EFFECT OF PROTEIN SOURCE OR TRACE MINERAL SOURCE ON LOW-QUALITY FORAGE UTILIZATION BY BEEF STEERS

A Thesis

by

JODI RAE COX

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Chair of Committee,	Tryon Wickersham
Committee Members,	Jason Sawyer
	David Anderson
Head of Department,	Cliff Lamb

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ABSTRACT

Studies were conducted to evaluate the use of pongamia seedcake (PSC) as a protein supplement and the interaction between mineral source and protein supplementation in cattle consuming low-quality forage. Five ruminally cannulated steers were used to determine the effects of PSC detoxification. Treatments consisted of a negative control (no supplemental protein, CON) and four supplemented treatments, each providing 100 mg of N/kg BW three of which contained PSC subjected to increasing levels of detoxification and a positive control supplement (0PSC). Two PSC supplements were mixed to contain 400 g/kg PSC with the PSC containing either 2 ppm karanjin (2PSC) or 49 ppm karanjin (49 PSC). The other PSC supplement (638PSC) contained 200 g/kg PSC with 638 ppm karanjin. Forage OMI and TDOMI was not greater for 0PSC than CON ($P > 0.11$). Supplementation with 2PSC and 49PSC tended to result in less FOMI (63.5 and 62.7 g/kg MBW, respectively; $P = 0.08$) than 0PSC (76.6 g/kg MBW). In experiment 2, thirteen ruminally cannulated steers were used to determine the effects of highly detoxified PSC (2 ppm karanjin) on forage utilization. Forage OMI, TOMI, and TDOMI were greater with supplementation of either 2PSC or 0PSC than CON ($P < 0.01$). Protein supplementation improved forage utilization by correcting the microbial N deficiency caused by forage low in ruminally degradable protein. Detoxification of PSC can result in a product comparable to conventional supplements for its ability to stimulate utilization of low-quality forage. Trial three used

eight steers 0 (CON) or 175 mg of N/kg BW as cottonseed meal (CSM) consuming a basal diet of low-quality hay (38g/kg CP). No mineral source × protein supplement interactions ($P \geq 0.64$) or significant effects of mineral source ($P \geq 0.06$) were observed for all measures of intake and digestibility. Protein supplementation increased all intake measurements ($P \leq 0.01$). There was a tendency ($P = 0.06$) for OMD to be greater (28 g/kg) for STM than HTM in both CSM and CON steers. Forage utilization increased in response to protein supplementation and there were minimal effects of trace mineral source on low-quality forage utilization by beef steers.

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CHAPTER I

INTRODUCTION

Protein Supplementation

Maximizing forage utilization is important for the success of ruminant production systems as global food demand increases with the growing world population. Factors, such as time of year, affect the quality of forage available for consumption which may limit utilization by decreasing intake and/or digestion. Supplementation in times of low forage quality can allow for greater utilization.

Protein supplementation is required when the basal forage lacks the N necessary to meet the requirements of the microbial population (< 6-8% CP; Tamminga, 1979; Kartchner, 1980; Cochran, 1996). Insufficient dietary N limits cell wall degradation by decreasing microbial activity (Tamminga, 1979). Limited N availability in the reticulorumen reduces microbial fermentation of carbohydrates and restricts synthesis of microbial protein and volatile fatty acids (Satter and Slyter, 1974). Diets consisting predominately of structural carbohydrates, such as in the case of low-quality forage, require the energy and protein produced from microbial fermentation in the reticulorumen to meet the animal's requirements. A ruminal N deficiency can be addressed by providing a supplemental source of N, whether with true protein or non-protein N (NPN).

Ruminal microbes acquire N from multiple sources. Nitrogen resulting from the deamination of amino acids of degraded feed and endogenous proteins, hydrolysis of urea from feedstuffs or from N recycling, and degradation of other N containing substances provide the microbial population with N for metabolism (Parker, 1995; Allison, 1969). Nitrogen from these sources accumulated in the rumen as ammonia. The ammonia pool is the most important source of N for microbes (Allison, 1969). Stern and Hoover (1979) reported that at least 40% of N used in the synthesis of amino acids (AA) in microbial crude protein (MCP) has passed through the ammonia pool prior to incorporation into an AA. This is in agreement with Mathison and Milligan (1971) who estimated that 50-65% of bacterial N originated from the ruminal ammonia pool, in addition to 31-55% of protozoal N.

Nitrogen from the ruminal ammonia pool in protozoa may be acquired by two mechanisms, direct utilization of N from the ammonia pool or indirectly from predation of bacteria (Mathison and Milligan, 1971). Over 80% of microbial species preferentially utilize ammonia as a source of N, versus AA in the ruminal fluid (Bryant and Robinson, 1962). Many cellulolytic bacterial species rely solely on N derived from the ammonia pool lack capacity to acquire N efficiently from preformed AA (Bryant and Robinson, 1962). For these reasons, synthesis of MCP is dependent upon adequate concentrations of ammonia in the reticulorumen, especially when cellulolytic bacteria dominate the microbial population (Allison, 1969; Mathison and Milligan, 1971; Stern and Hoover, 1979; Bryant and Robinson, 1962).

Satter and Slyter (1974) report the minimum ammonia concentration needed *in vitro* to allow for maximal growth of the microbial population to be close to 20 mg/L N. Below 20 mg N/L, MCP production and carbohydrate fermentation are expected to decrease. Satter and Slyter (1974) assumed microbial requirements in the reticulorumen are similar to those *in vitro;* however, recommend a minimum of 50mg/L N to ensure requirements are met. The amount of CP required in the supplement for optimal microbial growth and performance is dependent upon the availability of the N in the basal forage (Satter and Slyter, 1974).

Insufficient ruminally available N (RAN) for optimal forage utilization can be addressed by the addition of protein to the diet in the form of a supplement. Supplements with moderate to high concentrations of rumen degradable protein (RDP), such as cottonseed meal or soybean meal, increase ruminal ammonia concentrations (McCollum and Gaylean, 1985; Caton et al., 1988; Mathis et al., 2000). Urea, as well as other non-protein N (NPN) sources, increases ruminal ammonia concentrations (Kennedy and Milligan, 1978; Löest et al., 2001). Although NPN sources do not consist of true protein, the N is available to the microbial population much like the ruminally degradable true protein; accordingly, NPN is included in RDP fraction of CP. Increasing the supply of RAN, either by supplementation of RDP or endogenous N sources (i.e., N-recycling), improves MCP production, and therefore microbial efficiency is increased. Köster et al. (1996) reported an increase in microbial efficiency with the provision of soybean meal to beef cows consuming low-quality forage. In addition, an increase in microbial N flow to

the duodenum was reported. Additionally, VFA synthesis increases with additional RAN from greater fermentation. Mathis et al. (1999) reported a linear increase in total VFA concentration from 59.9 m*M* in steers not receiving supplement to 74.8 m*M* in steers receiving 0.5% BW as soybean meal. Köster et al. (1996) reported a quadratic increase in total VFA concentration where VFA concentrations substantially increased from 43.3 m*M* to 71.7 m*M* when RDP supplementation increased from 0 to 360 g/d RDP, but increasing supplemental RDP from 360 g/d to 720 g/d resulted in small additional increases from 71.7 m*M* to 76.4 m*M*. Supplementation of low-quality forage diets with sources rich in RDP has the potential to increase MCP, as well as VFA synthesis, ultimately leading to an improvement in the general nutritional status of the animal.

Supplementing N to cattle consuming low-quality forage (CP < 7%) has been evaluated using a wide variety of forages and sources of supplemental protein. When RAN is sufficient, such as when supplemental RDP is provided, forage intake increases. Campling et al. (1962) reported that cows being given 150g of urea daily consumed 39% more oat straw (2.9% CP) than cows receiving no supplemental N. Similarly, Bandyk et al. (2001) observed a 62% increase in organic matter intake (OMI) when steers were ruminally dosed with 400g/d of sodium caseinate, a source of RDP, compared to steers that received no protein supplementation. When steers were fed supplements containing various levels of RDP along with *ad libitum* dormant tallgrass-prairie forage (2.9% CP), forage intake increased by at least 40% in the groups given protein supplements to meet greater than 58% of the steer's CP requirement (DelCurto et al., 1990a). Klevesahl et al.

(2003) reported a 64% increase in forage OMI when RDP was supplemented at 0.123% of BW with a basal diet of grass hay (4.9% CP). Köster et al. (1996) reported a quadratic response of forage OM, total OM, digestible OM, and total N intake to increasing RDP supplementation, as casein, to cows fed *ad libitum* tallgrass prairie forage. The largest incremental increases were observed with the first increment of supplemental RDP (180g RDP/d; Köster et al.,1996). When Mathis et al. (1999) supplemented steers consuming tallgrass-prairie hay (5.3% CP) with SBM, FOMI and TOMI were increased by the increasing levels of supplement inclusion; however, FOMI plateaued when SBM supplementation reached 0.16% BW per day. Observation of a plateau in intake with increasing supplementation is likely caused by sufficient supply of RDP. When SBM was supplemented at 0.16% of BW, steers were receiving 94 g RDP/kg TDOMI (Mathis et al., 1999). Although the 2000 NRC recommended RDP be supplied at 130 g RDP/kg total digestible nutrients, requirements as low as 71 g RDP/kg TDOMI have been reported to maximize microbial efficiency (Hollingsworth-Jenkins et al., 1996). Another study by Mathis et al. (2000) observed no intake responses to forage that provided 82 g RDP/kg TDOMI; however, intake increased with supplementation when the basal forage diet provided only 59 g RDP/kg TDOMI. Intake was maximized when the low-quality forage was supplemented with 0.124%, ultimately providing 128 g RDP/kg TDOMI suggesting RDP requirements for maximum forage utilization fall somewhere between those requirements reported by the 2000 NRC and Hollingsworth-Jenkins et al. (1996). In all of the above trials provision of supplemental N, either in the form of true protein, urea, or sodium caseinate, to a basal diet of low-quality forage increased intake.

In addition to the intake responses, digestibility of low-quality forages is often increased with inclusion of supplemental RDP. Using a range of RDP levels, Köster et al. (1996) found that ruminal OM digestibility generally increased in response to supplemental RDP, from 43.3 to 47.4%. Apparent ruminal N digestibility increased, with the largest response occurring with the fist addition of supplemental RDP. Olson et al. (1999) reported a linear increase for DM, OM, and NDF digestibility (NDFD) with RDP supplementation. In a study conducted by Mathis et al. (1999), OMD was approximately 25% higher and NDFD increased 20% when SBM was supplemented at 0.5% of BW compared to cows not receiving a protein supplement. Guthrie and Wagoner (1988) reported an improvement in both OM and CP digestibility with increasing inclusion of SBM in the diet. Increases in NDF digestibility show that fiber digestion of the low-quality forage in the reticulorumen is improving (Guthrie and Wagoner, 1988). Campling et al. (1962) demonstrated that the provision of supplemental N in the form of urea, increased the digestion of oat straw (3% CP) from 44 to 53% in one trial and from 39 to 48% in a subsequent trial. Increases in digestibility were attributed to increased fiber digestion in the reticulorumen; however, increases in digestibility do not always occur with supplemental protein provision.

Increased intake resulting from protein supplementation may also increase ruminal outflow of digesta. Guthrie and Wagner (1988) reported a 67% increase in rate of particulate passage in heifers supplemented with 603 g SBM in comparison to unsupplemented heifers eating low-quality prairie hay (5.2% CP). Olson et al (1999)

also found a linear increase in ADIA and liquid passage rate as supplemental RDP was increased, and Klevesahl et al. (2000) found ADIA passage rate increased with the maximum rate occurring when steers were supplemented with RDP at 0.123% of BW. In a trial conducted by Mathis et al. (2000) steers were fed bermudagrass (8.2% CP), bromegrass (5.9% CP), or forage sorghum (4.3% CP) with increasing levels of RDP supplementation. Quality of hay affected the magnitude of changes in particulate passage rate with supplemental protein. Bermudagrass, the highest quality forage (8.2% CP) was not affected, but for bromegrass (5.9% CP) particulate passage increased 21%, and with forage sorghum (4.3% CP) particulate passage increased 50% with RDP supplementation at 0.124% of BW. In contrast, Drewery et al. (2014) observed no effect on liquid passage rate when CSM was supplemented at 100 mg N/kg BW compared to control steers receiving only oat straw (4.5% CP).

Some of the variation in the response of rate of passage to protein supplementation can, in part, be attributed to changes in reticulorumen volume. Egan (1965) demonstrated that improved N status, specifically true protein post-ruminally may allow greater ruminal fill. When Egan (1965) infused casein post-ruminally ruminal fill increased, which was not the case with post-ruminal infusion of urea. Increased ruminal capacity may prevent the observation of an increase in particulate passage rate, despite great total outflow per day.

In addition to supplementation with RDP, N made available through N recycling has the potential to improve utilization of low-quality forage (Wickersham et al., 2004).

The amount of N recycled to the rumen is related to NH_3 concentration and fermentable OM availability in the rumen, and to urea concentration in the blood (Kennedy and Milligan, 1980). Recycling of N to the rumen is most important when ruminal available N concentration is low, such as in the case of cattle consuming LQF (Cochran et al., 1998). Ruminally available N will be underestimated if the contribution of recycled N provided by rumen undegradable protein (RUP) is not considered in feeding systems. Ability of ruminants to utilize recycled N to alleviate a ruminal N deficiency or improve the overall N status of an animal may be significant. In a comparison of ruminal and post-ruminal protein supplementation to steers receiving a basal low-quality tallgrass-prairie hay (3.4% CP), Bandyk et al. (2001) reported RAN increased from 0.55 m*M* to 1.35m*M* ammonia N in steers post-ruminally dosed with 400g casein/d in comparison to those receiving no supplemental protein. This is in agreeance with Wickersham et al. (2004) who observed a 3X increase in RAN when steers received 0.87 $g \cdot kgBW^{-1} \cdot d^{-1}$ casein post-ruminally in comparison to control steers (5.3% CP tallgrass-prairie hay). Increases in RAN with supplemental protein provided post-ruminally agrees with the observation of Wickersham et al. (2008) that microbial incorporation of recycled N increases with RUP supplementation up to 124 mg N/kg BW in comparison to steers not receiving supplemental protein (47.7 and 13.9g/d, respectively). Although RDP provides more readily available ruminal N for microbes than RUP (3x higher, Bandyk et al., 2001), or post-ruminally dosed protein, correction of RAN deficiency and improvements of overall animal N status provided by RUP should not be overlooked.

