the four supplements, liquid supplements improved the utilization of low-quality forage by cattle, thus improving nutrient stewardship.

TABLE OF CONTENTS

Page

Page

LIST OF FIGURES

Page

LIST OF TABLES

Page

CHAPTER I
INTRODUCTION AND REVIEW OF LITERATURE

Protein supplements provide the ruminally available N required by ruminal microbes for synthesis of microbial crude protein, which is ultimately used by the host animal to address metabolizable protein requirements. Protein supplementation facilitates increased utilization of low-quality forage by cattle and increases animal performance (Martin and Hibberd, 1990; Hess et al., 1994; Bowman et al., 1995; Mathis et al., 2000; Bandyk et al., 2001; Wickersham et al., 2004). Evaluation and subsequent inclusion of new sources of supplemental protein is increasingly important, due to the increasing costs of traditional supplements. Karanja oilseed cake is a co-product from biofuel production high in CP (~30%; Ravi et al., 2000) supporting further evaluation of it as a protein supplement. Karanja seed comes from a drought and salt tolerant legume tree (*Pongamia pinnata*) growing on marginal soils; reducing the likelihood of it competing with existing food and feed production (Zahran, 1999). Yet, little is known about the nutritive value and the digestibility of karanja, or its ability to be incorporated into U.S. feeding systems.

Liquid and mineral supplements containing NPN (e.g., urea and biuret) have been developed as self-fed protein supplement options. Urea has proven to be beneficial to grazing cattle by improving their ability to address maintenance, reproduction, and growth requirements, through increased utilization of low-quality forage (Fonneskbeck et al. 1975). However, the use of a mineral mixture containing NPN has yet to be extensively researched. Mineral mixes are generally used to prevent or correct mineral

deficiencies, yet mineral supplementation programs are ineffective and uneconomical if basic protein and energy requirements are not addressed first. Intake of low-quality forage is inadequate due to low CP content and limited digestibility. Therefore, consumption of minerals from low-quality forage is likely to be inadequate as well (McDowell, 1996). However, if a mineral mixture containing small amounts of protein was provided, both nutrient deficiencies could be addressed with limited labor and supplement consumption. Similarly, provision of supplement fortified with required minerals could reduce the need for free choice minerals.

Characteristics of Protein

In the feed industry, protein values of feedstuff are reported as CP percentage, which is equal to N × 6.25 (NRC, 1996). Dietary CP can be fractionated by degradability of the protein in the rumen; thus giving us the terms degradable intake protein (DIP) and undegradable intake protein (UIP; NRC, 1996).

Undegradable intake protein is also commonly referred to as escape or bypass protein as microbes are incapable of utilizing it. Metabolizable protein (MP) is defined as the true protein absorbed by the intestine and is supplied by UIP and microbial crude protein (MCP). Microbial crude protein can supply 50 to 100% of the MP required (NRC, 1996). Undegradable intake protein addresses MP requirements by allowing the animal the first opportunity for enzymatic digestion and not allowing direct availability to the microbes. Undegradable intake protein is enzymatically digested and absorbed in the intestine. Once absorbed, UIP is transported to the liver where catabolism (producing carbon skeletons and ammonia) or anabolism (synthesizing proteins) occurs. Carbon

skeletons can be used to increase energy (glucose) production and availability, and ammonia can be detoxified to urea and recycled or excreted. Undegradable intake protein is capable of indirectly supplying N to the microbes through urea-N recycling.

Degradable intake protein is the portion of the CP that is not protected, but is directly degraded by ruminal microbes and includes both true protein and NPN (e.g. urea and biuret). Degradable intake protein directly supplies ruminally available N (RAN) to microbial populations that require a source of N to facilitate growth and VFA production. The NRC (1996) recommends 13% of TDN be delivered as DIP to meet microbial requirements for RAN. Addressing this requirement likely maximizes MCP production. However, this recommendation does not account for urea recycling and studies involving the consumption of low-quality forage by cattle have shown DIP requirements for maximizing forage utilization to be 11, 9, and 13.2% of TDN (Köster et al., 1996; Klevesahl et al., 2003; Wickersham et al., 2004; respectively). Additional work is required to solidify this requirement and the variables affecting it.

Response to Protein Supplementation

Nutritive value is used to indicate a feedstuff's contribution to addressing ruminal and animal requirements. Chemical analyses have been developed to quantify nutrient content (chemical composition) and in vitro and in situ procedures are used to describe digestibility. When a basal diet of low-quality forage is fed, evaluation of fermentable organic matter (energy) and protein are particularly important, as these are the primary drivers of animal performance.

Low-quality forage is utilized world-wide for ruminant production and includes dormant forages and crop residue. Wide availability of low-quality forage underscores the importance of evaluating available nutrients and accounting for the effects of forage quality on the response to protein supplementation. Low-quality forage is typically characterized by a high fiber content, low crude protein (< 7% CP), and limited digestibility (NRC, 1996). Ultimately, a basal diet of low-quality forage limits digestible energy intake and MP availability, culminating in decreased animal performance.

Low-quality forage contains a significant amount of TDN or potentially fermentable OM (energy), mainly from carbohydrates. Cattle grazing these types of roughages typically do not have sufficient voluntary intake to meet their nutrient requirements. Protein supplementation to cattle consuming low-quality forage effectively stimulates forage utilization and enhances animal performance (Hess et al., 1994; Bowman et al., 1995; Mathis et al., 2000; Bandyk et al., 2001; Wickersham et al., 2004). Protein supplementation allows more nutrients to be "unlocked" from the low-quality forage consumed, by providing a source of RAN. Increases in RAN benefits the microbes and increases microbial growth and fermentative activity. Increased fermentation increases voluntary forage intake, typically observed with N provision (Figure 1). Ultimately, protein supplementation stimulates intake of fermentable OM, improves energy status by increasing VFA production, and protein status by increasing microbial flow to the duodenum (DelCurto et al., 1990; Köster et al., 1996; McCollum and Galyean, 1985; Scott and Hibberd, 1990; Wickersham et al., 2008a). A relationship

between protein and energy exists, and balancing the requirements for each nutrient improves microbial fermentative capacity and ultimately, forage utilization.

Research on protein supplementation is often conducted as intake and digestion projects to allow for a number of comparisons and to limit the cost of research projects. Data from these projects is best summarized as total digestible OM intake (TDOMI) as this accounts for both intake and digestion, two of the critical responses to supplementation. As previously mentioned, low-quality forage is often singularly characterized by low CP. Yet, if CP and OM digestibility are combined, forage quality is able to be more completely described by it's TDN:CP or OMD:CP ratio, this value accounts for both available energy and CP. Organic matter digestion is a comparable measure to TDN with both being a measure of the energy availability. A better description of forage quality can yield more insight to various responses elicited by protein supplementation.

A lower OMD:CP ratio indicates the diet contains sufficient amounts of CP for the available fermentable OM, allowing microbial fermentation of OM to occur at a sufficient rate without supplemental protein. Thus, when supplemental protein is added, TDOMI response is lessened (Figure 2). Though not as encompassing, when forage nutritive value is expressed only as CP (Figure 3), similar TDOMI responses to protein supplementation are observed. Forages with adequate CP (> 7%) do not appear to benefit from a protein supplement, particularly supplements with large proportions of DIP (Moore et al., 1999). If forage CP is adequate enough to satisfy microbial and host requirements, protein supplementation has minimal value and energy supplementation

should be considered to improve animal performance. Butterworth et al. (1973) observed supplementation was most effective during the winter months when availability of quality forage was limited compared to high-quality forage in spring. Mathis et al. (2000) demonstrated the response to protein supplementation has limits, in part, set by the characteristics of the forage being consumed. Whether the nutrients (energy and protein) are provided adequately by the low-quality forage basal diet alone or need to be supplied by a supplement, once requirements are met responses to additional nutrients are diminished or non-existent.

As OMD:CP ratio increases, potential fermentable OM increases, but is not fermented because of limited availability of CP for microbial utilization. Thus, when forages of lower CP and high fermentable OM are fed, utilization of low-quality forage is increased to a greater extent with protein supplementation. DelCurto et al. (1990) determined the low-quality forage (2.9% CP) intake response in steers supplemented with energy and protein at various inclusions. Steers fed a high protein supplement with either low or high energy had greater forage intake over control steers. However, steers fed a low protein and high energy supplement, had reduced intake compared to control. Decreased forage intake may have resulted from a deficiency in protein being exacerbated with energy. These observations are consistent with Elliott (1967) who reported increased hay intake when protein was provided, but decreased intake when energy was supplemented.

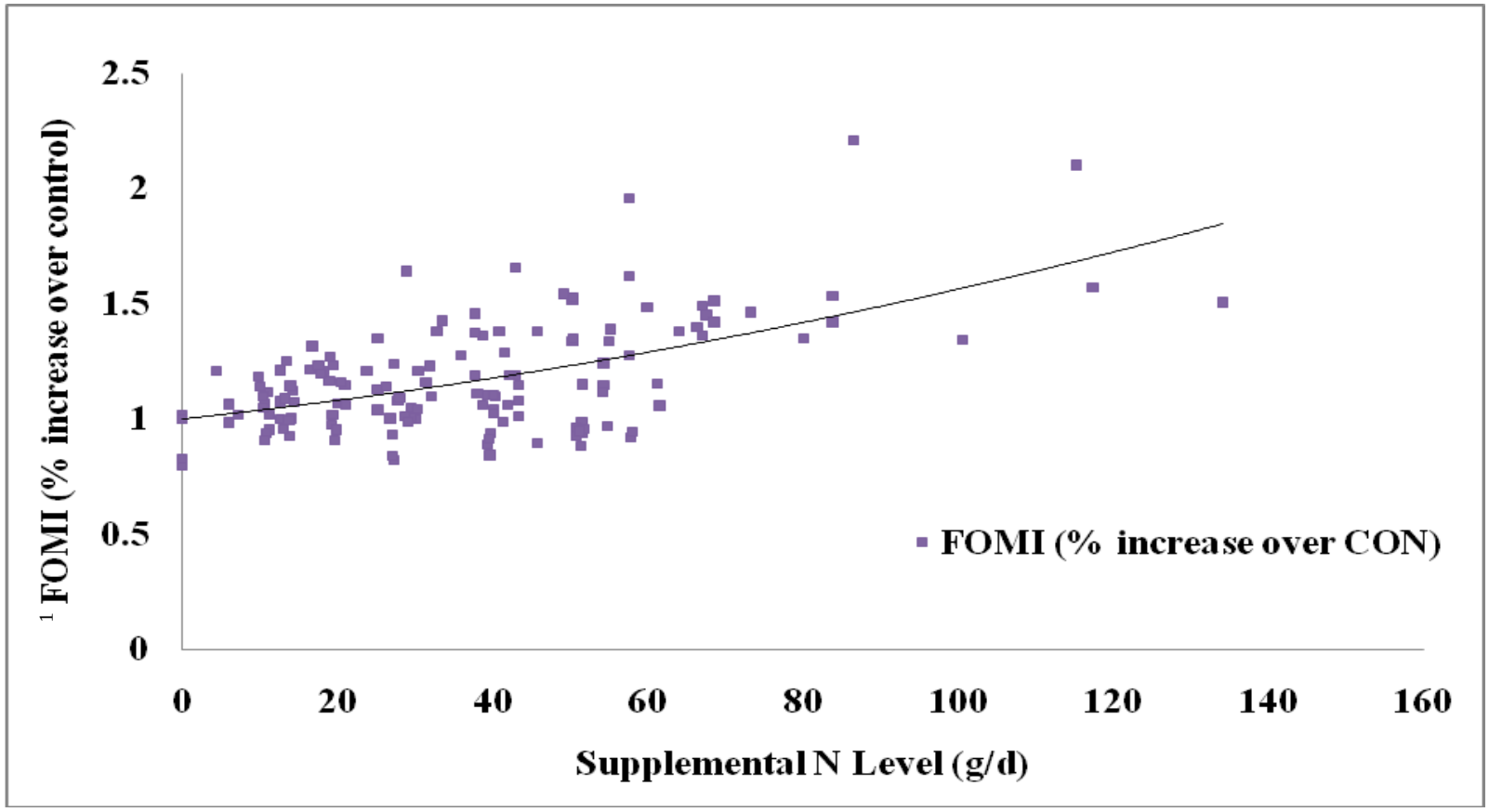

Figure 1. Summary of protein supplementation studies on the influence of supplemental N on forage OM intake as a percent increase over corresponding control treatment.

[1]Forage organic matter intake

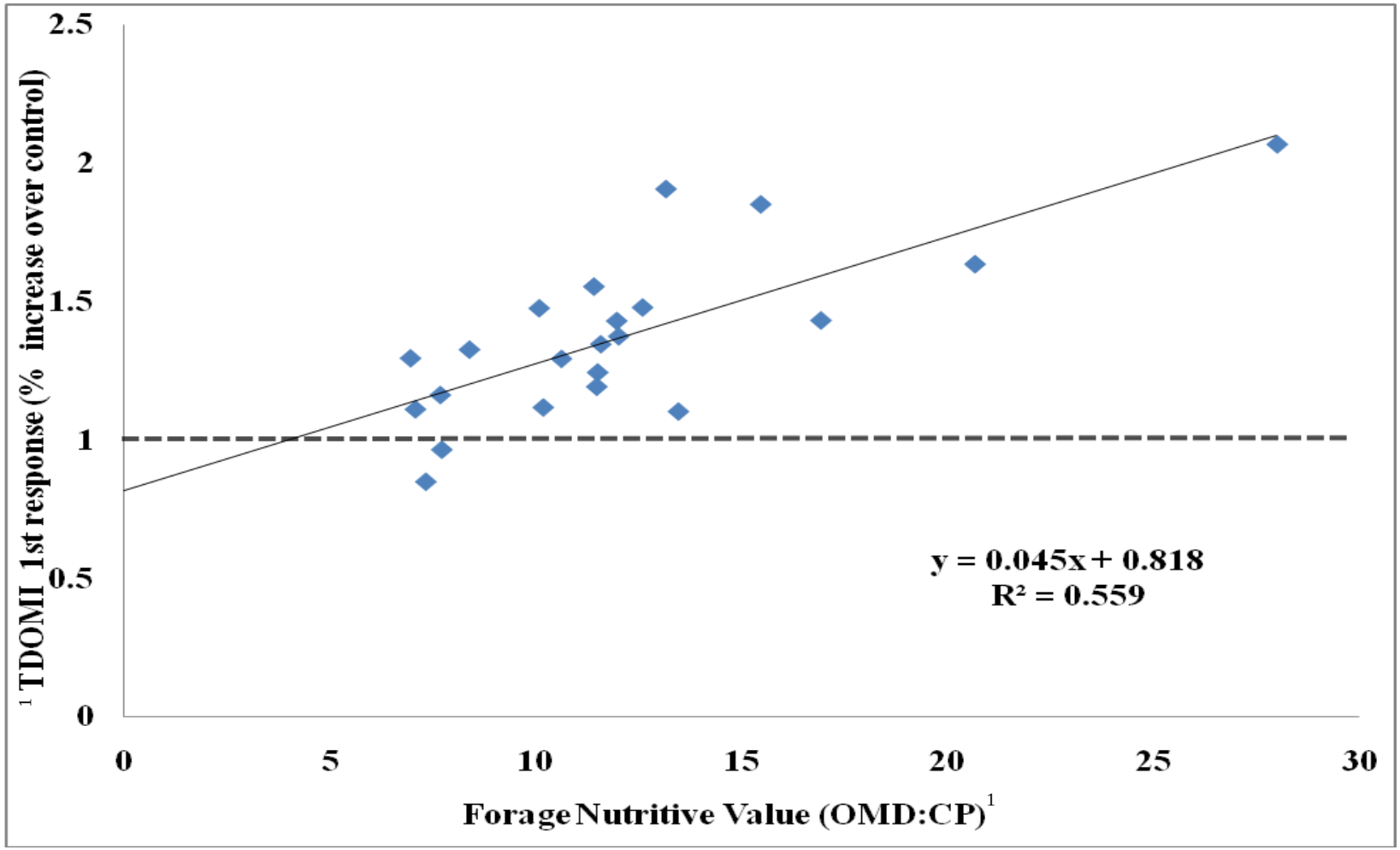

Figure 2. Summary of protein supplementation studies on the effect forage OMD:CP ratio has on first total digestible OM intake response to supplemental N as a percentage increase over their corresponding control treatments.

