EFFECTS OF ANTI-NUTRITIONAL COMPOUNDS IN PONGAMIA SEEDCAKE ON INTAKE, DIGESTION, AND RUMINAL FERMENTATION IN BEEF CATTLE

A Thesis

by

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ABSTRACT

Effects of increasing levels of the anti-nutritional compounds karanjin and pongamol on intake, nutrient utilization and ruminal fermentation were evaluated with a growing diet (trial I) and a forage diet (trial II).

Steers had *ad libitum* access to growing diet and Bermuda grass hay during trial 1 and trial 2, respectively. At the same time diets were fed, steers received their allocated doses of karanjin and pongamol via ruminal cannula to determine acceptable inclusion level of pongamia seedcakes containing varying levels of karanjin and pongamol, which are contained in the residual oil of commercially available pongamia seedcakes. Twelve steers in both studies were assigned to completely randomized block design consisting of a control (no karanjin and no pongamol) and one of 3 levels of karanjin and pongamol.

Dosing increasing levels karanjin and pongamol linearly ($P < 0.01$) decreased intake in Trial 1. No significant effects ($P \geq 0.12$) were observed for total DMD, OMD, NDFD, ADFD and CPD. Ruminal total VFA concentration and pH were not affected by inclusion levels ($P = 0.19$ and $P = 0.51$, respectfully). The lowest dose of karanjin and pongamol resulted in a greater ($P < 0.01$) molar proportion of propionate than other treatments, resulting in a reduction in acetate:propionate ratio ($P = 0.02$) for that treatment. Among all VFA molar concentration measurements, only isobutyrate exhibited a treatment × hour effect ($P = 0.02$).

When a forage diet was fed (Trial 2) increasing levels of karanjin and pongamol dosing resulted in a quadratic decrease ($P < 0.05$) in intake of total DM, OM, NDF and

ADF with the. Crude protein intake did not differ ($P = 0.14$) among treatments. There was no effect ($P \geq 0.22$) among treatments on DMD, OMD, NDFD and ADFD. Total VFA concentration and ruminal pH were not affected by inclusion level ($P = 0.59$ and 0.72, respectively. Therefore, acetate:propionate ratio also had no treatment effect ($P = 0.84$).

Increasing levels of karanjin and pongamol decrease intake, although digestibility is not impacted. Ruminal fermentation of steers fed with complete ration is slightly altered with karanjin and pongamol intake, however, none alteration was observed when steers were fed only Bermuda grass hay.

DEDICATION

First of all, to God Who blessed me with the wonderful life I have.

To my father Luiz Carlos Briani and my mother Heloisa Dágola Papin Briani, who always gave me support and love. I'll always remember mom helping me with my homework while dad was working hard to provide us the best.

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All work for the thesis was completed by the student, in collaboration with students of the 017B Laboratory of Ruminant Nutrition of the Department of Animal Science.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Biofuel

Biodiesel is a non-toxic, biodegradable, and environmentally benign fuel which does not contain any sulphur or aromatic compounds. Additionally, its combustion produces lower emissions of carbon monoxides, hydrocarbons, and particulates than conventional diesel (Karmakar et al., 2010). Trans-esterification, a biochemical process, is used to produce biodiesel from biological sources such as vegetable oils or animal fat (Karmakar et al., 2010). This process consists of catalyzation by an acid or base and forms esters and glycerol by the reaction of a triglyceride (fat/oil) with a bio-alcohol.

Growing environmental concerns about greenhouse gas emissions and global warming, increasing world energy demand due to economic development and population growth, and declining fossil fuel reserves have spawned interest in renewable fuels as energy sources for the future (Atabani et al., 2013; Prasad, 2014).Biodiesel is a promising alternative resource for diesel engines. Vegetable oils and animal fats were investigated well before the energy crisis of the 1970s (Andrade et al., 2011). Dr. Rudolf Diesel, who invented the diesel engine, demonstrated that an engine could run on 100% peanut oil (a biodiesel) at the World Exhibition in Paris in 1900. Subsequently, he made assertions that use of vegetable oils for fuel would help the global development of agriculture, and could become as important as petroleum(Agarwal, 2007).

Kumar and Sharma (2011) estimate that biodiesel could represent as much as 20-22% of all on-road diesel used in Brazil, Europe, China, and India by the year 2020. Demand for biodiesel is increasing due to rapid economic growth in India and China, and growth of other developed countries (Kumar and Sharma, 2011). High demand for renewable sources of energy has caused exponential growth of the biofuel industry. In parallel, the volume of co-products generated from biofuel production has grown dramatically (Abbeddou and Makkar, 2012).

One of the biggest challenges for biodiesel production is identification of feedstocks that are economically feasible, with sufficient yield and oil content, and eco-friendly (Singh et al., 2014). Use of edible vegetable oils for biodiesel production has resulted in increased food prices in recent years and has also given rise to the debate, "food versus fuel" (Chauhan et al., 2013). Accordingly, avoiding competition between biofuel production and human food production is desirable. A way to reduce such competition is to promote the use of plant species able to grow on non-arable land and under harsh climatic conditions (Abbeddou and Makkar, 2012). There are 3 different classifications of potential feed stocks for biodiesel production. They are first generation feed stocks or edible vegetable oils, second generation feed stocks or non-edible vegetable oils, third generation feedstock or microalgae and others including waste cooking oils. Second generation feedstock or non-edible vegetable oils are more attractive than first generation because they minimize competition with food production (Atabani et al., 2013). Second generation, non-edible oils are not appropriate/desirable/safe for human consumption due to their toxicity or low palatability. An example of a potential 2nd generation biofuel feedstock is karanja (*Pongamia pinnata*; Chauhan et al., 2013, Kaushik et al., 2015). Figure 1 represents a process flowchart of non-edible crops seed to biodiesel.

Pongamia pinnata, a member of the Papilionoideae subfamily, is an arboreal legume that can reach 15 to 25 m high, is valued for its provision of shade, ornamental character, seed oil, fodder, and green manure (Kaushik et al., 2015). Common names are 'karanja' and 'pongamia'. Pongamia originated from India and has been naturalized in Pakistan, Sri Lanka, Australia, Fiji and Japan (Takase et al., 2015).The plant has been introduced and is well established in several countries with humid, tropical lowlands

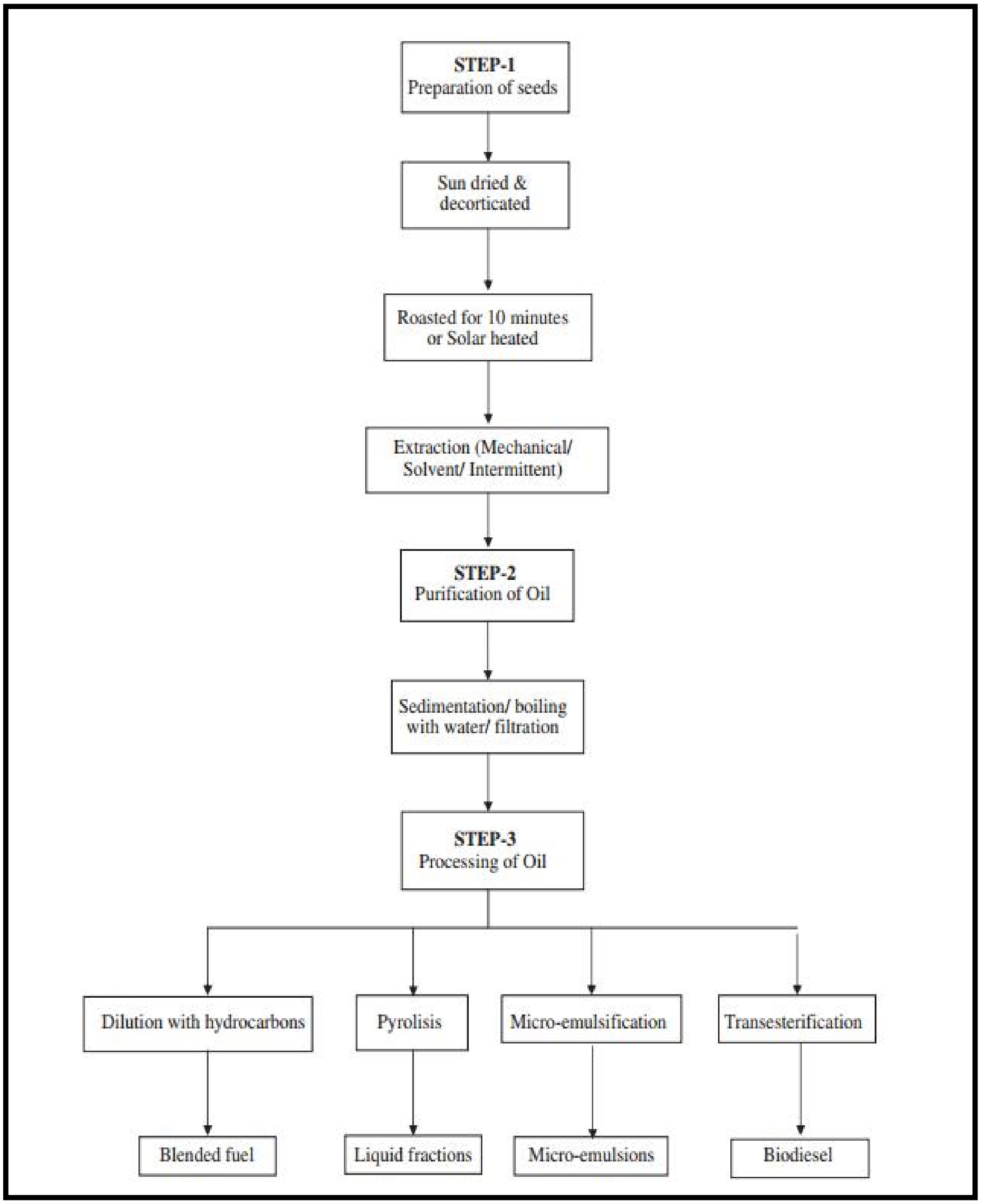

Figure 1. Process flowchart of non-edible crops seed to biodiesel. Reprinted from (Atabani et al., 2013).

such as parts of Australia, New Zealand and the USA (Scott et al., 2008).