A challenge associated with effective protein supplementation is the identification of a supplement that stimulates forage utilization at a relatively low price. Oilseed byproducts (e.g., cottonseed meal and soybean meal) have been commonly used as sources of supplemental protein. Additionally, urea included in dry supplements or as a liquid feed has been an effective source of supplemental ruminally available N. Despite the availability of widely used protein supplements, research into alternative protein supplements is warranted. Additionally, relatively little attention has been directed towards determining how protein supplementation effects or interacts with mineral supplementation programs.

Pongamia

Pongamia, also known as karanja, is the common name used to describe *Pongamia pinnata* (L.) Pierre, a shrub or tree indigenous to India and Malaysia. Known for its exceptional growth potential on marginal soils, specifically those with low moisture and high salinity levels, Pongamia plantations create an opportunity to utilize land not typically suitable for agricultural production (Murphy et al., 2012). Pongamia seeds consist of an outer hull portion (6% mass), and an inner kernel portion (94% mass) containing around 40% oil. Removal of the oil, either through pressing or solvent extracting, results in a residual meal containing 28-34% CP (Vinay and Kanya, 2008).

Because of its high oil content, Pongamia may be a viable option for deriving biofuel energy from a source non-competitive with food crops (Scott et al., 2008).

Processing pongamia for biofuel production results in a moderately high CP (25 - 34%; Panda et al., 2006) byproduct, pongamia seedcake (**PSC**).

Despite the moderate CP content of PSC, its use as a protein supplement for ruminants is limited due to high concentrations of tannins and toxins that have proven to reduce its suitability as a protein supplement. Specifically, two toxins, karanjin and pongamol, which are contained in high amounts (1900 - 3240 ppm karanjin) in the residual oil, decrease palatability, intake, and digestibility of the diet, as well as long-term average daily gain (**ADG**; Srivastava et al., 1990; Prabhu et al., 2001; Panda et al., 2006; Singh et al., 2006; Nagalakshmi et al., 2011; Housman et al., 2020). Various treatments such as water soaking, alkali, heat, de-oiling and autoclaving have been evaluated as means of removing the toxins to reduce the bitter and pungent taste of PSC (Gupta et al., 1981; Prabhu et al., 2001; Panda et al., 2006). Removal of oil for biofuel occurs by expeller-pressing PSC; however, residual oil (15-20%) remains in the seedcake (Vinay and Sindhu Kanya, 2008). Solvent-extraction removes residual oil by following expeller pressing with washing the cake in a chemical solvent, typically hexane, further reducing the concentration of oil from 11.5 to 2.7% and the toxins karanjin from 5667 to 1758 ppm, and pongamol from 2544 to 794 ppm (Housman et al., 2020).

Pongamia seedcake's potential role as a protein supplement has been evaluated in lambs, kids, and cattle with negative effects being reported in each species. When whole pongamia seeds were feed to lambs at 12% of the diet, DMI decreased 37% and total

digestible nutrient intake decreased 25%; however, OM digestibility increased 10% in comparison to the control protein supplement (Nagalakshmi et al., 2011). Decreases in intake were attributed to a dietary concentration of 3250 ppm of karanjin (Nagalakshmi et al., 2011). Housman et al. (2020) found total OMI was not different between steers receiving no protein supplement (5.25 kg/d) and steers fed supplements containing 20% expeller-pressed pongamia seedcake (E-PSC; 5.30 kg/d) and 40% solvent-extracted pongamia seedcake (S-PSC; 4.49 kg/d) supplements, but total OMI was less than steers receiving a protein supplement containing no PSC (6.70 kg/d). Steers receiving supplements with 40% E-PSC had significantly lower total OMI (4.13 kg/d) than all other treatments, including those not receiving a protein supplement. Decreases in OMI were attributed to the high levels of karanjin (1554 ppm) and pongamol (1058 ppm) present in the PSC.

In a study feeding goat kids E-PSC at 0, 20, 30, and 40% of a grower ration, DMD was not affected (Srivastava et al., 1990); however, the opposite was reported by Ravi et al. (2000) in lambs fed E-PSC at 24% of concentrate OMD decreased from 61.9 to 56.2%; however, OMD in lambs receiving S-PSC at 20% of concentrate did not differ (61.6%) from control. Ravi et al. (2000) also reported NDF digestibility was highest in lambs not eating PSC (55.9%) and lowest for the E- PSC diet (44.3%), with CON and E-PSC differing from each other but not from the S-PSC diet (49.4%). In contrast to work by Singh et al. (2006) using the same inclusion rates 24% E- and 20% S-PSC where they observed no differences in OMD or DMD between the PSC treatments and the control.

In addition, Singh et al. (2006) reported a 4% decrease in NDF digestibility when lambs received the E-PSC treatment in comparison to the control supplement, with S-PSC being intermediate (56%) and not different from either. Housman et al. (2020) also observed no differences in OMD or NDF digestibility when steers were fed up to 40% S-PSC (519 and 290 ppm karanjin and pongamol, respectively) and 20% E-PSC (900 and 578 ppm karanjin and pongamol, respectively) in a protein supplement fed to steers consuming a hay diet.

Long-term feeding studies with lambs, kids, and steers have reported effects of increasing inclusion of PSC on growth rate. After 155 days, ADG was more than 50% less (32.1 versus 68.9 g/d) in lambs receiving whole PSC in comparison to those receiving the control diet (Nagalakshmi et al., 2011). Average daily gain was 43% less in goat kids consuming a grower ration with 40% E-PSC; however, ADG in kids receiving 10 or 20% PSC containing rations did not differ from the control (Srivastava et al., 1990). Gupta et al. (1981) reported that calves could be fed a supplement with up to 24% S-PSC without affecting ADG; however, E-PSC at only 16% of the supplement reduced ADG by about 42%. Housman et al. (2020) reported decreased ADG in steers receiving 20% S-PSC (279 and 134 ppm karanjin and pongamol, respectively) and 20% E-PSC (733 and 391 ppm karanjin and pongamol, respectively) in their supplement in comparison to a control treatment (0.60 kg/d) but did not differ from each other (0.39 and 0.38 kg/d, respectively).

In addition to decreased intake, digestibility, and ADG, karanjin and pongamol are the likely culprits for poor palatability of PSC. In a palatability study (Housman et al., 2020) a decrease in total consumption, as well as rate of consumption, was reported in steers consuming E-PSC or S-PSC as inclusion level increased. Steers could tolerate up to 40% S-PSC and 20% E-PSC in the supplement before total consumption or rate differed from the control treatment. Therefore, treatments containing lower concentrations of pongamol and karanjin such as in the S-PSC may be consumed in larger quantities than treatments containing higher concentrations of the toxins (E-PSC).

Further reduction in the concentration of karanjin and pongamol may allow more PSC to be fed with results similar to conventional byproducts like CSM. Therefore, our hypothesis is that reducing the concentrations karanjin and pongamol, will allow substitution of commonly used oilseed by-products (such as SBM) with PSC to achieve the stimulatory effects of protein supplementation on low-quality forage utilization without the negative consequences observed in the presence of these toxins.

Mineral Supplementation

A nutrient is considered essential if, when removed from the diet, the animal becomes unable to maintain proper bodily function and reproduce (Graham, 1991). Trace minerals (**TM**) are typically low in bioavailability (Waghorn et al., 1990), but are required by cattle in microgram or milligram amounts to serve as components of metalloenzymes, hormones, and enzyme cofactors (NASEM, 2016). Trace minerals are consumed in a multitude of forms. Although minerals exist in feedstuffs,

supplementation may be necessary to meet requirements of the animal and, in some cases, the ruminal microbial population.

Supplementation with TM can occur with the use of mineral blocks, as fortification of another supplement or diet, or as loose mineral provided to the animal *ad libitum.* Due to their charged nature, free metal ions must be bound to a molecule that increases stability enough to be fed. The 'source' of a TM typically references the charged molecule to which it is bound. Trace minerals bound to a sulfate molecule (SO_4^-) are the most widely used in cattle diets.

The effectiveness or bioavailability of various alternative TM sources has been examined as a potential way to reduce undesirable mineral:mineral interactions and prevent excretion of unutilized TM. Following consumption, TM may be susceptible to interactions with microbial populations, as well as other compounds within the rumen. These interactions are dependent on the solubility of the TM in the slightly acidic rumen environment (pH 6.0 - 6.8) and the highly acidic abomasal environment (pH <3.0). Location and extent of solubility is critical when considering the animal's ability to utilize the TM. To utilize TMs, absorption must take place in the small intestine requiring mineral complexes to dissolve into their ionic forms (Waghorn et al., 1990). Although solubility is critical for absorption, minerals that dissociate in the rumen interact with ruminal microbes potentially decreasing nutrient utilization by decreasing microbial degradation and fermentation (Forsberg, 1978; Eryavuz et al., 2009). Early work, by Forsberg (1978) demonstrated than free Cu^{2+} inhibited *B. Succinogens*, *R.*

albus, and *E. Ruminantium* at concentrations as low as 21µg/ml. In an *in vitro* study, Eryavuz et al. (2009) observed that soluble Zn supplemented at 50 µg/ml killed microbes, therefore reducing cellulose digestion ($P < 0.01$) after 24 h of incubation. Martinez and Church (1970) found that additions of supplemental Zn and Mn at 20 and 100 ppm, respectively, reduced cellulose digestibility by 31 and 24%. Providing cattle with TM sources less soluble in the rumen should prevent the decrease in digestion and fermentation observed when provided rumen soluble TM sources.

Ruminal solubility of supplemental trace minerals (TM) sources ranges from 0.6% to 100% for $Cu_2(OH)_3Cl$ and $ZnSO_4$, respectively (Spears et al., 2004; Cao et al., 2000). High solubility of $CuSO_4$ (94.5 and 97%) in water and at pH 2.2 (98.3 and 100%) has been noted in two studies (Spears et al., 2004; Kegley and Spears, 1994; respectively). Similarly, Cao et al. (2000) compared the solubility of two forms of Zn finding $ZnSO_4$ to be 100% soluble in both water and 0.4% HCl. In contrast, Zn hydroxychloride ($Zn_5(OH)_8Cl_2 \cdot H_2O$) was 3% soluble in water and 100% soluble in 0.4% HCl (Cao et al., 2000). Spears et al. (2004) reported similar solubilities for Cu hydroxychloride ($Cu_2(OH)_3Cl$), being relatively insoluble in water (0.6%); however, 86.8% soluble at a pH of 2.2 compared to $CuSO_4$ (94.5 and 98.3% soluble, respectively). In agreement with Spears et al. (2004), Miles et al. (1998) found $Cu_2(OH)_3Cl$ to be less than 1% soluble in water and 100% soluble in 0.4% HCl. Kawshima et al. (1997) reported Co carbonate to be intermediate in solubility (11.4% soluble in water and 89.8% soluble in 0.4% HCl) compared to the high solubility of sulfate TMs, such as

$CuSO_4$, and low solubilities of hydroxychloride TMs, such as $Cu_2(OH)_3Cl$. Based on these findings,the reduced ruminal solubility of hydroxychloride forms may reduce the potential for negative effects on ruminal or microbial function, but their high solubility in the abomasum might allow them to become highly available for intestinal absorption.

Effects of decreased solubilities on intake and digestibility was evaluated in dairy cows fed high-quality forage and byproduct ingredients. Cows receiving hydroxy TM had greater NDF digestibility than those provided sulfate TM (48.5 and 46.4%, respectively; Faulkner and Weis, 2017). Similarly, when steers were provided hydroxy TM instead of sulfate TM on a corn silage-based diet, NDF digestibility tended to be about 4% higher (Caldera et al., 2019); however, a study by Genther and Hansen (2015) using similar diets and mineral treatments found no differences in digestibility caused by mineral source.

In addition to potential negative effects on fiber digestibility, TM complexes that are highly soluble in the rumen, such as sulfate bound TMs, may interact with antagonists leading to a decrease in bioavailability (Suttle, 1991). Some populations of ruminal microbes, such as *M. Elsdenii* and *S. Ruminantiam* utilize sulfate (SO_4^{2-}) as a readily available source of energy in the rumen, therefore producing sulfide (S^{2-}) as a waste product (Gawthorne and Nader; 1976). Sulfide can be absorbed, utilize H^+ in the rumen to produce H_2S that is eructated, or bind to TMs like Cu to form insoluble metal complexes that are not available to the animal (Suttle, 1974; Suttle, 1991; Spears, 2003).When goats received 0.34% dietary S, Qi et al. (1994) found S^{2-} and H_2S to be

30% higher in goats receiving 0.16% dietary S. Suttle (1974) reported that supplementation with $CuSO_4$ at 8 ppm diet increased rumen sulfide concentration 80% in ewes 2 h after feeding; however, plasma [Cu] decreased around 50% when the majority of Cu was in a CuS complex. In addition to insoluble sulfide complexes, abundance of S in the rumen (> 0.25% of diet DM; NASEM, 2016) in conjunction with Mo (> 2ppm DM) may interact with Cu resulting in insoluble thiomolybdate complex that will not release copper even under acidic conditions (Spears, 2003).

Despite the observed negative effects of some TM in the rumen, 3-13% of dietary Co is used for cobalamin synthesis (NASEM, 2016). Although Co itself has no known requirements in the body, cattle require Co to be supplied to the microbes at 0.15 ppm for sufficient generation of cobalamin (B_{12}; NASEM, 2016). Regardless of origin of B_{12}, either provided in the diet or synthesized by microbes, absorption by the animal is critical for functionality of methylmalonyl CoA mutase, an enzyme involved in metabolism of propionate from succinate, and methionine synthase, critical in the recycling of methionine in the brain (NASEM, 2016).