[1] TDOMI: Total digestible organic matter intake; OMD: Organic matter digestion; CP: Crude protein

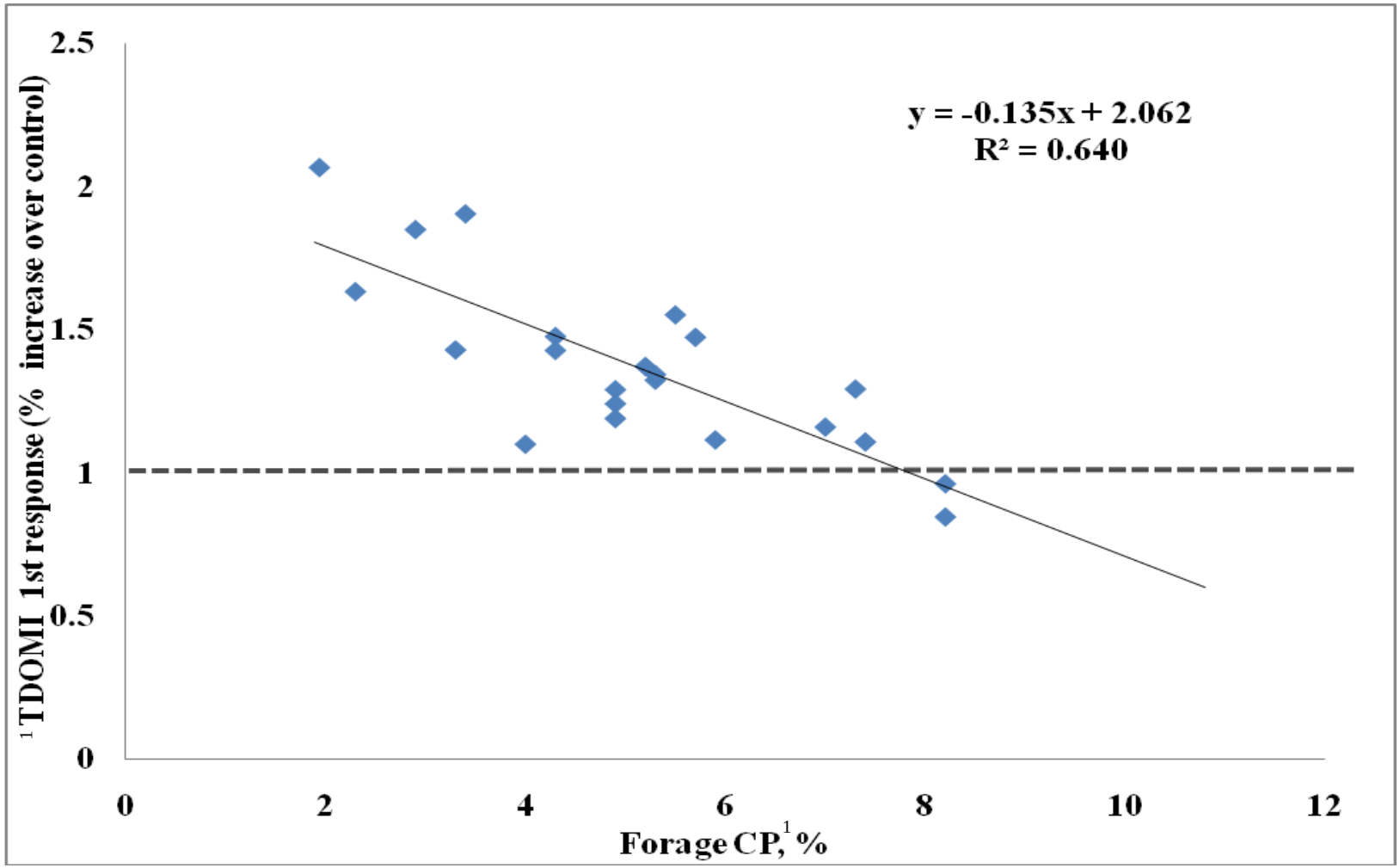

Figure 3. Summary of protein supplementation studies on the effect forage CP % has on first total digestible OM intake response to supplemental N as a percentage increase over their corresponding control treatments.

[1] TDOMI: Total digestible organic matter intake; CP: Crude protein

Increasing energy supply without a concomitant increase in protein supply does not typically increase forage utilization (Heldt et al., 1999; Olson et al., 1999; Klevesahl et al., 2003; Schroedger and Titgemeyer, 2007). This concept is supported by Figure 2; as supplemental energy would drive the OMD:CP ratio further to the right, creating a larger response surface for supplemental protein to increase fermentation.

Provision of supplemental protein to a basal forage with a high OMD:CP ratio; moves the OMD:CP ratio to the left, creating a smaller response surface for the next level of supplementation. This relationship has been documented as a quadratic increase in TDOMI to protein supplementation in multiple studies (Köster et al., 1996; Mathis et al., 2000; Wickersham et al., 2004). As additional increments of supplementation are added, forage utilization increases, albeit at a decreasing rate (Figure 4), until the requirement is reached. Trials evaluating levels of protein supplementation consistently report large increases in intake (at lower levels of N provision), which plateaus in response to higher levels of N provision (Hess et al., 1994; DelCurto et al., 2000; Wickersham et al., 2008b).

In contrast to the previously described quadratic relationship between forage utilization and protein supplementation, Wickersham et al. (2008a) reported linear responses in OM intake and TDOMI when steers were provided increasing levels of casein. Similarly, Olson et al. (1999) observed increases in TDOMI in response to various mixtures of casein and starch. These studies are examples of the DIP threshold not being reached by adding supplemental protein to low-quality forage diets. It is likely, had additional increments of supplementation been added, a quadratic effect would have

been observed. Whether the nutrients are provided by the forage (Figure 2) or a protein supplement (Figure 4), once requirements are met additional provision shows minimal improvement.

Non-Protein Nitrogen

Non-protein N supplements (e.g., urea- or biuret-containing) are beneficial to grazing cattle by providing fiber fermenting microbes with a source of ammonia-N, ultimately improving the utilization of low-quality forage (Toppo et al., 1997; Löest et al., 2001; Currier et al., 2004a, b, c). Currier et al. (2004a) reported improved OM intake in steers provided either urea (29.5 g total OM intake/kg BW) or biuret (29.5 g total OM intake/kg BW) over negative control steers (24.8 g total OM intake/kg BW). In a subsequent study, Currier et al. (2004b) ruminally infused the same NPN sources, and affirmed their initial discovery, as total OM intake increased when urea (17.7 g total OM intake/kg BW) or biuret (18.1 g total OM intake/kg BW) was compared to control (16.1 g total OM intake/kg BW). In another study, steers fed prairie hay (5.5% CP) and supplemented with either a urea molasses block or urea plus biuret molasses block, had increased forage utilization over the control (Löest et al., 2001). Intake and digestion parameters increased, which resulted in TDOMI of 53.1 and 50.7 (g/kg BW $^{0.75}$) for urea and urea plus biuret, over control (34.2 g/kg BW $^{0.75}$). However, Köster et al. (1997) compared diets formulated to provide increasing amounts of urea (0, 25, 50, 75, and 100% of the supplemental DIP) and a linear decrease in TDOMI was observed as supplemental DIP from urea increased, due to a decrease in OM intake and digestion.

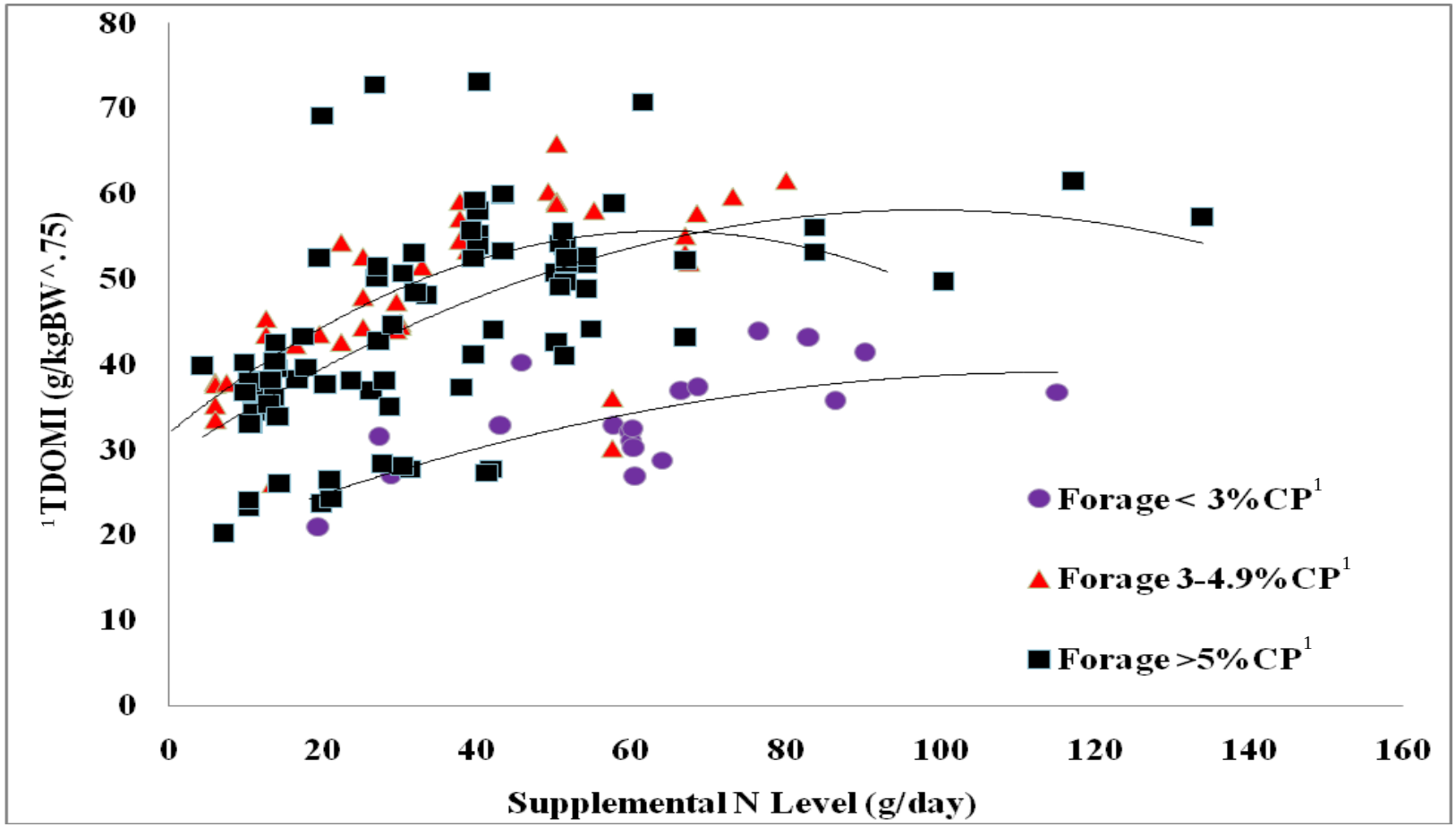

Figure 4. Summary of protein supplementation studies on the influence of supplemental N on total digestible OM intake response; fractioned by forage CP%.

1 TDOMI: Total digestible organic matter intake; CP: Crude protein

Responses such as these are likely due to urea being rapidly hydrolyzed to ammonia before the microbes could utilize the N (DelCurto et al., 2000). Addition of rapidly fermentable carbohydrates may allow for great capture of N and improve the effectiveness of urea provision. When NPN sources (typically urea) are incorporated, rapid hydrolysis of urea to ammonia may prevent optimum utilization of N and can result in urea toxicity.

Risk of toxicity can be reduced by synchronization of carbohydrate and N fermentation in the rumen (Huntington and Archilbeque, 1999) which would provide a source of readily available energy and protein. Biuret (slow-release urea) can more closely match the slow fermentative digestion rate of low-quality forage because of the low solubility of biuret. It is hydrolyzed to ammonia and CO_2 slower than urea (Ioset, 1969), which reduces the risk of toxic accumulation of ammonia in the blood (Fonnesbeck et al., 1975); consequently making biuret a safer option as well as, synchronizing OM fermentation and protein availability.

Microbes utilize ammonia N to synthesis amino acids for growth and maintenance, provided fermentable energy and carbon skeletons are available. Low-quality forages provide insufficient RAN to meet these requirements alone. Köster et al. (1996) reported ruminal ammonia concentration of 0.24 m*M* for unsupplemented steers, with other studies reporting comparable values of 0.34, 0.55, 0.33 and 0.27 m*M* for ruminants consuming basal diets of low-quality forage (Mathis et al., 2000; Bandyk et al., 2001; Wickersham et al., 2004 and 2008a, respectively). Ruminal ammonia N concentrations of 1.4 to 3.6 m*M*, established by Satter and Slyter (1974), are generally

accepted as the microbial “requirements”. However, these observations are from an in vitro project and need to be interpreted with caution.

Degradable intake protein is responsible for increased ruminal ammonia N concentrations because it provides a direct source of N to the ruminal microbes. Aforementioned data indicates low-quality forage fed with no supplementation results in ruminal ammonia N concentrations less than the 1.4 m*M*. Mathis et al. (2000) reported linear increases in ammonia N concentration with increasing quantities of DIP. Köster et al. (1996) reported similar findings with ammonia N concentrations that increased incrementally up to 6.87 m*M* for the highest level of casein provision. Oltjen et al. (1969) provided urea or biuret into a roughage diet and ruminal ammonia N was increased to 10.9 and 9.8 m*M*, respectively.

