EXPELLER PRESSED AND SOLVENT EXTRACTED PONGAMIA SEEDCAKE AS A PROTEIN SUPPLEMENT FOR CATTLE CONSUMING FORAGE

A Thesis

by

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ABSTRACT

Three studies evaluating palatability, nutrient utilization/N balance, and performance responses to long-term feeding of expeller-pressed (EKC) and solvent-extracted (SKC) Pongamia seedcake (PSC) were conducted. In Experiment 1, increasing amounts of either EKC or SKC mixed with wheat middlings (WM) were offered to steers to evaluate the palatability of PSC**.** Rate and extent of consumption were measured. Observations indicate that SKC is more palatable than EKC, and could be included at up to 40% of supplement without impacting consumption; 20% EKC may be effective for cattle consuming low-quality forage. In Experiment 2, isonitrogenous (100 mg of N/kg BW) amounts of supplements containing EKC or SKC were infused into steers consuming low-quality forage to compare effects of provision and level of PSC on forage utilization. Forage intake was not affected by 20% EKC or 40% SKC. An increase in forage intake was observed with the supplement when no PSC was included, but supplementation with 40% EKC decreased forage intake. Total tract digestion was not affected. All steers were positive for N balance. Nitrogen retention for all PSC-containing supplements was less than the positive control, but more than the negative control. Karanjin and pongamol intake and absorption was greatest for 40% EKC and least for 40% SKC when comparing PSC supplements. Based on these results, 40% EKC does not seem to be a viable option, but 40% SKC and 20% EKC could potentially be utilized as protein supplements for beef cattle on forage-based diets. In Experiment 3, fifteen steers were fed either a positive control (0% PSC), 20% EKC, or 20% SKC supplement for 126 days to determine the long-term effects of feeding PSC.

The control supplement resulted in greater OM intake compared to 20% EKC and 20% SKC. Average daily gain of steers on either PSC supplement was significantly lower than that of control steers, but similar to one another. Control steers were more efficient than PSC supplemented steers, and 20% EKC steers were more efficient than 20% SKC steers. Further research comparing performance of animals fed EKC and SKC should be conducted as PSC research in cattle is limited.

DEDICATION

To my parents and fiancé Justin, there's no way I ever could have gotten through this without y'all's constant love, support, and encouragement.

Phillipians 4:13

I can do all things through him who strengthens me.

Isaiah 40:28-31

[28]Have you not known? Have you not heard? The LORD is the everlasting God, the Creator of the ends of the earth. He does not faint or grow weary; his understanding is unsearchable. [29]He gives power to the faint, and to him who has no might he increases strength. [30]Even youths shall faint and be weary, and young men shall fall exhausted; [31]but they who wait for the LORD shall renew their strength; they shall mount up with wings like eagles; they shall run and not be weary; they shall walk and not faint.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Introduction

Pongamia is a common name typically used to describe *Pongamia pinnata* (L.) Pierre, a shrub or tree form legume indigenous to India and Malaysia. Due to the relatively high oil content of its seeds (25% to 40% by weight), and its ability to grow in marginal or degraded soils, it has been a subject of development as feedstock for biofuel production.

Extraction of Pongamia oil for biofuel results in the production of Pongamia seedcake (PSC). Because the meal of many oilseeds (e.g., soy, cotton) serves as a valuable feedstuff for livestock, there is interest in evaluating PSC as a protein supplement for beef cattle, particularly in Hawaii where Pongamia plantations are being developed. However, residual oil remaining in PSC contains anti-quality factors that decrease palatability, impacting intake, nutrient utilization, and animal performance (Gupta et al., 1981). Further processing of PSC by various methods has been shown to be effective in reducing these negative effects (Panda et al., 2006). Therefore, the palatability, nutrient utilization/N balance, and performance effects of long-term feeding of Pongamia seedcake, both with and without further processing, should be evaluated.

Protein

Protein is the most abundant substance in the body, other than water, and functions in repair/maintenance processes, can be an energy source, is active in cellular transportation and storage of certain molecules, and can function as hormones, enzymes

and antibodies. Proteins are long chains of amino acids, which primarily consist of carbon, hydrogen, oxygen, and nitrogen. Protein is one of six key nutrients, and is especially crucial in the diet of ruminants, as the nitrogen is required for growth and function of rumen microbes.

Protein values of feedstuffs are reported as percentage of crude protein (CP), which is equal to N × 6.25 (NRC, 2000). Dietary CP can be categorized into degradable intake protein (DIP) and undegradable intake protein (UIP) based on rumen degradability of the protein. Undegradable intake protein is often referred to as bypass protein and is not altered in the rumen because ruminal microbes are incapable of utilizing it. Metabolizable protein (MP) is defined as the true protein absorbed by the intestine, and is supplied by UIP and microbial crude protein (MCP; NRC, 2000). Microbial crude protein can supply 50 to 100% of the MP required (NRC, 2000). Undegradable intake protein addresses MP requirements by preventing direct availability of protein to the rumen microbes, and permitting it to bypass to the small intestine. In the small intestine, UIP is enzymatically digested, absorbed, and then transported to the liver to be either catabolized to carbon skeletons and ammonia or synthesized into other proteins. Carbon skeletons can be used to make glucose (energy), and the ammonia can be detoxified to urea and either recycled or excreted. Recycled urea is one way in which N is provided to the microbes.

Degradable intake protein is the portion of CP directly degraded by ruminal microbes. It can consist of both true protein and non-protein nitrogen (NPN; e.g. urea and biuret, nucleic acids, ionic salts of ammonia, etc.). Degradable intake protein

directly supplies ruminally available N (RAN) to microbial populations requiring a source of N to facilitate growth and VFA production. The NRC (2000) recommends DIP be provided at a rate of 13% of TDN amount in the diet in order to meet the microbial requirements for RAN, which will likely maximize production of MCP, increasing the amount of MP available to the animal. Mathis et al., (2000) provided varying levels of DIP (0.041, 0.082, and 0.124% BW) to cattle consuming low-quality bermudagrass, bromegrass, or forage sorghum, and reported significant variation among forages in amount of DIP needed to maximize intake and digestion. Wickersham et al., (2008) reports transfer of blood urea to the rumen contributes one-fourth to one-third of the N utilized by rumen microbes for MP production.

Supplementation

Supplementation is an addition of a feedstuff designed to complete/make up for a nutritional deficiency. Supplementation is recommended when the basal forage does not meet nutrient requirements, and can be in the form of protein, energy, mineral, or a combination. When a basal diet of low-quality forage (<7% CP) is fed, evaluation of energy and protein are particularly important since they are the primary drivers of animal performance and are typically the first limiting nutrients. The "ideal" supplement would be one that best meets the animal's nutritional needs, is easy to handle and distribute to the animal, and is the most economical to purchase and feed (DelCurto et al., 2000).

Protein supplementation

Protein supplementation is required when the amount of N in the forage is not meeting the requirements of rumen microbes (Kartchner, 1980), although some N may

be recycled and used to produce MCP. Under grazing conditions, response to protein supplementation is variable and depends on forage availability, forage quality, and environment, but is most beneficial when low-quality forage is readily available (Rittenhouse et al., 1970; Kartchner, 1980; Del Curto et al., 2000). Protein supplementation can increase BW and condition (or reduce rate of loss of BW and condition), forage and total OMI, and diet digestion in cattle consuming low-quality forage (Kartchner, 1980; DelCurto et al., 1990a; Sunvold et al., 1991; Bandyk et al., 2001; Wickersham et al., 2008). Increased forage intake as a result of protein supplementation is usually observed when forage has a CP content of less than 6-8% and supplements contain at least 20% CP (Kartchner, 1980). When CP of forage is greater than about 7%, protein supplementation has variable to little benefit (Mathis et al., 2000). When predicting performance responses to supplemental protein, forage availability, digestibility, stage/production requirements, CP content of the forage, and other limiting nutrients must be evaluated (DelCurto et al., 2000). Also, if forage availability is limited, responses to protein supplementation are blunted because forage intake is constrained.

Generally, protein supplementation increases intake when CP of forage is less than 7%, but has a variable effect on digestion. Increased intake is likely related to improved fiber digestion, indigestible fiber passage, increased tolerance to reticuloruminal fill due to improves fiber passage, and enhanced protein flow to the small intestine (DelCurto et al., 1990b; Bandyk et al., 2001). Supplemental DIP results in increased forage intake in cattle because as DIP is degraded in the rumen, N required

by the microbes is released, increasing cellulolytic activity. Both the quantity of protein provided by the supplement and the protein to energy ratio in the diet may affect how a supplement influences forage intake. Increasing MP supply to the animal by increasing microbial protein and (or) dietary UIP flow to the small intestine may stimulate intake (Egan, 1977; Bandyk et al., 2001; Wickersham et al., 2008).

An increase in intake typically decreases or has no effect on digestion; however, when protein is supplemented, the increased N available to the rumen microbes increases their cellulolytic activity, thus potentially increasing digestion. Caton el al. (1988), reported *in situ* NDF disappearance was greater in protein supplemented steers at 4,8,12, 18, and 36h post-supplementation, but overall was not affected by protein supplementation across hours. However, the literature shows variability in the digestion response to protein supplementation, which could be affected by type of forage. Wickersham et al., (2008) reported an increase in OM and NDF digestion as level of DIP increases when low-quality prairie hay was fed, Mathis et al., (2000) fed bermudagrass, bromegrass, or forage sorghum and reported no difference to a slight decrease in OM digestion when DIP was supplemented, and Rittenhouse et al., (1970) reported no difference in digestion between supplemented and unsupplemented cattle grazing mixed prairie range.

Animals consuming low-quality forage are primarily deficient in energy as a percent of requirement, but the first limiting nutrient for the microbes is typically N for ruminal microbial fermentation. Protein supplementation alleviates this deficiency. One of the most noted benefits of supplementing protein to ruminants consuming low-quality

forage is an improved ruminal N status (McCollum and Galyean, 1985). Protein supplementation increases ruminal ammonia N concentration, promoting microbial growth and forage degradation. Increased microbial activity and fermentation due to protein supplementation results in increased total VFA production. DelCurto et al. (1990a) reported a 30% increase in total VFA concentration when protein was supplemented, meaning there was increased energy available to the animal. Molar proportions of acetate decreased, and molar proportions of propionate and butyrate were not significantly affected by protein supplementation, however, these results varied between studies (DelCurto et al., 1990a; Mathis et al., 2000; Wickersham et al., 2008). Molar proportions of acetate are typically higher than propionate when cattle are consuming a forage based diet, so a decrease in molar proportions of acetate means a decrease in the acetate: propionate ratio, resulting in either more gluconeogenic propionate available for energy or just less total energy.

Protein supplements are effective because they provide the RAN required by ruminal microbes for synthesis of MCP, which is ultimately used by the animal to meet MP requirements. An inadequate supply of RAN limits microbial growth, thus reducing diet fermentation, digesta outflow, and intake. Addressing RAN deficiency should be first priority, and then consideration should be given to supplementing UIP when the MP requirements exceed that provided by MCP plus UIP. Until RAN requirements of the rumen microbes are met and the animal is maintained at a basal N status, DIP supplementation may be more effective than UIP supplementation in increasing intake of low-quality, protein-deficient roughages (Bandyk et al., 2001). Supplemental DIP or N

recycled from digested and absorbed UIP, mobilized tissue protein, or digested and absorbed microbial protein can both be used to meet microbial N requirements (Bandyk et al., 2001; Wickersham et al., 2008). Nitrogen from ruminally degraded protein is used to synthesize microbial protein by either incorporating free amino acids from proteolysis or incorporating ammonia N from deamination of amino acids (Hammond, 1992). Non-protein N can also be made into microbial protein in the rumen by incorporating ammonia from the enzymatic breakdown of NPN.

Microbial protein yield is maximized when the ratio of available energy to RAN is optimized (Hammond, 1992). Increasing energy intake while maintaining protein intake at a constant level (decreasing the protein : energy ratio) decreased blood urea nitrogen (BUN) in the study by Hammond, (1992), as BUN was 19.7 mg/dl at 75% of maintenance energy intake (MEI) and 5.6mg/dl at 150% of MEI. Satter and Slyter, (1974) determined the effect of ruminal ammonia concentration on microbial protein production by using continuous-culture fermenters charged with ruminal contents from steers dosed either a protein-free purified diet, an all- concentrate diet, or a forage-concentrate diet. They measured microbial protein yield as tungsic acid-precipitable N, which increased linearly with supplementary urea until ammonia began to accumulate in the incubating ruminal contents, and suggested increasing ruminal ammonia N concentrations to 5 mg/100 ml (3.6m*M*) will increase microbial protein production, but above this nothing is gained by further NPN supplementation (Satter and Slyter, 1974).