Similar to B_{12}, Cu^{2+}, Zn^{2+}, and Mn^{2+} must be absorbed in the small intestine to ensure proper metabolic function and prevent depressed intake, gain, and immunity (NASEM, 2016). Copper is utilized by cattle as an essential component of lysyl oxidase, cytochrome oxidase, superoxide dismutase, ceruplasmin, and tyrosinase (NASEM, 2016). Without sufficient Cu in the diet, cattle can experience anemia, reduced growth, weakened immune function, and reduced reproduction. Copper levels in the diet required

to prevent these symptoms ranges from 4 to 15 ppm; however, generally 10 ppm is recommended by the NRC (NASEM, 2016). Copper antagonists, Mo and S, at levels of 2 ppm and 0.25% of diet, respectively, have been found to increase the Cu requirement (Spears, 2003; Pogge et al., 2014). Although Cu requirements differ depending on intake of Mo and S, exceeding the maximum tolerable concentration of 40 ppm may cause Cu stored in the liver to suddenly be released causing hemolytic crisis, jaundice, tissue necrosis, or death (NRC, 1980).

Similar to Cu, Zn serves as an important component in metalloenzymes, specifically Cu-Zn superoxide dismutase, carbonic anhydrase, alcohol dehydrogenase, carboxypeptidase, alkaline phosphatase, and RNA polymerase. Proper function of these enzymes has been observed to affect metabolism of carbohydrates, lipids, protein, and nucleic acids (Graham, 1991). In addition and similarly to Cu, Zn plays a role in developing and maintaining a proper a well-functioning immune system (Graham, 1991). Concentrations necessary for proper function of the immune system and enzymes are reported by the NRC to be 30 ppm. Mayland et al. (1980) reported subclinical Zn deficiencies (7 to 17 ppm Zn) resulted in depressed intake, feed efficiency, and growth in calves. More severe Zn deficiencies may cause alopecia, excessive salivation, and parakeratotic lesions (Mills et al., 1967). Excessive dietary Zn (> 900 ppm Zn) may cause decreased weight gain, intake, and feed efficiency (Ott et al., 1966).

Manganese also serves as a component of enzymes, such as pyruvate carboxylate, arginase, and superoxide dismutase and as an activator for hydrolases,

kinases, transferases and decarboxylases (Hurley and Keene, 1987). Requirements for Mn in growing and finishing cattle are estimated to be around 20 ppm Mn; however, requirements double in lactating cows (Hansen et al., 2006a; Hansen et al., 2006b; NASEM, 2016). Hansen et al. (2006b) observed that 15.8 ppm Mn provided open heifers with sufficient Mn for growth; however, another study by Hansen et al. (2006a) reported a diet containing 16.6 ppm Mn was insufficient at meeting Mn requirements in gestating heifers. Inclusion of Mn at 50 ppm Mn allowed for 18% greater birthweights and higher plasma Mn concentrations in calves as well as their mothers (Hansen et al., 2006a).

Formation of insoluble metal complexes due to unwanted reactions in the rumen will prevent the animal from absorbing and benefiting from TM supplementation. Inadequate absorption of TMs may cause the symptoms of deficiency previously discussed. Feeding TMs that are not soluble in the rumen but remain highly soluble in the acidic environment of the abomasum may provide a way to maximize both digestibility and bioavailability. Spears et al., 2004 compared bioavailability of $CuSO_4$ and CuOHCl in diets with low and high levels of Mo fed to steers and observed that when antagonists, such as Mo, were available in the rumen, the higher solubility of $CuSO_4$ (94.5%) resulted in 96 and 32% lower liver and plasma Cu concentrations, respectively, than Cu provided by $Cu_2(OH)_3Cl$ that was not soluble in the rumen (0.6%).

Data evaluating the differences between hydroxy TM and sulfate TM sources fed to cattle consuming a basal low-quality forage diet with or without supplementation is not available. Our hypothesis is the protein supplementation will improve forage

utilization, and that we will observe greater digestibility in steers supplemented hydroxy TM than sulfate TM. It is likely that hydroxy TM will be more bioavailable to the animal than sulfate TM.

References

Allison, M. J. 1969. Biosynthesis of amino acids by ruminal microorganisms. *J. Anim. Sci*., 29: 797-807. https://doi.org/10.2527/jas1969.295797x

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. https://doi.org/10.2527/2001.791225x

Bryant, M. P., and I. M. Robinson. 1962. Some nutritional characteristics of predominant culturable ruminal bacteria. *J. Bacterio.* 84: 605-614.

Caldera, E., B. Weigel, V.N. Kucharczyk, K.S. Sellins, S.L. Archibeque, J.J. Wagner, H. Han, J.W. Spears, T.E. Engle. 2019. Trace mineral source influences ruminal distribution of copper and zinc and their binding strength to ruminal digesta. *J. Anim. Sci.* 97: 1852-1864. https://doi.org/10.1093/jas/skz072

Campling, R. C., M. Freer, and C. C. Balch. 1962. Factors affecting the voluntary intake of food by cows: 3. The effect of urea on the voluntary intake of oat straw. *Brit. J. Nutr.* 16: 115-124. https://doi.org/10.1079/BJN19620013

Cao, J., P. R. Henry, C. B. Ammerman, R. D. Miles, R. C. Littell. 2000. Relative bioavailability of basic zinc sulfate and basic zinc chloride for chicks. *J. Appl. Poult. Res.* 9: 513-517. https://doi.org/10.1093/japr/9.4.513

Caton, J. S., A. S. Freeman, and M. L. Galyean. 1988. Influence of protein supplementation on forage intake, in situ forage disappearance, ruminal fermentation and digesta passage rates in steers grazing dormant blue grama rangeland. *J. Anim. Sci.* 66: 2262-2271. https://doi.org/10.2527/jas1988.6692262x

Cochran, R.C., H.H. Köster, K.C. Olson, J.S. Heldt, C.P. Mathis, B.C. Woods. 1998. Supplemental protein sources for grazing beef cattle. 9th Florida Ruminant Nutrition symposium.

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. https://doi.org/10.2527/1990.682515x

Egan, A. R. (1965). Nutritional status and intake regulation in sheep. II. The influence of sustained duodenal infusions of casein or urea upon voluntary intake of low-protein roughages by sheep. *Aust. J. Agric. Res., 16:* 451-462. https://doi.org/10.1071/AR9650451

Eryavuz, A., B.A. Dehority. 2019. Effects of supplemental zinc concentration on cellulose digestion and cellulolytic and total bacterial numbers in vitro. *Anim. Feed Sci. Tech.* 151: 175-183. https://doi.org/10.1016/j.anifeedsci.2009.01.008

Faulkner, M. J., W. P. Weiss. 2017. Effect of source of trace minerals in either forage-or by-product–based diets fed to dairy cows: 1. Production and macronutrient digestibility." *J. Dairy Sci.* 100: 5358-5367. https://doi.org/10.3168/jds.2016-12095

Forsberg, C.W. 1978. Effects of heavy metals and other trace elements on the fermentative activity of the rumen microflora and growth of functionally important rumen bacteria. *Can. J. Microbiol.* 24: 298-306. https://doi.org/10.1139/m78-050

Gawthorne, J. M., and C. J. Nader. 1976. The effect of molybdenum on the conversion of sulphate to sulphide and microbial-protein-sulphur in the rumen of sheep. *Brit. J. Nutr.* 35: 11-23. https://doi.org/10.1079/BJN19760004

Genther, O. N., S. L. Hansen. 2015. The effect of trace mineral source and concentration on ruminal digestion and mineral solubility. *J. Dairy Sci.* 98: 566-573. https://doi.org/10.3168/jds.2014-8624

Graham, T. W. 1991. Trace element deficiencies in cattle. *Vet. Clinics North Amer.: Food Anim. Prac., 7*: 153-215. https://doi.org/10.1016/S0749-0720(15)30816-1

Gupta, B.S., J.P Srivastava, A.K. Tripathi, A.K. Verma, S. Thakur. 1981. Biological evaluation of Karanj (Pongamia glabra) cake. *Indian J. Anim. Health*,75–77.

Guthrie, M. J., and D. G. Wagner. 1988. Influence of protein or grain supplementation and increasing levels of soybean meal on intake, utilization and passage rate of

prairie hay in beef steers and heifers. *J. Anim. Sci.* 66: 1529-1537. https://doi.org/10.2527/jas1988.6661529x

Hansen, S. L., J. W. Spears, K. E. Lloyd, and C. S. Whisnant. 2006a. Feeding a low manganese diet to heifers during gestation impairs fetal growth and development. *J. Dairy Sci.* 89: 4305-4311. https://doi.org/10.3168/jds.S0022-0302(06)72477-8

Hansen, S. L., J. W. Spears, K. E. Lloyd, and C. S. Whisnant. 2006b. Growth, reproductive performance, and manganese status of heifers fed varying concentrations of manganese. *J. Anim. Sci.* 84: 3375-3380. https://doi.org/10.2527/jas.2005-667

Hollingsworth-Jenkins, K.J., T. J. Klopfenstein, D. C. Adams, J. B. Lamb. 1996 Ruminally degradable protein requirement of gestating beef cows grazing native winter sandhills range, *J. Anim. Sci.*, 74: 1343–1348, https://doi.org/10.2527/1996.7461343x

Housman, L. E., J.E. Sawyer, J. R. Cox, L.A. Redmon, T.A. Wickersham. 2020. Expeller-pressed and solvent-extracted Pongamia seedcake as a protein supplement for cattle consuming a basal diet of forage. *Anim. Feed Sci. Tech.* 114521. https://doi.org/10.1016/j.anifeedsci.2020.114521

Hurley, L.S., and C. L. Keen. 1987. Manganese. Pp: 185-223 in Trace Elements in Human and Animal Nutrition, Vol. 1, W. Mertz, ed. New York: Academic Press.

Kartchner, R. J. 1980. Effects of protein and energy supplementation of cows grazing native winter range forage on intake and digestibility. *J. Anim. Sci.*, 51: 432-438. https://doi.org/10.2527/jas1980.512432x

Kawashima, T., P. R. Henry, C. B. Ammerman, R. C. Littell, and J. Price. 1997. Bioavailability of cobalt sources for ruminants. 2. Estimation of the relative value of reagent grade and feed grade cobalt sources from tissue cobalt accumulation and vitamin B12 concentrations. *Nutr. Res.* 17: 957-974. https://doi.org/10.1016/S0271-5317(97)00061-4

Kegley, E. B., and J. W. Spears. 1994. Bioavailability of feed-grade copper sources (oxide, sulfate, or lysine) in growing cattle. *J. Anim. Sci.* 72: 2728-2734. https://doi.org/10.2527/1994.72102728x

Kennedy, P. M., and L. P. Milligan. 1980. The degradation and utilization of endogenous urea in the gastrointestinal tract of ruminants: a review. *Canadian J. Anim. Sci.,* 60: 205-221. https://doi.org/10.4141/cjas80-030

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. Tech.* 105: 5-20. https://doi.org/10.1016/S0377-8401(03)00057-9

Köster, H.H., R. C. Cochran, E. C. Titgemeyer, E. S. Vanzant, I. Abdelgadir, G. St-Jean. 1996. Effect of increasing degradable intake protein on intake and digestion of low-quality, tallgrass-prairie forage by beef cows, *J. Anim. Sci.* 74: 2473-2481. https://doi.org/10.2527/1996.74102473x

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. *Anim. Feed Sci. Tech.,* 94: 115-126. https://doi.org/10.1016/S0377-8401(01)00312-1

Martinez, A., and D. C. Church. 1970. Effect of various mineral elements on in vitro rumen cellulose digestion. *J. Anim. Sci.* 31: 982-990. https://doi.org/10.2527/jas1970.315982x

Mathis, C. P., R. C. Cochran, G. L. Stokka, J. S. Heldt, B. C. Woods, K. C. Olson. 1999. Impacts of increasing amounts of supplemental soybean meal on intake and digestion by beef steers and performance by beef cows consuming low-quality tallgrass-prairie forage. *J. Anim. Sci.* 77: 3156-3162. https://doi.org/10.2527/1999.77123156x

Mathis, C. P., R. C. Cochran, J. S. Heldt, B. C. Woods, I.E.O. Abdelgadir, K. C. Olson, E. C. Titgemeyer, E. S. Vanzant. 2000. Effects of supplemental degradable intake protein on utilization of medium-to low-quality forages. *J. Anim. Sci.* 78: 224-232. https://doi.org/10.2527/2000.781224x

Mathison, G.W., and L. P. Milligan. 1971. Nitrogen metabolism in sheep. *Brit. J. Nutr.*, 25: 351-366. https://doi.org/10.1079/BJN19710100

Mayland, H. F., R. C. Rosenau, and A. R. Florence. 1980. Grazing cow and calf responses to zinc supplementation. *J. Anim. Sci.* 51: 966-974. https://doi.org/10.2527/jas1980.514966x

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. https://doi.org/10.2527/jas1985.602570x

Miles, R. D., S. F. O'keefe, P. R. Henry, C. B. Ammerman, and X. G. Luo. 1998. The effect of dietary supplementation with copper sulfate or tribasic copper chloride on broiler performance, relative copper bioavailability, and dietary prooxidant activity. *Poult. Sci.* 77: 416-425. https://doi.org/10.1093/ps/77.3.416

Mills, C. F., A. C. Dalgarno, R. B. Williams, and J. Quarterman. 1967. Zinc deficiency and the zinc requirements of calves and lambs. *Brit. J. Nutr.* 21: 751-768. https://doi.org/10.1079/BJN19670076

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, and H. Brodie. 2012. A common view of the opportunities, challenges, and research actions for pongamia in Australia. *BioEnergy Research* 5: 778-800. 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 utilisation, immune response and carcass characteristics in Nellore lambs. *Small Rum. Res.* 97: 12-20. https://doi.org/10.1016/j.smallrumres.2011.02.003

National Academies of Sciences, Engineering, and Medicine (NASEM). 2016. Nutrient Requirements of Beef Cattle: Eighth Revised Edition. Washington, DC: The National Academies Press. doi: 10.17226/19014

NRC (National Research Council). 1980. Mineral tolerance of Domestic Animals. Washington, D.C: National Academy Press.