Undegradable intake protein can increase ruminal ammonia N via the recycling of urea N when intestinally absorbed amino acids or mobilized tissue amino acids are catabolized, resulting in ammonia being detoxified to urea and recycled. However, dependence on the recycling of UIP-N to meet ruminal ammonia is less effective than direct provision of DIP. Bandyk et al. (2001) reported ammonia N concentrations of 1.35 m*M* with UIP and 4.16 m*M* with DIP, compared to control; 0.55 m*M*. Similarly, Wickersham et al. (2004) reported DIP and UIP supplementation to a low-quality forage diet resulting in increased ruminal ammonia concentrations of 2.67 and 0.91 m*M* respectively, over control (0.52 m*M*).

Increases in ruminal ammonia can increase fermentation and as a result, we would expect total VFA concentrations to increase. Köster et al. (1996) observed an

increase in total VFA concentrations, with the largest increase occurring in response to the initial DIP infusion level. Similarly, Olson et al. (1999) observed total VFA concentration increases with increasing inclusion of casein. Increases in VFA concentrations are reported when DIP provision occurs due to microbes receiving supplemental N, which increases their fermentative activity; leading to increases in microbial production of VFA.

When provided as true protein, DIP provides precursors for branched-chain VFAs. However, both Köster et al. (1997) and Farmer et al. (2004) reported decreases in branch chain VFA (BCVFA) concentrations in response to urea supplementation. When supplemental protein is provided by NPN sources, branch chain amino acids (BCAA) are not provided, and BCAA are precursors to BCVFA.

While total VFA concentration increases in response to protein, changes in individual VFA concentrations vary in response to protein supplementation. Acetate tends to decline, while propionate increases, and butyrate slightly increase or remained generally unchanged; in response to increasing DIP provision (Köster et al., 1996; Mathis et al., 2000; Wickersham et al. 2008b). Wickersham et al. (2004, 2008a) observed linear decreases in molar proportions of acetate, along with increases in isobutyrate, valerate, and isovalrate in response to DIP provision. Bandyk et al. (2001) reported similar responses to ruminal infused protein in individual VFA concentrations. Protein supplementation increases total VFA concentrations and provokes various responses from individual VFAs.

Ruminal pH tends to decrease in response to protein supplementation (Oltjen et al., 1969; Hennessy and Nolan, 1988; DelCurto et al., 1990; Martin and Hibberd, 1990; Olson et al., 1999; Mathis et al., 2000; Wickersham et al., 2008b). If the rumen experiences a moderate depression in pH (approximately 6.0), a small decrease in fiber digestion can occur. Further decreases to 5.5 or 5.0 result in a depressed microbial growth rates and decreased fibrolytic microbial concentrations, eventually causing fiber digestion to be completely inhibited (Hoover, 1986). Mould et al. (1983) found cellulolysis started to be inhibited at a pH of 6.3, with total inhibition occurring when the ruminal pH fell below 6.0. "Cellulolysis threshold" is pH 6.0-6.1 and anything below that value would result in a negative response in ruminal cellulolytic activity (Mould et al., 1983). Köster et al. (1996) described a general decrease in ruminal pH with increasing levels of DIP; 6.92 to 6.52 for 720 g/d of DIP and control, respectively. It was thought ruminal fermentation continued to increase because all values were well within acceptable levels for cellulolytic bacteria. Similarly, Olson et al. (1999) witnessed a linear reduction in average ruminal pH levels when supplemented diets were compared to control (6.65 to 6.25). However, many studies do not witness any evidence towards changes in the ruminal pH (McCollum and Galyean, 1985; Bandyk et al., 2001; Currier et al., 2004c; Wickersham et al., 2004). Mathis et al. (2000) found no significant changes of pH in two out of three trials conducted, with the third showing only slight reductions. When low-quality forage is consumed, a decrease in pH when DIP is provided indicates increased fermentation and it is highly unlikely for ruminal pH to drop enough to hinder fiber fermentation.

Nitrogen Recycling

Ruminal N can come from a two sources, dietary and endogenous. Dietary sources of N are ingested, and the DIP fraction is degraded where amino acids can be incorporated into MCP or deaminated. Ammonia resulting from amino acid deamination is either incorporated into MCP or absorbed through the epithelium and transported to the liver, via the blood. Microbial crude protein and UIP are digested and absorbed from the small intestine and enter the portal blood for transport to the liver. Catabolism of amino acids produces a waste product, ammonia, which is detoxified to urea. In the large intestine, undigested proteins may be used by the microbes and produce ammonia which is absorbed and transported to the liver, and any N left is destined for excretion via fecal output.

Amino acids can either supply precursors for protein anabolism (tissue synthesis, enzyme, hormone, or metabolite synthesis) or undergo protein catabolism to supply carbon skeletons and ammonia N. Carbon skeletons can be used as an energy source and the ammonia N is detoxified to urea via the ornithine cycle. Once the N is in the form of urea, it can either be recycled or sent to the kidneys (in times of excess), where it can be excreted in the urine.

Urea can be recycled back into the saliva, the large intestines, or back into the rumen where it is immediately broken down upon diffusion to ammonia N by membrane-associated urease. Recycled N is integrated and can be utilized in the same manner, and has the same fate, as other sources of RAN. Urea recycled back through saliva eventually enters the rumen as a source of RAN.

Nitrogen recycling is increased by a number of factors; dietary N intake, dietary energy density, ruminal fermentation, salivary secretion rates, ruminal ammonia concentrations, OM availability, ruminal pH, blood ammonia or urea concentrations, and urease concentrations and activities. When low-quality forage is fed, N recycling becomes essential for ruminants to be able to meet microbial and host requirements. Consumption of a low-quality forage diet is the ideal situation for N recycling to be increased because of the availability of fermentable OM and low ruminal ammonia concentrations allowing the ammonia gradient to increase affinity for N recycling to the gut rather than being excreted as urea (Hammond, 1992; Wickersham et al., 2008a). The NRC (1996) describes a deficiency of ruminal ammonia encourages N recycling, and when ruminal ammonia is in excess of utilization, ammonia absorption from the rumen is encouraged.

Kennedy and Milligan (1978) fed a low-quality diet of brome grass to sheep and found a decreased ammonia concentration was accompanied by an increase in ruminal ammonia N being derived from recycled urea. Increases in OM and fermentation potential led to an increase in the N required by the microbes. Since the diet could not provide the required amount of N, the urea being recycled back into the gut increased to meet demands. Bunting et al. (1989) fed a low (66.5 g N/d) and high (126.1 g N/d) protein diet to beef heifers and reported findings indicating lower RAN was accompanied by a greater proportion of blood urea N (BUN) used in the rumen of the heifers given the low dietary protein treatment. There was an increased use in recycled

urea because of the escalated need for N due to increased OM combined with inadequate dietary protein intake, which was unable to satisfy the microbial needs.

Marini and Van Amburgh (2003) showed a linear increase in urea recycling to saliva with salivary urea N concentrations increasing from 1.0 to 10.0 m*M* for the control diet and supplemented diet, respectively. Yet, this effect was accompanied by decreased rate of bacterial usage of recycled N as the supplement increased. Likely, microbes were receiving enough N from dietary sources and needed less N from recycled urea. When urea was ruminally infused, the increased ruminal ammonia levels caused by the additional dietary N, decreased usage of ammonia N derived recycled N (Kennedy and Milligan; 1978). However, Wickersham et al. (2008a) reported increased urea recycled to the gut from 19.8 g of N/d for unsupplemented steers to 48.6 g of N/d for those receiving the highest provision of DIP. Even with protein supplementation, it is difficult to achieve ammonia levels limiting the ammonia gradient's pull and therefore; N recycling typically increases with protein supplementation. Ruminal ammonia levels necessary to limit the transfer of urea to the rumen were quantified by Vercoe (1969), who noted ruminal ammonia levels in cattle consuming low-quality forage. Ruminal ammonia levels of 3.79 to 4.3 m*M* were found to limit transfer of recycled urea through the rumen wall. Nitrogen recycling is an essential process allowing ruminants to increase forage utilization of low-quality forage.

Mineral Mixture Supplementation

Non-protein N (urea and biuret) are readily utilized as self-fed supplements. Similar to true protein sources, NPN improve production of grazing cattle through

increased utilization of low-quality forage (Fonneskbeck et al. 1975). Supplement programs including mineral mixes to correct deficiencies are ineffective and uneconomical if basic nutrient requirements are not addressed prior to program application. Because of this, if a mineral mixture containing a source of protein was fed, multiple nutrient deficiencies could be addressed with a single application, which would decrease labor and supplement consumption. Currently, there is limited research involving supplementing mineral mixes associated with NPN as a source of protein. Their effect on forage utilization by cattle consuming low-quality forage is still largely unknown.

Karanja

The "karanja" plant is a legume tree (*Pongamia pinnata*) originally from Asia; particularly India, produces an oilseed. This legume tree is drought and salt tolerant. Like all legume species, it has a symbiotic relationship with "rhizobia" bacteria which is responsible for N fixation (Biswas et al., 2011), and allows karanja to thrive in N poor soil conditions. Karanja's ability to thrive on marginal land reduces the likelihood of competing with existing crop land (Zahran, 1999). Karanja seed contains approximately 27 to 39 % oil with the furanoflavonoids making up 5 to 6 % (by weight) of the oil (Bringi and Mukerjee, 1987). Within the oil, karanja oilseed contains anti-nutrient components (such as glabrin, pongamol, tannins, and karanjin, a furanoflavonoid), making it unpalatable and toxic (Vinay and Kanya, 2008). Several studies have shown this significantly reduces feed intake and nutrient balance when animals are fed karanja seedcake meal (Vinay and Kanya, 2008; Singh et al., 2006; Soren et al., 2009). Yet,

numerous detoxification approaches have been developed to improve this co-product, karanja seedcake, to make it suitable for inclusion into ruminant diets (Vinay and Kanya, 2008). Recently, karanja seed oils have been utilized for biofuel production and the oilseed cake (co-product which is high in CP; ~30%; Ravi et al., 2000) has gained interest as a possible protein supplement, due to the increasing costs of traditional supplements, which are tied to the value of corn. However, nutritive value and digestibility of karanja, or its ability to be incorporated into large ruminant diets, is still largely unknown.

Several detoxification processes, including solvent extraction, water leaching, autoclaving, acid and alkali treatments, and expeller presses (Vinay and Kanya, 2008) enhanced palatability and decreased toxicity of karanja seedcake meal. Some detoxification methods have proven to be more efficient than others at removing undesirable toxins; such as those reported by Ravi et al. (2000). Lambs were fed a control diet, a diet containing expeller pressed seedcake (EKC), or solvent extracted karanja seedcake meal (SKC). An overall decrease in nutrient digestibility in the animals fed the EKC was observed, except for ether extract digestibility, which was most likely increased due to the increased oil content that accompanied the EKC meal. Average daily gain was negatively affected by EKC (48.8 g) when compared to the control (60.5 g). They concluded SKC could be fed up to 20% of a concentrated diet with no adverse effects on nutrient utilization, nutrient balance, growth rate, or feed conversion efficiency for up to 98 days (Ravi et al., 2000).

Similarly, Singh et al. (2006) also found ADG, BW, and DMI to be significantly decreased when EKC was fed to lambs. It was concluded SKC was superior to EKC; most likely because the toxic compounds are contained within the oil fraction and due to solvent detoxification causing a more complete extraction of the oil.

Conclusion

Ruminal available N requirements (for the microbes) and MP requirements (for the host) are not typically met by low-quality forage. Protein supplementation facilitates increased utilization of low-quality forage by cattle and increased animal performance (Bowman et al., 1995; Mathis et al., 2000; Wickersham et al., 2004). As long as low-quality forage is going to be utilized in an attempt to satisfy these two requirements, supplemental protein will be considered a necessity to optimize nutrient utilization of low-quality forage. Therefore, the objectives of our research are to established nutritive values and in situ degradation characteristics of various karanja seedcakes, and determine their value in cattle supplements. Additionally, we will determine the efficacy of four NPN-based protein supplements, all designed to be self-fed, for stimulating forage utilization in cattle consuming low-quality forage.

CHAPTER II

DETERMNING NUTRITIVE VALUE OF KARANJA (*PONGAMIA PINNATA*) SEEDCAKE TYPES FOR POTENTIAL SUPPLEMENTATION TO CATTLE

Synopsis

Karanja (*Pongamia pinnata*), is a drought-resistant nitrogen-fixing legume producing an oilseed currently being evaluated as a source of bio-diesel. Oil is extracted from the seed, resulting in a meal with potential as a protein supplement. Karanja contains anti-nutrient components (such as glabrin, pongamol, tannins, and karanjin; a furanoflavonoid) in its oil making it unpalatable and toxic. Nutritive value data and characterization of protein and OM degradability of karanja meal is limited. Accordingly, we determined nutritive value and in situ degradation characteristics of karanja meal. Karanja seedcake meal samples were: ISR (source: India, crusher: Willamette Biomass Processors Inc.; raw) and ISD (detoxified), IER (source: India, crusher: Komet DD 85 expeller; raw), and IED (detoxified), AER (source: Australia, crusher: expeller; raw), and AED (detoxified). Crude protein for raw samples ranged from 22.3 to 27.5% CP with the detoxified samples ranging from 21.4 to 27.2% CP. Karanja was highly degradable with 95.5, 91.0, and 83.2% DIP for ISR, IER, and AER; respectively, and decreasing to 90.5, 88.5, and 79.4% DIP for ISD, IED, and AED; respectively. Gross energy ranged from 4622 to 5674 cal/g, likely due to the various crushing methods. Crude protein and OM were fractionated into A, B, and C pools. Organic matter fraction A degradability ranged from 59.6 to 89% ($P < 0.05$) for all the karanja seedcake samples. Fraction A of CP ranged from 61.6 to 96.2% degradability for

all the samples ($P < 0.05$). Karanja seedcake is a source of highly degradable nutrients and our results indicate karanja seedcake may possibly be utilized in grazing cattle protein supplements, consequently increasing economic sustainability of biofuel production. Additional research is needed to test the palatability and optimal inclusion rate for cattle consuming low-quality forage.

Introduction

Supplemental protein is used to provide ruminally available nitrogen (RAN) required by ruminal microbes. Ruminally available N is necessary for synthesis of microbial crude protein, the primary source of metabolizable protein. Increased utilization of low-quality forage by cattle is commonly observed when protein supplementation occurs and ultimately leads to increases animal performance (Mathis et al., 2000; Wickersham et al., 2004). Investigations of new supplemental protein sources are increasingly important, as the cost of traditional supplements increases. Karanja oilseed cake (a co-product from biofuel production) is characteristically high in CP (~30%; Ravi et al., 2000), supporting its evaluation as a protein supplement.

Originating throughout Asia, karanja seed comes from a drought and salt tolerant legume tree, *Pongamia pinnata* (L), growing on marginal soils and typically does not complete for existing production land (Zahran, 1999). Yet, little is known about the nutritive value and digestibility of karanja seedcake, or its ability to be incorporated into U.S. feeding systems. Karanja oilseed contains anti-nutrient components (such as glabrin, pongamol, tannins, and karanjin; a furanoflavonoid) in the oil, making the seedcake unpalatable and toxic (Vinay and Kanya, 2008) if it contains significant

quantities of oil. Karanja seeds contain approximately 27 to 39% oil with furanoflavonoids representing up to 5 to 6% (by weight) of the oil (Bringi and Mukerjee, 1987). Presence of the furanoflavonoids significantly reduces feed intake, growth, overall health, and nutrient balance when animals are fed karanja seedcake meal (Vinay and Kanya, 2008; Singh et al., 2006; Soren et al., 2009). Therefore, detoxification methods have been developed in efforts to improve karanja to make it suitable for inclusion into ruminant diets.