Blood urea and ruminal ammonia concentrations serve as effective indicators of N utilization. When there is a dietary protein deficiency, ruminal NH_3-N concentrations

are low and urea recycling back to the rumen is increased. When there is excess N compared to energy in the rumen, ruminal NH_3-N concentrations increase (Hammond, 1992). Unused ruminal NH_3-N enters the portal blood through the rumen wall to be transported to the liver where it is detoxified and converted to urea. Urea circulates in the blood to the kidneys and is excreted in the urine or is recycled back into saliva, the rumen, or milk in lactating cows. As DIP level increases, the amount of urea entering the gut and returned to the ornithine (urea) cycle increased by about 83% (Wickersham et al., 2008). Blood urea nitrogen concentrations are highly correlated with ruminal NH_3-N concentrations, and are indicative of the protein:energy ratio in the diet (Egan and Kellaway, 1971; Hammond, 1983a and b). An increase in dietary protein solubility or degradability can increase ruminal ammonia concentrations, resulting in higher concentrations of circulating urea (Hammond, 1992). However, both increased level of feed and energy intake are reported to decrease BUN concentration because there is excess energy, throwing off the protein:energy ratio, so there is less ruminal ammonia being converted to urea and circulating in the blood (Hammond, 1992). Vercoe, (1967) reported as the level of intake increases, BUN tends to decrease by about 15-17%. When BUN concentrations were less than 7 mg/dl, ADG increased from 21-133% when protein was supplemented compared to control (Hammond, 1992). Blood urea nitrogen concentrations between 8 and 10 mg/dl indicate a relatively well balanced digestible protein and energy intake, but concentrations above 10 mg/dl generally indicate protein waste (Hammond, 1992).

Protein supplements are commonly derived from oil-seed by-products (e.g. soybean meal, cottonseed meal, sunflower meal, etc.). These sources are usually high in CP (greater than 20%) and have energy densities similar to those of cereal grains (greater than 3.00 Mcal ME/kg; DelCurto et al., 2000). Feeds high in UIP, such as feather meal, corn gluten meal, blood meal, and other "bypass" supplements, can be used, but they don't offer an advantage over more cost-effective feeds (defined as more benefit for the price paid, or cost per nutrient) with high levels of DIP (Alawa et al., 1986; Fleck et al., 1988). Non-protein nitrogen use has become increasingly common in replacing natural protein because of lowered cost greater N content, and if utilized with the same efficiency as natural protein, would yield substantial economic advantages. One kg of NPN provides as much nitrogen as 5-6 kg of plant source protein, with biuret containing N equivalent to 250% protein, and urea containing N equivalent to 260-280% protein (Fonnesbeck et al., 1975). However, NPN is usually not utilized as well as natural protein, and dietary plant proteins provide energy as well. Sources of NPN include urea, biuret, urea phosphate, uric acid, and others, with urea and biuret being the most commonly used today. Diets supplemented with NPN must contain a source of readily available energy (carbohydrate), vitamins, phosphorus and other essential minerals, and have a N:sulfur ratio of approximately 10:1 for best utilization as sulfur-containing amino acids are essential for building animal tissue proteins (Fonnesbeck et al., 1975). Non-protein nitrogen must not exceed more than one-third of the total protein in the diet, must not exceed more than 3% of the concentrate mix, and must be less than

1% of the total ration dry matter, cattle fed urea at these levels did not develop toxicity (Davis and Roberts, 1959; Chalupa, 1968**)**.

Oltjen (1969) reported growth rates and feed efficiency on purified diets containing NPN are only 65% of what they are on protein containing diets, but Gleghorn et al., (2004) reported linear increases in carcass-adjusted ADG, G:F, and carcass-adjusted G:F with increasing concentration of supplemental urea. Biuret and urea have approximately equal nutritional value, and in a nutritional study examining diets deficient in protein, both produced equivalent growth when fed medium-energy basal diets deficient in protein (Fonnesbeck et al., 1975). However, N retention was reduced by about 60% in steers fed urea compared to those fed isolated soy protein in a different study (Oltjen, 1969). Although NPN is more cost effective than providing naturally-sourced protein, it should not completely replace natural protein in the diet of ruminants.

Urea is rapidly hydrolyzed by to carbon dioxide and ammonia in the rumen, which elevates rumen pH, allowing ammonia to be more rapidly absorbed (Chalupa, 1968). Once ammonia is absorbed into the blood, it is transferred to the liver where it is converted back to urea, and either excreted in the urine or recycled back to the rumen via the saliva or by diffusion from the blood directly into the rumen. Toxicity may result if the liver is unable to convert all absorbed ammonia to urea, leaving ammonia present in the blood, increasing blood alkalinity (Chalupa, 1968; Fonnesbeck et al., 1975). The toxic level is about 20-30 g of urea/45.36 kg of BW, and symptoms appear when blood ammonia levels reach about 4.1-4.4 mg/L (Davis and Roberts, 1959; Oltjen et al., 1963). Signs of toxicity are uneasiness, staggering, kicking at flanks, incoordination, tetany,

prostration, severe convulsions, slobbering, bloating, regurgitation, coma, and death within 30-60 min after consuming a toxic dose (Davis and Roberts, 1959; Oltjen et al., 1963; Austin, 1967). Weak acids or a vinegar/water solution (acetic acid as a 5% solution or as vinegar) has been successfully used as oral treatments for ammonia toxicity by neutralizing the ammonia (weak base) produced (Davis and Roberts, 1959). Young cattle not previously exposed to urea or starving animals are more likely to consume a toxic amount, however, urea can limit voluntary intake due to its low palatability.

Biuret is hydrolyzed to ammonia and carbon dioxide in the rumen more slowly than urea, which helps to prevent toxic buildup of ammonia in the blood (ammonia toxicity). Biuret also has low solubility in water, which has two benefits: 1) it allows more biuret to be fed than urea because its low solubility allows it to remain in the rumen longer, and 2) it is able to withstand weathering effects such as rain, snow, and humid climate conditions (Fonnesbeck et al., 1975). Unlike urea, biuret shows no undesirable palatability characteristics (Oltjen et al., 1969). The microbial population in the rumen requires time to adapt to biuret in order to efficiently hydrolyze it into ammonia, about 21 days (Oltjen et al., 1969). Although both sources of NPN have their respective advantages and disadvantages, biuret and urea are both suitable nitrogen sources for ruminants when only replacing part of the protein in the diet.

Although oilseed supplements are most commonly used with low-quality forages, many others such as alfalfa, wheat middlings, and high-quality meadow hays can be used effectively. Protein supplementation is critical to the optimal use of low-

quality forage, but energy density may be important depending on status of body condition. In general, natural protein appears to be the most beneficial for high-fiber, low-quality forage diets in ruminants, as NPN tends to be less effective (DelCurto et al., 2000).

Energy supplementation

In contrast to protein supplements, energy supplements are reported to depress both intake and digestion of low-quality forage (Sanson et al., 1990). As quantity of corn increased, forage intake and hemicellulose and cellulose digestion decreased linearly by 42%, 56%, and 36% respectively (Chase and Hibberd, 1987; Caton and Dhuyvetter, 1997). Depressions in hay intake are most likely due to reduced passage rate and digestion may be explained by decreased rate of ruminal hay disappearance (Chase and Hibberd, 1987). Decreased fiber digestion may be due to low RAN values, which would inhibit microbial growth and activity. In a study by Chase and Hibberd, (1987), the mean ruminal NH_3-N concentrations were below the recommended minimum of 2-5 mg/dl required for maximum microbial growth suggested by Satter and Slyter, (1974) when supplemental corn was provided. Because of this, energy supplements should be formulated with adequate ruminal available protein.

Energy supplementation tends to substitute for low-quality forage intake, and as a result has little to no influence on cattle performance. When grain was supplemented at 1 kg/d or less, the substitution rate of supplement for forage was low, ranging from 1:0.5 to 1:1 (Rittenhouse et al., 1970; Kartchner, 1980). However, when grain was supplemented >1 kg/d, supplement intake displaced more forage, with ratios ranging

from 1:1.1 to 1:1.6 g of supplement per g of forage (Rittenhouse et al., 1970; Lusby et al., 1976). Energy supplementation depresses the molar proportion of acetate by about 10%, but increases butyrate by 33% (Chase and Hibberd, 1987). When the goal of the producer is to maximize performance on a high-fiber, low-quality forage basal diet, energy supplementation is not recommended. However, it becomes a viable solution when forage availability is limited because energy supplements substitute for forage intake. In digestion studies, increasing energy at low levels of protein supplementation has shown to decrease intake and digestion of low-quality forage, but at high levels of protein supplementation, increasing energy usually has little effect on intake and digestion of low-quality forage (DelCurto et al., 2000).

Pongamia

India has deficit livestock feedstuffs, and Pongamia is abundant in this area. Multiple studies have been conducted in India (primarily in sheep and goats, but some in cattle and buffalo) to evaluate PSC as a protein supplement for livestock. In previous research, Pongamia was fed as a mix with EKC replacing 4, 24, and 50% of conventional protein source in the diet, and SKC has replaced 12, 20, 24, 25, 30, 40, 50, 60, 75, 80, and 100% of a conventional protein source in the diet (Table 1).

Pongamia pinnata (karanja) is a drought and salt tolerant legume tree native to India and Asia. It produces an oilseed currently being investigated as an oil source for the biodiesel industry. The seed consists of an outer hull and inner kernel, and is 27-39% oil, 20-30% protein, and 5-6% furano-flavonoids (Vinay and Sindhu Kanya, 2008). After oil extraction, 2/3 of the weight of the seed is left as residual cake which ranges

from about 22.3-27.5% CP, 85% of which is DIP, making it a potential protein source for livestock. Complete extraction of the oil is recommended, as the oil is unpalatable and contains toxic anti-nutrients such as phytates, tannins, and protease inhibitors, and karanjin, pongamol (furanoflavonoids), glabrin and other polyphenolic compounds. Even after extraction, 15-20% of the oil still remains in the seed (Vinay and Sindhu Kanya, 2008). Karanjin and pongamol are the two main toxic components, which could explain the oil's antibacterial and insecticidal properties and bitter taste (Dutta et al., 2012). Mahli et al. (1989) reported the LD_{50} of karanjin in mice is 14.32 mg/kg, and of pongamol is 17.14 mg/kg. It is suggested karanjin may have a central nervous system stimulant activity because of its flavone ring system, while pongamol may be a central nervous system depressant due to its benzofuran ring (Mahli et al., 1989). Karanjin comprises about 1.25% of Pongamia oil, while pongamol is present at lower levels and

Table 1. Current knowledge on feeding Pongamia seedcake to livestock

Reference	Animal	Type of PSC[1]	% of Concentrate Mixture	Length of trial, d	Results/findings
Dutta et al., 1993	Crossbred lactating cows	SKC	10% of concentrate mixture	150	SKC can be safely fed to dairy animals up to 10% inclusion of the concentrate mixture
Konwar and Banerjee, 1987	Crossbred lactating cows	SKC	Replaced 50, 75, or 100% of groundnut cake in concentrate mixture	161	SKC can safely replace 50% of the deoiled groundnut cake in the diet of lactating cows
Soren et al., 2008	Growing lambs	SKC further treated with via water washing, lime treating, or binder treating	Replaced 50% of soybean meal in concentrate mixture	196	Water washed SKC can replace 50% of the soybean meal in the diet without negatively affecting carcass characteristics and sensory attributes
Soren et al., 2009	Growing lambs	SKC further treated with via water washing, lime treating, or binder treating	Replaced 50% of soybean meal in concentrate mixture	196	Water washed SKC can replace 50% of the soybean meal in the diet without negatively affecting performance, and karanjin concentration should be taken into account when formulating diets containing PSC

Table 1. Continued

Reference	Animal	Type of PSC[1]	% of Concentrate Mixture	Length of trial, d	Results/findings
Gupta et al., 1981	Buffalo calves, crossbred growing calves	EKC for palatability trial, SKC for performance study	EKC at 4% of concentrate mixture, SKC 40, 60, and 80%	365	SKC can replace mustard cake up to 60% in the diet of growing calves
Singh et al., 2006	Growing lambs	EKC and SKC	24% EKC, 20% SKC of concentrate mixture	238	ADG declined after 13 wk for both EKC and SKC. Long-term feeding of EKC and to some extent SKC can have deleterious effects on performance
Nagalakshmi et al., 2011	Growing lambs	PSC, type not specified	12% of concentrate mixture	155	PSC at 12% inclusion resulted in depressed performance and immunocompetence
Soren and Sastry, 2009	Growing lambs	SKC further treated with via water washing, lime treating, or binder treating	Replaced 50% of soybean meal in concentrate mixture	96	Feeding of processed SKC influenced the balance of N, Ca, P, and karanjin. Major routed of karanjin excretion are through feces and urine. Feeding of processed SKC did not cause deleterious effects on microbial protein synthesis

Table 1. Continued

Reference	Animal	Type of PSC[1]	% of Concentrate Mixture	Length of trial, d	Results/findings
Ravi et al., 2000	Growing lambs	EKC and SKC	Replaced 50% of deoiled groundnut cake in concentrate mixture	98	SKC at 20% inclusion can be fed for 98 d without affecting performance, but EKC at 24% is not recommended as it negatively affected intake and digestion of nutrients
Konwar and Banerjee, 1987	Crossbred bulls	SKC	Palatability study: 40, 50, 60, 80, and 100% replacement of groundnut cake Performance study: 50, 75, and 100% replacement of groundnut cake	42	Inclusion of SKC on isonitrogenous and almost isocaloric basis up to 100% replacement of deoiled groundnut cake had no negative effect on body weight in cross-bred bulls
Srivastava et al., 1990	Growing kids	SKC	20, 30, and 40% replacement of groundnut cake	266	SKC can replace up to 30% of deoiled groundnut cake in the diet of growing kids

[1]PSC = Pongamia seedcake; SKC = solvent-extracted Pongamia seedcake; EKC = expeller-pressed Pongamia seedcake

is more soluble in oils compared to karanjin (Dutta et al., 2012). When PSC replaced 50% of the soybean meal in the concentrate mixture, long-term exposure (196 days) to these compounds in lambs has resulted in histopathological lesions in the testes, epididymis, parathyroid, liver, and small intestine, reduced length of long bones, reduced radiographic density, thinner cortices and increased diameter of the medullary cavity when a binder-treated cake was fed, and thicker cortices and narrowing of the medullary cavity when lime-treated cake was fed, which is suggestive of poor bone mineralization (Soren, 2006).