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. https://doi.org/10.2527/1999.7741016x

Ott, E. A., W. H. Smith, R. B. Harrington, and W. M. Beeson. 1966. Zinc toxicity in ruminants. II. Effect of high levels of dietary zinc on gains, feed consumption and feed efficiency of beef cattle. *J. Anim. Sci.* 25: 419-423. https://doi.org/10.2527/jas1966.252419x

Panda, A.K., V.R.B. Sastry, A. Kumar, S.K. Saha. 2006. Quantification of karanjin, tannin and trypsin inhibitors in raw and detoxified expeller and solvent extracted

karanj (Pongamia glabra) cake. *Asian-Aust. J. Anim. Sci.* 19: 1776–1783. https://doi.org/10.5713/ajas.2006.1776

Parker, D. S., M. A. Lomax, C. J. Seal, and J. C. Wilton. 1995. Metabolic implications of ammonia production in the ruminant. *Proceedings of the Nutrition Society* 54: 549-563. https://doi.org/10.1079/PNS19950023

Pogge, D. J., M. E. Drewnoski, and S. L. Hansen. 2014. High dietary sulfur decreases the retention of copper, manganese, and zinc in steers. *J. Anim. Sci.* 92: 2182-2191. https://doi.org/10.2527/jas.2013-7481

Prabhu, T. M., C. Devakumar, V. R. B. Sastry, and D. K. Agrawal. 2003. Quantification of karanjin using high performance liquid chromatography in raw and detoxified karanj (Pongamia glabra vent) seed cake. *Asian-Aust. J. Anim. Sci.* 15: 416-420. https://doi.org/10.5713/ajas.2002.416

Prabhu, T. M., C. Devakumar, V. R. B. Sastry, and D. K. Agrawal. 2003. Quantification of karanjin using high performance liquid chromatography in raw and detoxified karanj (Pongamia glabra vent) seed cake. *Asian-Aust. J. Anim. Sci.* 15: 416-420. https://doi.org/10.5713/ajas.2002.416

Qi, K., C. D. Lu, F. N. Owens, and C. J. Lupton. 1994. Effects of sulfate supplementation on performance, acid-base balance, and nutrient metabolism in Angora kids. *Small Rum. Res.* 15: 19-29. https://doi.org/10.1016/0921-4488(94)90054-X

Ravi, U., P. Singh, A.K. Garg, D.K. Agrawal. 2000. Performance of lambs fed expeller pressed and solvent extracted karanj (Pongamia pinnata) oil cake. *Anim. Feed Sci.Technol.* 88: 121–128. https://doi.org/10.1016/S0377-8401(00)00173-5

Satter, L. D., and L. L. Slyter. 1974. Effect of ammonia concentration on rumen microbial protein production in vitro. *Brit.J. Nutr.*32: 199-208. https://doi.org/10.1079/BJN19740073

Scott, P.T., L. Pregelj, N.C. Johanna S. Hadler, M.A. Djordjevic, and P.M. Gresshoff. 2008. Pongamia pinnata: an untapped resource for the biofuels industry of the future. *Bioenergy Res.* 1: 2-11. DOI 10.1007/s12155-008-9003-0

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. *Anim. Feed Sci. Technol.* 126: 157-167. https://doi.org/10.1016/j.anifeedsci.2005.05.025

Spears, J. W. 2003. Trace mineral bioavailability in ruminants. *J. Nutr.* 133: 1506S-1509S. https://doi.org/10.1093/jn/133.5.1506S

Spears, J. W., E. B. Kegley, L. A. Mullis. 2004. Bioavailability of copper from tribasic copper chloride and copper sulfate in growing cattle. *Anim. Feed Sci. Tech.* 116:1-13. https://doi.org/10.1016/j.anifeedsci.2004.06.002

Srivastava, J. P., B. S. Gupta, S. Thakur, A. K. Verma. 1990. Utilization of deoiled karanj (Pongamia glabra) cake in kid grower rations. *Indian J. Anim. Nutr.* 7:15-20.

Stern, M.D., and W.H. Hoover. 1979. Methods for determining and factors affecting rumen microbial protein synthesis: a review. *J. Anim. Sci.*, 49: 1590-1603. https://doi.org/10.2527/jas1979.4961590x

Suttle, N. F. (1974). Effects of organic and inorganic sulphur on the availability of dietary copper to sheep. *Brit. J. Nutr.* 32: 559-568. https://doi.org/10.1079/BJN19740109

Suttle, N. F. 1991. The interactions between copper, molybdenum, and sulphur in ruminant nutrition. *Ann. Rev. Nutr.* 11: 121-140.

Tamminga, S. (1979). Protein degradation in the forestomachs of ruminants. *J. Anim. Sci.* 49: 1615-1630. https://doi.org/10.2527/jas1979.4961615x

Vinay, B. J., and T.S. Kanya. 2008. Effect of detoxification on the functional and nutritional quality of proteins of karanja seed meal. *Food Chem.* 106: 77-84. https://doi.org/10.1016/j.foodchem.2007.05.048

Wickersham, T. A., E. C. Titgemeyer, R. C. Cochran, E. E. Wickersham, and D. P. Gnad. 2008. 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. https://doi.org/10.2527/jas.2007-0325

Wickersham, T. A., R. C. Cochran, E. C. Titgemeyer, C. G. Farmer, E. A. Klevesahl, J. I. Arroquy, 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. Tech.* 115: 19-36. https://doi.org/10.1016/j.anifeedsci.2004.03.005

CHAPTER II

EFFECTS OF PONGAMIA SEEDCAKE AS A PROTEIN SUPPLEMENT ON UTILIZATION OF LOW-QUALITY FORAGE BY BEEF CATTLE

Overview

Two studies were conducted to evaluate the use of pongamia seedcake (PSC) as a protein supplement for beef cattle. Experiment 1 used five ruminally cannulated steers in a 5 × 5 Latin square to determine the effects of various levels of PSC detoxification on its utility as a protein supplement. The five treatments, dosed ruminally daily, consisted of a negative control (no supplemental protein, CON) and four supplemented treatments, each providing 100 mg of N/kg BW three of which contained PSC subjected to increasing levels of detoxification and a positive control supplement (0PSC). Two PSC supplements were mixed to contain 400 g/kg PSC with the PSC containing either 2 ppm karanjin (2PSC) or 49 ppm karanjin (49 PSC). The other PSC supplement (640PSC) contained 200 g/kg PSC with 638 ppm karanjin. Bermudagrass hay (68 g/kg CP) was provided *ad libitum*. Forage OMI and TDOMI was not greater for 0PSC than CON ($P >$ 0.11). Supplementation with 2PSC and 49PSC tended to result in less FOMI (63.5 and 62.7 g/kg MBW, respectively; $P = 0.08$) than 0PSC (76.6 g/kg MBW). In contrast, 638PSC tended to result in less TDOMI ($P = 0.08$) than 0PSC (35.4 versus 44.6 g/kg MBW) resulting from numerical decreases in OM intake and digestibility. Nitrogen retention was not significantly different ($P \geq 0.11$) between 0PSC and any of the PSC

containing treatments; however, CON was less than 0PSC ($P = 0.05$). In experiment 2, thirteen ruminally cannulated steers were used in a randomized complete block study to determine the effects of highly detoxified PSC (2 ppm karanjin) on forage utilization. Steers were blocked by weight. Three treatments consisting of a control (no supplement, CON) and two providing 75 mg of N/kg BW, one containing 400 g/kg pongamia seedcake (2PSC) and one containing no PSC (0PSC) were dosed ruminally each day. Bluestem hay (49 g CP/ kg DM) was provided *ad libitum* to determine effects of protein supplementation on forage utilization. Forage OMI, TOMI, and TDOMI were greater with supplementation of either 2PSC or 0PSC than CON ($P < 0.01$). Digestibility was not significantly affected by treatment ($P = 0.19$). Detoxification of PSC can result in a product comparable to conventional supplements for its ability to stimulate utilization of low-quality forage.

Introduction

Agricultural and food-industry by-products comprise approximately 30% of worldwide agricultural production (Ajila et al., 2012); therefore, effective utilization of these resources is essential. Agriculture byproducts are rich in many nutrients, such as protein, that can be used to feed animals in place of human-edible feed ingredients. Oilseed by-products, such as cottonseed meal (**CSM**) and soybean meal (**SBM**), have been widely used as sources of supplemental protein to improve utilization of low-quality forage (Caton et al., 1988; Guthrie and Wagner, 1988). Despite its use, SBM is not an ideal protein supplement for ruminants as it has a superior amino acid profile

more effectively utilized in monogastric food producing systems. Therefore, supplementation with human-inedible sources is preferred to enhance the sustainability of beef production (Baber et al., 2018, 2019a, 2019b). Human-inedible sources of protein allow the nutritional requirements of cattle to be met without directly competing with humans (Baber et al., 2019a, 2019b; Takiya et al., 2019).

While many byproducts are well characterized, novel feedstuffs are being developed from alternative biofuel sources, including post-extraction algal residue from algae (Drewery et al., 2014; Morrill et al., 2017a and 2017b) and pongamia seedcake (PSC) from pongamia (Gupta et al., 1981; Housman et al., 2020).

Using novel feedstuffs like PSC poses challenges such as overcoming naturally occurring antinutritional factors (Prabhu et al., 2002; Panda et al., 2006). A barrier to utilizing PSC is the high levels of toxins and tannins known to affect palatability, intake, and gain (Gupta et al., 1981; Konwar and Banjeree, 1987; Ravi et al., 2000; Housman et al., 2020). Of particular importance in pongamia products are the compounds karanjin and pongamol.

Accordingly, our hypothesis is that reducing concentrations of anti-nutritional factors, specifically karanjin and pongamol, will allow substitution of commonly used by-products such as SBM with PSC without reductions in the stimulatory effects of protein supplementation on low-quality forage utilization.

Materials and 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. Two experiments were conducted to evaluate the effects of PSC detoxification on its suitability as a source of supplemental protein.

Experiment 1

In experiment 1, five ruminally cannulated steers (217.0 ± 40.1 kg BW) were used in a 5 × 5 Latin square to determine the effects of various levels of PSC detoxification on its utility as a protein supplement. Five treatments consisting of a negative control (no supplemental protein, CON) and four supplemented treatments, each provided 100 mg of N/kg BW, three of which contained PSC subjected to increasing levels of detoxification and a positive control supplement (0PSC, 420 g/kg soybean meal, 380 g/kg cracked corn, 200 g/kg dried distillers' grains; Table 1). Two PSC supplements were mixed to contain 600 g/kg dried distillers' grains and 400 g/kg PSC; the PSC used contained either 2 ppm karanjin and 3 ppm pongamol (2PSC) or 49 ppm karanjin and 16 ppm pongamol (49 PSC). The other PSC supplement (638PSC) contained 200 g/kg SBM, 200 g/kg cracked corn, 400 g/kg dried distillers' grains and 200 g/kg PSC with karanjin and pongamol concentrations of 638 ppm and 76 ppm pongamol, respectively. To ensure complete consumption, supplements were dosed ruminally prior to feeding hay at 0600h each day. Bermudagrass (*Cynodon dactylon*) hay was chopped through a screen (76 × 76mm) and offered at 0600h daily at 130% of the

previous 4-d average consumption. Steers were housed in an enclosed barn and allowed *ad libitum* access to water and commercial trace mineralized salt (composition: ≥ 96% NaCl, 1,000 ppm Fe, 80 ppm Zn, 2,000 ppm Mn, 70 ppm I, 250 ppm Cu, 100 ppm Co; Roto Salt Company, Penn Yan, New York)**.**

The experiment consisted of five 14-d periods, each consisting of 8 d to adapt steers to treatments, 5 d to determine intake and digestion, and 1 d to evaluate ruminal fermentation. Steers were housed in individual pens (2.1 × 1.5 m) for the first 7 d of each period, and then moved to individual metabolism crates for the remainder of adaptation and throughout the collection period. Metabolism crates were designed such that feces and urine were collected into separate bins by gravity. Calculations of intake and digestion were made from observations on d 9 through 13. Hay, supplement, and ort samples were collected d 9 through 12 to correspond with fecal samples collected d 10 through d 13. Feces and urine collected over each 24-h period were thoroughly mixed and a portion of each (3% fecal matter, 3% urine) was sub-sampled before freezing at -20°C. Urine pH was maintained below 3 by adding 400 ml of 6 *M* HCl to urine bins prior to the initiation of each day's collection.

Experiment 2

In experiment 2, thirteen ruminally cannulated steers (424.6 ± 156.0 kg BW) were used in a completely randomized study to determine the effects of highly detoxified PSC (2 ppm karanjin and 3 ppm pongamol) on forage utilization. Steers were stratified by weight and assigned to one of three treatments. The three treatments consisted of a

control (no supplement, CON, n = 3 or 4) and two providing 75 mg of N/kg BW, one containing 400 g/kg pongamia seedcake (2PSC; with the remaining 600 g/kg provided as dried distillers' grains; n = 3 or 4) and one containing no PSC (0PSC; 420 g/kg SBM, 380 g/kg cracked corn, 200 g/kg DDG). To ensure complete consumption, supplements were dosed ruminally prior to feeding hay at 0600h each day. *Bothriochloa ischaemum v. songarica* (bluestem hay) was chopped through a screen (76 × 76 mm) and offered at 0600h daily at 130% of the previous 4-d average consumption. Steers were housed in an enclosed barn and allowed *ad libitum* access to water and commercial trace mineralized salt blocks as in Exp. 1.