There is limited research involving the use of karanja seedcake meal as a source of protein in cattle. Nutritive value and digestibility has not been well established and further research is needed. Therefore, our objectives were to determine nutritive value and in situ degradation characteristics of karanja seedcake.

Materials & Methods

The experimental protocol was approved by the Institutional Animal Care and Use Committee at Texas A&M University, and included the use of anesthesia when surgical procedures were performed.

Karanja seedcake samples were obtained from two different locations, India (I) and Australia (A) and subjected to two different crushing processes, screw press (S) and expelled (E). The screw press was located at Willamette Biomass Processors Inc. and was an unmodified horizontal screw press using a double press method with an electric heating element within the press cage. The expeller used was located at the Texas AgriLife Research Center in Lubbock, TX and was a Komet DD 85 expeller. Samples were divided and a portion of each was frozen at -20°C while the remainder was ground

with a Wiley mill (No. 4 Wiley Mill, Thomas Scientific; Swedesboro, NJ) to pass a 3-mm screen. A 1000 g was then mixed with 1000 ml of a 1.5% sodium hydroxide solution and allowed to dry at 55°C in a forced-air oven for 96 h to produce detoxified (D) seedcake (Panda et al., 2006), whereas raw (R) was not treated. Accordingly, samples represented were India screw pressed raw (ISR) and detoxified (ISD), India expelled raw (IER) and detoxified (IED), and Australia expelled raw (AER) and detoxified (AED). Detoxified and raw samples of each karanja seedcake meal were subsequently ground to pass a 2-mm screen for the in situ procedure. A portion of the samples were ground to pass a 1-mm screen for chemical analysis. To facilitate grinding, all samples were ground with pelleted dry ice.

Nutritive Value Determinations

Samples were dried in a forced-air oven at 105°C for 24 h for DM determination. Determination of OM was made by combusting samples in a muffle furnace at 450°C for 8 h. An Elementar Rapid N Cube (Elementar; Hanua, Germany) was used to measure N and CP was calculated as N × 6.25. Analysis for NDF and ADF was performed using an Ankom Fiber Analyzer (Model 200, ANKOM Technology; Macedon, NY) with sodium sulfite and amylase omitted and without correction for residual ash. SDK Laboratories (Hutchinson, KS) determined ether extract using the Goldfish Fat Extraction method with petroleum ether. Total starch, ether extract, and acid hydrolysis fat was also measured by SDK Laboratories (Hutchinson, KS) using α-amylase glucosidase method and acid hydrolysis, respectively. Gross energy content was determined using an adiabatic bomb calorimeter (Parr 1356, Parr Instrument Company; Moline, IL). Lignin

content was measured following the procedures outlined for a Daisy Incubator (Ankom Technology Corp., Macedon, NY). Condensed tannins were measured by Dr. Lambert at Texas Agri-Life Research (Stephenville, TX) using the procedures outlined by Wolfe et al. (2008). Complete macro and trace mineral analysis was performed on the karanja seedcake meal and included calcium (Ca), phosphorus (P), sulfur (S), potassium (K), magnesium (Mg), sodium (Na), zinc (Zn), copper (Cu), Iron (Fe), Aluminum (Al), Cobalt (Co), Molybdenum (Mo), Chromium (Cr), Nickel (Ni), and Manganese (Mn).

Degradable and undegradable intake protein was determined using *Streptomyces griseus* with the in vitro protease procedures (Mathis et al., 2001). These observations were compared to in situ protein degradabilities. In-vitro true digestibility (IVTD) was quantified using a Daisy Incubator (Ankom Technology Corp., Macedon, NY). Briefly, samples were incubated for 48 h in a mixture of buffer solution, rumen fluid, and CO_2 (pH = 6.8) with constant agitation at 39.5°C. Rumen fluid was obtained from a steer fed a diet of prairie hay, supplemented daily with cottonseed meal.

In Situ Procedures

Three ruminally-cannulated steers were used to determine in situ CP and OM disappearance of meal samples. Steers were adapted to a forage diet and supplemented with 0.5 kg of cottonseed meal delivered twice daily (Table 1) for 14 d prior to incubating the samples in the rumen. Five grams of each sample was placed in a Dacron bag (10 × 20cm; Ankom Technology Corp.; Macedon, NY) and subsequently heat sealed. Bags were pre-incubated in tepid water (39˚C), placed in a weighted mesh polyester bag (36 × 42 cm), and were allowed to incubate in the rumen for 4, 6, 12, 24,

48 and 72 h, respectively. To facilitate accurate time of incubation, bags were inserted in reverse order of incubation length (i.e., 72-h bags first). Samples from each time point were run in duplicate and replicated across animal. At removal, bags were subjected to 10 cold water rinses in a top-loading washing machine with each cycle consisting of 1 minute agitation and 2 minutes spin. Zero hour bags were not incubated, but were subjected to pre-incubation in tepid water and the rinsing processes. Bags were dried to a constant weight at 60°C in a forced-air oven, then allowed to air equilibrate for 24 h to determine dry matter disappearance. Residue was sub-sampled for DM determination and then combusted for 8 h at 450˚C in a muffle furnace for OM determination. Nitrogen remaining in the bags was determined by total combustion of the residue using an Elementar Rapid N Cube (Elementar; Hanua, Germany) and CP was calculated as N × 6.25.

Calculations

Ruminal degradation was described by the 1st-order disappearance model without time delay. Protein, OM, and DM were fractionated into A, B, and C fractions, where A = CP, OM, or DM instantaneously degraded after the rinsing process (0 h); B = potentially degradable intake CP, OM, or DM (4 – 48 h); and C = undegradable intake CP, OM, or DM escaping the rumen (72 h). The B fraction was calculated as 100% - A - C = B. To calculate the degradability of the B fraction, the proportion of the protein, OM, or DM classified as B was multiplied by kd / (kd + kp), where kd represents the rate of degradation measured as the slope of the regression of the natural logarithm of nutrients remaining over time and kp equals rate of passage 3% / h.

Table 1. Diet composition fed to trial steers.

Items	CSM[1]	Hay
Organic matter	91.4	93.5
Crude protein	28.9	6.2
Neutral detergent fiber	24.5	72.7
Acid detergent fiber	11.0	37.6

[1] CSM: cottonseed meal.

Statistical Analysis

The SAS System for Windows Release 9.2 (SAS Inst. Inc.; Cary, NC) was used for statistical analysis. In situ data was analyzed using the PROC MIXED procedure. Terms in the model included detoxification and location, with bag being the random term. Means for each location and standard deviations within location were calculated using the LSMEANS option.

Results & Discussion

Organic matter was decreased by an average of 2% following detoxification (Table 2). This reduction in OM is largely attributable to the addition of sodium (Table 3), which was added to the meal as sodium hydroxide during detoxification. Panda et al. (2006) reported 1.5% sodium hydroxide was effective at reducing anti-nutrients such as, karanjin, tannins, and trypsin inhibitors. Detoxification did not produce any appreciable changes in CP. However, method of oil extraction appeared to be a primary driver of CP as ISR and ISD contained more protein 27.5 and 27.2% CP, respectively then the other four sample types 22.3, 23.5, 21.4, and 25.3% CP for IER, AER, IED, AED, respectively. Our CP values are similar to those reported in the literature, which range

from 25 to 34% CP (Konwar et al., 1987; Gupta et al., 1981; Viney and Kanya, 2008; Soren and Sastry 2009; Nagalakshmi et al., 2011). In accordance, both measures of lipid content, ether extract and acid hydrolysis fat, were substantially less, 10.9 to 13.3% for ISD and ISR, than the other four sample types (≥ 22.7%). As expected, this difference in the lipid content resulted in gross energy values greater (> 5,371 cal/g) for IER, AER, IED, and AED, then for ISR and ISD (< 4,622 cal/g). Greater lipid content would be expected to increase the value of karanja seedcake due to the increased availability of energy. However, greater lipid content negatively affects palatability and increases the risk of toxicity due to anti-nutrients in the oil fraction. Currently, an impediment to utilizing karanja seedcake as a supplement is the need for removal of karanjin and other anti-nutritive compounds that reduce acceptability.

Table 2.Effect of source and detoxification on the nutritive value of Karanja seedcake.

Samples[1]	ISR	IER	AER	ISD[2]	IED[2]	AED[2]
Item						
Dry matter	91.8	94.8	91.7	89.4	91.8	95.7
Organic matter	95.9	96.7	96.5	94.0	95.0	94.1
Crude protein	27.5	22.3	23.5	27.2	21.4	25.3
Protein fractions, % CP[3]						
Degradable intake protein	95.5	91.0	83.2	90.5	88.5	79.4
Undegradable intake protein	4.4	8.9	16.7	9.4	11.4	20.5
Starch	6.7	6.2	6.7	6.8	6.4	5.5
In vitro true DM Digestion[4]	90.4	87.3	92.7	87.4	89.2	89.3
Fiber chemistry						
Neutral detergent fiber	12.0	22.5	14.6	14.0	13.6	18.7
Acid detergent fiber	6.8	18.0	9.1	8.2	7.7	9.4
Lignin	0.41	0.34	0.22	0.34	0.29	0.30
Condensed tannins	1.35	1.51	1.11	1.43	1.41	1.01
Gross energy, cal/g	4622	5674	5371	4485	5470	5582
Fat analysis						
Ether extract	12.8	28.2	26.2	10.9	29.1	27.9
Acid hydrolysis fat	13.3	28.5	22.7	11.7	29.1	28.3
US dollars/tonne[5]	335.5	421.3	420.1	336.2	421.0	429.9

[1]Used unmodified horizontal screw press using a double press method with an electric heating element within the press cage (Willamette Biomass Processors Inc) and a Komet DD 85 expeller (Texas AgriLife Research Center in Lubbock, TX). Karanja seedcake meal samples: India screw pressed raw (ISR) and detoxified (ISD), India expelled raw (IER) and detoxified (IED), and Australia expelled raw (AER) and detoxified (AED).

[2] Detoxification Method: 1000 g of Karanja seedcake mixed with 1000 mL 1.5% sodium hydroxide solution and dried at 55°C for 4 d.

[3] Degradable intake protein for hay was determined by Streptomyces griseus in vitro protease procedure.

[4] In vitro true DM digestion was determined by incubating samples for 48 h in a mixture of buffer, rumen fluid, and CO_2 with constant agitation at 39.5°C.

[5] Values for karanja samples calculated using a hedonic model (Bryant et al., 2012). Value was determined without accounting for the contribution of fat.

Table 3.Effect of source and detoxification on the mineral composition of Karanja seedcake.

Samples[1]	ISR	IER	AER	ISD[2]	IED[2]	AED[2]
Item						
Macro-minerals, % DM[3]						
Calcium	0.53	0.40	0.53	0.54	0.39	0.37
Phosphorus	0.39	0.30	0.41	0.40	0.3	0.36
Potassium	0.96	0.77	0.99	1.02	0.74	0.97
Magnesium	0.16	0.13	0.16	0.16	0.12	0.18
Sodium	0.01	0.03	0.02	0.91	0.65	0.94
Sulfur	0.28	0.22	0.28	0.29	0.22	0.25
Micro-minerals, ppm[3]						
Aluminum	13.6	14.0	7.17	15.6	17.6	5.3
Cobalt	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2
Copper	14.9	12.3	14.7	15.2	11.7	14.5
Iron	60.3	41.2	49.1	61.8	42.6	59.0
Manganese	29.5	24.8	29.7	29.9	22.8	41.6
Molybdenum	0.5	0.43	0.59	0.57	0.36	< 0.3
Zinc	37.3	30.9	37.3	39.1	30.4	56.0
Chromium	0.5	0.4	0.3	0.6	0.5	0.7
Nickel	3.0	3.2	3.8	3.7	3.1	2.5

[1]Used unmodified horizontal screws press using a double press method with an electric heating element within the press cage (Willamette Biomass Processors Inc) and a Komet DD 85 expeller (Texas AgriLife Research Center in Lubbock, TX). Karanja seedcake meal samples: India screw pressed raw (ISR) and detoxified (ISD), India expelled raw (IER) and detoxified (IED), and Australia expelled raw (AER) and detoxified (AED).

[2] Detoxification Method: 1000 g of Karanja seedcake mixed with 1000 mL 1.5% sodium hydroxide solution and dried at 55°C for 4 d.

[3] DM: Dry matter; ppm: parts per million

To date, most of the research on karanja seedcake has been conducted in India where expelling or pressing is the predominant process to extracting oil, similar to the processes used to generate samples for our project. Typically, oil extraction via this common method, leaves 15 to 20% oil in the seedcake (Murphy et al. 2012); a range that more closely represents the lipid content of our samples. Singh et al. (2006) reported expeller pressed karanja seedcake was not acceptable as a feedstuff and subsequent data supports this conclusion (Nagalakshmi et al., 2011).

However, a closer look revealed solvent extracted or deoiled karanja seedcake inclusion resulted in performance comparable to control treatments. Dutta et al. (1993) concluded 10% inclusion of deoiled karanj seedcake into a concentrate mixture was safe for dairy cows, and no unwanted effects were observed when solvent extracted karanj seedcake was included at 20% in a grain mix (Ravi et al., 2000). Similarly, Konwar et al. (1987) observed no difference in gain when deoiled karanja seedcake was included at a rate of 17%. In agreement, Gupta et al. (1981) reported no adverse effects on nutrient utilization when deoiled karanj seedcake made up 16% of the ration in growing calves. Seedcake in Konwar et al. (1987) and Gupta et al. (1981) contained 0.33 and 0.4% ether extract, respectively; which are in contrast to our high lipid content, 10.9 to 29.1%. Because karanjin is extracted with the oil (Viney and Kanya, 2008), these trials suggest solvent extraction offers the most acceptable path to feeding karanja seedcake. More complete extraction will increase the suitability and value of karanja seedcake as a protein supplement by diminishing toxicity concerns and increasing the CP content.

Additionally, extraction of a greater percentage of the lipid will increase biofuel yield per ton of harvested seed, further increasing the economic vitality of biofuel production.

Protein degradability was described in two ways, in vitro protease and in situ with both methods yielding similar results. Protein degradability using the protease procedure was greater in raw samples, 95.5, 91.0, 83.2 % for ISR, IER, and AER, respectively; than detoxified samples, 90.5, 88.5, and 79.4 % for ISD, IED, and AED, respectively (Table 2). In accordance with this observation, protein degradabilities determined by the in situ method were also greater for the raw versus detoxified samples; 96.4, 92.9, 79.5% for ISR, IER, and AER decreasing to 91.8, 83.7, and 72.5 % for ISD, IED, and AED (Table 4). In a collaborative project across multiple labs, Mathis et al. (2001) also found strong agreement between the two methods used in our project to determine protein degradability.

Table 4. Effect of source and detoxification on the digestibility of Karanja seedcake.