De-oiled Pongamia seedcake (PSC) is the leftover component of the seeds following oil extraction. Extraction is accomplished using solvent (usually hexane; SKC) or by expeller pressing (EKC). Solvent extraction tends to be more effective at removing oil. About 80% of the oil is removed when making EKC, but less than 0.5% of the oil remains when SKC is produced (Vinay et al., 2008). Multiple detoxification procedures have been tested to improve the seedcake, including soaking the cake in

water, autoclaving, dry heat, microbiological treatments, alkali treatment, and ether extraction (Scott et al., 2008). Protease inhibitors are eliminated by autoclaving the seedcake with lime, refluxing with 2% HCl, and then neutralizing with sodium hydroxide (Scott et al., 2008 and Panda et al., 2006). Vinay and Sindhu Kanya, (2008) report treating PSC with 2% HCl for one hour at room temperature was the best detoxification method, reducing the concentration of tannins by 54%, phytates by 72.5%, trypsin inhibitor activity by 74%, and resulted in karanjin content of 0.03% with efficient removal of oil. This method did not affect nutritional quality of the protein.

Reduced feed intake is a recurring issue in animals fed Pongamia (karanj) seedcake, probably due to palatability. Reduced intake results in decreased ADG and total gain (Nagalakshmi et al., 2011). Observed reductions in intake are greater with EKC because of the higher residual oil concentration, thus more of the anti-nutrients present in the cake; however, reductions have been observed with SKC (Ravi et al., 2000). Although nutrient intake tends to be lower in animals fed PSC, Nagalakshmi et al., (2011) reported no effect on DM and OM digestion or nutrient retention with SKC. Ravi et al., (2000) reported no adverse effects on nutrient utilization, nutrient balance, growth rate, and feed conversion efficiency when SKC was fed at 20% of the diet for 98 days. Existent data suggests SKC should not negatively impact nutrient utilization and growth; however, validation of this and a more complete understanding of its impact on intake are required.

Solvent extracted seedcake was fed at 20% for 98 days in lambs without deleterious effects on performance, nutrient utilization, immunity, balance, growth rate,

or feed conversion efficiency (Ravi et al., 2000; Vinay and Sindhu Kanya, 2008). Toxicity begins to take effect at approximately week 13 for both 24% EKC and 20% SKC in a study conducted by Singh et al., (2006), and Nagalakshmi et al., (2011) reported body weight decreasing at 120 days when 12% PSC was fed, and karanjin content was estimated to be 0.325%. In a palatability study by Gupta et al., (1981) with growing buffaloes, EKC (9.0% ether extract) was unpalatable even at a 4% level and the animals developed toxic symptoms such as loss of appetite, weight loss, frequent strong color urination, swelling of facial muscles and intermaxillary space, lameness, skin discoloration, loss of hair, and gangrene of the tail/sloughing. As a follow up to the palatability study Gupta et al., (1981) fed SKC (0.4% ether extract) to growing calves for 365 days at the 0,40,60 and 80% level and found SKC can be fed up to a 60% without negative effect on performance. Toxicity symptoms were observed with EKC, and Ravi et al. (2000) states EKC is not recommended as a feed stuff. However, Singh et al. 2006 reports there were no adverse effects on vital organs including brain and endocrine glands, except for mild degenerative changes in liver fatty infiltration from feeding either EKC or SKC. Based on the literature, PSC is safe to feed short term, not exceeding about 13-17 weeks.

Wheat middlings

About 25% of milled wheat remains as byproduct, wheat middlings (WM; Sunvold et al., 1991). Wheat middlings are fine particles of wheat bran, wheat shorts, wheat germ, wheat flour, and some of the offal from the "tail of the mill", obtained from the usual process of commercial milling, and must contain less than 9.5% fiber

(AAFCO, 2010). As a result of processing differences, there are "light" WM and "heavy" WM. "Light" WM have a higher proportion of bran and small amounts of starch, while "heavy" WM have a greater amount of flour attached to the bran and contain more of the starchy endosperm. Nutrient content of WM is thought to be influenced most by the amount of starch attached to the bran (Cromwell et al., 1992). Wheat bran contains considerably greater levels of CP, amino acids, NDF, Ca, and P than wheat flour. Based on this, the type most suitable and nutritious depends on the species, as "heavier" WM would be more suitable for monogastrics because of the greater starch content and "light" WM would be better for feeding ruminants because it contains more bran, which is more fibrous and more nutrient dense.

Wheat middlings are a good source of energy, amino acids, and P. Cromwell et al. (2000) reports WM are on average about 89.6% DM, 16.2% CP, 0.12% Ca, 0.97% P, and 36.9% NDF, while NRC, (2000) lists these values as 89% DM, 18.40% CP, 0.15% Ca, 1% P, and 35% NDF. According to Cromwell et al. (2000), on average contain 1.13% Arginine, 0.43% Histidine, 0.50% Isoleucine, 1.02% Leucine, 0.66% Lysine, 0.25% Methionine, 0.34% Cystine, 0.63% Phenylalanine, 0.54% Threonine, 0.19% Tryptophan, and 0.73% Valine.

Wheat middling-based supplements increased forage and total DMI, DM digestion, and NDF digestion by about 65%, 101%, 44%, and 20%, respectively compared to negative control when dormant bluestem hay was fed (Sunvold et al., 1991). This same study reported WM supplementation produced similar increases in intake, digestion, ruminal passage, and fermentation characteristics of low-quality forage

when compared to an oilseed meal/grain supplement of equal CP (21% CP) content when both fed to provide a similar amount of energy daily. Dalke et al., (1997) reported a 9.2% increase in DMI and a 10.1% increase in F:G as WM inclusion in the diet increased, but DMD, OMD, and starch digestion decreased. Heldt et al., (1998) and Cox et al., (1989) conducted studies on cows grazing native winter range, and both reported, in comparison to soybean meal, WM based supplements provided more supplemental energy and a similar amount of protein, thus increasing cow performance and precalving cow weight gains.

Wheat middlings are energetically less dense, requiring them to be fed in greater quantities compared to an oilseed meal/grain supplement. This allows for the potential for feeding higher levels to cattle on hay-based diets, and can result in better performance responses than corn and soybean meal at high levels of WM intake (Garces-Yepez et al., 1997). High levels of WM (7 Mcal of ME/d) showed the greatest volume of ruminal fluid, ruminal ammonia N concentrations, and decreased ruminal pH and acetate-to-propionate ratio (Sunvold et al., 1991). Increased forage intake with increasing amount of WM provided daily could be due to improved protein availability. It is suggested the rate of ruminal CP digestion is about 1.5 times faster in WM than SBM, which may result in ammonia N being released more rapidly in wheat middlings (Sunvold et al., 1991). Wheat middlings are effective supplements to low-quality forage, and when formulating a WM-based supplement, Sunvold et al. (1991) suggests the CP content should be 20% or greater to ensure adequate stimulation of forage intake and ruminal fermentation. Zobell et al., (2003) and Poore et al., (2002) reported WM

can be included up to 50% of diet DM in growing/finishing diets without adverse effects on production, carcass, and ruminal fermentation characteristics other than pH, as rumen pH decreased to about 5.5 due to the starch in WM being rapidly degraded in the rumen. Dalke et al., (1997) reported WM could replace 5% of dry-rolled corn in finishing diets, but could replace 50-100% of chopped alfalfa in finishing diets without negatively impacting feedlot steer performance.

In Pongamia studies, most mixed PSC with deoiled groundnut cake or mustard cake, but Gupta et al., (1981), Ravi et al., (2000), Singh et al., (2006), and Soren and Sastry (2009) included wheat bran in their test supplements. Although PSC has some issues with palatability, Dhuyvetter et al., (1999) states WM are very palatable and are readily consumed by all classes of cattle. Wheat middlings were selected to mix with PSC in this study not only because of their increased palatability, but also because they are readily available in Hawaii, the area of which this study was targeted for.

Conclusion

Protein is the most abundant substance in the body and has numerous functions in the body, but is increasingly important for ruminants as it provides N for the symbiotic rumen microbes. Therefore, supplementation is necessary when the basal forage does not meet nutrient requirements, typically when forage is <7% CP. Increased forage intake as a result of protein supplementation is usually observed when forage has a CP content of less than 6-8% and supplements contain at least 20% CP, however, energy supplements are reported to depress both intake and digestion of low-quality forage. Utilization of Pongamia oil as a biofuel has resulted in the production of

Pongamia seedcake, a by-product remaining after the extraction of the oil. Accordingly, there is interest in evaluating PSC as a protein supplement for beef cattle, particularly in Hawaii where Pongamia plantations are being developed. However, residual oil in PSC contains anti-quality factors that decrease palatability, which then impact intake and animal performance. Extraction is accomplished using solvent (usually hexane; SKC) or by expeller pressing (EKC), solvent extraction tends to be more effective at removing oil. Wheat middlings were selected to mix with PSC in this study not only because of their increased palatability, but also because they are readily available in Hawaii, the area of which this study was targeted for. As previous work comparing EKC and SKC, especially in cattle, is limited, these studies will be conducted comparing EKC to SKC in terms of palatability, nutrient utilization, and long-term feeding effects on animal performance. Based on previous studies on Pongamia, we have developed the following hypotheses: 1) Cattle will consume Pongamia seedcake (PSC) when blended with commercially available feed ingredients. However, at some level of inclusion PSC will decrease consumption. 2) Steers dosed PSC supplement at a greater percent of inclusion will have greater levels of forage intake and digestion than unsupplemented steers. However, steers dosed SKC will have greater levels of intake than those dosed EKC. 3) Solvent extraction will create a product that will not have deleterious effects on animal performance when fed as a protein supplement for 126 days.

CHAPTER II

PALATABILITY OF EXPELLER PRESSED AND SOLVENT EXTRACTED SEEDCAKE AS A PROTEIN SUPPLEMENT IN CATTLE

Overview

Seven steers (355.98 ± 38.3 kg of BW) were used in a 7 × 7 Latin square to evaluate the palatability of two types of Pongamia seedcake (PSC) included at varying levels to cattle consuming a basal diet of Bermudagrass (5.5% CP, 67% NDF). Treatments were 20, 40, and 60% of the supplement as expeller pressed Pongamia seedcake (EKC), or 20, 40, and 60% of the supplement as solvent extracted Pongamia seedcake (SKC), and a 100% wheat middlings (WM) control. Supplement (0.5 kg) was offered once daily prior to feeding hay at 0600h. Periods were 4 d long, each consisting of 3 d to feed the test supplement followed by a 1 d washout period when 100% WM supplement was fed. Rate and completeness of consumption were observed by allowing steers to eat for 10 min, then collecting and weighing the remaining supplement. Supplement was returned and the time required for complete consumption or until loss of interest was recorded.

There was a treatment × d interaction ($P < 0.01$) for amount of supplement consumed, and a trend ($P = 0.08$) for rate of consumption. For both PSC types the amount consumed linearly decreased ($P < 0.01$), and this decrease was more pronounced with EKC supplements than with SKC supplements, and as inclusion level of PSC increased. When comparing the two seedcake types at the same percent of inclusion, consumption of 60% EKC (185 g) was less than ($P < 0.01$) 60% SKC (385 g) and

consumption of 40% EKC (221 g) was significantly ($P < 0.01$) lower than 40% SKC (415 g), but consumption of 20% EKC (465 g) and 20% SKC (489 g) were not statistically different ($P = 0.53$). Rate of consumption and ten min consumption rate of steers fed the 40 and 60% levels of both EKC and SKC were significantly slower ($P < 0.01$) than the 100% WM control. Differences in consumption rate between SKC and EKC at the same levels of inclusion were not statistically significant at the 20% and 60% level, but at the 40% level SKC rate of consumption was significantly ($P < 0.01$) faster than EKC, 55.0 versus 14.2 g/min. Palatability issues were most likely associated with the anti-nutrient karanjin being present in the residual oil in the cake giving it a bitter, pungent odor and taste. Greater residual oil/karanjin concentrations in EKC (11.5% oil and 2855 ppm karanjin) than SKC (2.7% oil and 684 ppm karanjin), which in part explains the differences in palatability.