Oil pressed from the seeds of the karanja tree has been recognized for various medicinal properties (Takase et al., 2015). During the 16^{th} century, fruits of Pongamia tree were utilized for parasitic infections, urinary diseases, diabetes and hemorrhage; expressed oil from seeds was used as a laxative, for intestinal parasites and for skin afflictions. The oil has alleged antiseptic, stimulant, and healing properties, and is applied in skin diseases, scabies, sores and herpes (Badole and Bodhankar, 2011). Recently, interest in this tree has focused on the use of oil from its seed for biodiesel, (Kaushik et al., 2015). Pongamia has the capacity to grow in a variety of soil types ranging from stony to sandy to clayey, and is tolerant of high salinity. It can be found along waterways and seashores, with its roots in fresh or salt water. This plant can persist in an annual rainfall range between 500 to 2500 mm, elevations from sea level to an altitude of 1200m, soil pH from 6 to 9 with the optimum level between 6.5 – 8.5. Furthermore, it nodulates and fixes atmospheric nitrogen in the soil (Sangwan et al., 2010). Nitrogen fixing results from a symbiotic relationship with nitrogen-fixing bacteria, an obvious advantage that legumes have over other plants, (Scott et al., 2008). In contrast to other conventional biofuel crops, Pongamia can be used to improve soil fertility thus improving soils for future agricultural purposes (Kumar and Sharma, 2011).

To be an effective and economical promising biodiesel plant, the tree should possess important traits such as the capacity to produce a large number of high oil content seeds, with acceptable oil quality (Kesari et al., 2010). Pongamia mature seeds contain about 5% shell and 95% oleaginous kernel and an average weight 1.2 g (Badole and Bodhankar, 2011). Each fruit contains 1 to 2 kidney shaped brownish red kernels (Karmee and Chadha, 2005). Karanja seeds yield 35% of oil by weight. Seed oil is thick, yellow or reddish brown (Biswas et al., 2011), it has a disagreeable odor, bitter taste and becomes darker during storage (Badole and Bodhankar, 2011). Pongamia trees produce seed yield ranging from 10 kg to more than 90 kg per tree (Anon, 1969; Dutta et al., 2012). Based on this yield expectations are between 900 – 9000 kg seed/ha, assuming 100 trees/ha, resulting in an oil yield ranging between 225 – 2250 kg oil/ha (Karmee and Chadha, 2005). In India, annual production of non-edible oil from *Pongamia pinnata* yielded 55,000 t (Jain and Sharma, 2010).

Vegetable oils are made of hundreds of chemicals, most of them a mixture of triglycerides containing the glycerol backbone modified with various chain length fatty acids (Biswas et al., 2011). Pongamia oil is predominantly composed of oleic acid (51.8%), linoleic acid (17.7%), palmitic acid (10.2%), stearic acid (7%) and linoleic acid (3.6%; Scott et al., 2008, Karmee and Chadha, 2005). Currently, per annum worldwide, only 6% is being utilized out of 200 million t of seed (Takase et al., 2015). Accordingly, Pongamia oil has a significant potential to be exploited as an oil source more intensively.

Pongamia as a source of feed

Global bio-diesel production is set to reach 24 billion L in 2017 (Kesari et al., 2010). Extraction of non-edible oil results in the production of by-products, some of which can be used as feed for animals. Ruminants are capable of turning products that are not suitable for humans into animal products of high biological value, due to the microbial fermentative process that takes place in the reticulo-rumen (Carrera et al., 2012).

Ruminant nutritionists are constantly seeking inexpensive, readily available, non-competitive (with human food) industrial co-products for feeding ruminants. Historically, protein-rich feeds are one of the costliest feed portions of animal diets. Protein requirements are commonly met by providing oilseed coproducts (e.g. soybean meal, cottonseed meal, and distillers grains) as protein supplements (Dutta et al., 2012). To reduce diet cost, conventional protein sources may be partially or completely replaced by unconventional sources of protein, since conventional protein sources cost more (Panda et al., 2008b).

Because pongamia seeds contain 33 – 36% oil (Katekhaye et al., 2012), approximately 65 % of the original seed weight is left as a residual meal known as pongamia seedcake. Pongamia seedcake has been used for several purposes like fertilizer, fungicide and insecticide. Pongamia seedcake has been evaluated as animal feed (Gupta et al., 1981; Konwar et al., 1984; Konwar et al., 1987; Konwar, 1987; Konwar and Banerjee, 1987; Dutta et al., 1993; Singh et al., 2006; Soren et al., 2009; Nagalakshmi et al., 2011; Rao and Kumar, 2015).

Pongamia meal contains 28 – 34% crude protein (Vinay and Kanya, 2008). Different processes of oil extraction result in different protein concentrations in the seedcake. Crude protein content of rotary-pressed pongamia seedcake ranges from 6 - 24%, while it varies from 22 to 29 % in expeller-pressed cake (**EKC**) and 30 to 34% in solvent-extracted cake (**SKC)**; (Dutta et al., 2012).

Expeller pressing process leaves 15-20% of residual oil in the cake (Vinay and Kanya, 2008).According to Prabhu et al. (2002), EKC, contained 28.7% CP, 12.1 ether extract, 4% of crude fiber, 94.7% OM and 53.9% carbohydrate, and after further solvent extraction, SKC contained 33.5% CP, 0.3% EE, 5% crude fiber, 94. 7% OM and 61.0% carbohydrate. Greater removal of oil (EE) (as with solvent extraction versus expeller extraction) results in a proportionate increase in CP. Table 1 contains nutritional composition of kernels and de-oiled pongamia seedcake.

Saturated and unsaturated fatty acid composition of pongamia oil is 20.5% and 79.4% respectively. Oleic acid (46%) is the primary monounsaturated fatty acid whereas linoleic acid (27.1%) and linolenic acid (6.3%) are the primary polyunsaturated fatty acids (Sangwan et al., 2010). Table 2 contains fatty acid compositions from pongamia seed oil.

Table 1. Nutritional composition of Kernels, EKC and SKC. Reprinted from (Natanam, 1989).

Constituent	Kernels	EKC	SKC
CP (%)	20.5	24.3	26.9
EE (%)	33.2	14.2	1.7
CF (%)	3.8	3.9	5.5
NFE (%)	39.7	52	60.2
Carbohydrate (%)	33.3	26.2	19
Total ash (%)	2.8	5.6	5.7
ME (MJ/kg)	20.2	13.4	8.3
Tannins (%)	1.65	3.16	3.41
Trypsin inhibitors (%) protein	5.3	8.7	8.2
Major minerals (%)			
Ca	0.51	0.76	0.87
P	0.38	0.48	0.55
Na	0.29	-	-
K	0.27	0.23	0.49
Mg	0.47	0.27	0.2
Trace minerals (ppm)			
Cu	1.37	1.96	1.97
Fe	3.82	14.32	17.81
Co	0.27	0.09	0.15
Mn	35.78	76.21	70.82
Pb	0.11	0.73	1.00

Table 2. Fatty acid composition from seed oil of *Pongamia pinnata*.

Fatty acids	Structure	Composition (%)
Palmitic acid	16:0	10.8
Stearic acid	18:0	8.7
Oleic acid	18:1	46
Linoleic acid	18:2	27.1
Arachidic acid	20:0	0.8
Linolenic acid	18:3	6.3
Behenic acid	22:0	3.2
Myristic acid	14:0	0.23
Capric acid	10:0	0.1
Lauric acid	12:0	0.1
Total saturated fat	-	20.5
Total monounsaturated fatty acid	-	46
Total polyunsaturated fatty acid	-	33.4

Adapted from (Sangwan et al., 2010). Fatty Acid Composition from Seed Oil of *Pongamia pinnata.*

Anti-nutritional factor

Despite its apparent nutrient composition, pongamia seedcake may contain toxic and unpalatable components such as the furanoflavones karanjin and pongamol, polyphenolic compounds, and protease inhibitors (Scott et al., 2008). Other anti-nutrients such as phytates, tannins and an unusual amino acid named glabrin are also present in the meal (Vinay and Kanya, 2008) and all of these limit its utility as an animal feed. Additional chemical treatments and extraction techniques have been utilized in an effort to reduce these compounds in seedcake with varying success.

Protease inhibitors refer to proteins and polypeptides that specifically inhibit proteolytic enzymes (Rackis et al., 1986) and were discovered by Read and Haas (1938) who showed that defatted soy flour inhibited trypsin's ability to liquefy gelatin.. Protease inhibitors reduce digestibility of plant food proteins (Rackis et al., 1986; Rattansi and Dikshit, 1997). Pongamia seedcake contained a trypsin inhibitor at up to 8.7% of total protein, and tannins up to 3.4% respectively (Natanam, 1989; Panda et al., 2006). Trypsin and chymotrypsin inhibitors are found in the oily portion of pongamia seedcake (Rattansi and Dikshit, 1997; Dutta et al., 2012). Therefore, the nutritive value and protein digestibility of pongamia seedcakes generally are increased by processing to eliminate the antinutritional components (Mandal et al., 1985). .

Raw soybean meal which contained trypsin inhibitors was fed to chicks which developed hypertrophic pancreases which contained abnormally high concentrations of trypsinogen (Chernick et al., 1948; Lyman and Lepkovsky, 1957). Excreted feces of rats fed raw soybean meal contained excessive amounts of proteolytic activity, proving that an excess of proteolytic enzymes was being secreted by the pancreas due to the presence of trypsin inhibitors in the diet. Therefore, growth depression caused by trypsin inhibitor might be due to the loss of essential amino acids produced by over secretion of pancreatic proteolytic enzymes which are rich in sulfur containing amino acids, fundamental for body tissue synthesis, but irretrievably lost by excretion (Lyman and Lepkovsky, 1957).

Flavonoids are phenolic substances widely distributed in plants that are involved in plant self-protection against drought, salinity and pathogens (Sharma et al., 2011), often limit nutritive value, and may contribute to a bitter taste. Some are related to the condensed tannins which may also have anti-nutritive characteristics. Many flavonoids occur as glycosides (Van Soest, 1994).. The furanoflavones present in pongamia seed cake include karanjin, pongamol, pongapin, pongaglabron, kanjone and isopongaflavone lanceolatin B (Limaye, 1925; Roy et al., 1977; Dutta et al., 2012). The group of furano-flavonoides constitutes 5-6% by weight of the oil (Bringi, 1987; Vinay and Kanya, 2008), in which the main constituent is karanjin (Katekhaye et al., 2012). Karanjin and pongamol have been shown to have anti-bacterial activity against both gram-positive and gram-negative organisms (Badole and Bodhankar, 2011).

Karanjin and pongamol are the major flavones of the seeds, and are associated with the non- glyceride portion of the oil (Badole and Bodhankar, 2011). These two substances are the most toxic among the flavonoids, because of that the bitter taste of the seed cake is attributed to the presence of these two toxic compounds (Dutta et al., 2012).