Samples[1]	ISR	IER	AER	ISD[2]	IED[2]	AED[2]
Item						
Organic Matter, %						
Fraction A[ab]	89.0	73.0	59.6	86.8	70.5	60.0
Fraction B[abc]	5.4	21.7	35.5	9.8	25.3	34.6
Fraction C[abc]	5.6	5.3	4.9	3.4	4.1	5.3
Fraction B kd, %/ hr[a]	3.3	4.7	3.2	2.6	2.8	3.5
Estimated Degradability, total[b]	91.8	86.1	76.8	91.2	82.8	77.9
Crude Protein, %						
Fraction A	96.2	90.8	67.2	90.1	78.4	61.6
Fraction B	0.6	6.0	29.9	7.4	16.7	32.2
Fraction C	3.1	3.1	2.8	2.6	4.9	6.2
Fraction B kd, %/ hr	0.48	1.53	2.18	0.92	1.45	1.63
Estimated Degradability, total	96.4	92.9	79.5	91.8	83.7	72.5

[1]Used unmodified horizontal screws press using a double press method with an electric heating element within the press cage (Willamette Biomass Processors Inc) and a Komet DD 85 expeller (Texas AgriLife Research Center in Lubbock, TX). Karanja seedcake meal samples: India screw pressed raw (ISR) and detoxified (ISD), India expelled raw (IER) and detoxified (IED), and Australia expelled raw (AER) and detoxified (AED).

[2] Detoxification Method: 1000 g of karanja seedcake mixed with 1000 mL 1.5% sodium hydroxide solution and dried at 55°C for 4 d.

[a] Detoxification effect at $P < 0.05$

[b] Sample effect at $P < 0.05$

[c] Detoxification × Sample interaction at $P < 0.05$

Protein degradability determined by either method was greater in this project than anticipated. Paul et al. (1995) conducted a trial using the in situ method to determine degradability of multiple unconventional feed ingredients and observed an average CP degradability of 31.1% for deoiled karanj cake, with only 19.6% being degraded by 3 h and 45.6% by 20 h. In stark contrast to the observed A fraction in our project (61.6 to 96.2% A), the A fraction for soybean meal and cottonseed meal are 15 and 26.5%, respectively (NRC, 2001). The largest fraction for both conventional supplements is B for soybean and cottonseed meal, (84.4 and 55.5%; respectively). In contrast, our B fractions ranged from 0.6 to 32.2%. Rate of degradability for the B fraction of soybean and cottonseed meal were 7.5 and 6.8% / h, respectively, while the rate of degradability for our samples was found to be slower with and range from 0.48 to 2.18% / h. Chocolate byproduct (11.9% CP) is most comparable in its protein fractionation to karnaja with fractions of 74.1, 25.9, and 0.0% for A, B, and C (respectively; NRC, 2001). Along with protein degradability, OM degradability was highly degradable, with A fraction being the majority and the degradability ranging from 76.8 to 91.8%. Similarly, in vitro true DM digestion was high for our samples; 87.3 to 92.7%.

All measures of nutrient degradation within our samples were higher than expected and likely the result of a combination of factors. Samples from India, ISR, IER, ISD, and IED, were more degradable than samples from Australia, AER and AED. This difference could be attributable to differences in growing environment or tree genetics. Additionally, samples may have been processed differently in each country to facilitate

importation into the U.S. for extraction. Samples originating in India also differed in degradability when extracted using a screwpress versus the expeller. Higher lipid content and differences in particle size, is likely explained by oil extraction procedures and may explain the lower degradabilities in IER and IED versus ISR and ISD, due to a more complete extraction of oil, and consequently higher degree of initial break down of oilseed nutrients prior to receiving the sample. The oil nature of our samples required grinding procedures to be more aggressive and involved simultaneously grinding pelleted dry ice. It is likely the aggressive nature of the process contributed to producing smaller particle size, leading to high nutrient availability observed. According to Vinay and Kanya (2008), the natural state of karanja proteins could also likely be a factor with reports karanja seed proteins may not be complex, or bound to any constituents by glycosidic or ester linkages; therefore making them easily digestible, or in our case degradable.

Detoxification decreased OM degradability of samples originating from India; 91.8 and 86.1% for ISR and IER, respectively; compared to 91.2 and 82.8% for ISD and IED, respectively. Karanja seedcake was mixed with 1000 mL 1.5% sodium hydroxide solution and dried at 55°C for 4 d. It is possible that Maillard products may have formed during the drying process. Maillard reaction occurs at temperatures greater than 50 to 60°C and decrease the availability of nutrients (Van Soest et al., 1982). Additionally, CP degradability could have been affected by detoxification exposing samples to sodium hydroxide solution. In agreement, alkali treatment was seen to effectively protected protein from degradation in the rumen (Mir et al., 1984). Furthermore, Viney and Kanya

(2008) stated alkali treatments would lead to decreased nutritive value of proteins due to racemization, leading to partial formation and destruction of amino acids.

Our samples Ca concentrations ranged from 0.37 to 0.54% with P concentrations ranging from 0.41 to 0.30%, which is similar to previous studies measuring karanja nutritive value (Ravi et al. 2000; Soren and Sastry, 2009). Compared to soybean meal and cottonseed meal, which both display inverted ratios, karanja seedcake possesses a suitable Ca:P ratio for a protein supplement, with the exception of AED.

Highest and lowest average monetary values for the karanja seedcake samples over time is depicted in Figure 5 (Bryant et al., 2012). Values were calculated without contribution from fat because if karanja seedcake were to be fed to livestock, complete lipid extraction would be a necessity to remove toxins. As karanja seedcake increases in CP, it is expected to result in higher values because it is priced similarly to soybean meal. While residual oil increases the monetary value of karanja seedcake, according to the hedonic model used, in reality the oil toxins would significantly reduce (if not prevent) the use of karanja seedcake; thus solvent extraction using hexane is recommended for preparation of feeding to live animals on trial.

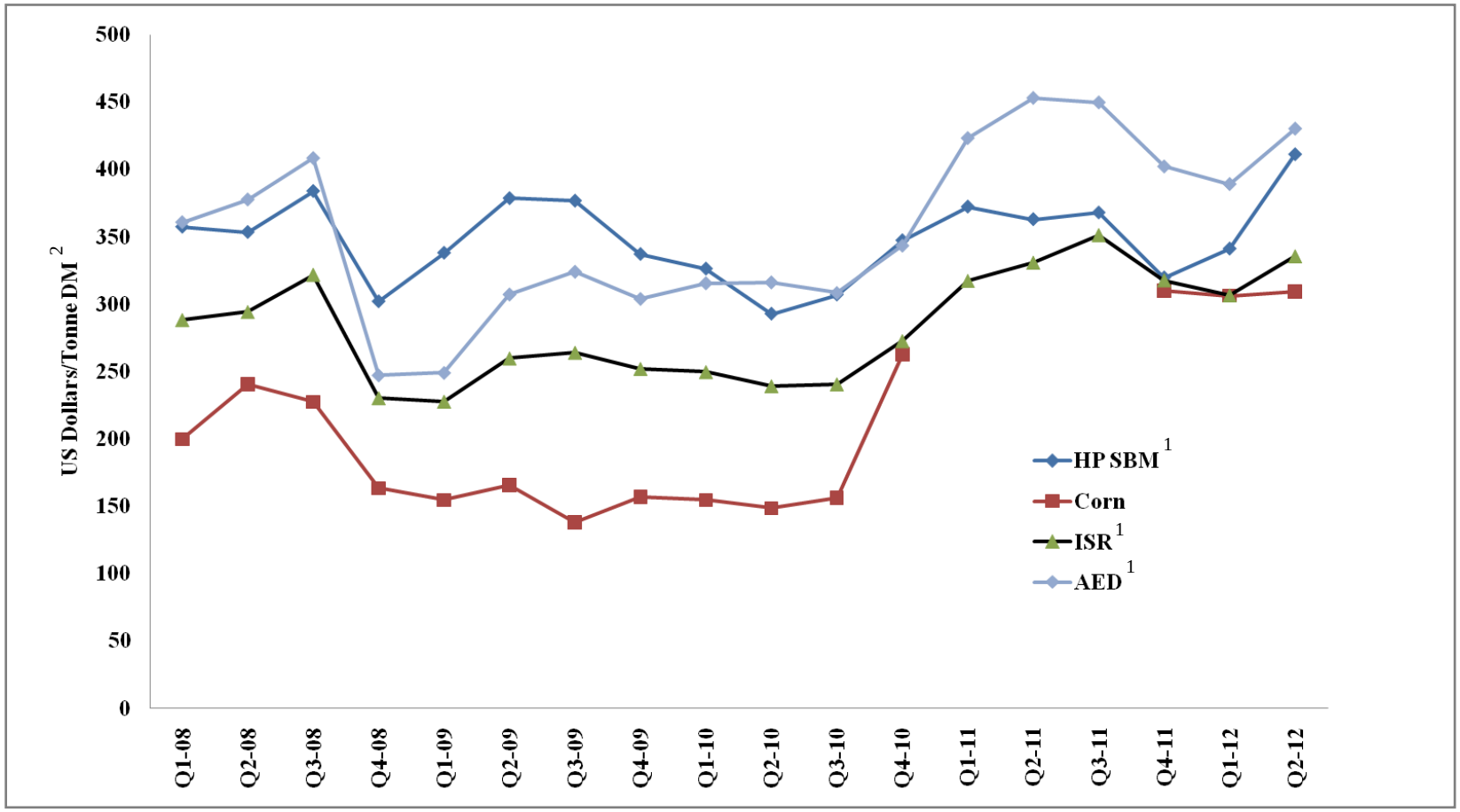

Figure 5. Hedonic values of specific Karanja samples compared to common protein sources.

[1] AED: Australia expelled detoxified; ISR: India screw-pressed raw; HP SBM: high protein soybean meal

[2] Values for karanja samples calculated using a hedonic model (Bryant et al., 2012). Value was determined without accounting for the contribution of fat.

Conclusion

Overall, karanja contains highly degradable nutrients readily available to livestock. However, extraction of the toxin containing oil fraction of karanja seedcake is necessary for inclusion into ruminant diets. Our data suggest nutritive values and degradability of the karanja seedcake meal varies due to extraction and detoxification methods. Research suggests solvent extraction to be the most effective, yet additional research is needed to test the palatability and optimal inclusion rate for cattle consuming low-quality forage. Karanja seedcake will likely contribute to the overall economic sustainability of biofuel production from karanja.

CHAPTER III

EVALUATION OF NON-PROTEIN NITROGEN BASED PROTEIN SUPPLEMENTS TO ENHANCE LOW QUALITY FORAGE UTILIZATION

Synopsis

We evaluated the efficacy of four NPN-based protein supplements for stimulating intake and digestion in cattle consuming low-quality forage. Five ruminally-cannulated Angus steers (initial BW 238 kg ± 9.1 kg) were used in a 5 × 5 Latin square and provided ad libitum access to low-quality forage (6.8% CP). Supplements were infused directly into the rumen prior to feeding and included a 40% CP mineral mix (40MM), 60% CP mineral mix (60MM), 25% CP liquid (25L), or 35% CP liquid (35L). Mineral mixes were provided at 114 g/d and liquids were administered at approximately 310 g/d. Periods were 14 d long with 8 d for adaptation to treatment and 6 d for sample collection. Forage OM intake tended to be greater for 25L ($P = 0.06$) and 35L ($P = 0.08$) then control. Total OM intake significantly increased ($P < 0.01$) for both liquid supplements, when compared to the control treatment. Total digestible OM intake was greater ($P < 0.01$) for 25L (3.4 kg/d) and 35L (3.36 kg/d) than control (2.94 kg/d). Neither forage nor total OM intake were significantly affected by 40MM or 60MM treatments. There were no significant differences ($P \geq 0.11$) between supplements and control for total tract digestion which ranged from 56.0 – 58.6% for OM and 55.8 – 59.2% for NDF. Ruminal ammonia was greater ($P < 0.01$) for all treatments then control, 0.33 m*M* versus 1.06, 1.96, 1.71, and 2.09 m*M*, for 40MM, 60MM, 25L, AND 35L; respectively. Total ruminal VFA concentrations increased from 84.7 to 98.7 m*M*

for control versus supplemented ($P < 0.06$). Supplementation with liquids had a significant effect on intake. Further investigation is warranted to discern if supplements would elicit a greater response if lower-quality forage was provided.

Introduction

Protein supplementation to cattle consuming low-quality forage (< 7% CP) is a routine production practice to increase voluntary forage intake, improve fiber digestion and ultimately, improve animal performance (Lee et al., 1987; Mathis et al., 2000; Wickersham et al., 2008). Köster et al. (1996) demonstrated the "first-limiting" factor to utilization of low-quality forage is degradable intake protein (DIP). Trials evaluating elevating levels of protein supplementation have consistently reported an increased intake (at lower levels of N provision), which plateaus in response to higher levels of N provision (Köster et al., 1996; DelCurto et al., 2000; Wickersham et al., 2008b).

Sources of NPN (urea, biuret, and other forms of slow-release urea) stimulate forage utilization (Fonneskbeck et al. 1975; Earley et al., 1999) by supplying a source of ruminally available N (RAN). Supplementing a mixture of NPN with a source of readily available carbohydrates, couples protein and energy supplementation to improve N utilization (Bowman et al., 1995). Toppo et al. (1997) demonstrated the efficacy of this model when they reported an increased total digestible OM intake (TDOMI) for cattle consuming low-quality forage with a urea-molasses supplement (1.88 kg/d) when compared to control (1.32 kg/d).

Mineral mixes are commonly provided to prevent or correct mineral deficiencies in grazing cattle. However, the incorporation of NPN into a mineral mixture has only

been explored by few (Coombe et al., 1971; Wu et al., 2005); neither of which involved cattle grazing low-quality forage. Blending of NPN and mineral supplements may prove to be a cost effective means of addressing not only mineral requirements, but also protein deficiencies.

Accordingly, this study was designed to determine the efficacy of four NPN-based protein supplements, two mineral mixes and two liquids designed to be self-fed supplements, for increasing forage utilization.

Materials & Methods

The experimental protocol was approved by the Institutional Animal Care and Use Committee at Texas A&M University, and included the use of anesthesia when surgical procedures were performed.

Five ruminally-cannulated Angus steers (average initial BW 238 kg ± 9.1 kg) were used in a 5 × 5 Latin square. Effects of four NPN-based protein supplements were determined on forage utilization and ruminal fermentation parameters. Steers were housed in an enclosed, climate controlled barn with continuous lighting and were provided ad libitum access to fresh water, a commercial trace mineral block (composition: ≥ 96.0% NaCl, 1.00% S, 0.15% Fe, 0.25% Zn, 0.30% Mn, 0.009% I, 0.015% Cu, 0.0025% Co, and 0.001% Se, United Salt Corp.; Houston, TX), and low-quality prairie hay (Table 5). Hay was fed once daily at 0600 h at 130% of the average voluntary intake of the previous 5 d to ensure that access to forage was not limited. Prairie hay was chopped through a 76 × 76 cm mesh screen prior to feeding.