Steers were fed treatments for 18 d: 11 d for adaptation, 6 d to determine intake and digestion, and 1 d to quantify ruminal fermentation. Steers were housed in individual pens (2.1 × 1.5 m) for the entirety of the study. Calculations of intake and digestion were made from observations on d 11 through 17. Hay, supplement, and ort samples were collected d 11 through 16 to correspond with fecal samples collected d 12 through d 17. Steers were fitted with fecal bags for total feces collection. Feces collected over each 24-h period were thoroughly mixed and sub-sampled before being dried at 55 degrees C in a forced air oven for 96 h.

Laboratory Analysis

Hay, fecal, and ort samples were dried in a forced-air oven for 96 h at 55°C, allowed to air-equilibrate, and weighed to determine partial DM. Hay and supplement samples were composited on an equal weight basis across days. Ort and fecal samples

were composited by steer across days within each period. Hay, ort, fecal and supplement samples were ground with a Wiley mill to pass a 1-mm screen and dried at 105°C for determination of DM. Organic matter was determined as the loss in dry weight upon combustion in a muffle furnace for 8 h at 450°C. Neutral detergent fiber (NDF) was performed using an Ankom Fiber Analyzer with amylase. Sodium sulfite was omitted and there was no correction for residual ash (Ankom Technology Corp., Macedon, NY). Ruminally degradable protein was determined by incubating forages and supplements in a buffer and *S. griseus* protease mixture for 48 h. Following incubation, samples were analyzed for N content using Kjeldahl method. Digestion was calculated by the following formula: [1-(output of nutrient/intake of nutrient)] × 100.

Statistical Analysis

Experiment 1

Intake, digestion, and N balance were analyzed using the MIXED procedure in SAS 9.2 (SAS Inst. Inc., Cary, NC). Terms in the model included treatment and period, with steer as a random effect. Least squares means were calculated by treatment and compared using contrasts were CON versus 0PSC, 2PSC versus 0PSC, 49PSC versus 0PSC, and 638 PSC versus 0PSC.

Experiment 2

Intake and digestion were analyzed using the MIXED procedure in SAS 9.2 (SAS Inst. Inc., Cary, NC). Terms in the model included treatment, with steer within treatment as

the random effect. The LSMEANS options was used to calculate treatment means and the pdiff function was used to separate treatment means.

For both experiments, significance was determined at $P \leq 0.05$ and a tendency between $P > 0.05$ and $P \leq 0.10$.

Results

Experiment 1

As per the design, supplement OM intake was greater ($P < 0.01$; Table 2) for 0PSC than CON, resulting in a tendency ($P = 0.08$) for greater total OM intake for 0PSC. However, this did not result in a significant difference ($P = 0.11$) in TDOMI, which is partially attributable to the lack of a difference ($P = 0.56$) in OM digestibility. There was at tendency (P = 0.08) for FOMI to be greater in 0PSC (76.6 g/kg MBW) supplemented steers than 2PSC and 49PSC (63.5 and 62.7 g/kg MBW, respectively). Total OM intake tended to be greater for 0PSC than 2PSC ($P = 0.10$), whereas supplementation with 49PSC and 640PSC did not result in a significant difference ($P \geq 0.11$) from 0PSC. Total digestible OM intake when steers were provided 2PSC and 49PSC supplements did not significantly ($P \leq 0.11$) from 0PSC. However, TDOMI tended ($P = 0.08$) to be greater in steers receiving 0PSC compared to those supplemented with 638PSC ($P \geq 0.08$), resulting from numerically greater TOMI and OMD for 0PSC than 638PSC. Digestion of NDF was less ($P = 0.02$) in steers supplemented with 638PSC compared to the 0PSC treatment (413 and 492 g/kg, respectively).

As expected, intakes of karanjin and pongamol increased as extent of PSC detoxification decreased ($P < 0.01$; Table 2).

All steers retained N (positive N balance); however, CON steers retained less ($P = 0.05$; Table 3) than 0PSC (6.8 versus 14.8 g of N/d, respectfully). For all other measures of N utilization, except forage N intake, 0PSC was greater ($P \leq 0.01$) than CON. Following FOMI, forage N intake was greater ($P = 0.05$) for 0PSC than 2PSC and tended to be greater ($P = 0.07$) for 0PSC than 49PSC, but was not different for 0PSC compared to other treatments. Accordingly, there was a tendency ($P \geq 0.09$) for total N intake to be greater for 0PSC than 2PSC and 49PSC, but not others. For all other measures of N utilization there were no significant differences between 0PSC steers and those receiving PSC containing supplements ($P \geq 0.11$).

Experiment 2

By design, supplement OM intake was greater ($P < 0.01$; Table 5) for 0PSC and 2PSC than CON. Forage, total OM, and total digestible OM intake were significantly greater for supplemented steers (0PSC and 2PSC) than CON ($P < 0.01$). Provision of supplemental protein did not significantly affect either OM or NDF digestion ($P \geq 0.19$). As expected, intake of karanjin and pongamol was higher for 2PSC (5.58 and 8.36 mg/kg MBW; $P < 0.01$) than both 0PSC and CON (0 mg/kg MBW; Table 5).

Discussion

Our hypothesis was that reducing the concentrations of karanjin and pongamol in PSC would enable PSC to be substituted for SBM as a supplemental source of protein in cattle consuming low-quality forage. The magnitude of response to supplemental protein, specifically to the positive control (0PSC), varied between the two experiments with FOMI being 31.8% greater for 0PSC than CON in experiment 2 while only 9.9% greater in experiment 1. Supplementation with 0PSC increased OMD by 7% in experiment 2 versus 3% in experiment 1. Ultimately, these two responses culminated in 52.5% greater TDOMI with protein supplementation in experiment 2 and 23.5% greater TDOMI in experiment 1. Approximately 73% of the TDOMI response observed in experiment 1 is attributable to the provision of a highly digestible (858 g/kg DM) supplement.

An attenuated forage utilization response to supplemental protein in cattle fed a basal diet of bermudagrass (82 g CP/kg DM) was previously observed by Mathis et al. (2000), who attributed the lack of supplemental protein response to sufficient ruminally available N (RAN) in the bermudagrass (82 g RDP/kg TDOMI). In that study, the forage protein content exceeded both the 70 g CP/kg DM threshold postulated by Moore and Kunkle (1991) and the 60-80 g CP/kg DM suggested by Cochran (1996). Additionally, the bermudagrass fed had low digestibility (517 g DOM/kg DM), limiting the response surface.

Although the 2000 NRC recommended RDP be supplied at 130 g RDP/kg total digestible nutrients, requirements as low as 71 g RDP/kg TDOMI have been reported as sufficient to maximize microbial efficiency (Hollingsworth-Jenkins et al., 1996). The bermudagrass hay fed in experiment 1 provided 68 g CP/kg DM which is very close to the aforementioned 70 g CP/kg DM threshold, below which a protein supplementation response is expected, thus the protein supplementation response surface was relatively small in experiment 1. In contrast, the King Ranch Bluestem fed in experiment 2 contained 49 g CP/kg DM and had a digestibility of 557 g DOM/kg OM creating the potential for a relatively large increase in forage utilization with the delivery of supplemental protein, which was observed.

Our observations of increased forage utilization in experiment 2, are consistent with other studies which have observed FOMI increases of 23 to 32% when utilizing forages with CP ranging from 43 to 53 g/kg DM (Mathis et al., 2000; Wickersham et al., 2004;, Drewery et al., 2014). Drewery et al. (2014) observed a 23% increase in FOMI when providing CSM to steers receiving a basal diet of straw. Similarly, sodium caseinate provision (0.87 g/kg BW) increased FOMI 32% in steers consuming grass hay (Wickersham et al., 2004).

In experiment 2, both PSC and conventional protein supplements increased forage utilization to a similar extent compared to the unsupplemented control. Blending PSC with conventional protein supplements has been previously reported by Ravi et al. (2000) and Srivastava et al. (1990), and they observed similar total DM intakes when

feeding conventional protein supplements and PSC-containing supplements to lambs (240 g PSC/kg DM of supplement) and goat kids (200, 300, and 400 g PSC/kg DM of supplement), respectively. In contrast, Housman et al. (2020) found that when feeding steers solvent-extracted PSC at 400 g/kg supplement, forage OMI, total OMI, and TDOMI were similar to unsupplemented steers, but were 20% lower than responses to a conventional protein supplement. Differences between PSC and the conventional protein supplement observed in Housman et al. (2020) were likely caused by higher intake of karanjin and pongamol (147.1 and 82.0 mg/kg MBW, respectively) in comparison to the toxin consumption in experiment 2 (5.58 and 8.36 mg/kg MBW, respectively).

Increases in digestibility are often reported in response to the inclusion of supplemental protein (21% increase, Köster et al., 1996; 13% increase, Wickersham et al., 2008). However, OMD was not measurably affected by protein supplementation in either experiment 1 or 2. Köster et al. (1996) observed increases in OMD when supplemental RDP was provided up to 58 g RDP/kg TDOMI, but additional RDP did not further increase OMD. In the present study, sufficient RDP in the control (86.8 g RDP/kg DOMI) or resistance of bermudagrass to digestion may have prevented any increase in OMD in experiment 1.

Although RDP remained under the requirement when supplemental protein was provided, OMD did not increase significantly in experiment 2. As with conventional protein supplements, PSC containing supplements did not affect OMD in either of the present experiments. Previous work using PSC as a source of supplemental protein

(Srivastava et al., 1990) to goat kids at 0, 200, 300, and 400 g PSC/kg of concentrate did not observe an increase in OMD. When lambs were fed solvent extracted PSC at 200 g PSC/kg DM or expeller pressed PSC at 240 g PSC/kg DM of concentrate, no change in OMD was found (Singh et al., 2006). Using the same diets as Singh et al. (2006), reported OMD was similar between the control supplement and solvent extracted PSC, but expeller pressed PSC supplementation decreased OMD by 5.5% (Ravi et al. (2000).

Antinutritional factors, pongamol and karanjin, have been suggested to reduce digestibility due to their known anti-bacterial and insecticidal properties, and to their phenolic structure (Kumar and Singh, 2002; Sajid et al., 2012). In experiment 1, reduced NDF digestibility in steers fed the 638PSC treatment compared to 0PSC may have resulted from the greater amounts of karanjin and pongamol (509.4 and 60.7 g/kg MBW) consumed by steers receiving the 638PSC treatment. Steers fed 2PSC and 49PSC consumed 6.4 and 155.2 g/kg MBW of karanjin and 9.5 and 41.2 g/kg MBW of pongamol, respectively and digestibility was not different than steers receiving 0PSC.

Singh et al. (2006) reported a 7% decrease in NDF digestibility in lambs fed expeller pressed PSC (29 g/kg EE) compared to those fed solvent extracted PSC (17 g/kg EE); a primary difference in these methods of extraction is the amount of karanjin and pongamol that is removed in the extraction processes (Panda et al., 2006). Ravi et al. (2000) also reported a 13.9% reduction in NDF digestibility when lambs were fed an expeller-pressed pongamia supplement compared to a conventional control supplement.

In contrast, Housman et al. (2020) found no differences in NDF digestibility between control and supplemented treatments. Similarly, no changes in NDF digestibility were observed in experiment 2 ($P = 0.53$). Expeller pressed PSC has generally been observed to contain more residual fat (90 – 115 g/kg DM; Gupta et al., 1981; Housman et al., 2020) and both karanjin (733 mg/kg DM) and pongamol (390 mg/kg DM).

In a N balance trial conducted by Srivastava et al. (1990), goat kids were fed PSC at 0, 200, 300, and 400 g PSC/kg of their total concentrate. Similar to experiment 1, all animals remained in a state of positive N balance. Nitrogen intake was not affected by level of PSC inclusion, ranging from 8.68-12.35 g/d N ($P < 0.05$). Nitrogen retention decreased with increasing inclusion of PSC, with 0 PSC and 200 PSC treatments not differing from one another ($P > 0.05$), 200 PSC and 300 PSC not differing ($P > 0.05$), and 300 and 400 PSC not being different; however, the 0 PSC diet provided greater N retention than the 300 and 400 PSC diets with 5.72, 4.35, and 3.25 g/d of N respectively. Housman et al. (2020) also reported positive N retention in steers consuming expeller pressed PSC at 200 g/kg of the supplement and solvent extract PSC at 400 g/kg (19.7 and 22.9 g/kg, respectively). As expeller pressed PSC inclusion increased in their work, retained N decreased; however, it was never lower than those steers not receiving supplemental protein. Supplements in experiment 1 and Housman et al. (2020) with no PSC had the greatest retained N similar to Srivastava et al. (1990).

Conclusion

When PSC has been well detoxified similar to the 2PSC treatment, PSC can be fed as a source of supplemental protein similar to a conventional source of protein. The PSC used in our supplements was solvent extracted and then subjected to further proprietary extractions to remove the karanjin and pongamol. To see a clear response to supplemental protein, forage must be of low enough quality to cause a deficiency in RAN.