Introduction

Pongamia pinnata (karanja) is a legume tree native to India and Asia producing an oilseed currently under investigation as source of biodiesel. The seed consists of an outer hull and inner kernel, and is 27-39% oil, 20-30% protein, and 5-6% furano-flavonoids (Vinay and Sindhu Kanya, 2008). After oil extraction, 2/3 of the weight of the seed is left as residual cake which ranges from 22.3-27.5% crude protein (CP), making it a potential protein source for livestock. Extraction of oil is recommended, as the oil is unpalatable and contains toxic anti-nutrients such as phytates, tannins, and protease inhibitors, and karanjin, pongamol (furanoflavonoids), glabrin and other

polyphenolic compounds. Karanjin and pongamol are the two main toxic components, which could be the cause of the oil's bitter taste (Dutta et al., 2012).

Our objective was to determine palatability of a supplement containing expeller pressed Pongamia seedcake (EKC) and solvent extracted Pongamia seedcake (SKC) to cattle consuming a forage diet.

Materials & methods

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

Seven steers (355.98 ± 38.3 kg of BW) were used in a 7 × 7 Latin Square experiment designed to evaluate the palatability of two types of Pongamia seedcake (PSC) at varying levels to cattle consuming a basal diet of Bermudagrass hay (Table 2). Supplements were formulated to be 20%, 40%, and 60% of the supplement as EKC and 20%, 40%, and 60% of the supplement as SKC, with the remainder being WM. The control supplement was 100% WM. Bermudagrass was chopped through a screen (76 × 76 mm) and offered at 0600 and fed at 2% of initial BW to facilitate complete consumption. Steers were housed in individual stalls in an enclosed barn and given continuous access to water and commercial trace mineral blocks (composition: ≥ 97% NaCl, 1,000 ppm Ca, 1,500 ppm Fe, 2,500 ppm Zn, 3,000 ppm Mn, 90 ppm I, 150 ppm Cu, 25 ppm Co, and 10 ppm S; United Salt Corporation, Houston, TX). Treatments were arranged as a 3 × 2 factorial plus a control (100% wheat middlings; WM). The first factor consisted of level of PSC at 20, 40, or 60%, and the second factor consisted

Table 2. Composition of expeller pressed Pongamia seedcake, solvent extracted Pongamia seedcake, wheat middlings, and Bermudagrass hay[1]

Item	EKC	SKC	WM	Hay
OM, %	95.3	94.8	93.6	93.1
NDF, %	14.2	14.4	41.7	66.6
ADF, %	12.4	11.0	14.4	38.8
CP, %	26.6	29.8	18.0	5.5
Fat (EE), %	11.5	2.7		
Karanjin, ppm	5667	1758		
Pongamol, ppm	2544	794		

[1]EKC = expeller-pressed Pongamia seedcake; SKC = solvent-extracted Pongamia seedcake; WM = wheat middlings

of PSC source; expeller pressed (EKC) or solvent extracted (SKC). The remaining portion of each PSC supplement was WM. Supplement (500 g), consisting of WM and PSC at various inclusion levels, was offered once daily prior to feeding hay at 0600 h.

Seven consecutive 4-d periods consisted of 3 d to feed the test supplements and 1 d washout period when the 100% WM supplement was fed to all steers. Rate and extent of consumption were observed by allowing steers to eat for 10 minutes, then collecting and weighing the remaining supplement. Immediately after weighing, supplement was returned and the time required for complete consumption or loss of interest was recorded.

Hay, EKC, SKC, and WM were dried in a forced-air oven for 96 h at 55^{o} C and allowed to air-equilibrate for determination of partial DM. Hay and supplements were pooled across day on an equal weight basis, then ground through a 1-mm screen using a Wiley mill and dried at 105^{o}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^{o}C. Nitrogen was measured using the Elementar rapid N cube (Elementar, Hanua, Germany) and CP was calculated as N × 6.25. Neutral detergent fiber (NDF) and ADF analysis was performed sequentially using an Ankom Fiber Analyzer with amylase. Sodium sulfite was omitted and there was no correction for residual ash (Ankom Technology Corp., Macedon, NY).

Statistical analysis

Palatability parameters were analyzed using the MIXED procedure in SAS 9.2 (SAS Inst. Inc., Cary, NC). Terms in the model included treatment, period, day, and

treatment × day, with steer and treatment × steer × period as the random terms. The repeated term was day, with treatment × steer as the subject.

Results

There was a day × treatment interaction ($P < 0.01$; Table 3) for amount of supplement consumed. Steers fed 100% WM, 20% SKC, and 60% SKC supplements had consistent intakes across all three days. Both 40% SKC and EKC resulted in gradual reductions in intake across the 3-d period from 479 to 379 ($P < 0.05$) and 238 to 210 g ($P > 0.05$), respectively. Consumption of the 20% EKC treatment increased on d 2, beginning at 456 g on d 1, increasing to 478 g on d 2, and decreasing to 459 g on d 3, but the differences were not significant ($P > 0.05$). Consumption of the 60% EKC supplement significantly decreased ($P < 0.05$) from 332 g on d 1 to 59 g on d 2 and then increased to 165 g on d 3. When averaged across days, there was a linear reduction ($P < 0.01$) in supplement consumption with the addition of either EKC or SKC. Consumption of supplements containing 20% EKC, and 20% SKC (465 and 489 g) were not significantly different ($P > 0.05$) than the 100% WM control. Consumption of 40% and 60% SKC were intermediate and averaged 415 and 385 g, respectively. Provision of 40% EKC and 60% EKC resulted in intakes of 221 and 185 g, which were significantly ($P < 0.05$) less than all other treatments.

A trend ($P = 0.08$, Table 3) for a treatment × d interaction was apparent for rate of supplement consumption (ROC). Steers fed 100% WM, 20% EKC, 20% SKC, 40% EKC, and 60% SKC supplements had consistent ROC across all three days. Steers fed 40% SKC resulted in gradual reductions in ROC across the 3-d period from 74.5 to 44.3

Table 3. Effect of supplement composition on the amount consumed and percent consumption[1]

	Consumption[3]	10 min ROC[4]	ROC[5]
Treatment[2]	g	(g/min)	(g/min)
100% WM	500[a]	89.0[a]	89.5[a]
20% EKC	465[a]	73.1[a,c]	71.3[a,c]
40% EKC	221[b]	38.5[b,d]	14.2[b,d]
60% EKC	185[b]	48.0[d]	17.6[d]
20% SKC	489[a]	83.3[a,c]	82.7[a]
40% SKC	415[a]	61.3[c,d]	55.0[c,e]
60% SKC	385[c]	48.0[d]	38.3[e,d]
SEM	32.8	11.24	12.02
Treatment × d, *P*-value	< 0.01	0.21	0.08
EKC Linear, *P*-value	< 0.01	< 0.01	< 0.01
SKC Linear, *P*-value	< 0.01	< 0.01	< 0.01

[1]Within each row, means with differing superscripts differ at ($P < 0.05$)
[2]WM= wheat middlings, EKC= expeller pressed Pongamia, SKC= solvent extracted Pongamia
[3]Amount consumed = supplement consumed on average, out of 500 g.
[4]10 min ROC = rate of consumption during the first 10 min after offering the supplement
[5]ROC = rate of consumption until the supplement was completely consumed or the steer lost interest

g/min ($P < 0.05$). Rate of consumption of the 60% EKC supplement significantly decreased ($P < 0.05$) from 29.9 g/min on d 1 to 3.4 g/min on d 2 and then increased to 19.6 g/min on d 3.

When averaged across days, there was a linear reduction ($P < 0.01$, Table 3) in supplement ROC with the addition of either EKC or SKC. Rate of consumption of supplements containing 20% EKC, and 20% SKC (71.3 and 82.7 g/min) were not significantly different ($P = 0.14$ and $P = 0.59$, respectively) than the 100% WM control. Rate of consumption of 40% and 60% SKC were intermediate and averaged 55.0 and 38.3 g/min, respectively. Provision of 40% EKC and 60% EKC resulted in ROC of 14.2 and 17.6 g/min, which were significantly ($P < 0.05$) slower than all other treatments. There was not a significant treatment × d interaction ($P = 0.21$) for 10 min ROC. Rate of consumption and 10 min ROC both decreased linearly ($P < 0.01$) when either EKC or SKC made up increasing proportions of the supplements. Rate of consumption of 20% EKC (71.2 g/min) was significantly ($P < 0.01$) faster than 40% EKC and 60% EKC (14.1 and 17.6 g/min, respectively), but 40% EKC and 60% EKC were not different ($P = 0.77$). Rate of consumption of 20% SKC (82.7 g/min) was ($P \leq 0.03$) greater than 40% SKC and 60% SKC (55.0 and 38.3 g/min), but consumption rate of 40% SKC was not different than 60% SKC ($P = 0.18$). Differences in rate of consumption between Pongamia types at the same level of inclusion were not statistically significant at either the 20% or 60% level, but at the 40% level SKC rate of consumption was ($P < 0.01$) greater than EKC.

Ten minute consumption rate of 40% EKC, 40% SKC, 60% EKC, and 60% SKC were all significantly slower ($P < 0.01$, Table 3) than the 100% WM control. The ten minute consumption rate of 20% EKC was significantly ($P < 0.01$; $P = 0.03$) faster than 40% EKC and 60% EKC, but the difference between 40% EKC and 60% EKC was not statistically different ($P = 0.38$). For SKC, ten minute consumption rate of 20% tended to be faster than 40% ($P = 0.06$), 40% was not statistically different from 60% ($P = 0.22$), and 20% had a statistically faster ten minute rate of consumption than 60% ($P < 0.01$). Differences in ten minute consumption rate between Pongamia types at the same level of inclusion were not statistically significant at the 20% and 60% level, but at the 40% level SKC ten minute rate of consumption was significantly ($P = 0.04$) faster than EKC.

Discussion

When included at higher levels (60%) in the supplement, PSC reduced palatability of the supplements. At the 40 and 60% level, SKC was more palatable than EKC, most likely due to the greater oil/karanjin concentration in the EKC (5667 vs 1758 ppm karanjin). Pongamia seedcake was not offered at 100% of the supplement because this was not a viable option as it would not be readily consumed, with anti-quality factors karanjin and pongamol likely being the cause of the bitter taste making PSC less palatable (Ravi et al., 2000; Dutta et al., 2012). In this study, 2.34% residual oil remained in the production of SKC. Vinay and Sindhu Kanya, (2008) reported treating PSC with 2% HCl for one hour at room temperature was the best detoxification method, and resulted in karanjin content of 0.03% with efficient removal of oil. Nagalakshmi et al., (2011) reported karanjin content was estimated to be 0.325% of the supplement

when SKC was fed at 12% of the concentrate mixture. Karanjin content of raw SKC (1758 ppm, 0.18%,) used for supplements in this study was intermediate to the values reported by Vinay and Sindhu Kanya, (2008) and Nagalakshmi et al., (2011). Although previous work regarding palatability of Pongamia protein supplements is limited, Gupta et al., (1981) reported EKC was unpalatable even at 4% of the concentrate mixture (500g) when mixed with only salt and mineral, but SKC can replace up to 60% of conventional protein sources in the supplement, equivalent to a 24% SKC supplement. However, in the current study, 20% EKC was relatively palatable, but consumption of the 60% SKC supplement was not high enough to be considered a viable option. Konwar and Banerjee, (1987) fed bulls SKC at 0, 40, 50, 60, 80, and 100% of the concentrate mixture with the remaining being deoiled groundnut cake, and reported decreased concentrate consumption when SKC was included at 60% or greater, which is consistent with observations in the present study. In our study, palatability of PSC supplements containing 20% or 40% PSC was comparable to control supplements containing 0% PSC. Ravi et al., (2000) reported similar results, with no difference in concentrate intake when EKC and SKC were included at 24% and 20% of the diet respectively.

Using similar methodologies to ours, Drewery (2012) evaluated the palatability of post-extraction algal residue mixed with one of two dry carriers: dried distillers' grains (DDG) or cottonseed meal (CSM). Regardless of carrier ingredient, both EKC and SKC supplements were consumed less readily than PEAR supplements at all levels of inclusion. In the study by Drewery (2012), both control supplements, 100 DDG and

100 CSM, were consumed almost twice as fast as WM. However, Drewery (2012) offered 1000 g vs the 500 g offered in this study, which may have made it easier to consume the supplement. Further investigation should be conducted in which EKC and SKC are mixed with DDG or CSM to see if it improves palatability. Rate of consumption of PEAR supplements was also faster than PSC supplements. Again, this poor palatability is thought to be due to the bitterness coming from karanjin present in the cake.

Conclusions

Pongamia seedcake, when used as a protein supplement for livestock, must be mixed with a carrier in order to facilitate consumption. Palatability issues are most likely associated with the anti-nutrient karanjin being present in the residual oil in the cake giving it a bitter, pungent odor and taste. Since residual oil/karanjin concentrations are higher in EKC, more issues with palatability were expected. Even following further processing of the cake (solvent extraction), karanjin concentration is still high enough to present palatability problems when SKC is fed at a higher inclusion level (60% and greater). These data suggest SKC is more palatable than EKC and can be included in a protein supplement up to a 40% level. However, 20% EKC inclusion may be a viable option for cattle consuming low-quality forage.