Treatments were arranged in a 1-way structure with four treatments plus a negative control. Treatments consisted of four unique protein supplements; 40% CP mineral mix (40MM), 60% CP mineral mix (60MM), 25% CP liquid (25L), and 35% CP liquid (35L; Table 5 and 6). All supplements were dosed directly into the rumen just prior to feeding to ensure complete consumption. Mineral mixes were provided at 114 g and liquid supplements were provided at 1.3 g/kg BW (approximately 310 g/d). Level of mineral mix provision was based on estimated intake of 114 g/d for a grazing animal. Levels of liquid supplementation were based on estimated intake of 0.65 kg/day for a 500 kg cow. Ratios of N to sulfur in the supplements were below 10:1, with the exception of 60MM (18:1). Experimental periods were 14 d long, including 8 d for adaptation to treatment and 6 d for sample collection. Throughout all five periods, steers were housed in individual pens (2.1 m × 1.5 m). Calculations of intake and digestion were made from observations on d 9 through 13. Hay, supplement, and ort samples were collected beginning on d 9 through 12 to correspond to fecal grab samples which were collected on d 10 through 13. Hay and supplement were sampled as they were fed, with approximately 300 g of hay being retained and 50 g of each supplement being retained for analysis. Orts were removed and weighed and approximately 300 g of each were retained and dried at 55°C for 96 h prior to analysis. Fecal grab samples (approximately 300 g on wet basis) were collected every 8 h starting at 0 h on 10 d and advanced 2 h each day through 13 d. Fecal samples were immediately frozen at -20°C. On 14 d, ruminal fluid samples were collected by suction strainer just before treatments were administered (0 h) and at 4, 8, 12, 16, and 20 h after feeding. Immediately after

sampling, ruminal pH was determined using a portable pH meter with a combination electrode (Symphony, VWR; Radnor, PA). Subsamples of ruminal fluid were prepared by combining 8 mL of rumen fluid and 2 mL of 25% m-phosphoric acid for analysis of VFA and ruminal ammonia concentrations.

Table 5.Chemical composition of forage and supplements.

Items	Prairie Hay	40MM[1]	60MM[1]	25L[1]	35L[1]
Dry matter, %	91.4	96.5	95.9	51.2	55.7
	----------% DM----------				
Organic matter	92.6	30.2	41.7	81.1	81.5
Crude protein	6.8	42.8	76.9	51.0	61.0
Degradable intake protein (%CP)[2]	58	98	98	85	90
Undegradable intake protein (%CP)[2]	42	2	2	15	10
Neutral detergent fiber	71.5	–	–	–	–
Acid detergent fiber	36.5	–	–	–	–

[1] 40MM = received 114 g of 40% crude protein mineral mix, 60MM = received 114 g of 60% crude protein mineral mix, 25L = received 1.3g/kg BW of 25% crude protein liquid, 35L = received 1.3g/kg BW of 35% crude protein liquid

[2] DIP for prairie hay was determined by Streptomyces griseus in vitro protease procedures (Mathis et al., 2001), and was calculated for the supplements based on the ingredients values from the NRC, (1996).

Table 6. Mineral composition of protein supplements.

Items	40MM[1]	60MM[1]	25L[1]	35L[1]
	----------% DM[2]----------			
Macro-minerals (%)				
Calcium (Ca)	18.7	15.4	0.0	0.2
Phosphorus (P)	4.4	4.4	2.0	1.8
Potassium (K)	0.2	0.2	0.6	1.4
Magnesium (Mg)	0.6	0.6	0.6	0.6
Sodium (Na)	4.5	4.8	3.4	2.0
Sulfur (S)	0.7	0.7	1.9	1.6
Micro-minerals (ppm)[2]				
Aluminum (Al)	2400.0	2400.0	–	–
Cobalt (Co)	36.5	36.5	8.9	8.1
Copper (Cu)	886.0	886.0	404.5	369.9
Iron (Fe)	5607.2	5606.4	711.6	884.2
Manganese (Mn)	2402.5	2402.5	1487.6	1351.8
Zinc (Zn)	3405.2	3405.2	1185.7	1076.2

[1] 40MM = received 114 g of 40% crude protein mineral mix, 60MM = received 114 g of 60% crude protein mineral mix, 25L = received 1.3g/kg BW of 25% crude protein liquid, 35L = received 1.3g/kg BW of 35% crude protein liquid
[2] DM: Dry matter; ppm: parts per million

Laboratory Analysis

Fecal and ort samples were composited for each animal within each period. Representative samples of the hay and supplements were composited on an equal weight basis within each period. Hay, orts, and fecal samples were dried at 55°C in a forced-air oven at for 96 h, air-equilibrated, and weighed to determine partial DM. All samples were ground with a Wiley mill (No. 4 Wiley Mill, Thomas Scientific; Swedesboro, NJ) to pass a 1-mm screen for chemical analysis. Dry matter of hay, orts, fecal, and supplements were determined by drying for 24 h at 105°C in a forced-air oven, and OM was determined as loss in dry weight upon combustion for 8 h at 450°C in a muffle

furnace. Hay, orts, and supplements were analyzed for N using an Elementar Rapid N Cube (Elementar; Hanua, Germany) and CP was calculated as N × 6.25. Analysis for NDF and ADF were performed using an Ankom Fiber Analyzer (Model 200, ANKOM Technology Corp.; Fairport, NY) with sodium sulfate and amylase omitted and without correction for residual ash. To determine acid detergent insoluble ash (ADIA) of hay, orts, supplements, and fecal samples, Ankom bags containing ADF residues were combusted for 8 h at 450°C in a muffle furnace.

Ruminal fluid samples were thawed and centrifuged at 20,000 × *g* for 5 min. Ruminal VFA concentrations were determined using gas chromatography as described by Vanzant and Cochran (1994). Colorimetric determination of ruminal ammonia concentrations were measured using a UV/VIS (Sigma Diagnostics; St. Louis, MO) as described by Broderick and Kang (1980).

Calculations

Total tract digestion coefficients for DM, OM, and NDF were calculated, using the procedures described by Cochran and Galyean (1994) using the formula: [1-(output of nutrient/ intake of nutrient)] × 100. Acid detergent insoluble ash was used as an internal marker to calculate total fecal output by determining ADIA fed and dividing by ADIA in the fecal.

Statistical Analysis

Intake and digestion were analyzed using the MIXED procedure of SAS (SAS Inst. Inc.; Cary, NC). Terms in the model included treatment and period with steer included as a random effect. Preplanned contrasts were used to determine significance

and are: 1) control vs 40MM, 2) control vs 60MM, 3) control vs 25L, 4) control vs 35L. Treatment means was calculated using the LSMEANS option. Ruminal fermentation parameters were analyzed using the MIXED procedure of SAS. The repeated term in this model were hour, with treatment × steer serving as the subject and the same contrasts as above were used.

Results

Forage OM intake (FOMI) was greater ($P \leq 0.08$) for the 25L (5.56 kg/d) and 35L (5.53 kg/d) supplements than control (5.20 kg/d); however, 40MM and 60MM did not increase FOMI (Table 7). Total OM intake (TOMI) significantly increased ($P < 0.01$) for both liquid supplements, when compared to the control treatment. Total digestible OM intake (TDOMI) increased significantly ($P < 0.01$) from 2.94 kg/d for control to 3.40 and 3.36 kg/d for 25L and 35L, respectively. In accordance with FOMI, there was not a significant change ($P \geq 0.27$) in TDOMI with either mineral mix. Compared to control, digestible NDF intake was significantly greater ($P \leq 0.05$) with liquid supplementation.

As designed, total N intake and DIP intake was greater ($P \leq 0.01$) with supplemental protein. No significant differences ($P \geq 0.11$) were observed for total tract digestion of either OM or NDF. Total tract OM digestion ranged from 56.0 to 58.6 %.

A significant ($P < 0.01$) treatment × time interaction was observed for ruminal ammonia concentration (Table 8). This interaction was largely due to the magnitude of difference between treatments across time rather than significant changes in treatment rankings. Supplemental protein regardless of source significantly increased ($P \leq 0.01$)

ruminal ammonia concentrations over the control. Average ruminal ammonia concentrations for control, 40MM, 60MM, 25L, and 35L were 0.33, 1.06, 1.96, 1.71, and 2.09 m*M*; respectively. Ruminal ammonia peaked at 4 h for all supplements (Figure 6). Ruminal pH was lower ($P \leq 0.02$) in steers provided supplemental protein, compared to control; however, this decrease was small and was unlikely to impact fiber digestion. Total VFA concentrations increased ($P \leq 0.02$) for 40MM (94.2 m*M*) and 60MM (94.7 m*M*) when compared to control (84.7 m*M*). Liquid treatment 35L increased ($P < 0.01$) total VFA concentration to 98.7 m*M* and 25L increased ($P = 0.06$) total VFA concentration to 92.0 m*M*.

Molar proportions of major VFA (acetate, propionate, and butyrate) were not affected by protein supplementation. However, valerate proportions were affected significantly by the liquid supplements (25L and 35L), but the increase was minimal with 0.42 m*M* for control to 0.47 and 0.49 for 25L and 35L, respectively. All supplements significantly ($P \leq 0.06$) decreased isobutyrate. However, only the 60MM treatment significantly decreased ($P = 0.04$) isovalerate when compared to control.

Table 7.Effect of protein supplement source on intake and digestion of steers fed low quality prairie hay.

	Treatment[1]						Contrast *P*-value[3]			
Item[4]	CON	40MM	60MM	25L	35L	SEM[2]	CON v 40MM	CON v 60MM	CON v 25L	CON v 35L
OM intake, kg/d										
Forage	5.20	5.34	5.13	5.56	5.53	0.30	0.44	0.71	0.06	0.08
Supplement	–	0.03	0.05	0.25	0.26	–	–	–	–	–
Total	5.20	5.38	5.18	5.81	5.79	0.30	0.33	0.92	< 0.01	< 0.01
TDOMI	2.94	3.03	3.06	3.40	3.36	0.19	0.43	0.27	< 0.01	< 0.01
NDF intake, kg/d										
Total	4.06	4.18	4.03	4.35	4.30	0.23	0.41	0.83	0.06	0.11
Digestible	2.26	2.35	2.40	2.47	2.45	0.14	0.30	0.12	0.03	0.05
N intake, g/d										
Forage	60.2	61.9	59.7	64.5	64.6	3.7	0.46	0.84	0.08	0.07
Supplement	–	7.5	14.2	12.9	16.8	0.3	< 0.01	< 0.01	< 0.01	< 0.01
Total	60.2	69.4	73.9	77.5	81.4	3.7	< 0.01	< 0.01	< 0.01	< 0.01
DIP intake	34.6	42.9	48.2	48.1	52.0	2.1	< 0.01	< 0.01	< 0.01	< 0.01
Total tract digestibility, %										
OM	56.7	56.0	58.6	58.6	58.4	1.31	0.68	0.34	0.33	0.39
NDF	55.8	56.0	59.2	56.8	57.1	1.38	0.91	0.11	0.62	0.51

[1] 40MM = received 114 g of 40% crude protein mineral mix, 60MM = received 114 g of 60% crude protein mineral mix, 25L = received 1.3g/kg BW of 25% crude protein liquid, 35L = received 1.3g/kg BW of 35% crude protein liquid

[2] Standard error of the mean

[3] CON vs 40MM = control vs. 40% crude protein mineral mix; CON vs 60MM = control vs. 60% crude protein mineral mix; CON vs 25L = control vs. 25% crude protein liquid; CON vs 35L = control vs. 35% crude protein liquid.

[4] OM: Organic matter; TDOMI: Total digestible organic matter intake; NDF: Neutral detergent fiber; DIP: Degradable intake protein

Table 8. Effect of treatment on ruminal fermentation in steers grazing prairie hay.

Item	Treatment[1]					SEM[2]	Contrast *P*-value[3]			
	CON	40MM	60MM	25L	35L		CON vs 40MM	CON vs 60MM	CON vs 25L	CON vs 35L
Ruminal Ammonia, m*M*	0.33	1.06	1.96	1.71	2.09	0.26	< 0.01	< 0.01	< 0.01	< 0.01
pH	6.68	6.53	6.53	6.54	6.48	0.04	0.01	0.01	0.02	< 0.01
Total VFA, m*M*	84.69	94.22	94.67	91.96	98.66	3.38	0.02	0.01	0.06	< 0.01
VFA, mol/100mol										
Acetate	72.31	72.45	72.46	72.09	71.66	0.47	0.81	0.80	0.72	0.30
Propionate	17.94	18.14	17.89	18.43	18.31	0.42	0.66	0.92	0.30	0.42
Butyrate	8.21	7.91	8.29	8.01	8.54	0.28	0.28	0.76	0.47	0.23
Isobutyrate	0.66	0.61	0.55	0.59	0.59	0.02	0.06	< 0.01	0.02	0.01
Valerate	0.42	0.44	0.43	0.47	0.49	0.02	0.46	0.71	0.04	0.01
Isovalerate	0.47	0.45	0.38	0.41	0.41	0.03	0.68	0.04	0.15	0.17

[1] 40MM = received 114 g of 40% crude protein mineral mix, 60MM = received 114 g of 60% crude protein mineral mix, 25L = received 1.3g/kg BW of 25% crude protein liquid, 35L = received 1.3g/kg BW of 35% crude protein liquid

[2] Standard error of the mean

[3] CON vs 40MM = control vs. 40% crude protein mineral mix; CON vs 60MM = control vs. 60% crude protein mineral mix; CON vs 25L = control vs. 25% crude protein liquid; CON vs 35L = control vs. 35% crude protein liquid.