Table 1. Composition of low-quality hay and protein supplements[1]

		Supplements[2]			
Item	Hay	0PSC	2PSC	49PSC	638PSC
Chemical composition, g/kg of DM					
OM	927	950	947	947	948
NDF	734	175	372	366	346
CP	68	297	312	310	308
RDP	63	69	51	55	56
Karanjin, ppm			0.8	19.6	126.6
Pongamol, ppm			1.2	6.4	15.2
Supplement composition, g/kg					
SBM		420	0	0	200
Corn		380	0	0	200
DDG		200	600	600	400
PSC		0	400	400	200

[1]SBM= soybean meal; DDG= dried distillers' grain; PSC= pongamia seedcake
[2]CON = no supplement; 0PSC = no Pongamia seedcake included; 2PSC = 400 g of 2 ppm karanjin Pongamia seedcake/kg of supplement 49PSC = 400g of 49 ppm karanjin Pongamia seedcake/kg of supplement; 638PSC = 200 g of 638 ppm karanjin Pongamia seedcake/kg of supplement

Table 2: Effect of feeding Pongamia seedcake on intake and digestion in cattle consuming low-quality forage[1]

Item	Treatments[2]									
	CON	0PSC	2PSC	49PSC	638PSC	SEM[3]	CON v 0PSC	2PSC v 0PSC	49PSC v 0PSC	638PSC v 0PSC
No. of Obs	5	5	5	4	5					
OM Intake, g/kg MBW[4]										
Supplement	0.0	7.1	7.1	7.1	7.1	0.1	<0.01	0.94	0.79	0.98
Forage	69.7	76.6	63.5	62.7	65.1	6.7	0.32	0.08	0.08	0.16
Total	69.7	83.7	70.6	69.8	72.2	6.7	0.08	0.10	0.11	0.15
Digestible	36.1	44.6	36.9	35.4	35.4	4.4	0.11	0.14	0.11	0.08
Intake, mg/kg MBW										
Karanjin	0	0	6.4	155.2	509.4	8.4	1.00	0.54	<0.01	<0.01
Pongamol	0	0	9.5	41.2	60.7	0.6	1.00	<0.01	<0.01	<0.01
Total Tract Digestion, g/kg										
OM	517	533	521	502	487	23.7	0.56	0.67	0.32	0.11
NDF	505	492	472	477	413	26.4	0.67	0.54	0.67	0.02

[1]Within each row, means with differing superscripts differ at (P< 0.05) level of significance

[2]CON = no supplement; 0PSC = no Pongamia seedcake included; 2PSC = 400 g of 2 ppm karanjin Pongamia seedcake/kg of supplement 49PSC = 400g of 49 ppm karanjin Pongamia seedcake/kg of supplement; 638PSC = 200 g of 638 ppm karanjin Pongamia seedcake/kg of supplement

[3]SEM = standard error of the mean

[4]MBW = metabolic body weight (initial BW$^{\wedge 0.75}$)

Table 3: Effect of feeding Pongamia seedcake on N retention in cattle consuming low-quality forage

Item	Treatments[1]									
	CON	0PSC	2PSC	49PSC	638PSC	SEM[2]	CON v 0PSC	2PSC v 0PSC	49PSC v 0PSC	638PSC v 0PSC
No. of Obs	5	5	5	4	5					
N, g/d										
Total Intake	47.8	72.0	62.7	62.3	64.5	6.0	< 0.01	0.09	0.10	0.16
Forage Intake	47.8	51.7	41.5	41.5	44.2	5.3	0.41	0.05	0.07	0.13
Supplement Intake	0.0	20.3	21.2	20.9	20.3	1.1	< 0.01	0.17	0.43	0.97
Fecal	27.8	37.7	32.8	32.7	34.4	3.5	0.01	0.17	0.21	0.35
Urinary	13.2	19.5	21.7	20.8	19.8	2.0	< 0.01	0.24	0.52	0.83
Absorbed	20.0	34.3	30.0	29.4	30.1	3.7	< 0.01	0.25	0.22	0.26
Retained	6.8	14.8	8.3	8.7	10.3	3.4	0.05	0.11	0.11	0.25

[1]CON = no supplement; 0PSC = no Pongamia seedcake included; 2PSC = 400 g of 2 ppm karanjin Pongamia seedcake/kg of supplement 49PSC = 400g of 49 ppm karanjin Pongamia seedcake/kg of supplement; 638PSC = 200 g of 638 ppm karanjin Pongamia seedcake/kg of supplement
[2]SEM = standard error of the mean

Table 4: Composition of low-quality forage and protein supplements[1]

Item	Hay	0PSC	2PSC
Chemical composition, g/kg of DM			
OM	894	951	946
NDF	761	205	400
CP	49	315	336
RDP	66	51	55
Karanjin, ppm			0.8
Pongamol, ppm			1.2
Supplement composition, g/kg			
SBM		420	0
Corn		380	0
DDG		200	600
PSC		0	400

[1]SBM = soybean meal; DDG = dried distillers' grain; PSC = pongamia seedcake

Table 5: Effects of feeding Pongamia seedcake on intake and digestion in cattle consuming low-quality forage[1]

Item	Treatments[2]			SEM[3]	*P*-value
	CON	0PSC	2PSC		
No. of Observations	4	4	5		
OM Intake, g/kg MBW[4]					
Supplement	0.0[a]	6.2[b]	6.2[b]	0.14	<0.01
Forage	55.9[a]	73.7[b]	67.5[b]	3.03	<0.01
Total	55.9[a]	79.9[b]	73.7[b]	3.02	<0.01
Total digestible	31.2[a]	47.6[b]	42.2[b]	1.90	<0.01
Intake, mg/kg MBW					
Karanjin	0[a]	0[a]	5.58[b]	0.15	<0.01
Pongamol	0[a]	0[a]	8.36[b]	0.23	<0.01
Total Tract Digestion, g/kg					
OM	557	596	574	19.3	0.19
NDF	569	586	563	18.9	0.53

[1]Within each row, means with differing superscripts differ at ($P< 0.05$) level of significance
[2]CON= no supplement; 0PSC = contained no PSC; 2PSC = contained 400 g/kg DM as 2 ppm karanjin Pongamia seedcake
[3]SEM = standard error of the mean
[4]MBW = metabolic body weight (initial BW^0.75)

CHAPTER III

EFFECTS OF TRACE MINERAL SOURCE AND PROTEIN SUPPLEMENTATION ON UTILIZATION OF LOW-QUALITY FORAGE BY BEEF STEERS

Overview

Eight steers (484 ± 44.9 kg of BW) were used in replicated 4 × 4 Latin Squares to evaluate the interaction between mineral source and protein supplementation in cattle consuming a basal diet of King Ranch Bluestem hay (38g/kg CP, 746 g/kg NDF). Treatments were arranged as a 2 × 2 factorial with steers receiving either 0 (CON) or 175 mg of N/kg BW provided as cottonseed meal (CSM; 441 g/kg CP, 1.21 kg DM/d). To control for Ca intake, steers on CON received supplemental dicalcium phosphate (76 g/d). For the second factor steers received either sulfate trace mineral (STM; 99 g/d) or hydroxy trace mineral (HTM; 99 g/d; IntelliBond Vital 4; Micronutrients LLC, Indianapolis, IN). Four 21-d periods were conducted, consisting of 13 d to adapt steers to treatments, 6 d to measure intake and digestion, 1 d to complete a ruminal fermentation profile, and 1 d to determine ruminal fill. No mineral source × protein supplement interactions ($P \geq 0.64$) or significant effects of mineral source ($P \geq 0.06$) were observed for all measures of intake and digestibility. Protein supplementation increased all measures of intake ($P \leq 0.01$) and more than doubled total digestible OM intake (TDOMI). However, NDF digestion (NDFD) was not significantly affected ($P = 0.35$) by protein supplementation. There was a tendency ($P = 0.06$) for OMD to be greater (28 g/kg) for STM than HTM in both CSM and CON steers. There was a tendency ($P = 0.07$) for a mineral source × protein supplement interaction for

rate of passage, resulting from similar passage rates for CON regardless of mineral sources and increased passage rate with protein supplementation with a greater increase for HTM steers. Additionally, there were tendencies (P = 0.07 and 0.08) for mineral source × protein supplement interaction for ruminal DM and ADF fill. Rumen DM and ADF fill responded similarly with fill increasing with supplemental protein for steers fed STM, and decreasing with supplemental protein in steers fed HTM. Forage utilization increased in response to protein supplementation and there were minimal effects of trace mineral source on low-quality forage utilization by beef steers.

Introduction

Protein supplementation is required to mitigate performance losses when the basal forage lacks the N necessary to meet the requirements of the microbial population (Kartchner, 1980). Low-quality forage, containing less than 6-8% CP, may not provide sufficient ruminally available N to allow for maximum forage utilization (Tamminga, 1979; Cochran, 1998). A limited supply of dietary ruminally available N limits cell wall degradation, decreasing digestion (Tamminga, 1979), and the amount of energy available to the animal. Supplemental protein stimulates microbial activity augmenting low-quality forage utilization by increasing consumption of digestible organic matter (Köster et al., 1996; Drewery et al., 2014; Housman et al., 2020).

Other factors may also influence diet utilization. Minerals that dissociate in the rumen interact with ruminal microbes potentially decreasing nutrient utilization by decreasing microbial degradation and fermentation (Forsberg, 1978; Eryavuz et al., 2009) Early work, by Forsberg

(1978) demonstrated than free Cu^{2+} inhibited *B. Succinogens*, *R. albus*, and *E. Ruminantium* at concentrations as low as 21µg/ml. In an *in vitro* study Eryavuz et al. (2009) observed that soluble Zn supplemented at 50 µg/ml killed microbes, therefore reducing cellulose digestion ($P < 0.01$) after 24 h of incubation.

Ruminal solubility of supplemental trace minerals (TM) sources ranges from 0.6% to 100% for $Cu_2(OH)_3Cl$ and $ZnSO_4$, respectively (Spears et al., 2004; Cao et al., 2000). Specifically, Cao et al. (2000) compared the solubility of two forms of Zn finding $ZnSO_4$ to be 100% soluble in both water and 0.4% HCl. In contrast, Zn hydroxychloride ($Zn_5(OH)_8Cl_2 \cdot H_2O$) was 3% soluble in water and 100% soluble in 0.4% HCl. Spears et al. (2004) reported similar solubilities for Cu hydroxychloride ($Cu_2(OH)_3Cl$), being relatively insoluble in water (0.6%); however, 86.8% soluble at a pH of 2.2 compared to $CuSO_4$ (94.5% soluble in water and 96.8% soluble at a pH of 2.2). Effects of those decreased solubilities on intake and digestion were evaluated in dairy cows fed high-quality forage and byproducts in which cows receiving hydroxy TM (HTM) had greater NDF digestion than those provided sulfate TM (48.5 and 46.4%, respectively; Faulkner and Weis, 2017). These observations suggest that the low solubility of HTM in the rumen may prevent a decrease in NDF digestion caused by soluble STM.

Data comparing HTM and sulfate TM (STM) fed to cattle consuming a basal low-quality forage diet with or without protein supplementation are not present in the literature. Our objective was to determine the effects of trace mineral source with or without supplemental protein on forage utilization in cattle consuming low-quality forage. Our hypothesis is the protein supplementation will improve forage utilization and we will observe greater digestibility in steers supplemented HTM than STM.

Materials and 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.

Eight steers (484 ± 44.9 kg of BW) were used in replicated 4 × 4 Latin Squares to evaluate the interaction between mineral source and protein supplementation in cattle consuming a basal diet of King Ranch Bluestem hay (*Bothriochloa ischaemum* var *songarcia,* 38 g CP/kg DM; Table 1). Treatments were arranged as a 2 × 2 factorial with steers receiving either 0 (CON) or 175 mg of N/kg BW provided as cottonseed meal (CSM; 1.21 kg DM/d). For the second factor steers received either sulfate trace mineral (STM; 99 g/d with Cu, Zn, Mn, and Co provided as $CuSO_4$, $ZnSO_4$, $MnSO_4$, $CoSO_4$, respectively) or hydroxy trace mineral (HTM; 99g/d) with Cu, Zn, Mn, and Co provided as $Cu_2(OH)_3Cl$, $Zn_5(OH)_8Cl_2{\cdot}H_2O$, $Mn_2(OH)_3Cl$, and $CoCO_4$, respectively (IntelliBond® VITAL4..; Micronutrients LLC, Indianapolis, IN). Mineral supplements were formulated to supply Cu, Zn, Mn, Co, Se, and I at twice the animal's recommended daily requirements (NASEM, 2016). To control for Ca intake, steers on CON received supplemental dicalcium phosphate (76 g/d).

To ensure complete consumption, all supplements were dosed ruminally prior to feeding at 0600h. Hay was chopped through a screen (76 mm × 76 mm) and offered at 0600 h daily at 130% of the previous 4-d average consumption. Steers were housed in individual metabolism pens (2.1 m × 1.5 m) within an enclosed climate controlled barn and allowed *ad libitum* access to water.

Four 21 d periods were conducted, consisting of 13 d to adapt steers to treatments, 6 d to determine intake and digestion, 1 d to quantify ruminal fermentation, and 1 d to determine ruminal fill. Steers were fitted with fecal bags for total feces collection. Calculations of intake and digestion were made from observations on d 13 through 19. Hay, supplement, and ort samples were collected d 13 through 18 to correspond with fecal samples collected d 14 through d 19. Feces collected over each 24-h period was thoroughly mixed and sub-sampled before being dried.

On d 21, ruminal DM and liquid contents were measured by manual evacuations prior to feeding (0 h) and 4 h after feeding. Immediately after removal ruminal contents were thoroughly mixed, sampled thrice for subsequent determination of DM and acid detergent insoluble ash (ADIA), and contents were returned to the rumen.

Hay, fecal, ort, and ruminal digesta samples were dried in a forced-air oven for 96 h at 55°C, allowed to air-equilibrate, and weighed to determine partial DM. Hay and supplement samples were composited on an equal weight basis across days. Ort and fecal samples were composited by steer across days within each period. Hay, ort, fecal, digesta, and supplement samples were ground with a Wiley mill to pass a 1-mm screen. Samples were dried at 105°C 24 h for determination of DM. Organic matter was determined as the loss in dry weight upon combustion in a muffle furnace for 8 h at 450°C. Crude protein contents for the forage and protein supplements was determined as 6.25 × Kjeldahl N. These samples were also analyzed for neutral detergent fiber (aNDF) using an Ankom Fiber Analyzer with sodium sulfite and amylases omitted and without correction for residual ash (Ankom Technology Corp. Macedon, NY). Acid detergent fiber (ADF) was also determined using an Ankom Fiber Analyzer and acid detergent

insoluble ash (ADIA) was determined by combusting ANKOM bags containing the ADF residues for 8 h at 450°C in a muffle furnace. Ruminally degradable protein was determined by incubating forage and CSM in a buffer and *S. griseus* protease mixture for 48 h. Following incubation, samples were analyzed for N content using Kjeldahl method. Digestion was calculated by the following formula: [1- (output of nutrient/intake of nutrient)] × 100. Particulate passage was estimated by dividing the rate of ADIA consumption by average amount of ADIA in the rumen (Waldo et al., 1972). As a proxy for ruminal solubility, 10g of each mineral was mixed with 0.1L deionized water at room temperature and stirred. After stirring for 25 min, the sample was allowed to rest for 5 min before being extracted through a 0.45µg syringe filter. Metal not solubilized in the deionized water, remaining on the filter, was considered insoluble and quantified using ICP-MS.