CHAPTER III

EFFECT OF LEVEL OF PONGAMIA ON NUTRIENT UTILIZATION IN CATTLE CONSUMING FORAGE

Overview

Five ruminally cannulated steers (362.4 ± 40.3 kg BW) were used in a 5 × 5 Latin square to determine the effects of provision and level of either solvent extracted or expeller pressed Pongamia seedcake (SKC and EKC, respectively) on forage utilization. The five treatments consisted of a control (no supplement, NOSUPP) and four supplemented treatments providing 100 mg of N/kg BW containing different levels of PSC. One supplement provided 0% of the N as PSC (0PSC), one provided 40% of the N as SKC (40SKC), and two provided either 20 or 40% of the N as EKC (20EKC and 40EKC, respectively). Five 14-d periods were conducted, each consisting of 8 d to adapt steers to treatment, 5 d to determine intake and digestion, and 1 d to quantify ruminal fermentation. During the project, 3 steers on 40EKC were removed because of extremely low intake (≤ 1.30 kg/d or 0.36 % of BW), and this response was attributed to the treatment. Compared to all other treatments, 0PSC had the greatest forage OMI, total OMI, and TDOMI ($P < 0.01$). Intakes when the 40SKC and 20 EKC were used were not different from NOSUPP nor each other ($P > 0.05$), but all measures of intake were lower for 40EKC ($P < 0.05$, n=2). Supplementation did not increase total tract digestion ($P \geq 0.79$). All steers retained N, but 0PSC retained (34.7 g/d) more N than all other treatments ($P < 0.05$), most likely related to the differences in N intake and energy availability. Steers receiving NOSUPP retained the least amount of N (7.0 g/d) which

was not different (P = 0.17) from 40EKC (15.5 g/d). Steers dosed 20EKC and 40SKC retained similar amounts of N (22.9 and 19.7 g/d, respectively; P > 0.05) and were not different than 40EKC. Steers supplemented with PSC absorbed more pongamol and karanjin than either NOSUPP or 0PSC (P < 0.05). All PSC treatments differed from each other (P < 0.05) for both intake and absorption of pongamol and karanjin. Of PSC supplemented steers, provision of 40SKC resulted in the lowest absorption of both pongamol and karanjin (219 and 393 mg/d, respectively), intermediate was 20ECK (435 and 678 mg/d, respectively) and the greatest was 40ECK (789 and 1132 mg/d, respectively). Based on this study, 40EKC does not seem to be a viable option as a protein supplement for cattle. However, 40SKC and 20EKC could potentially be utilized as protein supplements for beef cattle on forage based diets, with SKC being a more suitable option.

Introduction

Pongamia pinnata (karanja) is a drought and salt tolerant legume tree native to India and Asia (Scott et al., 2008). Pongamia produces a large seed consisting of an outer hull and inner kernel, and is 27-39% oil, 20-30% protein, and 5-6% furano-flavonoids (karanjin and pongamol; Vinay and Sindhu Kanya, 2008). Pongamia is currently being investigated as an oil source for the production of biodiesel. After oil extraction, 2/3 of the weight of the seed is left as residual cake which ranges from about 22.3-27.5% CP, suggesting it had potential as a protein source for livestock. However, residual oil is unpalatable and contains toxic anti-nutrients, so nearly complete oil extraction is recommended (Vinay and Sindhu Kanya, 2008). Karanjin and pongamol

are the two main toxic components, and my result in the oil's antibacterial and insecticidal properties and bitter taste (Dutta et al., 2012).

Protein supplementation can increase body weight and condition, forage intake, and diet digestion in cattle consuming low-quality forage (Kartchner, 1980; DelCurto et al., 1990a; Sunvold et al., 1991; Bandyk et al., 2001; Wickersham et al., 2008). Reduced feed intake is a reoccurring issue (Konwar and Banerjee, 1987; Konwar et al., 1987; Singh et al., 2006; Soren and Sastry, 2009; Soren et al., 2009; Nagalakshmi et al., 2011) in animals fed PSC, most likely due to palatability. Observed reductions in intake are greater when the fed PSC contains greater concentrations of residual oil and anti-nutrients, but reductions have been observed at relatively low levels of residual oil (3.0%; Ravi et al., 2000).

Our objective was to determine the effect of increasing levels of Pongamia seedcake inclusion on nutrient utilization, N balance, karanjin and pongamol balance, and ruminal fermentation characteristics in cattle consuming a basal diet of low-quality forage.

Materials & methods

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

Five ruminally cannulated steers (362.4 ± 40.3 kg BW) were used in a 5 × 5 Latin square to determine the effects of provision and level of two types of PSC (SKC and EKC) on forage utilization. The five treatments consisted of a control (no

supplement, NOSUPP) with the other four providing 100 mg of N/kg BW containing various levels of PSC. One supplement provided 0% of supplemental N as PSC (0PSC), one provided 40% of supplemental N as SKC (40SKC), and two provided either 20 or 40% of supplemental N as EKC (20EKC and 40EKC, respectively; Table 4). To ensure complete consumption, supplements were dosed ruminally prior to feeding hay at 0600h each day. Formulation of the protein supplements (Table 4) and treatment design were based on the observations from Project 1 to create supplements cattle were likely to consume and were viable as commercial protein supplements. Bermudagrass 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 an enclosed barn and allowed *ad libitum* access to water and commercial trace mineralized salt blocks (composition: ≥ 96.5% NaCl, 1,600 ppm Fe, 4,000 ppm Zn, 1,200 ppm Mn, 100 ppm I, 260 ppm Cu, 40 ppm Co; North American Salt Company, Overland Park, Kansas).

Five 14-d periods were conducted, each consisting of 8 d to adapt steers to treatment, 5 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 first 4-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

Table 4. Composition of forage and supplements[1]

Item	Hay	0PSC	20EKC	40EKC	40SKC
Chemical composition, % of DM					
OM	92.0	93.4	93.8	94.0	93.7
NDF	67.4	24.2	21.3	17.7	19.7
ADF	41.8	9.8	10.2	10.6	9.8
CP	6.2	29.7	28.6	27.9	28.5
Karanjin, ppm		40	900	1554	519
Pongamol, ppm		20	578	1058	290
Diet composition, % of DM					
WM		47.5	37.5	27.5	27.5
SBM		47.5	37.5	27.5	27.5
EKC or SKC		0	20	40	40
Molasses		5	5	5	5
Macrominerals, %					
Ca		0.47	0.4	0.42	0.43
P		0.73	0.64	0.53	0.57
K		1.60	1.48	1.36	1.44
Mg		0.35	0.3	0.26	0.28
Na		0.04	0.05	0.03	0.09
S		0.24	0.27	0.24	0.24
Microminerals, ppm					
Co		0.49	0.62	0.44	0.6
Cu		12.7	13.3	14.4	17.8
Fe		134	146	190	213
Mn		77.2	68.5	62.9	97.3
Se		< 1.50	1.95	< 1.50	1.96
Zn		64.9	60.7	58.2	78.8
Cr		< 0.30	0.43	0.76	0.69
Mo		2.54	1.79	1.71	1.51
Ni		4.44	5.07	5.75	5.91

[1]Supplements consisted of wheat middlings (WM), molasses, soybean meal (SBM), and expeller pressed Pongamia or solvent extracted Pongamia (EKC/SKC) at 0, 20, or 40% of N

and a portion of each (3% of fecal matter, 1.5% of 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.

On d 14 of each period, ruminal fermentation parameters were measured. A suction strainer (Raun and Burroughs, 1962; 19 mm diameter, 1.5 mm mesh) was used to collect rumen fluid samples prior to feeding (0 h), and at 4, 8, 12, 16, and 20 h after feeding. A portable pH meter with a combined electrode (VWR SympHony) was used to measure the pH of each sample at the time of sampling. Subsamples of rumen fluid were prepared and frozen at -20°C for later determinations of VFA and ammonia N analysis. Prior to freezing, 8 ml of rumen fluid were combined with 2 ml of 25% *m*-phosphoric acid for VFA analysis. Nine ml of rumen fluid were combined with 1ml of 1 N HCl for NH_3 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 period. Hay, ort, fecal, and supplement samples were ground with a Wiley mill to pass a 1-mm screen and analyzed for DM, OM, NDF, ADF, and N using procedures outlined in Project 1. Organic matter was determined as the loss in dry weight upon combustion for 8 h at 450° F. Nitrogen was measured using the Elementar rapid N cube (Elementar, Hanua, Germany) and CP was calculated as N × 6.25. Analysis for NDF was performed using an Ankom Fiber Analyzer with sodium sulfite and amylase omitted and without correction for residual

ash (Ankom Technology Corp., Macedon, NY). Acid detergent fiber was also determined using an Ankom Fiber Analyzer. Digestion was calculated by the following formula: [1- (output of nutrient/intake of nutrient)] × 100.

Fecal and supplement samples were analyzed for karanjin and pongamol by the Office of the Texas State Chemist using liquid chromatography-mass spectrometry (LC/MS). Five grams of ground sample were extracted with 50 mL of 100% methanol and put on a shaker for 1 hour. The extract was filtered through Whatman #1 paper, diluted with 50% acetonitrile in water (Dilution factor=5), and purified by 0.2 um syringe filter before being injected into LC/MS.

Rumen fluid samples were thawed and centrifuged at 20,000 × *g* for 20 min. Volatile fatty acid concentrations were measured using a gas chromatograph with methods described by Vanzant and Cochran (1994). Ammonia concentrations were measured using a UV-VIS with colorimetric procedures as described by Broderick and Kang (1980).

Statistical analysis

Intake, digestion, N balance, and karanjin/pongamol 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. Volatile fatty acid and ruminal ammonia were analyzed using the MIXED procedure. Terms in the model were treatment, period, hour, and hour × treatment, with steer and treatment × period × steer included as random terms. The repeated term was hour, with treatment × steer as the subject. Treatment means were calculated using the LSMEANS option.

Results

Compared to all other treatments 0PSC steers had the greatest ($P < 0.01$) forage OMI, total OMI, and TDOMI (6.12, 6.70, and 3.91 kg/d, respectively; Table 5). No supplement, 20EKC, and 40SKC steers were similar to one another ($P > 0.05$) in observed forage OMI (5.25, 4.71, and 4.88 kg/d, respectively), total OMI (5.25, 5.30, and 5.49 kg/d, respectively) and TDOMI (3.06, 3.11, and 3.21 kg/d, respectively). Provision of 40EKC resulted in the lowest intake ($P < 0.05$) for forage OMI, total OMI, and TDOMI (3.57, 4.13, and 2.46 kg/d, respectively). Total tract digestion was similar for steers on all treatments ($P \geq 0.79$).

All steers were positive in N balance (Table 5), but steers receiving the 0PSC supplement had a greater ($P < 0.05$) amount of total N intake (112.3 g/d) than those on all other treatments as forage intake of these steers were greater than all other steers. Steers receiving the 20EKC and 40SKC supplement were similar in total N intake ($P = 0.58$; 93.2 and 95.7 g/d, respectively) and were greater than NOSUPP and 40EKC steers (65.3 and 77.3 g/d, respectively), but were still significantly lower than the 0PSC steers ($P > 0.05$). Steers receiving the 40EKC treatment was similar to NOSUPP steers ($P = 0.09$), and had significantly lower total N intake than those receiving 0PSC, 20EKC, and 40SKC ($P < 0.05$). The 0PSC steer had significantly greater forage N intake (76.6 g/d) compared to those receiving all other treatments ($P < 0.05$). Steers on 20EKC and 40SKC treatments (58.6 and 61.2 g/d, respectively) were similar to NOSUPP steers in forage N intake ($P > 0.05$). The steers on the 40EKC supplement had significantly lower

Table 5. Effect of Pongamia seedcake on intake, digestion, and N balance in cattle consuming forage[1]

Item	Treatments[2]						
	NOSUPP	0PSC	20EKC	40EKC	40SKC	SEM[3]	*P*-value
No. of Obs[4]	5	5	5	2	5		
OM intake, kg/d							
Forage	5.25[a]	6.12[b]	4.71[a]	3.57[c]	4.88[a]	0.36	< 0.01
Total	5.25[a]	6.70[b]	5.30[a]	4.13[c]	5.49[a]	0.36	< 0.01
Digestible	3.06[a]	3.91[b]	3.11[a]	2.46[c]	3.21[a]	0.23	< 0.01
Total Tract Digestion, %							
OM	58.3	58.1	58.7	61.3	58.7	2.32	0.79
NDF	58.4	57.2	57.9	58.1	57.0	2.01	0.91
N, g/d							
Intake							
Total	65.3[a]	112.3[b]	93.2[c]	77.3[a]	95.7[c]	5.46	< 0.01
Forage	65.3[a]	76.6[b]	58.6[a,c]	45.8[c]	61.2[a]	5.66	< 0.01
Supplement	0.0	35.7	34.5	32.6	34.4		
Fecal	38.8	47.0[a]	37.6	28.7[b]	41.1	6.02	0.18
Urinary	19.6[a]	30.7[b]	32.6[b,c]	34.9[b,c]	34.9[c]	2.68	< 0.01
Absorbed	26.5[a]	65.4[b]	55.5[b,c]	48.5[c]	54.6[c]	5.54	< 0.01
Retained	7.0[a]	34.7[b]	22.9[c]	15.5[a,c]	19.7[c]	5.25	< 0.01

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

[2]NOSUPP = no supplement control; 0PSC = 0% of N from Pongamia; 20EKC = 20% of N from expeller pressed Pongamia seedcake; 40EKC = 40% of N from expeller pressed Pongamia seedcake; 40SKC = 40% of N from solvent- extracted Pongamia seedcake; all supplements were dose ruminally at 100 mg N/kg BW

[3]SEM = standard error of the mean

[4]Only 2 observations for 40EKC are included as 3 steers were removed from the project because of low intake

forage N intake ($P < 0.05$; 45.8 g/d) than those on all other treatments except those on 20EKC ($P = 0.08$).