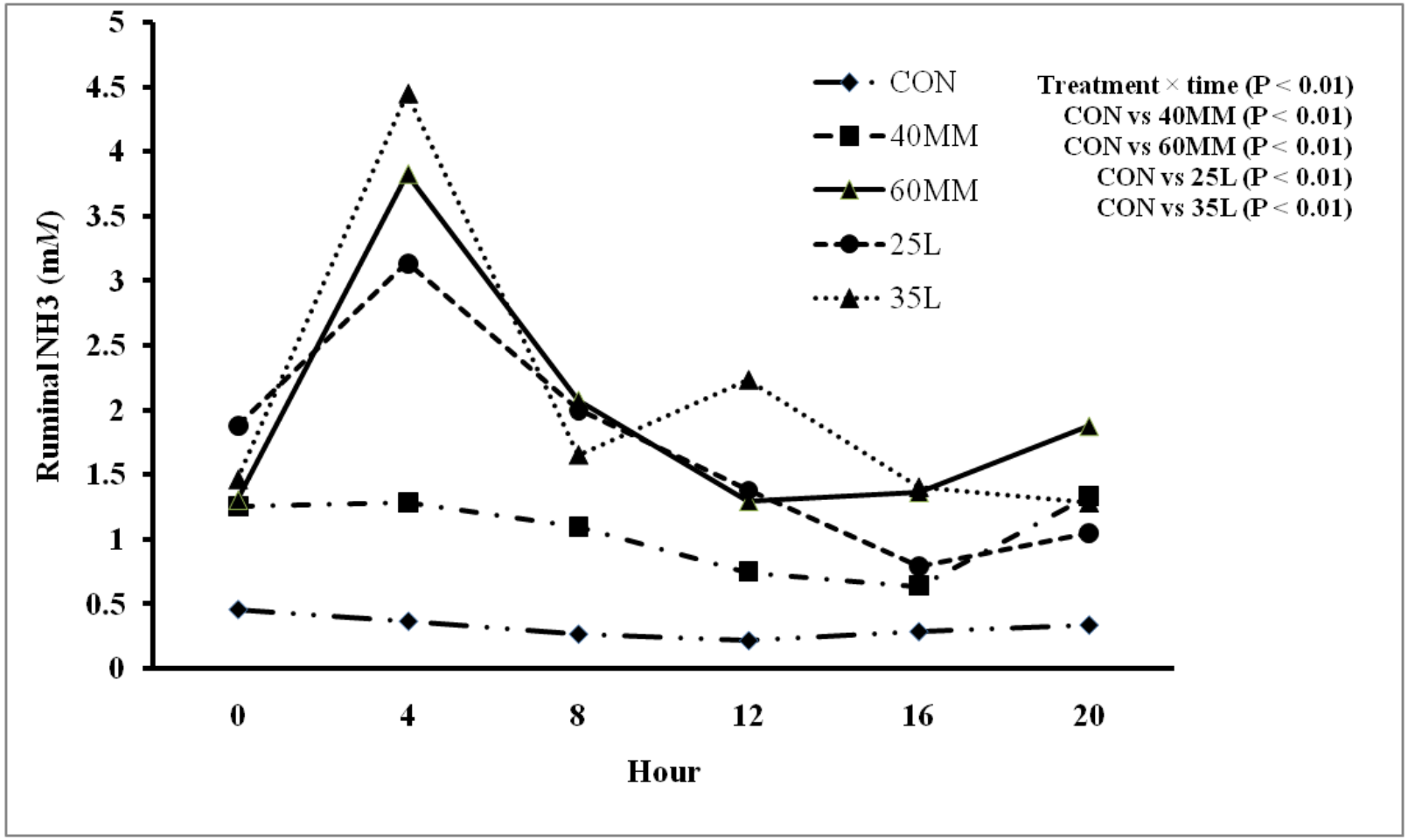

Figure 6. Effect of treatment on ruminal ammonia concentrations in steers fed low quality prairie hay. 40MM = received 114 g of 40% crude protein mineral mix, 60MM = received 114 g of 60% crude protein mineral mix, 25L = received 1.3g/kg BW of 25% crude protein liquid, 35L = received 1.3g/kg BW of 35% crude protein liquid.

Discussion

Our protein supplements differed in both source of NPN (urea or biuret) and their carriers (molasses versus minerals). Urea is the most commonly used source of NPN, which provides N readily available for incorporation by ruminal microbes due to its immediate hydrolysis to ammonia N. In contrast, biuret is a slow-release form of urea, synthesized by heating urea to form a larger molecule. Unlike urea (167 g/100ml at 40°C), biuret has low water solubility (2.2 g/100ml at 37°C; Tiwari, 1972) and it is hydrolyzed to ammonia at a slower rate than urea (Kistiakowsky and Rosenberg, 1952; Bauriedel, 1971). Treatments were not provided isonitrogenously nor was biuret provided with a source of readily available carbohydrates. In contrast, urea was provided with molasses, a readily available source of carbohydrate.

Measures of intake (FOMI and TDOMI) were greater than control with provision of urea containing liquid protein supplements (25L and 35L). In agreement, urea-containing liquid supplements were fed to cattle grazing low-quality forage and intake was greater for supplemented compared to nonsupplemented cattle (Earley et al., 1999). Similarly, Sowell et al. (2003) fed liquid protein supplements to beef cows grazing native grass and reported increased forage intake over control. Yet, when Köster et al. (1997) compared isonitrogenous supplements containing increasing proportions of urea-N (0, 25, 50, 75, and 100% of N as urea) at the expense of true protein, a linear decrease in TDOMI was observed as supplemental DIP from urea increased. This response was largely driven by OM intake and a slight reduction in digestion. Responses such as these likely resulted from urea being rapidly hydrolyzed to ammonia prior to microbial capture

of ammonia in microbial crude protein. Rapidly fermentable carbohydrates may be beneficial, by providing fermentable energy with the ammonia to increase capture. This suggestion is supported by Ernst et al. (1975), who supplemented molasses, urea, or molasses plus urea to cattle consuming low-quality forage (2.9% CP). All supplemented steers had greater forage OM intake and TDOMI than control; however, the greatest response occurred when energy and N were provided concomitantly. Similarly, Currier et al. (2004a) observed increased total OM intake over control when urea supplement contained readily available carbohydrates. In our project, improvements in forage utilization when urea containing liquid supplement was provided, is likely the result of coupling a source of highly fermentable carbohydrates via molasses with the protein supplement. In this scenario, ruminal microbes are supplied with both energy and N to increase fermentative capacity, synthesis of microbial crude protein and consequently, forage utilization.

Improvements over control in forage utilization were not observed when biuret containing mineral mix supplements (40MM and 60MM) were provided. Similar observations were reported when urea-mineral lick blocks were supplemented and roughage intake was not significantly affected (Wu et al., 2005). In contrast, Ammerman et al. (1972) reported biuret supplementation increased forage intake when compared to control. However, this trial provided biuret with a source of readily available energy in the form of citrus pulp. Similarly, in a project using the same N sources as our project, Currier et al. (2004a) observed greater OM intake in biuret supplemented steers (29.5 g/kg BW) over control steers (24.8 g/kg BW). However, Currier et al. (2004a) also

incorporated the NPN into a pelleted supplement containing energy in the form of soybean hulls and dried molasses. In our project, forage utilization likely did not respond to biuret supplementation because no readily available carbohydrates, such as those contained within the urea liquid supplements, were provided. Even though 60MM provided more N than 25L, without accompaniment of energy, the mineral mixtures could not provide the necessary nutrients required to increase forage utilization. Further research is warranted to determine if mineral mixes are effective at stimulating utilization of lower quality forages.

Along with the noted differences between our supplements, the basal forage quality (6.8 % CP) created a small response surface for us to observe possible forage utilization responses with protein supplementation. Forage fed in this trial was near the CP percentage defining low-quality forage, (7% CP; NRC, 1996). As forage quality approaches higher CP, RAN is present in adequate levels to meet microbial and host requirements; thus rendering protein supplementation of minimal value. Therefore, forage utilization is not likely to benefit from a protein supplement, particularly supplements with large proportions of DIP due to microbial population's requirement for RAN being satisfied by the forage alone. Mathis et al. (2000) reported the response to protein supplementation has limits, in part, set by the characteristics of the forage being consumed.

Forage quality can be described by a TDN:CP ratio. Moore et al. (1999) reported supplementation increased forage intake only when forage TDN:CP ratio was > 7, which describes a forage deficient in N relative to the available energy. Forages containing

lower TDN:CP ratios, contain enough CP to support the digestion of the potential fermentable OM; allowing adequate microbial fermentation to occur without supplemental protein or energy.

Our basal forage had an approximate TDN:CP ratio of 8.3, (OM digestion percentage was used as proxy for TDN), suggesting the potential response to supplemental N may be small. As previously discussed, both 35L and 25L provided energy and N, which improved forage utilization by correcting the small imbalance of the TDN:CP ratio, but more importantly by providing both energy and N to "jump start" microbial fermentation; thus aiding to increase intake.

When biuret containing mineral mixes were provided (in addition to the basal forage diet containing near adequate amounts of nutrients), only N was supplied, resulting in lowering the average TDN:CP ratio of the diet to 7.9. By only providing N with no additional fermentable OM (as done with 40MM and 60MM), microbial utilization of N was not improved and consequently, neither was forage utilization.

Adequate ruminal ammonia concentration is essential for forage utilization because cellulolytic microbes utilize ammonia N to synthesize amino acids. In the present study, ruminal ammonia concentrations ranged from 0.33 to 2.09 m*M*. Wickersham et al. (2004) reported similar ruminal ammonia concentrations in unsupplemented (0.33 m*M*) and supplemented (2.67 m*M*) steers. Similarly, ruminal ammonia concentrations were observed to increase from 1.36 m*M* for control to 2.57 and 2.73 m*M* for urea and biuret, respectively (Currier et al., 2004c). A wider range was observed by Köster et al. (1996); reporting ruminal ammonia concentration of 0.24 m*M*

for nonsupplemented steers and incrementally increased to 6.87 m*M* for the highest level of DIP. Even still, Oltjen et al. (1969) observed higher ruminal ammonia concentrations of 10.9 (for urea) and 9.8 m*M* (for biuret) in steers. Although, our ruminal ammonia concentrations were low, it is unlikely fermentative fiber digestion was hindered by insufficient RAN, as there was a modest increase in digestibility and increased VFA concentrations suggest some improvement in fermentation.

Peak ruminal ammonia concentrations were observed at h 4 for all treatments. Currier et al. (2004c) reported similar ruminal ammonia levels peaking at h 3 with daily administration of urea or biuret. Likely, 35L had the highest peak due to urea being highly degradable within the rumen. Ruminal ammonia concentrations for 60MM surpassed 25L at h 4 because this treatment provided the second highest level of N. This is somewhat surprising as biuret is hydrolyzed to ammonia and carbon dioxide at a slower rate than urea (Ioset, 1969; Fonnesbeck et al., 1975). However, provision of molasses with urea (as with our liquid supplements) may have tempered increases in ruminal ammonia by capturing ammonia N in microbial crude protein. Additionally, it is possible if rumen fluid samples were collected at h 2, we would have observed a greater peak for 25L and 35L. Ruminal microbes likely capitalized on the limited amount of N provided by 25L to assist with the increased fermentative demand set by the newly provided readily available carbohydrates. Similar reports observed decreased ruminal ammonia concentrations when lower levels of supplemental N were provided with energy compared to supplemental N alone (Slyter et al., 1971; Olson et al., 1999; Klevesahl et al., 2003). Accordingly, when supplemental protein and energy are

provided, ruminal ammonia concentrations will reflect lower peaks when compared to concentrations provoked by protein supplementation alone.

We observed increases in total VFA concentrations from 84.7 to 98.7 m*M* in response to additional N. Similarly, Köster et al. (1996) observed an increase in total VFA concentrations, with the largest increase occurring in response to the initial DIP infusion level. Olson et al. (1999) observed total VFA concentrations to increase with increasing inclusion of protein. In our project, the highest total VFA concentration was observed when 35L was provided. Ernst et al. (1975) reported increased intake when molasses plus urea were provided over control, and stated molasses contributed to the microbial crude protein production, which resulted in higher VFA concentrations above when urea is supplemented alone. Hemsley and Moir (1963) observed similar results when molasses was added to urea supplemented diets and found increased microbial protein concentrations causing increased VFA concentrations compared to control.

Increases in total VFA concentrations occur when DIP provision occurs due to microbes receiving supplemental N, which increases their fermentative activity; leading to increases in microbial production of VFA.

Conclusion

Protein supplementation with urea containing liquid supplements improved forage utilization, while biuret containing mineral mixture supplements did not significantly affect intake or digestion of low-quality forage. Noted differences between the supplements along with basal forage quality were main contributors influencing utilization responses. Providing fermentable OM in accordance with supplemental N

appeared to increase forage utilization more so than N provision alone. Further investigation is needed with poorer quality forage than used in the present project, to discern the true potential of all four protein supplements in cattle consuming low-quality forage.

LITERATURE CITED

Ammerman, C.B., G.J. Verde, J.E. Moore, W.C. Burns, and C.F. Chicco. 1972. Biuret, urea and natural proteins as nitrogen supplements for low quality roughage for sheep. J. Anim. Sci. 35:121-127.

Bandyk, C.A., R.C. Cochran, T.A. Wickersham, E.C. Titgemeyer, C.G. Farmer and J.J. Higgins. 2001. Effect of ruminal vs postruminal administration of degradable protein on utilization of low-quality forage by beef steers. J. Anim. Sci. 79:225-231.

Bauriedel, W.R. 1971. Hydrolysis of ^{14}C-biuret by *in vitro* rumen fermentation and crude biuretase preparations. J. Anim. Sci. 32(4):704-710.

Biswas, B., P.T. Scott and P.M. Gresshoff. 2011. Tree legumes as feedstock for sustainable biofuel production: Opportunities and challenges. J. Plant Phys. 168:1877-1884.

Bowman, J.G.P., Sowell, B.F., and J.A. Paterson. 1995. Liquid supplementation for ruminants fed low-quality forage diets: A review. Anim. Feed Sci. and Technol. 55:105-138.

Bringi, N.V. and S. K. Mukerjee. 1987. Karanja seed oil. Pages 143-166 in Non-traditional Oilseeds and Oils in India. N. V. Bringi, ed. Oxford and IBH Pub. Co., New Delhi, India.

Bryant, H.L., I. Gogichaishvili, D. Anderson, J.W. Richardson, J. Sawyer, T. Wickersham, M. Drewery. 2012. The value of post-extracted algae residue. Algal Res. In press.

Bunting, L.D., J.A. Boling and C.T. MacKown. 1989. Effect of dietary protein level on nitrogen metabolism in the growing bovine: I. Nitrogen recycling and intestinal protein supply in calves. J. Anim. Sci. 67:810-819.

Butterworth, M.H., E.L. Aguirre, A.C. Aragon and D.L. Huss. 1973. Use of molasses containing urea as a supplement to pangolagrass pastures in Northeast Mexico. J. Range Manage. 26:269-271.

Cochran, R.C. and M.L. Galyean. 1994. Measurements of in vivo forage digestion by ruminants. Pages 613-643 in Forage Quality, Evaluation, and Utilization. G.C. Fahey, Jr., M.C. Collins, D.R. Mertens and L.E. Moser, ed. ASA-CSSA-SSSA, Madison, WS.

Coombe, J.B., K.R. Christian and M.D. Holgate. 1971. The effect of urea on the utilization of ground, pelleted roughage by penned sheep: III. Mineral supplements. J. Agric. Sci. 77(1):159-174.

Currier, T.A., D.W. Bohnert, S.J. Falck and S.J. Bartle. 2004a. Daily and alternate day supplementation of urea or biuret to ruminants consuming low-quality forage: I. Effects on cow performance and the efficiency of nitrogen use in wethers. J. Anim. Sci. 82:1508-1517.

Currier, T.A., D.W. Bohnert, S.J. Falck and S.J. Bartle. 2004b. Daily and alternate day supplementation of urea or biuret to ruminants consuming low-quality forage: II.

Effects on site of digestion and microbial efficiency in steers. J. Anim. Sci. 82:1518-1527.

Currier, T.A., D.W. Bohnert, S.J. Falck and S.J. Bartle. 2004c. Daily and alternate day supplementation of urea or biuret to ruminants consuming low-quality forage: III. Effects on ruminal fermentation characteristics in steers. J. Anim. Sci. 82:1528-1535.

DelCurto, T., R.C. Cochran, D.L. Harmon, A.A. Beharka, K.A. Jacques, G. Towne and E.S. Vanzant. 1990. Supplementation of dormant tallgrass-prairie forage: I. Influence of varying supplemental protein and(or) energy levels on forage utilization characteristics of beef steers in confinement. J. Anim. Sci. 68:515-531.