Statistical Analysis

Intake, digestion, rate of passage, and ruminal fill were analyzed using the MIXED procedure in SAS 9.4 (SAS Inst. Inc. Cary, NC). Terms in the model included treatment, period, and square, with steer as a random effect. Treatments were calculated with the LSMEANS option and the pdiff function was used to separate treatment means when the overall F test was significant. Significance was determined at $P \leq 0.05$ and a tendency between $P > 0.05$ and $P \leq 0.10$.

Results

Intake and Digestibility

No mineral source × protein supplement interactions ($P \geq 0.68$; Table 2) or significant effects of mineral source ($P \geq 0.06$) were observed for any measure of digestibility or intake. However, there was a tendency (P = 0.06) for OMD to be greater (4 and 9%) for STM than HTM in both CSM and CON steers respectively.

Protein supplementation increased total OM intake (TOMI) and OM digestion ($P < 0.01$), resulting in a more than two-fold increase in total digestible OM intake (TDOMI) and digestible energy (DE) intake. However, NDF digestion (NDFD) was not affected ($P = 0.35$) by protein supplementation.

Rate of Passage

There was a tendency ($P = 0.07$; Table 3) for a mineral source × protein supplement interaction for rate of passage. In steers receiving no protein supplement, passage rates were similar among mineral sources. In steers receiving protein supplement (CSM), passage rate increased relative to unsupplemented controls ($P < 0.01$), but the increase was greater ($P = 0.07$) in steers receiving HTM than those receiving STM.

Additionally, there were tendencies ($P = 0.07$ and 0.08) for mineral source × protein supplement interaction effects on ruminal DM and ADF fill. Ruminal fill increased with supplemental protein for steers fed STM, and decreased with supplemental protein in steers fed HTM.

Mineral Intake

No mineral source × protein supplement interactions ($P \geq 0.57$; Table 5 and 6) were observed for either macromineral or micromineral intake. Steers receiving protein supplementation received increased ($P < 0.01$) amounts of of K, Mg, N, and S. Due to the augmentation of the diet for steers not receiving protein supplements, Ca intake was not different between CON and CSM ($P = 0.91$). An increase in phosphorus intake ($P < 0.01$) was detected for steers receiving CON; however, the magnitude of the difference was small (approximately 1 g/d).

Macromineral intakes were not affected by mineral source ($P \geq 0.13$) with the exception of Na ($P = 0.03$), which was 0.5 g/d less in STM than HTM. Microminerals measured were all provided in excess of steer requirements (NASEM 2016).

All microminerals, with the exception of Fe, were consumed in greater quantities in steers receiving protein supplement ($P \leq 0.03$; Table 6), resulting from the combination of CSM intake and greater forage intake, both of which provided microminerals. Steers fed CSM consumed less Fe ($P < 0.01$) than steers fed CON because the dicalcium phosphate provided with CON contained 12,100 mg/kg Fe. Mineral source result in significant differences ($P < 0.01$) in Co, Cu, Fe, and Mn intake. Intake of Cu and Mn was greater in STM (26.2 and 7.7%, respectively) than HTM. In contrast, intake of Co and Fe was greater in HTM (33.4 and 5.5%, respectively) than STM due to ingredient variability and mixing.

Discussion

Intake

Our objective was to determine the effects of trace mineral source on forage utilization, specifically digestibility, in beef steers fed low-quality forage, with or without protein supplementation. Protein supplementation is required to sustain animal performance (Mathis et al., 1999), generally prevent body condition score and weight loss when the basal forage lacks the N necessary to meet microbial requirements (Kartchner, 1980). Supplementation of protein, specifically ruminally degradable protein (RDP), provides a source of ruminally available N (RAN) to increase forage utilization (Köster et al., 1996; Hollingsworth-Jenkins et al., 1996; Mathis et al., 2000; Wickersham et al., 2004). Relieving a ruminal protein deficit may result in a secondary factor, becoming a limitation; alternately, interactions of other nutrients with microbial function may limit the response to protein. Forage offered in this experiment failed to supply sufficient RDP to meet microbial requirements, providing 48 g RDP/kg TDOMI. The requirement in cattle fed low-quality forage has been observed to range from 71 to 130 g RDP/kg TDOMI (Hollingsworth-Jenkins et al., 1996; NASEM, 2016). Accordingly, provision of supplemental protein increased intake of FOM, TDOM, and TDNDF by 38, 44, and 42%, respectively, which is similar to previous observations (Köster et al., 1996; Mathis et al., 2000; Wickersham et al., 2004; Drewery et al., 2014; Housman et al., 2020).

Digestion

Protein supplementation increased OMD by 10%, which is in accordance with previous observations (Köster et al., 1996; Wickersham et al., 2004; Drewery et al., 2014) and accounts

for a portion of the TDOMI response to supplementation. However, NDFD was not greater in supplemented steers which is in contrast to previous work (Köster et al., 1996; Wickersham et al., 2004; Drewery et al., 2014). Supplementation lead to increased passage rate potentially preventing an increase in NDFD because the ruminal microbes has less time to degrade consumed NDF. Similar to our experiment, in a study examining the effects of supplemental protein on ruminal capacity and site and extent of digestion, Hannah et al. (1991) observed increased OMD with no effect on ruminal NDFD when steers were provided supplemental protein. Hannah et al. (1991) attributed the similarity in NDFD between supplemented and unsupplemented steers to have in part been caused by the 41% increase in rate of digestion and the 28% increase in passage rate that occurred when steers received supplemental protein. Although rate of digestion was not measured in our study, the faster rate of passage and increased OMD suggests that ruminal digestion of easily degradable nutrients, including the supplement itself, happened more quickly with protein supplementation; however, increased rate of digesta passage from the rumen prevented observable increases in NDF digestion.

In contrast to our hypothesis, STM tended to ($P = 0.06$) to result in greater OMD than HTM. Our hypothesis was based on the observation that HTM are less soluble in the rumen than STM (9.7 versus 14.0%, respectively; Genther and Hansen, 2015) thus preventing a reduction in digestibility that is caused by free metals interacting with ruminal microbes (Eryavuz et al., 2009). However, the insolubility of the STM used in this project suggest that they had limited ruminal solubility. The lack of solubility observed in these ingredients does not agree with the previous work showing that metals bound to sulfate molecules, such as $CuSO_4$, are easily dissociated in neutral environments such as water, or the reticulorumen (solubility ranging from

94.5 to 100%; Cao et al., 2000; Spears et al., 2004; Caldera et al., 2019). In contrast, the solubility of HTM fed in this project was in the solubility range (0.6 – 3%) observed previously (Cao et al., 2000; Genther and Hansen, 2015; Caldera et al., 2019).

Formation of insoluble metal complexes, such as cupric sulfide (CuS), is credited for decreasing fiber digestion and mineral bioavailability typically observed when STM are provided to cattle. When sulfate minerals like $CuSO_4$ dissociate, free metals may bind or adsorb to microbes or charged compounds known as trapping agents (Suttle, 1991). Trapping agents such as S^{2-}, MoO_4^{2-}, and Fe_2O_3, have a strong affinity for free cations and may cause TM like Cu to become insoluble even in acidic environments (Suttle, 1991).

Some populations of ruminal microbes, such as *Desulfovibrio*, utilize sulfate (SO_4^{2-}) as a terminal electron receptor in the creation of energy, producing sulfide (S^{2-}) as a waste product (Huisingh et al., 1974). Sulfide can be absorbed, reduced by H^+ in the rumen to produce H_2S that is eructated, or bind to TMs to form insoluble sulfide metal complexes that are not available to the animal (Suttle, 1974; Suttle, 1991; Spears, 2003). Suttle (1974) reported that supplementation with $CuSO_4$ at 8 mg/kg diet increased rumen sulfide concentration 80% in ewes 2 h after feeding; however, plasma Cu concentrations decreased about 50% when the majority of Cu was in a CuS complex.

In addition to insoluble sulfide complexes, abundance of S in the rumen (> 0.25% of diet DM; NASEM, 2016) in conjunction with Mo (> 2 mg/kg DM) may interact with Cu resulting in insoluble thiomolybdate complex that will not release copper even under acidic conditions (Spears, 2003). Suttle (1974) found low sulfur diets (1.0 g S/kg DM) did not affect

bioavailability of Cu when Mo was present at 4.5 mg Mo/kg DM; however, when Mo remained at 4.5 mg Mo/kg DM, diets containing more S (4.0 g S/kg DM) reduced Cu bioavailability by 30-60%. Reducing dissociation and formation of insoluble complexes in the rumen with HTM allows greater bioavailability of mineral to the animal. Other potential insoluble complexes such as cupric phosphate, cupric sulfide, zinc phosphate, and zinc sulfide may be formed when TMs are soluble in the rumen. Ruminal insolubility of STM in this study suggests some of these insoluble metal complexes may have formed prior to feeding. To our awareness, this observation has not been reported in the literature, but results on this study indicate that this question warrants further investigation. If, in fact, the STM minerals were rendered insoluble due to storage, then it is likely that they were not available to be utilized by the animal post-ruminally.

Conclusions

In this study supplemental protein increased OM, DE, and N intake as expected based on previous literature; however, mineral source had no effect. A tendency for the STM treatment to increase OM digestibility in comparison to the HTM treatment disagrees with previous literature comparing hydroxychloride and sulfate bound minerals (Caldera et al., 2019). However, the STM used in the project did not have the anticipated solubility characteristics, suggesting future work on the effects of mineral storage are warranted. Furthermore, sulfate TM that did not dissociate in the rumen was likely not able to be utilized by the animal post-ruminally.

Table 6: Composition of low-quality hay, cottonseed meal, sulfate mineral, and hydroxy mineral[1]

Item	Hay	CSM	STM	HTM	Dical
Chemical composition, g/kg of DM					
DM	924	908	980	982	980
OM	896	920	--	--	--
NDF	746	203	--	--	--
ADF	490	142	--	--	--
ADIA	59	12.4	--	--	--
CP	38	441	--	--	--
RDP	66	57	--	--	--
Solubility, mg/L					
Cu	--	--	0	2.94	38
Mn	--	--	0	24.4	--
Zn	--	--	0	0	--
Mineral, g/kg					
Ca	3.03	3.83	142.75	135.50	211.00
P	0.15	10.40	82.98	83.13	186.00
K	15.15	14.78	14.4	13.90	1.80
Mg	0.78	6.53	30.35	27.35	2.60
Na	0.23	2.33	101.75	107.50	2.50
S	0.95	4.78	11.05	5.88	9.50
Microminerals, mg/kg					
Co	0.20	0.67	22.60	37.88	5.72
Cu	2.24	14.30	1895.00	1442.50	8.59
I	5.00	5.00	83.10	73.00	5.00
Fe	37.78	162.39	4917.50	5607.50	12100.00
Mn	33.31	21.93	3380.00	2920.00	594.00
Mo	1.30	1.18	11.68	11.50	21.8
Se	0.13	4.91	16.43	14.5	5.59
Zn	11.19	73.10	5512.50	5207.5	90.20

[1]CON= no supplement; CSM = cottonseed meal; STM = sulfate mineral; HTM = hydroxy mineral

Table 7: Effect of sulfate and hydroxy mineral types on intake and digestion in cattle consuming low-quality forage

Item	Treatments[1]				SEM[2]	Supplement	Mineral	S×M
	CON		CSM					
	STM	HTM	STM	HTM				
No. of Observations	8	8	7	8				
Intake, kg/d								
Supplement OM	0	0	1.12	1.11	-	-	-	-
Forage OM	5.61	5.69	9.11	9.00	0.42	<0.01	0.96	0.75
Total OM	5.61	5.69	10.23	10.11	0.43	<0.01	0.95	0.75
TDOM	3.03	2.87	6.10	5.80	0.24	<0.01	0.28	0.73
NDF	4.64	4.72	7.83	7.72	0.35	<0.01	0.97	0.72
TDNDF	2.57	2.48	4.48	4.23	0.21	<0.01	0.35	0.68
Total Tract Digestion, g/kg								
OM	543	510	594	571	15.0	<0.01	0.06	0.70
NDF	555	532	570	544	17.0	0.35	0.11	0.90
DE Intake, Mcal/d	12.16	11.34	25.40	23.92	0.98	<0.01	0.20	0.71
N Intake, g/d	37.8	38.0	147.0	145.4	3.6	<0.01	0.83	0.75

[1]CON= no supplement; CSM = cottonseed meal; STM = sulfate mineral; HTM = hydroxy mineral
[2]SEM = standard error of the mean

Table 8: Effect of sulfate and hydroxy mineral types on rate of passage and rumen fill[1]

Item	Treatments[2]				SEM[3]	Supplement	Mineral	S×M
	CON		CSM					
	STM	HTM	STM	HTM				
No. of Observations	8	8	7	8				
Passage Rate, %/h	1.60[c]	1.50[c]	2.06[b]	2.54[a]	0.19	<0.01	0.22	0.07
Rumen DM Fill, kg	14.4	18.7	19.6	13.8	3.7	0.89	0.70	0.07
Rumen ADF Fill, kg	7.70	9.70	9.91	6.88	1.59	0.83	0.71	0.08

[1]Within each row, means with differing superscripts differ at ($P< 0.05$) level of significance
[2]CON= no supplement; CSM = cottonseed meal; STM = sulfate mineral; HTM = hydroxy mineral
[3]SEM = standard error of the mean