Karanjin is the principal furanoflavonoid in pongamia oil; it is a non-fatty component that has a molecular formula $C_{18}H_{12}O_4$ and a melting point of 158.5°C (Limaye, 1925; Prabhu et al., 2002). The oil contains 1.25% karanjin (Badole and Bodhankar, 2011). According to (Prabhu et al., 2002), raw, expeller-pressed seedcake (EKC) contains 0.19% of karanjin. Solvent extraction of residual pongamia oil reduces this to 0.01% karanjin in the seed cake, increasing the value of the seedcake by decreasing the amount of the anti-nutritional compound.

Pongamol is a crystalline compound from the oil of Pongamia seeds and is the second important toxic component which has a molecular formula $C_{18}H_{14}O_4$. Pongamol belongs to the flavone group of compounds and contain a hydroxyl, a methoxyl and an ethylene double bond (Rangaswami and Seshadri, 1942). This non-fatty component of the oil has a melting point of 128-29°C and constitutes about 0.85% of the karanj oil (Badole and Bodhankar, 2011). Karanjin and pongamol in whole seed are found in a ratio of approximately 1:0.6 respectively (Mandal et al., 1985; Kumar, 2011).

Glabone is a furanoflavone ($C_{18}H_{12}O_4$) which is also present in Pongamia seedcake and has a melting point 170-171°C (Das Kanungo et al., 1987). Glabrin (4,5 dihydroxy-N-methyl pipecolinic acid) is a nitrogenous substance that is a complex amino acid present in the seeds of *Pongamia glabra* which has the empirical formula $C_{21}H_{12}O_{12}N_3$ and melts at 290°C (Mandal et al., 1986).

As the furanoflavonoids oil soluble, extraction of oil reduces the concentration of these phenolic compounds in the seedcake; better oil extraction typically removes more antinutritional compounds, (Susarla et al., 2012). Secondary treatments of seedcake have been explored as methods to detoxify and extract the residual oil present in the pongamia seed cake after expeller pressing. Secondary oil extraction methods include solvent extraction, water washing and pressure cooking. Chemical detoxification methods include sodium hydroxide treatment, calcium hydroxide treatment, urea ammoniation and acid treatments (Prabhu et al., 2002). Solvent extraction yields a better quality of seed oil, with lower amounts of karanjin in the meal after treatment, than other secondary extraction methods (Prabhu et al., 2002; Badole and Bodhankar, 2011). Solvent extraction can be accomplished using numerous solvents (absolute methanol, aqueous methanol, absolute ethanol, aqueous ethanol, absolute acetone, aqueous acetone, deionized water) (n-Hexane, petroleum ether, ethyl acetate, isopropanol), mixtures of ethyl acetate (91.53%) + water (8.47%) and isopropanol (87.8%) + water (12.2%) (Sajid et al., 2012). Among all of them, n-Hexane solvent yielded best results, providing a seedcake with less amounts of toxic compounds.

Various methods of processing and/or secondary treatment result in a range of reported values of karanjin and pongamol in expeller seedcake (Table 3). Variance in these concentrations, and the linkage of their presence with negative performance outcomes, results in difficulty in predicting the effects of feeding a given lot of seedcake.

Effects of feeding pongamia seedcake

Karanjin and pongamol present in pongamia seedcake are responsible for lowering performance of livestock species (Table 4). Previous research reported that poultry fed pongamia seedcake had reduced feed intake (Mandal and Banerjee, 1974; Natanam et al., 1989a), decreased body weight gain (Mandal and Banerjee, 1974;

Natanam et al., 1989a; Panda et al., 2008b), poorer feed efficiency (Natanam et al., 1989b; Panda et al., 2008b), poorer feed conversion ratio and harmful effects on vital organs (Panda et al., 2008a), lower egg production and quality (Natanam et al., 1989c) and mortality (Mandal and Banerjee, 1974; Natanam et al., 1989b). In swine, feed intake and body weight gain was lowered when pongamia seedcake was fed (Samanta et al., 1986).

Ruminants are also negatively impacted due to the presence of karanjin and pongamol in pongamia seedcake. Previous research feeding pongamia seedcake to sheep reported lower feed intake (Ravi et al., 2000; Singh et al., 2006; Soren and Sastry, 2009; Soren et al., 2009; Soren et al., 2010; Nagalakshmi et al., 2011; Rao and Kumar, 2015), lower body weight gain (Ravi et al., 2000; Singh et al., 2006; Soren et al., 2008; Dineshkumar et al., 2013; Rao and Kumar, 2015), lighter carcass weight (Singh et al., 2006; Soren et al., 2008; Rao and Kumar, 2015), decreased meat quality resulting in lower acceptability after sensory panel, and reduced juiciness and tenderness (Soren et al., 2008), decreased efficiency of nutrient utilization (Nagalakshmi et al., 2011), decreased digestion (Ravi et al., 2000; Singh et al., 2006; Rao and Kumar, 2015), reduced N retention(Ravi et al., 2000; Singh et al., 2006; Soren and Sastry, 2009), reduced calcium retention (Ravi et al., 2000), reduced phosphorus retention (Soren and Sastry, 2009), compromised testicular function (Singh et al., 2006; Dineshkumar et al., 2013), depressed immune response (Nagalakshmi et al., 2011) and compromised bone development(Singh et al., 2006). Research conducted with cattle demonstrated a reduction in body weight gain (Konwar, 1987) and decreased feed intake (Konwar et al., 1987; Konwar and Banerjee, 1987).

Among existing studies, none have measured and reported the specific effects of consumption of karanjin and pongamol. Therefore, there is no method to predict the impact of karanjin and pongamol consumption on animal performance.

Conclusion

The anti-nutritional compounds present in pongamia seedcake limit its utilization by the beef cattle industry. The amounts of karanjin and pongamol on pongamia seedcake are highly variable, according to the literature (table 3). Understanding the relationship between exposure to these compounds and animal performance would allow any given lot of seedcake to be assessed and an appropriate recommendation for use made. Therefore, the aim of the present study is to evaluate the impact of karanjin and pongamol on intake, digestion and ruminal fermentation on beef cattle.

Table 3. Current knowledge of Karanjin amounts in SKC and EKC fed to livestock

Reference	Type of PSC	Karanjin content (mg/kg)
(Mandal et al., 1986)	EKC	3500
(Eipeson et al., 2010)	EKC	5300
(Susarla et al., 2012)	EKC	3600

Table 3. Continued. Current knowledge of Karanjin amounts in SKC and EKC fed to livestock

(Nagalakshmi et al., 2011)	EKC	3250
(Prabhu et al., 2002)	EKC	1900
(Panda et al., 2006)	EKC	3240
(Soren et al., 2007)	EKC	2850
(Panda et al., 2004)	EKC	3240
(Panda et al., 2004)	EKC	3250
(Vinay and Kanya, 2008)	SKC	300
(Eipeson et al., 2010)	SKC	500
(Soren and Sastry, 2009)	SKC	480
(Soren and Sastry, 2009)	SKC	740
(Soren and Sastry, 2009)	SKC	430
(Panda et al., 2006)	SKC	1320
(Prabhu, 2002)	SKC	100
(Rao and Kumar, 2015)	SKC	300
(Dineshkumar et al., 2013)	SKC	300
(Soren et al., 2010)	SKC	950

Table 3. Continued. Current knowledge of Karanjin amounts in SKC and EKC fed to livestock

(Soren et al., 2010)	SKC	980
(Soren et al., 2010)	SKC	1040
(Soren et al., 2009)	SKC	950
(Soren et al., 2009)	SKC	980
(Soren et al., 2009)	SKC	1040
(Soren et al., 2009)	SKC	1230
(Punj and Devendra, 1988)	SKC	100-150
(Soren, 2006)	SKC	100-1320

Table 4. Literature review of pongamia seedcake fed to livestock

Reference	Animal	Description/results
(Natanam et al., 1989a)	Poultry	Study measured growth and feed consumption of 4 broiler chicks. Results indicated that including 5% of raw pongamia seedcake in the diet significantly reduced growth from 444 g/d to 208 g/d and feed intake from 833 g/d to 419 g/d. Study demonstrated that pongamia kernels decreased performance by nearly 50% and should not be included in chicks diets even at the 5% level whether raw, oven dried, auto-claved or water-extracted.
(Natanam et al., 1989b)	Poultry	Study evaluated in a feeding trial the effect of pongamia oil at 1 and 2% inclusion level and incorporations of 10, 20 and 40% of EKC in broiler chicks' diet. Experiment consisted of six treatments groups including the control. Treatment with 40% level of EKC had 100% mortality. Body weight gain of treatments fed EKC at 10 and 20% levels ranged from 75 to 211g/d and differed significantly from control group, which resulted in 444g/d gain. Moreover, treatments 1 and 2% oil were significantly lower than control, and consisted of 217 and 69 g/d, respectively. Same authors made a second trial that attempted to evaluate effect of feeding autoclaved and water soaked EKC in chicks' performance. Levels of 10 and 20% inclusion of both processed cakes resulted on a lower weight gain compared to control group and the 20% water soaked

Table 4. Continued. Literature review of pongamia seedcake fed to livestock

		treatment resulted on 40% of mortality. Authors concluded that even at minimum levels of 1% of oil and 10% of raw EKC, pongamia oil should not be included in poultry diets.
(Natanam et al., 1989c)	Poultry	Experiment was conducted on a long-term feeding trial with 22 weeks length, using ninety White Leghorn pullets distributed between 5 treatments differing pongamia seedcakes processed in different oil extraction methods and included in diets at 10% level. Results demonstrated that body weight and maturity did not differ among treatments. Groups fed pongamia seedcake had significant lower egg production and poorer feed efficiency compared to control diet treatment. Birds fed pongamia seedcake produced significantly poorer quality eggs compared to the control group.
(Mandal and Banerjee, 1974)	Poultry	Study consisted of 3 experiments aimed to evaluate pongamia seedcake inclusion poulty ration. First Experiment attempted to substitute black till cake by pongamia seedcake. Trial utilized 120 one day old pure White Leghorn chicks of mixed sexes. Birds were fed from 0 – 28 days of age at 5 levels of substitutions, 0, 25, 50, 75 and 100%. Results demonstrated that pogamia seedcake should not be included even at 25% level due to significant lower body weight gain, feed consumption and mortality rate. Treatments 25, 50, 75 and 100% pongamia seedcake substitution consisted on mortalities rates of 54.1, 58.3, 58.3 and 71.8% respectively. The high