DelCurto, T., B.W. Hess, J.E. Huston, and K.C. Olson. 2000. Optimum supplementation strategies for beef cattle consuming low-quality roughages in the western United States. J. Anim. Sci. 77:1-16.

Dutta, K.K., B.S. Gupta, S.Thakur and J.P. Srivastava. 1993. Effect of feeding deoiled cake on milk yield in lactating cows. Indian J. Anim. Hlth. 32:51-53.

Earley, A.V., B.F. Sowell and J.G.P. Bowman. 1999. Liquid supplementation of grazing cows and calves. Ani. Feed Sci. and Technol. 80:281-296.

Elliott, R.C. 1967. Voluntary intake of low-protein diets by ruminants: I. Intake of food by cattle. J. Agri. Res. 16:463.

Ernst, A.J., J.F. Limpus and P.K. O'Rourke. 1975. Effect of supplements of molasses and urea on intake and digestibility of native pasture hay by steers. Aust. J. Exp. Agri. Anim. Husb. 15:451-455.

Farmer, C.G., B.C. Woods, R.C. Cochran, J.S. Heldt, C.P. Mathis, K.C. Olson, E.C. Titgemeyer, and T.A. Wickersham. 2004. Effects of supplementation frequency and supplemental urea level on dormant tallgrass-prairie hay intake and digestion by steers and prepartum performance of beef cows grazing dormant tallgrass-prairie. J.Anim. Sci. 82:884-894.

Fonneskbeck, P.V., L.C. Kearl, and L.E. Harris. 1975. Feed grade biuret as a protein replacement for ruminants. J. Anim. Sci. 40:1150-1184.

Gupta, B.S., J.P. Srivastava, A.K. Tripathi, A.K. Verma and S. Thakur. 1981. Biological evaluation of karanja (*Pongamia glabra*) cake. Indian J. Anim. Hlth. 6:75-77.

Hammond, A.C. 1992. Use of blood urea nitrogen concentration to guide protein supplementation in cattle. Pages 9-18 in Proc. 3rd Ann. Florida Ruminant Nutr.. Sym. Gainesville, Fl.

Heldt, J.S., R.C. Cochran, G.L. Stokka, C.G. Farmer, C.P. Mathis, E.C. Titgemeyer, and T.G. Nagaraja. 1999. Effects of different supplemental sugars and starch fed in combination with degradable intake protein on low-quality forage use by beef steers. J. Anim. Sci. 77:2793-2802.

Hemsley, J.A. and R.J. Moir. 1963. The influence of higher volatile fatty acids on the intake of urea-supplemented low quality cereal hay by sheep. Aust. J. Agric. Res. 14:509-517.

Hennessy, D.W. and J.V. Nolan. 1988. Nitrogen kinetics in cattle fed a mature subtropical grass hay with and without protein meal supplementation. Aust. J. Agric. Res. 39:1135-1150.

Hess, B.W., K.K. Park, L.J. Krysl, M.B. Judkins, B.A. McCracken and D.R. Hanks. 1994. Supplemental protein for beef cattle grazing dormant intermediate wheatgrass pasture: Effects on nutrient quality, forage intake, digesta kinetics, grazing behavior, ruminal fermentation, and digestion. J. Anim. Sci. 72:2113-2123.

Hoover, W.H. 1986. Chemical factors involved in ruminal fiber digestion. J.Dairy Sci. 69:2624.

Huntington, G.B. and S.L. Archilbeque. 1999. Practical aspect of urea and ammonia metabolism in ruminants. Proc. Am. Soc. Anim. Sci. www.asas.org/symposia/proceedings/filename Accessed Aug. 13, 2012.

Ioset, R. M. 1969. Kedlor feed grade biuret – a new non-protein nitrogen for beef cattle and sheep. Page 26 in Proc. Montana Nutr. Conf. Mont. State Univ. Bozeman, Mt.

Kennedy, P.M. and L.P. Milligan. 1978. Transfer of urea from the blood to the rumen of sheep. Br. J. Nutr. 40:149-154.

Kistiakowsky, G.B. and A.J. Rosenberg. 1952. The kinetics of urea hydrolysis by urase. J. Anim. Chem. Sci. 74(20):5020-5025.

Klevesahl, E.A., R.C. Cochran, E.C. Titgemeyer, T.A. Wickersham, C.G. Farmer, J.I. Arroquy and D.E. Johnson. 2003. Effect of a wide range in the ratio of supplemental rumen degradable protein to starch on utilization of low-quality, grass hay by beef steers. Anim. Feed Sci. and Technol. 105:5-20.

Konwar, B.K., G.C. Banerjee and L. Mandal. 1987. Effect of feeding deoiled karanja (*Pongamia glabra vent.*) cake on the quantity and quality of milk in cross-bred cows. Indian Vet. J. 64:62-65.

Köster, H.H., R.C. Cochran, E.C. Titgemeyer, E.S. Vanzant, I. Abdelgadir and G. St Jean. 1996. Effect of increasing degradable intake protein on intake and digestion of low-quality, tallgrass-prairie forage by beef steers. J. Anim. Sci. 74:2473-2481.

Köster, H.H., , R.C. Cochran, E.C. Titgemeyer, E.S. Vanzant, T.G. Nagaraja, K.K. Kreikemeier and G. St Jean. 1997. Effect of increasing proportions of supplemental nitrogen from urea on intake and utilization of low-quality, tallgrass-prairie forage by beef steers. J. Anim. Sci. 75:1393-1399.

Lee, G.J., D.W. Hennessy, J.V. Nolan and R.A. Leng. 1987. Responses to nitrogen and maize supplements by young cattle offered a low-quality pasture hay. Aust. J. Agric. Res. 38:195-207.

Löest, C. A., E.C. Titgemeyer, J.S. Drouillard, B.D. Lambert, and A.M. Trater. 2001. Urea and biuret as nonprotein nitrogen sources in cooked molasses blocks for steers fed prairie hay. Anin. Feed Sci. and Technol. 94:115-126.

Marini, J.C. and M.E. Van Amburgh. 2003. Nitrogen metabolism and recycling in Holstein heifers. J. Anim. Sci. 81:545-552.

Martin, S.K. and C.A. Hibberd. 1990. Intake and digestibility of low-quality native grass hay by beef cows supplemented with graded levels of soybean hulls. J. Anim. Sci. 68:4319-4325.

Mathis, C.P., R.C. Cochran, J.S. Heldt, B.C. Woods, I.E. Abdelgadir, K.C. Olson, E.C. Titgemeyer and E.S. Vanzant. 2000. Effects of supplemental degradable intake protein on utilization of medium-to low-quality forages. J. Anim. Sci. 78:224-232.

Mathis, C. P., R.C. Cochran, E.S. Vanzant, I.E.O. Abdelgadir, J.S. Heldt, K.C. Olson, D.E. Johnson, J. Caton, D. Faulkner, G. Horn, S. Paisley, R. Mass, K. Moore, and J. Halgerson. 2001. A collaborative study comparing an in situ protocol with single time-point enzyme assays for estimating ruminal protein degradability of different forages. Anim. Feed Sci. and Technol. 93:31-42.

McCollum, F.T. and M.L. Galyean. 1985. Influence of cottonseed meal supplementation on voluntary intake, rumen fermentation and rate of passage of prairie hay in beef steers. J. Anim. Sci. 60:570-577.

McDowell, L.R. 1996. Feeding minerals to cattle on pasture. Anim. Feed Sci. and Technol. 60:247-271.

Mir, Z., G.K. MacLeod, J.G. Buchanan-Smith, D.G. Grieve and W.L. Grovum. 1984. Methods for protecting soybean and canola proteins from degradation in the rumen. Can. J. Anim. Sci. 64:853-865.

Moore, J. E., M.H. Brant, W. E. Kunkle and D.I. Hopkins. 1999. Effect of supplementation on voluntary forage intake, diet digestibility, and animal performance. J. Anim. Sci. 77:122-135.

Mould, F.L., E.R. Ørstov and S.O. Mann. 1983. Associative effects of mixed feeds. I. Effects of type and level of supplementation and the influence of the rumen fliud

pH on cellulolysis in vivo and dry matter digestion of various roughages. Anim. Feed Sci. and Technol. 10:15-30.

Murphy, H.T., D.A. O'Connell, G. Seaton, R.J. Raison, L.C. Rodriguez, A.L. Braid, D.J. Kriticos, T. Jovanovic, A. Abadi, M. Betar, H. Brodie, M. Lamont, M. McKay, G. Muirhead, J. Plummer, N. L. Arpiwi, B. Ruddle, S. Saxena, P.T. Scott, C. Stucley, B. Thistlethwaite, B. Wheaton, P. Wylie, P.M. Gresshoff. 2012. A common view of the opportunities, challenges, and research actions for pongamia in Australia. Bioenerg. Res. doi: 10.1007/s12155-012-9190-6

Nagalakshmi, D., K. Dhanalakshmi and D. Himabindu. 2011. Replacement of groundnut cake with sunflower and karanj seed cakes on performance, nutrient utilization, immune response and carcass characteristics in Nellore lambs. Small Ruminant Res.. 97:12-20.

NRC. 1996. Nutrient Requirements of Beef Cattle. 7th rev. J.G. Buchanan-Smith, ed. Natl. Acad. Press, Washington, DC.

NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

Olson, K.C., R.C. Cochran, T.J. Jones, E.S. Vanzant, E.C. Titgemeyer and D.E. Johnson. 1999. Effects of ruminal administration of supplemental degradable intake protein and starch on utilization of low-quality warm-season grass hay by beef steers. J. Anim. Sci. 77:1016-1025.

Oltjen, R.R., E.E. Williams, Jr., L.L. Slyter and G.V. Richardson. 1969. Urea versus biuret in a roughage diet for steers. J. Anim. Sci. 29:816-822.

Panda, A.K., V.R.B. Sastry, A. Kumar, and S.K. Saha. 2006. Quantification of karanjin, tannin and trypsin inhibitors in raw detoxified expeller and solvent extracted karanj (Pongamia glabra) cake. Asian-Aust. J. Anim. Sci. 19:1776-1783.

Paul, B.N., B.S. Gupta, J.P. Srivastava and A.K. Verma. 1995. In sacco dry matter and protein degradability of certain unconventional feed ingredients. Indian Vet. J. 72:943-945.

Ravi, U., P. Singh, A.K. Garg and D.K. Agrawal. 2000. Performance of lambs fed expeller pressed and solvent extracted karanj (*Pongamia pinnata*) oil cake. Anim. Feed Sci. and Technol. 88:121-128.

Satter, L.D., and L.L. Slyter. 1974. Effects of ammonia concentration on rumen microbial protein production in vitro. Br. J. Nutr. 32:199-208.

Schroeder, G.F. and E.C. Titgemeyer. 2007. Interaction between protein and energy supply on protein utilization in growing cattle: A review. Livest. Sci. 114:1-10.

Scott, R.R., and C.A. Hibberd. 1990. Incremental levels of supplemental ruminal degradable protein for beef cows fed low-quality native grass hay. Okla. Agric. Exp. Sta. Res. MP- 129:57-63.

Singh, P., V.R.B. Sastry, A.K.Garg, A.K. Sharma, G.R. Singh and D.K. Agrawal. 2006. Effect of long term feeding of expeller pressed and solvent extracted karanj (*Pongamia pinnata*) seed cake on the performance of lambs. Ani. Feed Sci. and Technol. 126:157-167.

Slyter, L.L., R.R. Oltjen, E.E. Williams, Jr. and R.L. Wilson. 1971. Influence of urea, biuret and starch on amino acid patterns in rumional bacteria and blood plasma

and on nitrogen balance of steers fed high fiber purified diets. J. Nutr. 101:839-846.

Soren, N.M. and V.R.B. Sastry. 2009. Replacement of soybean meal with processed karanj (*Pongamia glabra*) cake on the balances of karanjin and nutrients, as well as microbial protein synthesis in growing lamb. Anim. Feed Sci. Technol. 149:16-29.

Soren, N.M., V.R.B. Sastry, S.K. Saha, U.D. Wankhade, M.H. Lade and A. Kumar. 2009. Performance of growing lambs fed processed karanj (*Pongamia glabra*) oil seed cake as partial protein supplement to soybean meal. J. Anim. Phys. And Anim. Nutr. 93:237-244.

Sowell, B.F., J.G.P. Bowman, E.E. Grings and M.D. MacNeil. 2003. Liquid supplement and forage intake by range beef cows. J. Anim. Sci. 81:294-303.

Tiwari, A.D. 1972. Metabolism of biuret by ruminants: In vitro and in vivo studies. PhD Diss. Univ. of Illinois, Urbana-Chanpaign.

Toppo, S., A.K. Verma, R.S. Dass, and U.R. Mehra. 1997. Nutrient utilization and rumen fermentation pattern in crossbred cattle fed different planes of nutrition supplemented with urea molasses mineral block. Anim. Feed Sci. Technol. 64:101-112.

Van Soest, P.J. 1982. Nutritional Ecology of the Ruminant. Cornell Univ. Press, Ithaca, NY.

Vinay, B.J. and T.C. Kanya. 2008. Effect of detoxification on the functional and nutritional quality of proteins of karanja seed meal. Food Chem. 106:77-84.

Vercoe, J.E. 1969. The transfer of nitrogen from the blood to the rumen in cattle. Aust. J. Agric. Res. 20:191-197.

Wickersham, T.A., R.C. Cochran, E.C. Titgemeyer, C.G. Farmer, E.A. Klevesahl, Arroquy, J.I., D.E. Johnson and D.P. Gnad. 2004. Effect of postruminal protein supply on the response to ruminal protein supplementation in beef steers fed a low-quality grass hay. Anim. Feed Sci. and Technol. 115:19-36.

Wickersham, T.A., E.C. Titgemeyer, R.C. Cochran, E.E. Wickersham and D.P. Gnad. 2008a. Effect of rumen-degradable intake protein supplementation on urea kinetics and microbial use of recycled urea in steers consuming low-quality forage. J. Anim. Sci. 86:3079-3088.

Wickersham, T.A., E.C. Titgemeyer, R.C. Cochran, E.E. Wickersham, and E.S. Moore. 2008b. Effect of frequency and amount of rumen-degradable intake protein supplementation on urea kinetics and microbial use of recycled urea in steers consuming low-quality forage. J. Anim. Sci. 86:3089-3099.

Wolfe, R.M., T.H. Terrill, and J.P. Muir. 2008. Drying method and origin of standard affect condensed tannin (CT) concentrations in perennial herbaceous legumes using simplified butanol-HCl CT analysis. J. Sci. Food Agric. 88:1060–1067.

Wu, Y., W. Hu and J. Liu. 2005. Effects of supplementary urea-minerals lick block on the kinetics of fibre digestion, nutrient digestibility and nitrogen utilization of low quality roughages. J. Zhejiang Univ. Sci. 6B(8):793-797.

Zahran, H.H. 1999. Rhizobium-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. Mol. Biol. Rev. 63:968-989.