Table 9: Macromineral intake with sulfate or hydroxy mineral supplementation

Item	Treatments[1]				SEM[2]	Supplement	Mineral	S×M
	CON		CSM					
	STM	HTM	STM	HTM				
No. of Observations	8	8	7	8				
Intake g/d								
Ca	48.8	48.3	49.0	48.3	1.6	0.91	0.51	0.88
P	23.0	23.0	22.1	22.0	0.4	<0.01	0.75	0.90
K	96.7	97.6	173.2	171.5	7.4	<0.01	0.94	0.80
Mg	7.9	7.8	18.8	18.3	0.4	<0.01	0.28	0.57
Na	11.4	12.0	15.1	15.5	0.20	<0.01	0.03	0.70
S	7.8	7.4	16.5	15.8	0.5	<0.01	0.13	0.64

[1]CON= no supplement; CSM = cottonseed meal; STM = sulfate mineral; HTM = hydroxy mineral
[2]SEM = standard error of the mean

Table 10: Micromineral intake with sulfate or hydroxy mineral supplementation

Item	Treatments[1]				SEM[2]	Supplement	Mineral	S×M
	CON		CSM					
	STM	HTM	STM	HTM				
No. of Observations	8	8	7	8				
Intake mg/d								
Co	3.88	5.40	5.04	6.50	0.53	0.03	<0.01	0.96
Cu	199	156	225	180	3.1	<0.01	<0.01	0.71
I	40.0	39.4	65.0	63.3	2.4	<0.01	0.53	0.71
Fe	1631	1701	1046	1124	34	<0.01	<0.01	0.83
Mn	583	542	695	645	17.7	<0.01	<0.01	0.73
Mo	10.9	11.0	15.7	15.5	0.6	<0.01	0.95	0.76
Se	2.85	2.65	8.47	8.78	0.87	<0.01	0.93	0.70
Zn	612	586	724	708	26	<0.01	0.39	0.83

[1]CON= no supplement; CSM = cottonseed meal; STM = STMfate mineral; HTM = hydroxy mineral
[2]SEM = standard error of the mean

References

Aijla, C.M., S.K. Brar, M. Verma, R.D. Tyagi, S. Godbut, J.R. Valéro. 2012. Bio-processing of agro-byproducts to animal feed. *Critical Reviews in Biotech.* 32: 382-400. doi: 10.3109/07388551.2012.659172

Baber, J.R., J. E. Sawyer, T.A. Wickersham. 2018. Estimation of human-edible protein conversion efficiency, net protein contribution, and enteric methane production from beef production in the United States. *Translational Anim. Sci.* 2: 439–450. doi:10.1093/tas/txy086

Baber, J.R., J. E. Sawyer, T.A. Wickersham. 2019a. Evaluation of net protein contribution, methane production, and net returns from beef production as duration of confinement increases in the cow-calf sector. *J. Anim. Sci.* 97: 2675–2686. doi: 10.1093/jas/skz145

Baber, J.R., J. E. Sawyer, B.P. Holland, K.J. Karr, A.B. Word, T.A. Wickersham. 2019b. Net protein contribution of beef feedlots from 2006 to 2017. *Translational Anim. Sci.* 3: 1575–1584. doi: 10.1093/tas/txz142

Caldera, E., B. Weigel, V.N. Kucharczyk, K.S. Sellins, S.L. Archibeque, J.J. Wagner, H. Han, J.W. Spears, T.E. Engle. 2019. Trace mineral source influences ruminal distribution of copper and zinc and their binding strength to ruminal digesta. *J. Anim. Sci.* 97: 1852-1864. https://doi.org/10.1093/jas/skz072

Cao, J., P. R. Henry, C. B. Ammerman, R. D. Miles, R. C. Littell. 2000. Relative bioavailability of basic zinc sulfate and basic zinc chloride for chicks. *J. Appl. Poult. Res.* 9: 513-517. https://doi.org/10.1093/japr/9.4.513

Caton, J. S., A. S. Freeman, and M. L. Galyean. 1988. Influence of protein supplementation on forage intake, in situ forage disappearance, ruminal fermentation and digesta passage rates in steers grazing dormant blue grama rangeland. *J. Anim. Sci.* 66: 2262-2271. https://doi.org/10.2527/jas1988.6692262x

Cochran, R.C., H.H. Köster, K.C. Olson, J.S. Heldt, C.P. Mathis, B.C. Woods. 1998. Supplemental protein sources for grazing beef cattle. 9th Florida Ruminant Nutrition symposium.

Drewery, M.L., J. E. Sawyer, W. E. Pinchak, T. A. Wickersham. 2014. Effect of increasing amounts of postextraction algal residue on straw utilization in steers, *J. Anim. Sci.* 92: 4642–4649, https://doi.org/10.2527/jas.2014-7795

Eryavuz, A., B.A. Dehority. 2019. Effects of supplemental zinc concentration on cellulose digestion and cellulolytic and total bacterial numbers in vitro. *Anim. Feed Sci. Tech.* 151: 175-183. https://doi.org/10.1016/j.anifeedsci.2009.01.008

Faulkner, M. J., W. P. Weiss. 2017. Effect of source of trace minerals in either forage-or by-product–based diets fed to dairy cows: 1. Production and macronutrient

digestibility." *J. Dairy Sci.* 100: 5358-5367. https://doi.org/10.3168/jds.2016-12095

Forsberg, C.W. 1978. Effects of heavy metals and other trace elements on the fermentative activity of the rumen microflora and growth of functionally important rumen bacteria. *Can. J. Microbiol.* 24: 298-306. https://doi.org/10.1139/m78-050

Genther, O. N., S. L. Hansen. 2015. The effect of trace mineral source and concentration on ruminal digestion and mineral solubility. *J. Dairy Sci.* 98: 566-573. https://doi.org/10.3168/jds.2014-8624

Gupta, B.S., J.P Srivastava, A.K. Tripathi, A.K. Verma, S. Thakur. 1981. Biological evaluation of Karanj (Pongamia glabra) cake. *Indian J. Anim. Health*,75–77.

Guthrie, M.J., D. G. Wagner. 1988. Influence of Protein or Grain Supplementation and Increasing Levels of Soybean Meal on Intake, Utilization and Passage Rate of Prairie Hay in Beef Steers and Heifers. *J. Anim. Sci.* 66:1529–1537, https://doi.org/10.2527/jas1988.6661529x

Hannah, S. M., R. C. Cochran, E. S. Vanzant, D. L. Harmon. 1991. Influence of protein supplementation on site and extent of digestion, forage intake, and nutrient flow characteristics in steers consuming dormant bluestem-range forage. *J. Anim. Sci.* 69: 2624-2633. https://doi.org/10.2527/1991.6962624x

Hollingsworth-Jenkins, K.J., T. J. Klopfenstein, D. C. Adams, J. B. Lamb. 1996 Ruminally degradable protein requirement of gestating beef cows grazing native winter sandhills range, *J. Anim. Sci.*, 74: 1343–1348, https://doi.org/10.2527/1996.7461343x

Housman, L. E., J.E. Sawyer, J. R. Cox, L.A. Redmon, T.A. Wickersham. 2020. Expeller-pressed and solvent-extracted Pongamia seedcake as a protein supplement for cattle consuming a basal diet of forage. *Anim. Feed Sci. Tech.* 114521. https://doi.org/10.1016/j.anifeedsci.2020.114521

Huisingh, J., J. J. McNeill, G. Matrone. "Sulfate reduction by a Desulfovibrio species isolated from sheep rumen." *Appl. Environ. Microbiol.* 28, no. 3 (1974): 489-497.

Kartchner, R. J. 1980. Effects of protein and energy supplementation of cows grazing native winter range forage on intake and digestibility. *J. Anim. Sci.*, 51: 432-438. https://doi.org/10.2527/jas1980.512432x

Konwar, B.K., Banerjee, G.C., 1987. Deoiled karanj cake (Pongamia glabravent.) A new feed ingredient in cattle ration. Indian vet. J. 64: 500–504.

Köster, H.H., R. C. Cochran, E. C. Titgemeyer, E. S. Vanzant, I. Abdelgadir, G. St-Jean. 1996. Effect of increasing degradable intake protein on intake and digestion of low-quality, tallgrass-prairie forage by beef cows, *J. Anim. Sci.* 74: 2473-2481. https://doi.org/10.2527/1996.74102473x

Kumar, M., R. Singh. 2002. Potential of Pongamia glabra vent as an insecticide of plant origin. *Biological Agriculture & Horticulture* 20: 29-50. https://doi.org/10.1080/01448765.2002.9754947

Mathis, C. P., R. C. Cochran, G. L. Stokka, J. S. Heldt, B. C. Woods, K. C. Olson. 1999. Impacts of increasing amounts of supplemental soybean meal on intake and digestion by beef steers and performance by beef cows consuming low-quality tallgrass-prairie forage. *J. Anim. Sci.* 77: 3156-3162. https://doi.org/10.2527/1999.77123156x

Mathis, C. P., R. C. Cochran, J. S. Heldt, B. C. Woods, I.E.O. Abdelgadir, K. C. Olson, E. C. Titgemeyer, E. S. Vanzant. 2000. Effects of supplemental degradable intake protein on utilization of medium-to low-quality forages. *J. Anim. Sci.* 78: 224-232. https://doi.org/10.2527/2000.781224x

Moore, J.E., W.E. Kunkle. 1991. Forage Quality and the need for protein and energy supplements. Florida Beef Cattle Short Course 40.

Morrill, J. C., J. E. Sawyer, S. B. Smith, R. K. Miller, J. R. Baber, T. A. Wickersham. 2017a. Post-extraction algal residue in beef steer finishing diets: I. Nutrient utilization and carcass characteristics. *Algal research* 25: 584-588. https://doi.org/10.1016/j.algal.2017.05.026

Morrill, J. C., J. E. Sawyer, S. B. Smith, R. K. Miller, M. D. Johnson, and T. A. Wickersham. 2017b. Post-extraction algal residue in beef steer finishing diets: II.

Beef flavor, fatty acid composition, and tenderness. *Algal research* 25: 578-583. https://doi.org/10.1016/j.algal.2017.07.007

National Academies of Sciences, Engineering, and Medicine (NASEM). 2016. Nutrient Requirements of Beef Cattle: Eighth Revised Edition. Washington, DC: The National Academies Press. doi: 10.17226/19014

Panda, A.K., V.R.B. Sastry, A. Kumar, S.K. Saha. 2006. Quantification of karanjin, tannin and trypsin inhibitors in raw and detoxified expeller and solvent extracted karanj (Pongamia glabra) cake. *Asian-Aust. J. Anim. Sci.* 19: 1776–1783. https://doi.org/10.5713/ajas.2006.1776

Prabhu, T. M., C. Devakumar, V. R. B. Sastry, and D. K. Agrawal. 2003. Quantification of karanjin using high performance liquid chromatography in raw and detoxified karanj (Pongamia glabra vent) seed cake. *Asian-Aust. J. Anim. Sci.* 15: 416-420. https://doi.org/10.5713/ajas.2002.416

Ravi, U., P. Singh, A.K. Garg, D.K. Agrawal. 2000. Performance of lambs fed expeller pressed and solvent extracted karanj (Pongamia pinnata) oil cake. *Anim. Feed Sci.Technol.* 88: 121–128. https://doi.org/10.1016/S0377-8401(00)00173-5

Sajid, Z.I., F. Anwar, G. Shabir, G. Rasul, K.M. Alkharfy, A.H. Gilani. 2012. Antioxidant, antimicrobial properties and phenolics of different solvent extracts from bark, leaves and seeds of Pongamia pinnata (L.) Pierre. *Molecules* 17: 3917-3932. https://doi.org/10.3390/molecules17043917

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. *Anim. Feed Sci. Technol.* 126: 157-167. https://doi.org/10.1016/j.anifeedsci.2005.05.025

Spears, J. W. 2003. Trace mineral bioavailability in ruminants. *J. Nutr.* 133: 1506S-1509S. https://doi.org/10.1093/jn/133.5.1506S

Spears, J. W., E. B. Kegley, L. A. Mullis. 2004. Bioavailability of copper from tribasic copper chloride and copper sulfate in growing cattle. *Anim. Feed Sci. Tech.* 116:1-13. https://doi.org/10.1016/j.anifeedsci.2004.06.002

Srivastava, J. P., B. S. Gupta, S. Thakur, A. K. Verma. 1990. Utilization of deoiled karanj (Pongamia glabra) cake in kid grower rations. *Indian J. Anim. Nutr.* 7:15-20.

Suttle, N. F. (1974). Effects of organic and inorganic sulphur on the availability of dietary copper to sheep. *Brit. J. Nutr.* 32: 559-568. https://doi.org/10.1079/BJN19740109

Suttle, N. F. 1991. The interactions between copper, molybdenum, and sulphur in ruminant nutrition. *Ann. Rev. Nutr.* 11: 121-140.

Takiya, C. S., C.M. Ylioja, A. Bennett, M.J. Davidson, M. Sudbeck, T.A. Wickersham, M.J. Vandehaar, B.J. Bradford. 2019. Feeding Dairy Cows with “Leftovers” and

the Variation in Recovery of Human-Edible Nutrients in Milk. *Front. in Sustain. Food Syst. 3*: 114. https://doi.org/10.3389/fsufs.2019.00114

Tamminga, S. (1979). Protein degradation in the forestomachs of ruminants. *J. Anim. Sci.* 49: 1615-1630. https://doi.org/10.2527/jas1979.4961615x

Waldo, D. R., L. W. Smith, E. L. Cox. 1972. Model of cellulose disappearance from the rumen. *J. Dairy Sci.* 55: 125-129. https://doi.org/10.3168/jds.S0022-0302(72)85442-0

Wickersham, T. A., R. C. Cochran, E. C. Titgemeyer, C. G. Farmer, E. A. Klevesahl, J. I. Arroquy, 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. Tech.* 115: 19-36. https://doi.org/10.1016/j.anifeedsci.2004.03.005

Wickersham, T. A., E. C. Titgemeyer, R. C. Cochran, E. E. Wickersham, and D. P. Gnad. 2008. 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. https://doi.org/10.2527/jas.2007-0325