Supplemental N intake, per design, was similar for steers on all treatments other than NOSUPP ($P > 0.05$). Urinary N was lower for NOSUPP steers (19.57 g/d) than those receiving all other treatments ($P < 0.05$). Positive control (0PSC), 20EKC, and 40EKC steers (30.7, 32.6, and 34.9 g/d, respectively) were similar in amount of urinary N ($P > 0.05$), and those on 40SKC (34.90 g/d) was comparable to 20EKC and 40EKC steers in urinary N ($P > 0.05$).

No supplement steers were significantly lower ($P < 0.05$; 26.5 g/d) in amount of apparent absorbed N than those on all treatments. The steer receiving the 20EKC supplement was similar to 0PSC ($P = 0.06$; 55.5 and 65.4 g/d, respectively), and steers provided 40EKC and 40SKC (48.5 and 54.6 g/d, respectively) were similar to 20EKC steers in apparent absorbed N ($P > 0.05$).

The NOSUPP steers were also significantly lower in retained N ($P < 0.05$; 7.0 g/d), while those on 0PSC were significantly higher in amount of retained N ($P < 0.05$; 34.7 g/d), most likely related to the differences in amount of total N intake. The steers on the 40EKC (15.5 g/d) treatment was similar to those provided NOSUPP ($P = 0.17$), and those receiving 20EKC and 40SKC (22.9 and 19.7 g/d, respectively) supplements were similar to 40EKC steers in amount of retained N ($P > 0.05$).

No supplement and 0PSC steers were similar in both karanjin and pongamol intake, and were significantly (Table 6; $P < 0.05$) lower than steers on all other treatments. The 0PSC steers were expected to be zero for karanjin and pongamol intake

Table 6. Effect of Pongamia seedcake on karanjin and pongamol absorption in cattle consuming forage[1]

	Treatments[2]						
Item	NOSUPP	0PSC	20EKC	40EKC	40SKC	SEM[3]	*P*-value
No. of Obs[4]	5	5	5	2	5		
Karanjin, mg/d							
Intake	0[a]	37[a]	678[b]	1132[c]	393[d]	45	< 0.01
Fecal	0	0	0	0	0	0	0.52
Absorbed	0[a]	37[a]	678[b]	1132[c]	393[d]	45	< 0.01
Pongamol, mg/d							
Intake	0[a]	15[a]	435[b]	789[c]	219[d]	28	< 0.01
Fecal	0	0	0	0	0	0	0.80
Absorbed	0[a]	15[a]	435[b]	789[c]	219[d]	28	< 0.01

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

[2]NOSUPP = no supplement control; 0PSC = 0% of N from Pongamia; 20EKC = 20% of N from expeller pressed Pongamia seedcake; 40EKC = 40% of N from expeller pressed Pongamia seedcake; 40SKC = 40% of N from solvent- extracted Pongamia seedcake; all supplements were dose ruminally at 100 mg N/kg BW

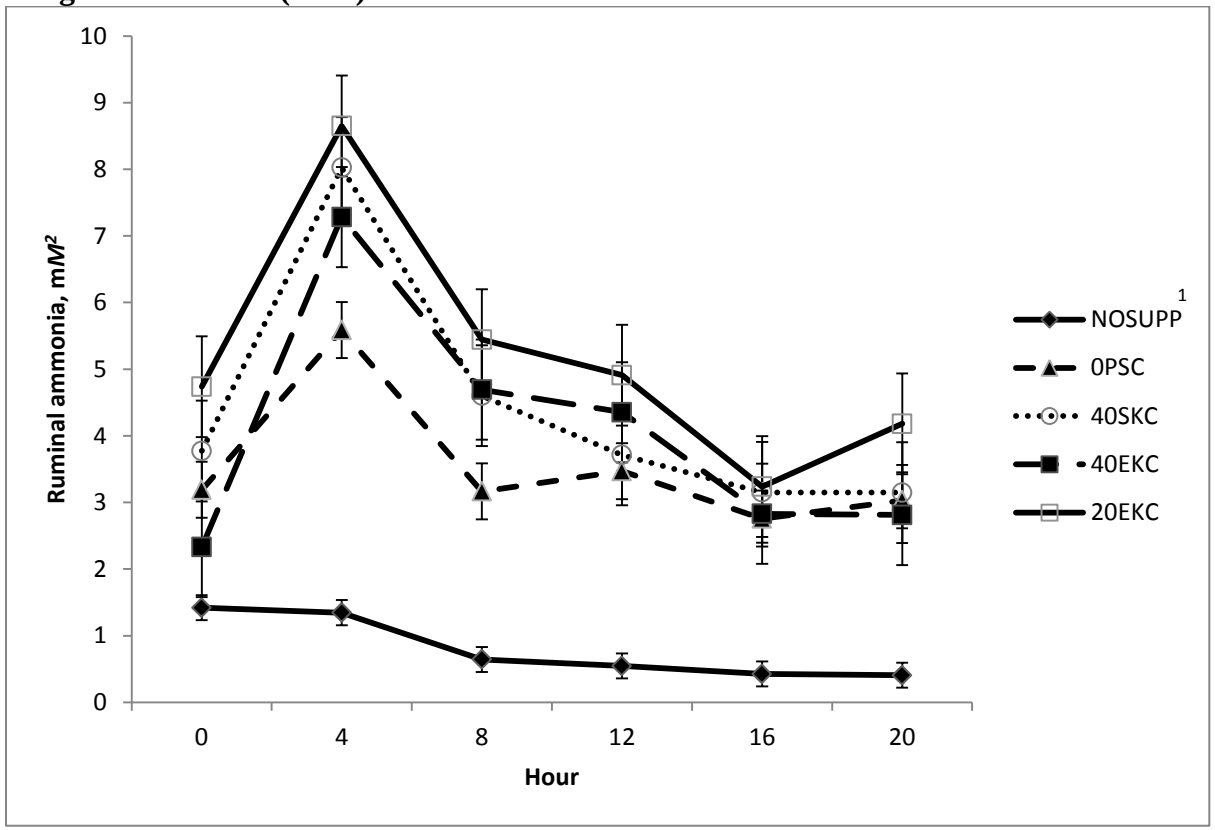
[3]SEM = standard error of the mean

[4]Only 2 observations for 40EKC are included as 3 steers were removed from the project because of low intake

and absorption as well since there was no PSC mixed in it, but an error in laboratory technique or with the machinery must have occurred at some point. Steers on the 40EKC were significantly higher ($P < 0.05$; 1132 and 789 mg/d, respectively) in karanjin and pongamol intake than those provided all other treatments. Those receiving 20EKC were next highest in karanjin and pongamol intake (678 and 435 mg/d), but were significantly different ($P < 0.05$) from steers on all other treatments. Steers receiving 40SKC were significantly different ($P < 0.05$) from steers receiving all other treatments and were third in terms of karanjin and pongamol intake (393 and 219 mg/d), but was the lowest of the PSC-containing treatments. Fecal karanjin and pongamol was similar for steers on all treatments as none appeared in the feces. Apparent absorbed karanjin and pongamol was similar for NOSUPP and 0PSC steers, and was significantly lower ($P < 0.05$) than steers dosed all other treatments. Again 0PSC was expected to be zero, but an error must have occurred in either lab technique or the machine. Steers given all three PSC containing supplements were significantly different ($P < 0.05$) from each other and steers on all other treatments, with 40EKC steers being highest in amount absorbed, followed by 20EKC and 40SKC steers respectively.

There was a treatment × time interaction ($P < 0.01$) for ruminal ammonia concentration (Figure 1). This interaction resulted from a relatively large increase in ruminal ammonia concentrations between h 0 and 4 for steers on all treatments except NOSUPP followed by a return to baseline by h 16. The NOSUPP steers had significantly lower ruminal ammonia (0.80 m*M*, Table 7) than those given all other treatments, but there was no significant difference ($P > 0.05$) between 0PSC, 40SKC,

Figure 1. Effect of protein supplementation with expeller-pressed Pongamia seedcake (EKC) or solvent extracted Pongamia seedcake (SKC) on ruminal ammonia concentration



[1]NOSUPP = no supplement control; 0PSC = 0% of N from Pongamia; 20EKC = 20% of N from expeller pressed Pongamia seedcake; 40EKC = 40% of N from expeller pressed Pongamia seedcake; 40SKC = 40% of N from solvent- extracted Pongamia seedcake; all supplements were dose ruminally at 100 mg N/kg BW

[2]Treatment × hour interaction, $P < 0.01$

Table 7. Effect of protein supplementation level with expeller-pressed Pongamia seedcake (EKC) or solvent extracted Pongamia seedcake (SKC) on ruminal fermentation parameters[1]

	Treatment[2]					
Item	NOSUPP	0PSC	20EKC	40EKC	40SKC	SEM[3]
No. of observations[4]	5	5	5	2	5	
Ammonia, m*M*[5]	0.80[a]	3.53[b]	5.19[b]	4.05[b]	4.40[b]	1.10
Total VFA, m*M*[6]	81.00[a]	87.57[a,b]	89.87[b]	92.18[a,b]	84.16[a,b]	4.66
Molar percentages						
Acetate	66.71	66.26	66.38	65.82	65.55	2.17
Propionate	19.80	19.83	19.71	21.37	21.46	1.55
Butyrate	9.60	10.15	9.95	9.79	9.79	0.73
Isobutyrate	1.02	0.99	1.05	0.89	0.84	0.12
Isovalerate	1.97[a]	1.84[a,b]	1.86[a,b]	1.78[a,b]	1.46[b]	0.24
Valerate	0.89	0.93	1.06	0.87	0.91	0.14
pH[7]	6.41	6.35	6.36	6.39	6.34	0.06

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

[2]NOSUPP = no supplement control; 0PSC = 0% of N from Pongamia; 20EKC = 20% of N from expeller pressed Pongamia seedcake; 40EKC = 40% of N from expeller pressed Pongamia seedcake; 40SKC = 40% of N from solvent- extracted Pongamia seedcake; all supplements were dose ruminally at 100 mg N/kg BW

[3]SEM = standard error of the mean

[4]Only 2 observations for 40EKC are included as 3 steers were removed from the project because of low intake

[5]Treatment × time: $P < 0.01$, effect of time: $P < 0.01$

[6]Treatment × time: $P = 0.97$, effect of time: $P = 0.98$

[7]Treatment × time: $P = 0.75$, effect of time: $P < 0.01$

40EKC, and 20EKC steers (3.53, 4.40, 4.05, and 5.19 m*M*, respectively). There was not a treatment × time interaction ($P = 0.97$) or effect of time ($P = 0.98$) on total VFA concentration. No supplement, 0PSC, 40SKC, and 40EKC steers were all similar in total VFA concentration, and 20EKC steers were similar to those receiving 40SKC and 40EKC. There was no significant difference in molar percentages of acetate, propionate, butyrate, isobutyrate, or valerate between steers on all treatments. Molar percentages of isovalerate were similar between NOSUPP, 0PSC, 40EKC, and 20EKC steers, and 40SKC steers were similar to those provided 0PSC, 40EKC, and 20EKC. There was no treatment × time interaction ($P = 0.75$) for rumen pH, but there was an effect of time ($P < 0.01$). There was no significant difference in rumen pH between steers in response to any of the treatments.

Discussion

When supplemental protein was provided, responses to intake in this study were variable based on type of supplement. Total OMI, forage OMI, and TDOMI were all stimulated by the 0PSC supplement, decreased by 40EKC, and showed no significant difference between 20EKC and 40SKC in comparison to control. The increase in intake, observed with 0PSC when a conventional source of supplemental protein was provided is in agreement with McCollum and Galyean, (1985), DelCurto el al., (1990a), Bandyk et al., (2001), and Wickersham et al., (2008). The increase seen in this project was larger than that reported by Bandyk et al., (2001), but was smaller than that reported by Wickersham et al., (2008), most likely due to the quality of the forage and the severity of the N deficiency. Intake values for all treatments were within the range reported by the

previously mentioned sources. The no difference in intake when 20EKC and 40SKC supplements were dosed were also observed by Paul et al., (1994) and Srivastava et al., (1990) when calves and kids were fed SKC at 0, 15, and 30% and 0, 20, 30, and 40% of the concentrate mixture respectively. Ravi et al., (2000) reported a similar decreased intake when EKC was fed at 24% of the diet. The decrease in intake with the 40EKC supplement is thought to be due to the increased level of anti-nutrients (i.e. karanjin and pongamol) still present in the seedcake due to the higher residual oil concentration of the EKC. Intakes for 40EKC steers would likely be even lower had the three steers removed from the 40ECK treatment been able to finish the period as their intakes were lower than the mean (forage OMI = 3.57 kg/d) when they were removed. Their removal is attributed to the provision of EKC because their intake consistently decreased as the number of days on 40EKC increased, and affected steers returned to acceptable levels of intake within a few days of being removed from the 40EKC treatment. Total tract digestion in this study was not affected by protein supplementation, which is in agreement with Srivastava et al., (1990). Singh et al., (2006) reported no difference in DM and OM digestion between control, EKC, and SKC, lower NDF digestion with EKC, and reduced ADF digestion when EKC and SKC supplements were fed. Ravi et al., (2000) reported decreased DM, OM, NDF, and ADF digestion when EKC was fed. Values for digestion are within ranges reported by Ravi et al., (2000), Singh et al., (2006), and Nagalakshmi et al., (2011), ranging from 58.1 to 61.3 for OM digestion and 57.0 to 58.4 for NDF digestion.