Table 4. Continued. Literature review of pongamia seedcake fed to livestock

		mortality was attributed to presence of toxic components in the pongamia seedcake. Second experiment utilized one hundred and eighty one day old pure White Leghorn chicks of mixed sexes. Pongamia seedcakes were subjected to eight different methods of processing and were included in the respective treatment rations. Average body gain of all treatments was significantly lower compared to control treatment. Third experiment utilized 40 one day old White Leghorn chicks of mixed sexes fed for 4 weeks. Trial consisted of 2 treatments, control and 25% pongamia seedcake inclusion in the diet. Average daily gain and feed efficiency ratio did not differ between treatments. Based on the results of all 3 studies, pongamia seedcake can be included up to 25% on protein equivalent basis in replacement of til cake. Authors reported that retarding factors lies on the oil portion of pongamia seedcake.
(Panda et al., 2007)	Poultry	Study evaluated effect of dietary incorporation of SKC replacing soybean meal at level of 12.5% nitrogen. Serum haemato-biochemical profile, humoral immune response and skeletal status of broiler chickens between 0 to 8 weeks old were evaluated. Results demonstrated that incorporation of SKC did not differ significantly serum concentration of hemoglobin, glucose, protein, albumin, globulin, uric acid, Ca and P. Bone ash, leg score and bone breaking strength

Table 4. Continued. Literature review of pongamia seedcake fed to livestock

		also had no significant difference.
(Panda et al., 2008a)	Poultry	Study utilized 520 broiler chicks one day old assigned into 13 treatments consisted of 40 birds each and pongamia seedcake inclusion in the diet was evaluated. Control diet consisted of soybean meal as the major protein source. Twelve isonitrogenous and isocaloric diets were formulated incorporating SKC and EKC differing on several extraction treatments. Soybean meal 50% replaced by pongamia seedcake. Results demonstrated significant poorer feed conversion ratio in all pongamia seedcake fed treatments. Authors concluded that daily consumption of 14.15 mg of karanjin and above results in harmful effects on vital organs of broiler chicks.
(Panda et al., 2008b)	Poultry	Research evaluated raw pongamia seedcake and SKC on growth performance and carcass characteristics of broiler chickens during 0 to 6 weeks of age. Control diet was based on soybean meal as the major protein source. Six isonitrogenous and isocaloric diets were formulated including 3 SKC differing on methods of extraction. Each different SKC replaced soybean meal at 12.5 and 25% level. Signicantly lower weight gain and poorer feed efficiency was observed in all pongamia fed treatments compared to control. At 25% level, breast yield lowered significantly.

Table 4. Continued. Literature review of pongamia seedcake fed to livestock

(Samanta et al., 1986)	Swine	Study consisted of 2 experiments. First trial evaluated different inclusion levels of pongamia seedcake on twenty four large white Yorkshire piglets. Control treatment consisted of a concentrate mixture of maize, groundnut cake, til cake and fish meal and was replaced by pongamia seedcake at 50, 75 and 100%. Results demonstrated that increasing pongamia seedcake inclusion in pig diets progressively and significantly decline feed intake and body weight gain. Second trial utilized forty White Yorkshire piglets (average 27.8 kg BW) randomly assigned into 4 treatments. During growth phase pigs were fed a growth diet up to reach 35kg of body weight and up to slaughter animals were fed a finishing diet. During growth phase, groundnut cake (13% of the diet) of control diet was replaced by pongamia seedcake at 25, 50 and 75% level on isonitrogenous basis. Results demonstrated that increasing pongamia seedcake in the diet significantly progressively declined body weight gains compared to control treatment.
(De et al., 1998)	Rat	Study evaluated liver's lipid profile and body growth of rats. Sixteen male albino rats were divided into two treatments and individually caged. Treatments were fed the same diet, apart from the oil used (20% of the diet). One treatment was fed pongamia oil and the other, groundnut oil. Growth and feed efficiency ratio were not significant different between

Table 4. Continued. Literature review of pongamia seedcake fed to livestock

		treatments. Serum and liver lipid parameters were not significantly different as well as fatty acid. Results demonstrated that treatments have nearly the same liver fatty acid composition in serum and liver tissue.
(Mahli et al., 1989)	Rat	Study evaluated pharmacological effects of karanjin and pongamol isolated and administered intraperitoneally in Albino mices. Karanjin was administered at 11.5, 13.5, 15.5 and 17.5 mg/kg BW to 4 different groups. Similarly, 4 different groups were administered 4 dose levels of pongamol at 15.0, 16.5, 18.0 and 20 mg/kg BW.. Additionally, pure oil was administered to 7 different groups at levels of 2.5, 3.5, 4.5, 5.5, 10, 15 and 20 ml/kg BW. Karanjin and pongamol had impacts on central nervous system, however, rats administered pure oil remained without behavioral changes. Responses indicated marked differences between karanjin and pongamol. Pongamol administration resulted on decreased sensitivity to sound and tough whereas karanjin increased. Muscle grip and tone decreased after pongamol administration but were normal after karanjin administration. Pongamol sedative effect was further confirmed by its hypnotic potentiating activity. Results demonstrated that pongamol had caused depression on the central nervous system and karanjin indicated central nervous stimulant activities.

Table 4. Continued literture review of pongamia seedcake fed to livestock

(Singh and Pandey, 1996)	Rat	Study evaluated anti-inflammatory activity of pongamia seed extracts in rats followed by individual chemical insults. Experiment consisted of 7 treatments, one as a control group. Treatments consisted of different solvent fractionated extracts. Rats were chemically induced right hind paw inflammation. Results demonstrated that polar constituents present in the seeds appear to have anti-inflammatory principles. All pongamia seeds extract fractions exhibited an anti-inflammatory effect.
(Vismaya et al., 2011)	Rat	Study evaluated karanjin anti-ulcerogenic properties in male albino rats. Animals were submitted to swim and ethanol stress-induced models. Ten and 20 mg/kg BW karanjin was administered. Serum, stomach and liver-tissue were analyzed for biochemical parameters. Results demonstrated that karanjin has the potential to inhibit ulcers. Animals treated with 10 and 20 mg/kg BW had ulcers inhibited by 50 and 74%, respectively.
(Srivastava et al., 1990)	Goat	Study evaluated utilization of pongamia seedcake on kid grower ration. Experiment consisted of 0, 20, 30 and 40% pongamia seedcake levels replacing de-oiled groundnut cake on an isonitrogenous basis. Dry matter intake, weight gain and nutrient digestion were observed. No significant difference on dry matter intake. Weight gain between 0, 20 and 30% treatments did not differ and ranged from 33 to 38 g/d. However 40% of inclusion resulted in a significant

Table 4. Continued. Literature review of pongamia seedcake fed to livestock

		lower gain, 22 g/d. Crude protein digestion, and production cost were significantly lower in 40% of inclusion group. Results demonstrated that, on a nitrogen basis, pongamia seedcake could replace groundnut cake up to 30% level on a kid grower diet.
(Chandra sekaran et al., 1989)	Sheep	The nutritive value of *Pongamia glabra* cake for sheep was assessed by digestion trials. Three digestion trials were conducted using 3 adult ewes to evaluate the nutritive value of pongamia seedcake. Study concluded that pongamia seedcake can be included in the concentrate mixture for sheep in a short-term feeding period.
(Dimri and Sharma, 2004)	Sheep	Sarcoptic mange is a disease that causes a negative impact in the sheep industry, leading to losses in wool and meat production. Study evaluated control of sarcoptic mange with several vegetable oils including Pongamia oil. Animal treated with Pongamia oil had a greater weight gain, enhanced liver function and increasing wool yield production. Results demonstrated that pongamia oil is an effective option for sarcoptic mange in sheep.
(Dineshk umar et al., 2013)	Sheep	Study evaluated replacement of soybean meal by pongamia seedcake on testicular architecture and semen production of rams. Animals were divided in three groups and fed different 0, 50 and 75% pongamia seedcake. Results demonstrated significant lower semen production and spermatozoa concentration of 75% treatment compared to 50% and control treatments.

Table 4. Continued. Literature review of pongamia seedcake fed to livestock

		Significant reduction of IGF-I expression was observed in 50 and 75% replacement groups compared with the control. There was a significant reduction of LHR expression of 75% treatment compared with the others. Animals fed 75% pongamia seedcake had significant lower body weight. Results indicated that pongamia seedcake could be included up to 50% of soybean replacement without affecting body weight. However, feeding pongamia seedcake for a long term may affect testicular function in sheep.
(Krishna moorthy et al., 2014)	Sheep	Study evaluated feeding pongamia seedcake on immunity and pathology of growing lambs. Twenty-four male lambs aging between 4-6 months were divided in 4 groups. Treatments consisted of pongamia seedcake replacing soybean meal at 0, 25, 50 and 75% levels for 140 days. Impact on humoral and cell immunity was assessed by antibody titer against *Peste des petites ruminants* (PPR). A significant decrease in humoral immunity was observed in 75% treatment. On histopathology, lambs in the 75% treatment had severe changes in intestine, testis and mesenteric lymph node compared to other treatments. Results demonstrated that pongmia seedcake could be safely fed up to 50% of protein replacement levels in lambs growing ration, with slightly adverse effect in immunity and pathology.

Table 4. Continued. Literature review of pongamia seedcake fed to livestock

(Nagalakshmi et al., 2011)	Sheep	Study evaluated impact of groundnut cake, sunflower seedcake and pongamia seedcake on performance, nutrient utilization, immune response and carcass characteristics in growing lambs. Experiment consisted of 3 treatments differing on protein ingredients. Three isonitrogenous and isocaloric complete diets consisted of 13% groundnut cake, 14.5% sunflower and 12% pongamia seedcake as protein ingredients. Dry matter and nutrient intake of animals fed pongamia seedcake were significant lower compared with other treatments. Dry matter, OM, EE, and NDF digestion of pongamia seedcake treatment were significantly greater due to lower intake. Significant lower growth rate was observed on pongamia fed treatment. Phosphorous balance was comparable between the treatments, however calcium and nitrogen balance was lower in pongamia seedcake treatment. Both humoral and cell-mediated immune responses were significantly depressed in lambs fed pongamia seedcake. Carcass characteristics did not differ between treatments.
(Rao and Kumar, 2015)	Sheep	Study evaluated substitution effect of soybean meal by pongamia seedcake on nutrient digestion, growth and carcass characteristics of sheep. Twenty-four male lambs were divided into four groups consisting of 6 animals each and fed different 0, 25, 50 and 75% levels of pongamia seedcake. Results demonstrated that as the level of pongamia seedcake increased in the diet, live

Table 4. Continued. Literture review of pongamia seedcake fed to livestock

		weight, hot carcass weight, DM intake, N-retention, OM, CP, ADF digestion decreased. Results demonstrated pongamia seedcake can be safely replace up to 25% of soybean meal content in the diet.
(Ravi et al., 2000)	Sheep	Study evaluated growth and nutrient utilization of lambs fed SKC and EKC. Animals were equally divided into three treatments which consisted of control diet, SKC and EKC. Control was fed a concentrate mixture contained 30% de-oiled groundnut cake, SKC and EKC treatments replaced 50% of de-oiled groundnut cake, consisting of 20 and 24% of the total concentrate mixture, respectively. Oat hay was fed *ad libitum* as a roughage source to lambs. Dry matter digestion, OM, CP, NDF, ADF were significantly lower in EKC group compared. Balance of nitrogen and calcium of EKC treatment were significantly lower compared to other treatments. Crude protein and TDN intake of EKC fed animals was significantly lower. Animals fed EKC had a significant lower average daily gain. Authors concluded SKC can be fed up to 20% of the diet to lambs without affecting the performance. However, 24% EKC inclusion should not be recommended to growing lambs due to intake nutrient digestion decrease.
(Ravi et al., 2001)	Sheep	Study evaluated feeding SKC and EKC on rumen and blood parameters of growing lambs. Animals were equally divided into three treatments. Control treatment consisted of a concentrate

Table 4. Continued. Literature review of pongamia seedcake fed to livestock

		mixture containing de-oiled groundnut cake, SKC and EKC treatments replaced groundnut cake at 50% level, consisting of 20 and 24% of the total concentrate mixture, respectively. Oat hay was fed *ad libitum* as a roughage source. Results demonstrated that feeding either expeller pressed and solvent extracted karanj cake had no adverse effects on concentration of total volatile fatty acids in the rumen, total nitrogen and TCA-ppt-N. Ruminal pH was significantly greater in EKC. Significantly lower ammonia nitrogen concentration was observed in both groups fed pongamia seedcake, indicating slow rate of deamination. The blood glucose concentration was comparable between all treatments. However, blood urea nitrogen level was significantly higher in EKC treatment. Results demonstrated that 20% SKC in the concentrate mixture did not affect rumen and blood parameters. However, EKC significantly increase blood urea-N and ruminal pH.
(Singh et al., 2006)	Sheep	Study evaluated impact of long term feeding SKC and EKC on lamb performance. Animals were equally divided into three treatments. Control treatment consisted of a concentrate mixture containing de-oiled groundnut cake, SKC and EKC treatments replaced groundnut cake at 50% level, consisting of 20 and 24% of the total concentrate mixture, respectively. Wheat straw was fed *ad libitum* as a roughage source. Dry matter, OM and EE digestion was comparable between

Table 4. Continued. Literature review of pongamia seedcake fed to livestock

		all treatments. However SKC and EKC treatments had a significant lower ADF and total carbohydrates digestion. Nitrogen balance, CP, TDN intake and body weight gain was significantly lower in SKC and EKC groups. Ruminal VFA concentrations were significantly lower on SKC and EKC treatments. Animals fed EKC had a significant lower fasted live weight and lower hot carcass weight. Results demonstrated that animals fed SKC and EKC had significantly thinner and less dense cortices, widened medullary cavity and lower spermatogenesis activity. Authors concluded that long term feeding SKC and EKC causes deleterious effects on lamb performance.
(Soren et al., 2008)	Sheep	Study evaluated carcass characteristics and meat sensory attributes of fattening lambs fed different processed SKC on isonitrogenous diets Treatments consisted of a soybean meal based diet control, SKC water washed, SKC 2.5% lime treated and 0.4% binder treated replacing 50% soybean meal of control diet. Animals had *ad libitum* chaffed oats straw. Results demonstrated that pre-slaughter weight and empty body weight of treatments SKC lime treated and binder treated were significantly lower compared to control and SKC water washed. Carcass length and carcass weight were significantly lower in SKC binder treated treatment. Liver yield was significantly greater in lambs fed SKC lime treated. Poor juiciness score, tenderness,

Table 4. Continued. Literature review of pongamia seedcake fed to livestock

		acceptability and organoleptic traits were significantly lower on SKC binder treated fed lambs. Authors concluded SKC water washed treated could be incorporated to replace up to 50% of soybean meal protein in concentrate mixture for sheep without negatively effects on carcass characteristics and sensory attributes.
(Soren and Sastry, 2009)	Sheep	Study evaluated replacement of soybean meal with different processed SKC on microbial protein synthesis, balances of karanjin and nutrients in growing lambs. Treatments consisted of a soybean meal based diet control, SKC water washed, SKC 2.5% lime treated and 0.4% binder treated replacing 50% soybean meal of control diet. Animals had *ad libitum* chaffed oats straw. Intake of all nutrients on SKC based diets was lower compared to control group, however, digestibility was comparable between all treatments. Nitrogen retention was significantly lower in SKC binder treated group. Lamb fed SKC water washed retained significantly more Ca compared to control, and P retention was significantly lower in all SKC treatments. Between SKC treatments, karanjin intake ranged from 3.6 to 4.4 mg/kg BW. Binder treatment retained significantly lower karanjin concentration compared to water washed and lime treatments. Microbial CP synthesis was similar between treatments. Authors concluded that feeding lambs different processed SKC influenced N, Ca, P and karanjin balances. However, it had no effect

Table 4. Continued. Literature review of pongamia seedcake fed to livestock

		on the microbial protein synthesis.
(Soren et al., 2009)	Sheep	Study evaluated replacement of soybean meal with different processed SKC on intake, nutrient digestion and wool yield in growing lambs. Treatments consisted of a soybean meal based diet control, SKC water washed, SKC 2.5% lime treated and 0.4% binder treated replacing 50% soybean meal of control diet. Animals had *ad libitum* chaffed oats straw. Treatments containing SKC had significantly lower dry matter intake. Nutrient digestion was comparable between all treatments, except for total carbohydrate, which was significantly lower in SKC lime SKC binder treatments. Feed: gain ratio, average daily gain and total gain did not differ between control and SKC water washed diets but was significantly lower in SKC lime and SKC binder treatments. Treatment SKC binder had significant lower wool yield. Crude protein, total digestible nutrients and dry matter conversion efficiency was comparable between control and SKC water washed diet but lambs on SKC lime and SKC binder diets had lower conversion efficiency. Authors concluded that soybean meal could be replaced by SKC water washed processed by up to 50% of nitrogen level of inclusion without affecting growth performance of lambs.

Table 4. Continued. Literature review of pongamia seedcake fed to livestock

(Soren et al., 2010)	Sheep	Study evaluated replacement of soybean meal with different processed SKC on ruminal fermentation, immune response and blood biochemical profile. Treatments consisted of a soybean meal based diet control, SKC water washed, SKC 2.5% lime treated and 0.4% binder treated replacing 50% soybean meal of control diet. Animals had *ad libitum* chaffed oats straw. Immune response was assessed through cell-mediated immune (CMI) response and humoral immune response (HI). Lambs were sensitized Brucella abortus antigen and antibody (Ab) titre was measured at 0 and 21d. CMI response was measured through skin thickness (mm) after intra dermal inoculation of phytohaemagglutin-P. Results showed that Ab titre was lower in SKC binder treated group after 21d. CMI response was comparable between all treatments. Blood glucose, urea nitrogen, albumin, globulin and haemoglobin concentration was similar between all treatments. However, total protein was significantly lower in SKC lime treated group and cholesterol was significantly higher in SKC binder treated group compared to other treatments. Ruminal pH, total nitrogen and ammonia nitrogen did not differ between treatments; however, total VFA and TCA-ppt nitrogen were significantly lower in SKC binder treated treatment. Body weight was comparable between control and SKC water washed groups; however, lower in SKC lime and SKC binder treated treatments. Control group had significantly higher DM, CP

Table 4. Continued. Literature review of pongamia seedcake fed to livestock

		and TDN intake compared to SKC fed treatments. Authors concluded that feeding SKC lime treated and binder treated through concentrate mixture to lambs affected growth and rumen fermentation, however, no such adverse effect was detected when SKC water washed was fed to lambs in the concentrate.
(Dutta et al., 1993)	Bovine	Study evaluated effect of feeding pongamia seedcake on milk yield of lactating cows. Twelve cows were divided into two treatments consisted of 6 individuals. Treatments consisted of control concentrate mixture containing 40% of mustard cake and 6 kg of non-legume green fodder. Animals were fed *ad libitum* chaffed paddy straw. Pongamia seedcake treatment had pongamia seedcake replacing 25% mustard cake protein, resulting in 10% inclusion of concentrate mixture. Experimental feeding length consisted of 150 days. Dry matter intake did not significantly differ between treatments as well as efficiency of feed utilization. Average daily milk production and milk composition was comparable between treatments. Milk fat, solid not fat, protein, lactose, total solid, total ash, calcium and phosphorous content were comparable between treatments. Authors concluded pongamia seedcake can be safely included in concentrate mixture of dairy cows up to 10 per cent.