In comparison, total N intake values, thus all N balance values, in this study are substantially higher than those reported by Srivastava et al., (1990), Ravi et al., (2000), Singh et al., (2006), Soren and Sastry, (2009), and Nagalakshmi et al., (2011), most likely because all of these studies were conducted in sheep and goats while this study was conducted in steers, therefore total intakes would naturally be lower in the studies using sheep and goats. When expressed as percent of N retained, the value in this study for 0PSC steers (30.9%) were lower than those reported by Srivastava et al., (1990), Ravi et al., (2000), Singh et al., (2006), and Soren and Sastry, (2009) (46.3%, 42.6%, 41.6%, and 39.0%, respectively). The percentage of N retained for 20EKC and 40EKC steers in this study (24.6% and 20.1%) was greater than that reported by Singh et al., (2006) (9.1% on EKC at 24% of concentrate mixture), but lower than that reported by Ravi et al., (2000) (32% on EKC at 24% of concentrate mixture). The percentage of N retained for 40SKC steers (20.6%) in this study was less than those reported by Srivastava et al., (1990), Ravi et al., (2000), Singh et al., (2006), and Soren and Sastry, (2009) (37.4-50.9%, 39.3%, 27.2%, and 25-37%, respectively). Paul et al., (1994) conducted a study in which an unconventional feed ingredient mixture (containing PSC) was fed at 0, 15, and 30% to calves. They measured N balance, and reported a positive balance for all treatments, and no difference between treatments for N intake, fecal N, urine N, or balance. This disagrees with results from this study, as N intake was significantly different ($P < 0.05$) for NOSUPP, 0PSC, 20EKC, and 40SKC steers due to the differences in forage N intake. Fecal N was similar except 0PSC and 40EKC steers were significantly different, and urinary N was different for NOSUPP steers. The values

in this study are less than those reported by Paul et al., (1994). Wickersham et al., (2008) measured N balance in cattle with increasing levels of DIP and found as DIP increased, amount of retained N did as well. In this study, N retained by the 0PSC steers was significantly higher than all other treatments, but steers receiving all three PSC-containing supplements were not different from each other even as inclusion level of PSC increased. Nitrogen balance values in this study are within the ranges reported by Wickersham et al., (2008).

Since no karanjin or pongamol appeared in the feces, it is thought all the karanjin and pongamol consumed was absorbed or metabolized (preventing detection in the feces) although we did not test urine for karanjin and pongamol. Soren and Sastry (2009) conducted a study in which lambs were fed SKC further processed using different methods, water washed, lime treated, and binder treated, in which karanjin balance was determined. Values for karanjin intake reported by Soren and Sastry (2009) were much lower than those in this study, most likely due to the further processing removing even more of the residual oil containing karanjin. However, Soren and Sastry (2009) reported minute amounts of karanjin in the feces and urine with a majority being retained due to high karanjin intake, which is in agreement with this study. Pongamol balance has not been previously studied. Mahli et al. (1989) reported the LD_{50} of karanjin in mice is 14.32 mg/kg, and of pongamol is 17.14 mg/kg. Intake of karanjin and pongamol in this study (3.12 and 2.18 mg/kg, respectively) was less than the LD_{50} of mice reported by Mahli et al. (1989). About 80% of the oil is removed when making EKC, but less than 0.5% of the oil remains when SKC is produced. In this study, 2.34% residual oil

remained in the production of SKC. Vinay and Sindhu Kanya, (2008) reported treating PSC with 2% HCl for one hour at room temperature was the best detoxification method, and resulted in karanjin content of 0.03% with efficient removal of oil. Nagalakshmi et al., (2011) reported karanjin content was estimated to be 0.325% when SKC was fed at 12% of the concentrate mixture. Karanjin content of raw SKC (0.18%) used for supplements in this study was intermediate to the values reported by Vinay and Sindhu Kanya, (2008) and Nagalakshmi et al., (2011).

In this study, ruminal ammonia concentrations were similar between 0PSC, 20EKC, 40EKC, and 40SKC steers. This is in agreement with Singh et al., (2006) who reports no difference in ruminal ammonia in lambs when fed a control groundnut cake supplement, and EKC supplement, and an SKC supplement. Singh et al., (2006) also reports no difference in rumen pH between the three treatments, which agree with this study. The rumen pH values in this study are lower than those reported by Singh et al., (2006), but are within the ranges reported by Mathis et al., (2000) and Bandyk et al., (2001). None of the Pongamia studies previously conducted measured molar percentages of VFA, but Soren et al., (2010) reported total VFA in lambs fed SKC further processed by different methods, including water washing, lime treating, and binder treating. In comparison with that study, our values for total VFA are higher. Compared to other protein supplementation studies, the total VFA concentrations in this study are slightly higher than those reported by Mathis et al., (2000), Bandyk et al., (2001), and Wickersham et al., (2008), but are similar to those reported by McCollum and Galyean, (1985). In this study, there was no difference in molar percentages of

acetate, propionate, butyrate, isobutyrate, and valerate between treatments, but 40SKC steers were significantly lower than NOSUPP steers in molar percentage of isovalerate. This disagrees with Mathis et al., (2000), and Wickersham et al., (2008) who report a linear decrease in acetate with an increasing amount of DIP. Molar percentages of acetate in this study are slightly lower than those reported by McCollum and Galyean, (1985), Mathis et al., (2000), Bandyk et al., (2001), and Wickersham et al., (2008). Molar percentages of propionate and butyrate are both similar across treatments in this study, which is in agreement with Mathis et al., (2000) and Bandyk et al., (2001). Isobutyrate was also not significantly affected by treatment in this study, which Bandyk et al., (2001), and Wickersham et al., (2008) also report. In this study, valerate was also not significantly affected by treatment, which disagrees with Mathis et al., (2000), Bandyk et al., (2001), and Wickersham et al., (2008) who all report an increase in valerate with increased DIP. Wickersham et al., (2008) reports no difference in isovalerate, which is similar to the results of this study as the only difference in molar percentage of isovalerate among treatments was 40SKC steers being significantly lower than NOSUPP steers only.

Conclusions

Protein supplementation with 20EKC or 40SKC did not elicit a difference in forage OMI, total OMI, or TDOMI when compared to an un-supplemented control. However, an increase in intake was observed when a commercially available 0PSC supplement was provided. Supplementation with 40EKC decreased forage OMI, total OMI, or TDOMI. Total tract digestion was not affected by any of the treatments. Steers

receiving all treatments were positive for N balance, but N retention was intermediate to NOSUPP and 0PSC steers for all PSC-containing supplements. Karanjin and pongamol intake and absorption was greatest for 40EKC steers and least for 40SKC steers when comparing PSC supplements. Ruminal ammonia was not different for steers on any of the treatments in which supplement was dosed. Total VFA was similar across treatments, except 20EKC steers were significantly higher than both NOSUPP and 0PSC steers. Rumen pH and molar percentages of VFA were similar across treatments, except 40SKC steers were significantly lower in isovalerate than NOSUPP steers. Based on these results, 40EKC does not seem to be a viable option as a protein supplement for cattle. However, 40SKC and 20EKC could potentially be utilized as protein supplements for beef cattle on forage based diets, with SKC being a more suitable option.

CHAPTER IV

LONG-TERM EFFECTS OF PONGAMIA SEEDCAKE AS A PROTEIN SUPPLEMENT IN CATTLE CONSUMING FORAGE AND CONCLUSIONS

Overview

Fifteen steers (253.27 ± 64.18 kg initial BW) were used in a randomized complete block for 126 d to determine the long-term effects of feeding two types of PSC, EKC and SKC. Five steers were used as controls (provided a commercially available protein supplement containing 0% Pongamia), five received a 20% EKC supplement, and five received a 20% SKC supplement. Intake was monitored throughout the project. Prior to project initiation and at wk 9 and 18, BW and diet digestion were determined and liver biopsies were collected. When scaled to metabolic BW, steers on the control supplement had significantly greater ($P < 0.05$) OMI per kg $BW^{0.75}$ than steers receiving the 20SKC supplement; however, the 20EKC was intermediate to the two other treatments and did not differ significantly ($P > 0.05$) from either. Total tract digestion was not significantly different between treatments ($P > 0.05$). Final BW of steers on the control supplement (330 kg) were significantly greater ($P < 0.05$) than those on either EKC or SKC (296 and 302 kg, respectively). Total gain from wk 0 to wk 18 was greater ($P < 0.05$) for control steers than both PSC treatments, but again there was no significant difference ($P > 0.05$) between PSC treatments (49.6 and 48.1 kg for EKC and SKC, respectively). Steers on the control supplement had significantly greater ($P < 0.05$) ADG (0.60 kg/d) than those receiving PSC supplements, but ADG was similar ($P > 0.05$) for 20% EKC and 20% SKC steers (0.39 and 0.38 kg/d,

respectively). Further research comparing performance of animals fed EKC and SKC should be conducted, as steers fed EKC performed better than expected in this study.

Introduction

Pongamia pinnata (karanja) is a drought tolerant legume tree native producimg an oilseed currently being investigated as a potential source of biodiesel. The seed consists of an outer hull and inner kernel, and is 27-39% oil, 20-30% protein, and 5-6% furano-flavonoids (Vinay and Sindhu Kanya, 2008). After oil extraction, 2/3 of the weight of the seed is left as residual cake, Pongamia seedcake (PSC), which ranges from about 22.3-27.5% CP, making it a potential protein source for livestock.

Protein supplementation can increase BW and condition, forage intake, and diet digestion in cattle consuming low-quality forage (Kartchner, 1980; DelCurto et al., 1990a; Sunvold et al., 1991; Bandyk et al., 2001; Wickersham et al., 2008). However, reduced feed intake is a reoccurring issue in animals fed PSC, thus resulting in decreased ADG and total gain (Nagalakshmi et al., 2011). Observed reductions in intake are greater in expeller pressed seedcake (EKC) because of the higher residual oil and anti-nutrient concentration, but reductions have been observed with solvent extracted seedcake (SKC) as well (Ravi et al., 2000).

Solvent extracted seedcake was fed at 20% of the concentrate mixture for 98 d to lambs without deleterious effects on performance, nutrient utilization, immunity, balance, growth rate, or feed conversion efficiency (Ravi et al., 2000; Vinay and Sindhu Kanya, 2008). However, Singh et al., (2006) and Nagalakshmi et al., (2011) report toxicity began to take effect at approximately wk 13 for both EKC and SKC, with BW

decreasing after 120 d of PSC supplementation. If fed long-term or in excess, studies have shown SKC can have deleterious effects on performance or the development of toxic symptoms such as loss of appetite, weight loss, frequent strong color urination, swelling of facial muscles and intermaxillary space, lameness, skin discoloration, loss of hair, and gangrene of the tail/sloughing (Gupta et al., 1981). Toxicity symptoms were observed more frequently with EKC than SKC, and Ravi et al. (2000) recommended EKC not be fed. Based on the literature, PSC is safe for short-term feeding, not exceeding 13-17 wk. Therefore, our objective was to determine the long-term effects of feeding Pongamia seedcake to cattle consuming a basal diet of medium-quality forage for more than 17 wk.

Materials & methods

All experimental protocols were approved by the Institutional Animal Care and Use Committee at Texas A&M University.

Fifteen steers (253.27 ± 64.18 kg initial BW) were used in a randomized complete block for 126 d to determine the long-term effects of feeding the two types of PSC. Five steers were used as controls (provided a commercially available protein supplement containing 0% Pongamia), five received a 20% EKC supplement, and five received a 20% SKC supplement (Table 8). Each steer was fed 1 kg of supplement prior to feeding hay. Bermudagrass hay was chopped through a screen (76 mm × 76 mm) and offered at 0600 h and 1100 h daily at 130% of the previous day consumption. Steers had *ad libitum* access to water for the duration of the trial. Steers were housed in pens in an outdoor barn, fitted with ball keys, and fed using a Calan gate system. Intake was

Table 8. Composition of forage and supplements fed long- term[1]

Item	Bermudagrass Hay	0% PSC[1]	20% EKC	20% SKC
Chemical composition, %				
DM	92.2	90.2	89.9	89.8
OM	91.9	89.7	90.9	91.9
NDF	73.8	44.1	34.9	40.3
ADF	43.0	27.4	24.5	30.1
CP	7.0	26.0	25.7	26.0
Diet composition, %				
EKC/SKC		0	20	20
Molasses		5	5	5
DDG		20	20	20
SBM		36	30	28
WM		39	25	27
Karanjin, ppm		5.0	733.0	279.2
Pongamol, ppm		1.0	390.7	134.3

[1]Supplements consisted of wheat middlings (WM), dried distillers' grains (DDG), molasses, soybean meal (SBM), and expeller pressed Pongamia (EKC) or solvent extracted Pongamia (SKC) at 20% respectively

monitored throughout the project. At wk 9 and 18, body weights were determined and liver biopsies were collected. Hay, ort, and fecal samples were collected at wk 13. Fecal samples were collected at 0400, 1600, 0800, 2000, 1200, and 2400 h across 3 consecutive days. Steers were moved to individual pens, the freshest sample was picked up off the ground, the collected pile of feces was marked, and collected samples were composited across day.