Table 4. Continued. Literature review of pongamia seedcake fed to livestock

(Gupta et al., 1981)	Bovine	Study evaluated pongamia seedcake as livestock feed. Calves were randomly assigned into five groups consisted of 3 calves over a feeding period consisted of 365 days. Control treatment was fed basal ration containing mustard cake. Treatments consisted of mustard cake replaced by SKC at 40, 40 autoclaved SKC, 60 and 80% levels. All animals were offered between two and three kg of green fodder and fed *ad libitum* paddy straw. Dry matter consumption was comparable between treatments. Control, 40 and 60% SKC had no significant difference in body weight gain. However, 40% SKC autoclaved and 80% treatments had significantly lower body weight gain compared to other treatments. Authors concluded that SKC could replace mustard cake up to 60% level without affecting profitability.
(Konwar et al., 1984)	Bovine	Study consisted of two research experiments to evaluate the nutritive value of pongamia seedcake on nutrient digestion, nitrogen and mineral balances in adult cattle. Digestion and nutrient balance studies were conducted on 4 adult young bulls. First experiment was fed groundnut cake as a single concentrate to find the digestible crude protein (DCP) and total digestible nutrients (TDN) contents of pongamia seedcake as a prerequisite for second trial, when TDN and DCP in pongamia seedcake were calculated after feeding ration composed of groundnut cake and pongamia seedcake at ratio 70:30, respectively. Bulls were fed paddy straw

Table 4. Continued. Literature review of pongamia seedcake fed to livestock

		as basal roughage and both TDN and DCP of the ingredient were known. Therefore, TDN and DCP of pongamia seedcake were determined by elimination method. Digestible CP and TDN content of pongamia seedcake were found to be 26.1 and 42.5%, respectively, on dry matter basis. Results demonstrated crude protein digestion the second trial was slightly lower (4%) compared to the first and might be due to some anti-nutritional factor(s) present in pongamia seedcake such as protein binders. No significant difference of phosphorous retention in both trials was observed; however bulls fed pongamia seedcake had slightly lower nitrogen retention and higher calcium balance. Authors concluded there is a possibility of including pongamia seedcake in young bulls ration.
(Konwar et al., 1987)	Bovine	Study evaluated consumption of pongamia seedcake by cows. Twelve cross-bred cows were randomly divided into four groups in a randomized block design. Previous milk yield production was taken into consideration for grouping the animals into treatments. Control treatment was fed with de-oiled groundnut cake at 25% level of the ration. Other treatments consisted of replacing 50, 75 and 100% de-oiled groundnut cake nitrogen by pongamia seedcake. Concentrations of pongamia seedcake in diets were 0, 16.58, 24.87 and 33.16%, respectively. All treatments had their concentrate mixture fed along with 10kg of green para grass and *ad libitum* paddy straw.

Table 4. Continued. Literature review of pongamia seedcake fed to livestock

		Average DM consumption had a progressive reduction with increasing pongamia seedcake in diets from 9.35 to 7.19 kg/d. Control and 25% treatment did not significantly differ; however, 50 and 75% treatments significantly decreased DM intake compared with other treatments. Authors concluded that lower dry matter intake of 50 and 75% treatments could be attributed to the poor palatability of pongamia seedcake which is invariably associated with depression on feed consumption.
(Konwar, 1987)	Bovine	Study was conducted to evaluate replacement of de-oiled groundnut cake by pongamia seedcake on growing calves ration. Fifteen growing male calves of 6 months age and average live weight 74 kg were divided into three treatments. Control treatment was fed conventional concentrate mixture containing 25% de-oiled groundnut cake. Other treatments received concentrate mixture with 50 and 75% replacement of de-oiled groundnut cake by pongamia seedcake. Total amount of pongamia seedcake in the supplement of the control treatment, 50 and 75% was 16.6 and 24.9%, respectively. All 3 diets were isonitrogenous, and calves were fed ad libitum paddy straw as roughage source. Body weight gain of control group and 50% treatment did not differ; however, 75% pongamia seedcake inclusion had significantly lower. Dry matter consumption was comparable between all treatments. Authors concluded that pongamia seedcake could be

Table 4. Continued. Literature review of pongamia seedcake fed to livestock

		safely incorporated up to 16.6% (replacing 50% of de-oiled groundnut cake) in the ration of growing calves.
(Konwar and Banerjee, 1987)	Bovine	Study consisted of 2 research projects to evaluate palatability of pongamia seedcake in cattle. First experiment trial consisted of twenty four young bulls. Animals were randomly assigned into 6 treatments differing on rations. Control ration contained 100% de-oiled groundnut cake, and the other treatments had different levels of pongamia seedcake replacing de-oiled groundnut cake. The 5 pongamia seedcake treatments were consisted of 60:40%, 50:50%, 40:60%, 20:80% and 0:100% de-oiled groundnut:pongamia seedcake ratio. Bulls were fed *ad libitum* paddy straw. Results demonstrated that increasing levels of pongamia seedcake significantly decreased DM intake, therefore, authors concluded pongamia seedcake is not palatable and should be mixed with other more palatable ingredients to avoid depression on feed intake. Second trial was conducted with 20 bulls randomly assigned into four isonitrogenous treatment diets. Treatments differed on replacing levels of de-oiled groundnut cake by pongamia seedcake. Experiment was conducted for 6 weeks and bulls had *ad libitum* paddy straw as roughage. Treatments were consisted of 0, 50, 75 and 100% pongamia seedcake which represented 0, 13.3, 19.9 and 26.5% of the concentrate mixture. Results demonstrated that pongamia seedcake did

Table 4. Continued. Literature review of pongamia seedcake fed to livestock

		not affect bulls' body weight gain replacing up to 100% de-oiled groundnut cake (26.5% of pongamia seedcake of diet dry matter).
(Paul et al., 1994)	Bovine	Study evaluated effect of feeding unconventional cakes and poultry manure mixture on voluntary intake of feed and nutrient utilization in growing cross-bred calves. Eighteen male calves were divided into three groups in a randomized block design experiment. Treatments consisted of replacing the concentrate mixture of control treatment by unconventional feed ingredients mixture at 15% and 30% levels. Control concentrate mixture ingredients proportion was 30% groundnut cake, 30% wheat bran, 37% maize, 2% mineral mixture and 1% common salt. Unconventional feed ingredients mixture was composed of 30% Kosum cake (*Schleichera oleosa*), 15% Karanj cake (*Pongamia glabra*), 20% Niger cake (*Guizotia abyssinica*), 15% Salseed meal (*Shorea robusta*) and 20% of dried poultry manure. Calves were fed 2kg/head concentrate mixture along with 2kg/head para grass and *ad libitum* wheat straw. Concentrates mixture of treatments did not differ on CP, CF, NFE and ash concentration. Both levels of unconventional feed ingredients mixture incorporation did not significantly affect growth as well as DM, OM, EE, CF, NFE and total carbohydrates digestion; however CP digestion of 15% treatment was significantly lower compared to the other treatments. The DCP, TDN, DE and

Table 4. Continued. Literature review of pongamia seedcake fed to livestock

		ME did not differ between treatments. Results demonstrated unconventional feed ingredients mixture up to 30%replacement of conventional concentrate mixture did not affect nutrient digestion.
(Paul et al., 1995)	Buffalo	Study using three crossbred cannulated adult male buffaloes evaluated *in sacco* dry matter and protein digestibility of pongamia seedcake. Buffaloes were fed concentrate mixture, green fodder and *ad libitum* wheat straw. Nylon bags were taken out after rumen fermentation for 3, 6, 12 and 20 hours. Results demonstrated that DM and protein ruminal degradability of pongamia seedcake was 39.11 and 31.11%, respectively.
(Brijesh et al., 2006)	Microbes	Study evaluated crude decoction of dried leaves of pongamia for antimicrobial properties and effect on production and action of enterotoxins responsible for infectious diarrhea. Results demonstrated that decoction of pongamia reduced production of cholera toxin resulting in selective antidiarrheal action with efficacy against cholera and enteroinvasive bacterial strains that causes bloody diarrhea.
(Sharma et al., 2011)	Microbes	Study demonstrated that flavonoids present in pongamia have significant activity against bacterial and fungal strains. Results reported that flavonoids produced by pongamia plants have active properties against microorganisms, which is becoming a research subject for possessing

Table 4. Continued. Literature review of pongamia seedcake fed to livestock

		antibacterial, antifungal and antiviral activity.
(Sajid et al., 2012)	Microbes	Results demonstrated that *Pongamia pinnata* have strong isolation and antioxidant properties and microbial agents for pharmaceutical and food uses.

CHAPTER II

EFFECTS OF ANTI-NUTRITIONAL COMPOUNDS PRESENT IN PONGAMIA SEEDCAKE ON INTAKE, DIGESTION, AND RUMINAL FERMENTATION OF GROWING DIET FED BEEF CATTLE

Synopsis

Pongamia seedcake, a coproduct of biodiesel production, is currently being evaluated as a potential source of livestock feed. However, it contains karanjin and pongamol (furanoflavonoids) that negatively affect utilization of pongamia seedcake as a feedstuff. Variable concentrations of both are found in pongamia seedcake; therefore, quantification of their effects on intake, digestion, and ruminal fermentation in beef cattle fed a growing diet is needed to establish thresholds for inclusion of seedcake in cattle diets.

Twelve ruminally cannulated Angus × Hereford steers (483 ± 14.5 kg of BW) were used in a completely randomized block design to determine the effects of 4 levels of karanjin (0, 3.0, 4.5, and 6.0 mg·kg $BW^{-1}\cdot d^{-1}$) and pongamol (0, 1.8, 2.7, and 3.6 mg·kg $BW^{-1}\cdot d^{-1}$) on intake, digestion, and ruminal fermentation. Steers had *ad libitum* access to a 0.94 Mcal/kg NEg diet. Steers were adapted for 8 d, intake and digestion was determined over 5 d, and ruminal fermentation was quantified on 1 d.

Intake decreased linearly ($P < 0.01$) from 10.23 to 5.60 kg of DM/d, respectively, as karanjin and pongamol dose increased. However, no effect of dose ($P \geq 0.12$) on any measure of digestion was observed. Total VFA concentration and ruminal pH were not

affected (P = 0.19 and 0.51, respectively) by treatment. Molar proportion of propionate was greater ($P \leq 0.02$) in steers receiving karanjin and pongamol at 3.0 and 1.8 mg·kg $BW^{-1} \cdot d^{-1}$, respectively, than in other treatments, resulting in a lower acetate:propionate ratio (P = 0.02).

Increasing levels of karanjin and pongamol decreased intake, although digestion was not impacted. Ruminal fermentation profile of steers fed with a complete ration was slightly, but not materially altered.

Introduction

A recent interest has emerged in Pongamia (*Pongamia pinnata*) oilseeds as a feedstock for biodiesel, (Kaushik et al., 2015). Pongamia seeds contain 33 – 36% oil (Katekhaye et al., 2012). Following oil extraction of the pongamia seed, approximately two thirds by weight of the original seed is left as pongamia seedcake. Pongamia seedcake has been used for several purposes including fertilizer, fungicide, and insecticide. Utilization of this protein seedcake as an animal feed has been conducted in cattle (Gupta et al., 1981; Konwar et al., 1984; Konwar et al., 1987; Konwar, 1987; Konwar and Banerjee, 1987; Dutta et al., 1993), goats (Srivastava et al., 1990), buffaloes (Paul et al., 1995) and sheep (Chandrasekaran et al., 1989; Ravi et al., 2000; Ravi et al., 2001; Dimri and Sharma, 2004; Singh et al., 2006; Soren, 2006; Soren et al., 2008; Soren and Sastry, 2009; Soren et al., 2009; Soren et al., 2010; Nagalakshmi et al., 2011; Krishnamoorthy et al., 2014; Rao and Kumar, 2015). Pongamia seedcake contains anti-nutritional components in the oil remaining after extraction (i.e. karanjin and pongamol), which decreases performance when fed to ruminants (Konwar et al., 1987; Konwar, 1987; Konwar and Banerjee, 1987; Srivastava et al., 1990; Ravi et al., 2000; Singh et al., 2006; Soren et al., 2008; Soren and Sastry, 2009; Soren et al., 2009; Soren et al., 2010; Nagalakshmi et al., 2011; Dineshkumar et al., 2013; Rao and Kumar, 2015). Therefore, the present research aims to further investigate the use of pongamia seedcake as a protein ingredient in beef cattle diets by dosing increasing levels of the anti-nutrients karanjin and pongamol. Results obtained in this study will enable the development of a response surface to pongamol and karanjin inclusion in a supplement. Ultimately, this data can be used to determine the inclusion level of pongamia seedcakes containing varying levels of anti-nutrients in the residual oil present in commercially available supplements.

Material & methods

The experimental protocol was approved by the Institutional Animal Care and Use Committee at Texas A&M University.

Twelve ruminally cannulated Angus steers (483 ± 14.5 kg of BW) were used in a complete randomized block design experiment with 4 treatments and 3 blocks to evaluate the effect of different levels of karanjin and pongamol intake on intake, digestion and ruminal fermentation in cattle consuming a growing diet (Table 5). Steers were blocked in 3 groups according to the relative level of voluntary intake of the growing diet measured prior to the beginning of the trial. Steers were housed in individual stalls in an enclosed barn and given *ad libitum* access to growing diet and water.

Table 5. Diet formulation and chemical composition of the total mixed ration (TMR) fed to steers receiving increasing amounts of karanjin and pongamol.

Ingredient	% DM basis
Bermuda grass hay	43.00
Alfalfa hay	14.80
Corn	22.80
Dried distillers'	8.40
Soybean meal	4.20
Molasses	6.30
Limestone	0.50
Total	100.00
	TMR[1]
Dry matter, %	89.40
Organic matter	92.75
NDF	47.51
ADF	25.59
CP	13.30
ME[2]	2.56
NEm[2]	1.55
NEg[2]	0.94

[1]TMR: Total mixed ration.
[2] Values were calculated based on the ingredient values from the NRC (2016).

Treatments consisted of a control (no karanjin and pongamol; **CON**) and three levels of purified karanjin and pongamol (INDOFINE Chemical Company, Inc., Hillsborough, NJ). A ratio of 1.0:0.6 karanjin to pongamol (Mandal et al., 1985; Kumar, 2011) and the level of dosing were determined from previous research in our laboratory and other published sources (Soren and Sastry, 2009; Soren et al., 2009; Rao and Kumar, 2015). Karanjin and pongamol were provided daily, such that steers received 3.0, 4.5, and 6.0 mg·kg BW^{-1} $\cdot d^{-1}$ of karanjin and 1.8, 2.7 and 3.6 mg·kg BW^{-1} $\cdot d^{-1}$ of pongamol, respectively.

Karanjin and pongamol powder were weighed into gel capsules individually for each steer according to individual weight and treatment assignment (mg/kg BW). Gel capsules were dosed directly via ruminal cannula daily, immediately prior to feeding, for the duration of the project.

After establishment of baseline diet intake, treatment application was initiated, and 8 d were allowed to expose steers to treatment, 5 d to determine intake and digestion, and 1 d to quantify ruminal fermentation.

Calculations of intake and digestion were made from observations on d 9 through 13. Feed refusals were collected and weighed prior to feeding 0600 h and a 200g sample was retained for analysis. Diet samples were composited across days on an equal weight basis. Orts samples were composited within steer across days. Diet samples were collected d 9 through 12 to correspond with fecal samples collected d 10 through d 13.

Acid detergent insoluble ash (ADIA) was used as an internal marker to estimate fecal production. On d 10 through d 13 fecal samples were collected every 8 h, with sample timing 2 h prior the day before so that 12 samples were obtained over a 4 d collection period. Fecal samples collected were composited and frozen at -20°C until analysis. Prior to analysis, each sample was allowed to thaw, was thoroughly mixed, and a representative subsample was collected for analysis.

On d 14 of each period, ruminal fermentation parameters were measured. A suction strainer (19 mm diameter, 1.5 mm mesh; Raun and Burroughs, 1962) was used to collect rumen fluid samples prior to feeding (0 h), and at 2, 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^{o}C for later determinations of VFA analysis. Prior to freezing, 8 ml of rumen fluid were combined with 2 ml of 25% *m*-phosphoric acid for VFA analysis.

Diet, ort and fecal 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 ration samples were composited on an equal weight basis across days. Ort and fecal samples were composited by steer across days within period. Diet, ort and fecal samples were ground with a Wiley mill to pass a 1-mm screen and analyzed for DM, OM, NDF, ADF and CP. Organic matter was determined as the loss in dry weight upon combustion for 8 h at 450^{o} F. 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. Hay, ration, ort and fecal samples were sent to a commercial laboratory (SDK Labs, Hutchinson, KS) for CP analysis. Fecal production was calculated by difference of ADIA concentration from the amount present on feed by the amount present on feces. ADIA quantity was obtained upon combustion of ADF samples of hay, ration, orts and feces for 8 h at 450°F. Digestion was calculated by the following formula: [1-(output of nutrient/intake of nutrient)]×100.

Intake, digestion and ruminal fermentation parameters were analyzed using the MIXED procedure of SAS 9.3 (SAS Inst. Inc., Cary, NC) for completely randomized block design with animal as the experimental unit. Steers were blocked by intake measured prior the treatment application. Orthogonal polynomial contrasts were used to determine linear or quadratic responses.. Ruminal fermentation parameters were analyzed using repeated measures techniques, with hour as the repeated variable and steer as the subject.

Results

All intake measurements decreased linearly (Table 6; $P < 0.01$) in response to increasing dosing of karanjin and pongamol. Total DM intake decreased from 10.23 kg/d for control (0 mg·kg $BW^{-1} \cdot d^{-1}$) to 7.80 kg/d for 9.6 mg·kg $BW^{-1} \cdot d^{-1}$. Similarly, OMI decreased from 9.49 to 7.22 kg/d with increasing karanjin and pongamol and TDOMI decreased from 7.26 kg/d for control (0 mg·kg $BW^{-1} \cdot d^{-1}$) to 5.60 kg/d for 9.6 mg·kg $BW^{-1} \cdot d^{-1}$.

Table 6. Effect of increasing dosing of anti-nutrients compounds; karanjin and pongamol on intake of steers fed medium quality growing ration.

Item[2]	Treatments[1]				SEM[3]	*P* – value	
	0 mg/kg BW	4.8 mg/kg BW	7.2 mg/kg BW	9.6 mg/kg BW		Linear	Quadratic
TDMI	10.23	9.57	8.72	7.80	0.45	< 0.01	0.77
OMI	9.49	8.87	8.09	7.22	0.42	< 0.01	0.77
TDOMI	7.26	6.60	5.96	5.60	0.30	< 0.01	0.64
NDFI	4.63	4.40	4.02	3.48	0.20	< 0.01	0.46
DNDFI	3.33	3.06	2.75	2.53	0.13	< 0.01	0.85
ADFI	2.47	2.36	2.15	1.87	0.11	< 0.01	0.45
CPI	1.38	1.30	1.18	1.07	0.06	< 0.01	0.81

[1] 0 mg/kg BW = control treatment were not dosed karanjin and pongamol, 4.8 mg/kg BW = dosed with 3.0 and 1.8 mg/kg BW of karanjin and pongamol, respectively, 7.2 mg/kg BW = dosed with 4.5 and 2.7 mg/kg BW of karanjin and pongamol, respectively, 9.6 mg/kg BW = dosed with 6.0 and 3.6 mg/kg BW of karanjin and pongamol, respectively.

[2] TDMI: Total dry matter intake; OMI: Organic matter intake; TDOMI: Total digestible organic matter intake; NDFI: Neutral detergent fiber intake; DNDFI: Digestible neutral detergent fiber; ADFI: Acid detergent fiber intake; CPI: Crude protein intake

[3] Standard error of the mean.

Digestion was not significantly affected by treatments (Table 7; linear, ($P \geq 0.65$; quadratic $P \geq 0.12$). Dry matter digestion ranged between 72.4 and 76.9%. Organic matter digestion averaged 75.6%. A similar response was observed in ADF digestion, and CP digestion.

Table 7. Nutrient digestion in steers fed with growing ration.

	Treatments[2]					*P* – value	
Item[1]	0 mg/kg BW	4.8 mg/kg BW	7.2 mg/kg BW	9.6 mg/kg BW	SEM[3]	Linear	Quadratic
DMD	75.4	73.4	72.4	76.9	2.58	0.67	0.12
OMD	76.4	74.4	73.6	78.0	2.53	0.66	0.14
NDFD	71.9	69.6	68.4	73.3	1.57	0.77	0.15
ADFD	65.3	63.1	61.8	66.9	2.61	0.80	0.22
CPD	71.3	71.3	66.0	75.0	5.36	0.68	0.17

[1]DMD: Dry matter digestion; OMD: Organic matter digestion; NDFD: Neutral detergent fiber digestion; ADFD: Acid detergent fiber digestion; CPD: Crude protein digestion.
[2] 0 mg/kg BW = control treatment were not dosed karanjin and pongamol, 4.8 mg/kg BW = dosed with 3.0 and 1.8 mg/kg BW of karanjin and pongamol, respectively, 7.2 mg/kg BW = dosed with 4.5 and 2.7 mg/kg BW of karanjin and pongamol, respectively, 9.6 mg/kg BW = dosed with 6.0 and 3.6 mg/kg BW of karanjin and pongamol, respectively.
[3] Standard error of the mean.

Ruminal pH was not significantly affected by treatment ($P = 0.50$) and there was no treatment × hour interaction ($P = 0.97$). Total ruminal VFA concentrations were minimally affected by treatment ($P = 0.18$) and there was no treatment × hour interaction ($P = 0.42$), however there was an hour effect ($P < 0.01$). Total VFA concentrations went up following feeding, and then returned to baseline levels (figure 2).