Hay, fecal, and ort samples were dried in a forced-air oven for 96 h at 55^{o}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 period. Hay, ort, fecal, and supplement samples were ground with a Wiley mill to pass a 1-mm screed and analyzed for DM, OM, NDF, ADF, and N using procedures outlined in Projects 1 and 2.

Calculations

Digestibilities were calculated by the following formula: [1- (output of nutrient/intake of nutrient)] × 100.

Statistical analysis

Intake, digestion, and animal performance were analyzed using the MIXED procedure in SAS 9.2 (SAS Inst. Inc., Cary, NC). Terms in the model included treatment, with block as a random effect.

Results

Steers receiving the control supplement had significantly greater ($P < 0.05$) total OMI (6.79 kg, Table 9) than both 20% EKC and 20% SKC steers (5.69 and 5.42 kg,

Table 9. Effect of long-term exposure to Pongamia seedcake on intake and digestion in cattle consuming forage[1]

	Treatments[2]				
Item	0% PSC	20% EKC	20% SKC	SEM[3]	*P*-value
No. of Observations	5	5	5		
Intake, kg/d					
Supplement OM	1.00[a]	0.67[b]	1.00[a]	0.07	<0.01
Forage OM	6.02[a]	5.16[a,b]	4.65[b]	0.48	0.05
Total OM	6.79[a]	5.69[b]	5.42[b]	0.46	0.02
TDOMI	4.18	3.23	3.34	0.31	0.10
Intake, g/kg $BW^{0.75}$					
Total OM	90.13[a]	81.25[a,b]	74.77[b]	5.58	0.09
TDOMI	55.42	46.51	45.40	3.69	0.16
Intake, mg/kg					
Karanjin	0.02	2.03	1.10		
Pongamol	0	1.08	0.53		
Total Tract Digestion, %					
OM	62.6	57.9	60.4	3.10	0.56
NDF	60.9	57.5	58.9	3.10	0.70

[1]Within each row, means with differing superscripts differ at ($P < 0.05$) level of significance
[2]Control = 0% of N from Pongamia; 20% EKC = 20% of N from expeller pressed Pongamia seedcake; 20% SKC = 20% of N from solvent- extracted Pongamia seedcake
[3]SEM = standard error of the mean

respectively). However, there was no difference in total OMI between steers fed 20% EKC and 20% SKC ($P > 0.05$). There was no significant different ($P > 0.05$) between treatments for forage OMI, and TDOMI. When put on a metabolic BW scale, steers on the control supplement had significantly higher OMI per kg $BW^{0.75}$ (90.13 kg, Table 9) than steers receiving the 20% SKC supplement (74.77 kg) ($P = 0.03$), but 20% EKC (81.25 kg) was not different from 0PSC ($P = 0.18$). Total OMI per kg $BW^{0.75}$ for 20% EKC was similar to 20% SKC ($P = 0.33$). There was no difference ($P > 0.05$) in TDOMI per kg $BW^{0.75}$ between the three treatments. None of the treatments were statistically different ($P > 0.05$) from each other for total tract digestion.

Initial BW of steers was similar among all treatments; however, end weight of steers on the 0% PSC supplement (330.18 kg, Table 10) were significantly higher ($P < 0.05$) than those on either PSC supplement (296.04 on 20% EKC and 301.83 on 20% SKC). End weights of steers receiving the 20% EKC and 20% SKC supplements were not different from each other ($P = 0.64$). Control (0% PSC) steers had significantly greater ($P < 0.05$) gain (64.61 kg) from wk 0 to wk 9 than both 20% EKC and 20% SKC steers (40.15 and 44.69 kg, respectively), but 20% EKC and 20% SKC steers were not different from each other ($P = 0.42$). There was no significant difference in gain from wk 9 to wk 18 among all three treatments ($P > 0.05$). Total gain from wk 0 to wk 18 was significantly ($P < 0.05$) higher for 0% PSC steers (75.13 kg) than both PSC treatments (49.62 kg for 20% EKC and 48.11 kg for 20% SKC), but again the two PSC treatments were not different from each other ($P = 0.72$). Steers on the 0% PSC supplement had significantly higher ($P < 0.05$) ADG (0.60 kg/d) than those receiving

Table 10. Effect of long-term exposure to Pongamia seedcake on animal performance and efficiency[1]

	Treatments[2]				
Item	0% PSC	20% EKC	20% SKC	SEM[3]	*P*-value
Body weight, kg					
Initial	254	246	254	16.5	0.75
Final	330[a]	296[b]	302[b]	18.0	0.03
Gain, kg					
Week 0-9	64.6[a]	40.2[b]	44.7[b]	3.74	< 0.01
Week 9-18	10.7	9.2	3.2	3.34	0.20
Total	75.1[a]	49.6[b]	48.1[b]	3.30	< 0.01
ADG, kg/d	0.60[a]	0.39[b]	0.38[b]	0.03	< 0.01
Gain:Feed					
Week 0-9[4]	0.16[a]	0.11[b]	0.09[b]	0.01	< 0.01
Week 9-18[5]	0.02	0.02	0.01	0.01	0.23
Total	0.08[a]	0.06[b]	0.05[c]	0.003	< 0.01

[1]Within each row, means with differing superscripts differ at ($P < 0.05$) level of significance
[2]Control = 0% of N from Pongamia; 20% EKC = 20% of N from expeller pressed Pongamia seedcake; 20% SKC = 20% of N from solvent- extracted Pongamia seedcake
[3]SEM = standard error of the mean
[4]Wk 0-9 average temperature = approximately 13˚C
[5]Wk 9-18 average temperature = approximately 24˚C

20% EKC and 20% SKC (0.39 and 0.38 kg/d respectively). Average daily gain was similar for 20% EKC and 20% SKC steers ($P = 0.72$).

From wk 0 to wk 9, 0% PSC steers were significantly ($P < 0.05$) more efficient (0.16) having a greater G:F than steers on either 20% EKC or 20% SKC (0.11 and 0.09, respectively). Feed efficiency of 20% EKC and 20% SKC steers was similar from wk 0 to wk 9 ($P = 0.18$). Feed efficiency from wk 9 to wk 18 was similar among all treatments ($P > 0.05$). Control steers (0% PSC) had a significantly ($P < 0.05$) higher (0.08) total G:F (wk 0 to wk 18) than both PCS treatments (0.06 for 20% EKC and 0.05 for 20% SKC). Total G:F of 20% EKC steers was significantly higher ($P < 0.05$) than those on 20% SKC ($P = 0.03$). Total G:F of steers on 20% SKC was significantly lower ($P < 0.05$) than all other treatments, making them the least efficient treatment group in this study.

Discussion

Intake responses to protein supplementation differed between supplement types. Total OMI was greater for the 0% PSC supplemented steers than those on 20% EKC and 20% SKC; however, responses in intake are hard to determine without having an unsupplemented control treatment to compare. In Project 2, the 20% EKC supplement resulted in no difference in intake response. Paul et al., (1994) and Srivastava et al., (1990) reported no difference in intake when calves and kids were fed SKC at 0, 15, and 30% and 0, 20, 30, and 40% of the concentrate mixture respectively, but Ravi et al., (2000) reported a reduced intake when 24% EKC was fed and an increased intake when 20% SKC was fed compared to a control deoiled groundnut cake supplement. When put

on a metabolic body weight basis, 20% SKC steers were significantly less than 0% PSC steers, and those on 20% EKC were similar to both for total DMI. Singh et al., (2006) reports significantly lower DMI per kg $BW^{0.75}$ for both EKC and SKC. Ravi et al., (2000), however, reports no significant difference in DMI per kg $BW^{0.75}$ between control 50% EKC, and 50% SKC. Values in these two studies were lower than those in this study, most likely due those studies being conducted with lambs as opposed to steers. Differences in gain between wk 0 and 9 vs gain from wk 9 to 18 could be due to differences in intake affected by temperature. Intake of steers was typically higher from wk 0 to 9 when the average temperature was approximately 13˚C, as opposed to that from wk 9 to 18 when the average temperature was warmer (approximately 24˚C). Total tract digestion was not affected by source of supplemental protein, which is in agreement with Project 2, Srivastava et al., (1990), and Soren and Sastry (2009). Srivastava et al., (1990) reported an 11% decrease in DM digestion as level of SKC increased from 0, to 20, 30, and 40% of the concentrate mixture, and Soren and Sastry (2009) reported a 2% decrease in DM digestion and a 3% decrease in OM digestion between control and SKC further processed by lime treating and binder treating. Singh et al., (2006) reported no difference in DM and OM digestion between control, EKC, and SKC, but NDF and ADF digestion was reduced by 2-7% compared to deoiled groundnut cake control when EKC and SKC supplements were fed. Ravi et al., (2000) reported decreased DM, OM, NDF, and ADF digestion when EKC was fed. Values for digestion are similar to those in Project 2, and are within ranges (58.1 to 61.3 for OM digestion and 57.0 to 58.4 for NDF digestion) reported by Ravi et al., (2000), Singh et al., (2006), and Nagalakshmi et al.,

(2011), although forages were different in the cited studies. Mahli et al. (1989) reported the LD_{50} of karanjin in mice is 14.32 mg/kg, and of pongamol is 17.14 mg/kg. Intake of karanjin and pongamol in this study (2.03 and 1.08 mg/kg, respectively, Table 8) was less than the LD_{50} of mice reported by Mahli et al. (1989). About 80% of the oil is removed when making EKC, but less than 0.5% of the oil remains when SKC is produced. In this study, 2.34% residual oil remained in the production of SKC. Vinay and Sindhu Kanya, (2008) reported treating PSC with 2% HCl for one hour at room temperature was the best detoxification method, and resulted in karanjin content of 0.03% with efficient removal of oil. Nagalakshmi et al., (2011) reported karanjin content was estimated to be 0.325% when SKC was fed at 12% of the concentrate mixture. Karanjin content of raw SKC (0.18%) used for supplements in this study was intermediate to the values reported by Vinay and Sindhu Kanya, (2008) and Nagalakshmi et al., (2011).

In this study, ADG of steers on 20% EKC and 20% SKC were significantly lower than that of control steers, which is in agreement with Singh et al., (2006) who reports a 59 and 49% decrease in ADG in lambs fed EKC and SKC respectively. Values in this study, however, were higher than in the Singh et al., (2006) study because it was conducted with steers. In contrast, Ravi et al., (2000) reports no significant difference in ADG between lambs fed deoiled groundnut cake control, EKC at 24% of the concentrate mixture, and SKC at 20% of the concentrate mixture. When expressed as a percent of control, ADG of steers on 20% EKC in this study (65.0% of control) was intermediate to those reported by Ravi et al., (2000) and Singh et al., (2006) when fed 24% EKC (80.7%

and 41.3% of control, respectively). Average daily gain of steers on 20% SKC in this study (63.3%) is again intermediate to those reported by Ravi et al., (2000) and Singh et al., (2006) when fed 20% SKC (98.5% and 50.7% of control, respectively). Control steers (0% PSC) in this study were significantly more efficient having a higher G:F than PSC supplemented steers, and 20% EKC steers were significantly more efficient than 20% SKC steers. This disagrees with Ravi et al., (2000) who reports no difference in F:G between control, 24% EKC, and 20% SKC. Previously conducted Pongamia studies report feed conversion (F:G) as opposed to feed efficiency (G:F), but when converted to (G:F) the values in this study are within the range (0.05 to 0.10) of that reported by Soren et al., (2009) when lambs were fed SKC further processed by water washing, lime treating, or binder treating. Feed efficiency values (0.06 for EKC and 0.05 for SKC) in this study were lower than those reported by Ravi et al., (2000) who reported G:F of 0.09 for EKC and 0.11 for SKC supplements when lambs were fed 24% EKC and 20% SKC. Further research comparing performance of animals fed EKC and SKC should be conducted, as steers fed EKC performed better than expected in this study even though they consumed less supplement and more karanjin and pongamol.

Conclusions

Total OMI was stimulated by the control supplement when compared to 20% EKC and 20% SKC, but responses in intake by 20% EKC and 20% SKC are hard to determine without having a negative control treatment to compare to. When put on a metabolic body weight basis, 20% SKC steers were significantly less than 0% PSC steers, and those receiving 20% EKC were similar to both other treatments for total

DMI. Total tract digestion was not affected by protein supplementation. Average daily gain of steers on 20% EKC and 20% SKC were significantly lower than that of 0% PSC steers, but were similar to each other. Control steers in this study were significantly more efficient having a higher G:F than PSC supplemented steers, and 20% EKC steers were significantly more efficient than 20% SKC steers. Further research comparing performance of animals fed EKC and SKC should be conducted.

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