There were no significant ($P \geq 0.17$) treatment × hour interactions for any of the other ruminal fermentation responses measured (table 8). The effect of hour was significant ($P < 0.01$) for all of responses measured and was the result of offering feed once daily and the corresponding increases in ruminal fermentation occurring within 2h of feeding, expect for acetate which peaked 4h after feeding. Additionally, there were no significant linear ($P \geq 0.12$) or quadratic ($P \geq 0.10$) effects for any of the other ruminal fermentation measurements. Treatment significant ($P < 0.01$) affected propionate concentration, treatment 4.8 mg·kg BW^{-1} ·d^{-1} was greater ($P \leq 0.02$) compared to other treatments resulting with an A:P smaller ($P \leq 0.02$) for 4.8 mg·kg BW^{-1} ·d^{-1} compared to other treatments. However no linear or quadratic effects were observed ($P \geq 0.12$).

Table 8. Effect of treatment on ruminal fermentation in steers fed medium-quality total mixed ration.

	Treatments[1]					*P* – value		
Item	0 mg/kg BW	4.8 mg/kg BW	7.2 mg/kg BW	9.6 mg/kg BW	SEM[2]	Treatment[3]	Hour[4]	Treat × hour[5]
pH	6.26	6.35	6.24	6.31	0.05	0.51	< 0.01	0.98
Total VFA, m*M*	101.64	101.04	109.32	99.78	3.85	0.19	< 0.01	0.43
VFA, mol/100mol								
Acetate, %	69.34	68.67	70.53	69.98	0.65	0.22	< 0.01	0.32
Propionate, %	15.92	16.84	15.52	15.70	0.28	< 0.01	< 0.01	0.57
Butyrate, %	11.05	10.86	10.42	10.66	0.35	0.63	< 0.01	0.17
Isobutyrate, %	1.17	1.15	1.11	1.18	0.06	0.87	< 0.01	0.64
Isovalerate, %	1.55	1.54	1.51	1.62	0.08	0.79	< 0.01	0.63
Valerate, %	0.94	0.94	0.89	0.86	0.03	0.12	< 0.01	0.61
A:P	4.40	4.12	4.58	6.31	0.10	0.02	< 0.01	0.73

[1] 0 mg/kg BW = control treatment were not dosed karanjin and pongamol, 4.8 mg/kg BW = dosed with 3.0 and 1.8 mg/kg BW of karanjin and pongamol, respectively, 7.2 mg/kg BW = dosed with 4.5 and 2.7 mg/kg BW of karanjin and pongamol, respectively, 9.6 mg/kg BW = dosed with 6.0 and 3.6 mg/kg BW of karanjin and pongamol, respectively.

[2] Standard error of the mean.

[3] Treatment effect

[4] Hour effect

[5] Treatment × hour effect

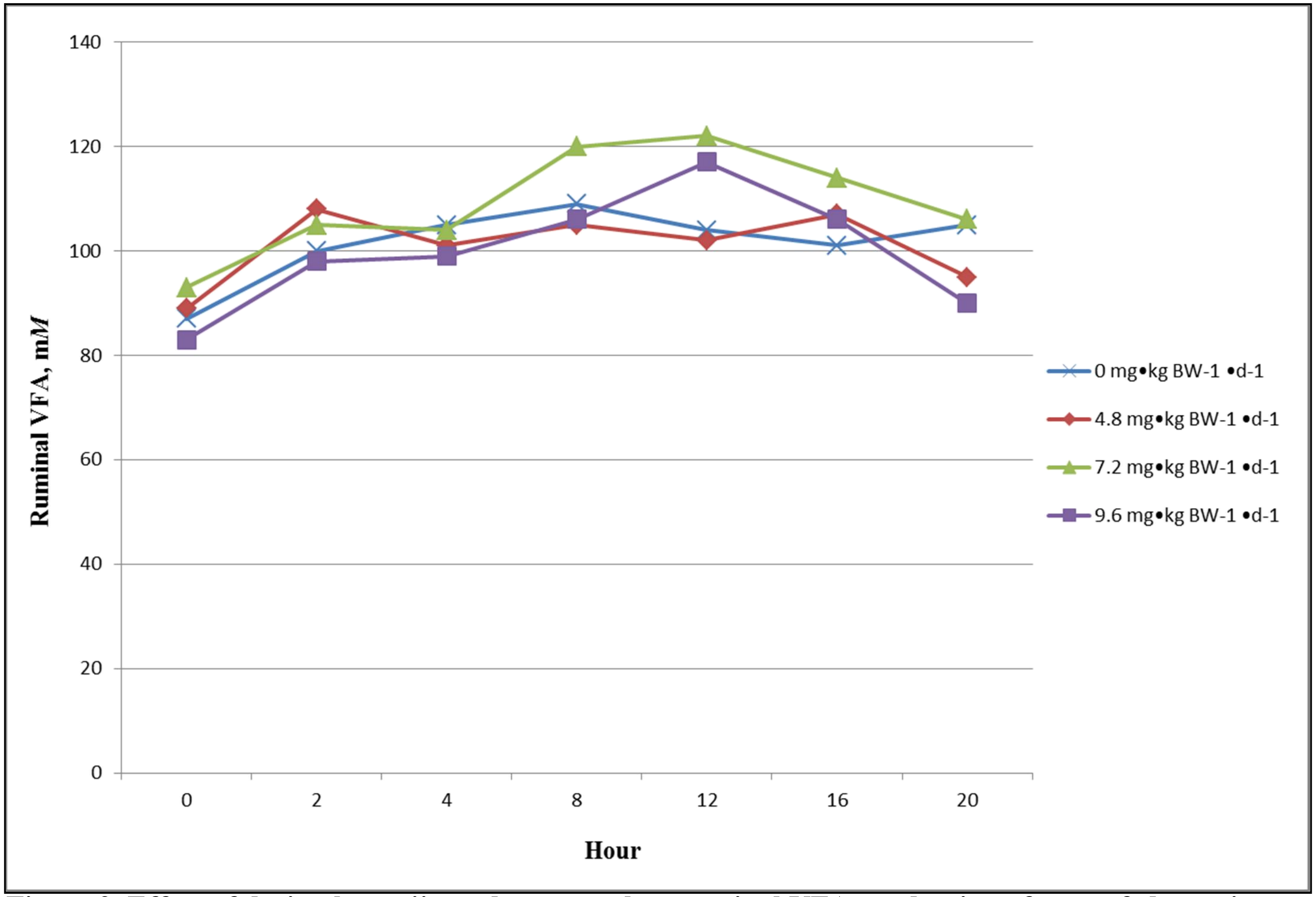

Figure 2. Effect of dosing karanjin and pongamol on ruminal VFA production of steers fed growing ration.
Treatment effect $P < 0.19$
Hour effect, $P < 0.01$
Treatment × hour interaction, $P = 0.43$

Discussion

Effects of increased dosing of karanjin and pongamol on intake, nutrient digestion and ruminal fermentation were determined. Our goal is to collect sufficient data across experiments to create a response curve describing the isolated effects of dosing karanjin and pongamol on diet utilization in cattle In the current study, inclusion of increasing levels of karanjin and pongamol linearly ($P < 0.01$) decreased intake. Bohlen (2015) conducted the first research study aimed at evaluating the acceptability of pongamia seedcake to cattle. Bohlen (2015) observed increased pongamia seedcake intake decreased ($P < 0.01$) feed consumption when levels of EKC and SKC ranged from 20 to 60% of the supplement. Rate and completeness of consumption were observed by allowing steers to eat for 10 min, then collecting and weighing the remaining supplement. Controls all of the supplement (500g) each day.. In contrast, EKC included at 60% providing 1.8 and 0.8 mg·kg BW^{-1} $\cdot d^{-1}$ karanijn and pongamol, respectively resulted in intake of 185 g/d whereas SKC at 60% providing 1.7 and 1.1 mg·kg BW^{-1} $\cdot d^{-1}$resulted in intake of 396 g/d. There were greater residual oil/karanjin concentrations in EKC (11.5% oil, 5667 ppm karanjin and 2544 ppm pongamol) than SKC (2.7% oil, 1758 ppm karanjin and 794 ppm pongamol), which in part explains the differences in palatability, since the bad taste of pongamia seedcake has mostly been attributed to karanjin and pongamol remaining in the residual oil of the cake (Ravi et al., 2000; Dutta et al., 2012).

Soren and Sastry (2009); Soren et al. (2009); Soren et al. (2010) conducted study in sheep which evaluated 50% nitrogen replacement of soybean meal in the supplement on an isonitrogenous basis with three different processed methods of pongamia seedcake, which consisted of water washed, lime treated and binder treated. Lambs were fed *ad libitum* oat straw and had karanjin intake ranging from 3.6 to 4.4 mg/kg BW among pongamia seedcake fed treatments. Control treatment total DM intake was 0.51 kg/d, which was greater than all 3 treatments that contained pongamia seedcake, which ranged from 0.38, to 0.42 kg/d, and depressed DMI from 23 to 31% compared to control group. While the intention was not explicity to evaluate palatability of pongamia seedcake, they demonstrated that feeding treated pongamia seedcake to lambs, lowered consumption irrespective of processing method. Even when present in minute quantities, karanjin concentration should be taken into consideration as limiter of intake when pongamia seedcake is included in the diet.

Mahli et al. (1989) administered karanjin and pongamol intraperitoneally in mice to investigate pharmacological effects. Pongamol showed infusion increased sensitivity to sound, touch, muscle grip and tone whereas karanjin resulted in decreased sensitivity to sound and touch. Pongamol was reported as having hypnotic effects confirming its sedative effect due to depression of central nervous system. However, their results suggest that karanjin stimulated the central nervous system. Additionally, pure oil was administered and mice showed normal behavior, strengthen the hypothesis that karanjin and pongamol are the substances present in the pongamia oil that alters behavior.

In a study conducted by Bohlen (2015) to evaluate nutrient digestion of supplements containing different levels of SKC and EKC. Supplements were dosed ruminally prior feeding hay. Animals had karanjin infusion from 1.1 to 3.2 mg·kg BW^{-1} $\cdot d^{-1}$ and pongamol from 0.6 to 2.2 mg·kg BW^{-1} $\cdot d^{-1}$. Amounts of karanjin and pongamol dosed were lower compared to the present study. Steers had lower TDOMI intake, from 3.91 to 2.46 kg/d with pongamia seedcake infusion in her work. In accordance with our study, decreased intake Bohlen's work cannot be attributed to reduced palatability of pongamia seedcake reported by Ravi et al. (2000) and Dutta et al. (2012), because it was dosed ruminally, but rather a potential metabolic effect of karanjin and pongamol.

Additionally, Bohlen (2015) reported that the karanjin and pongamol dosed as seedcake was either absorbed or metabolized in the gut, preventing detection in the feces.

In our study, nutrient digestion was not significantly different between treatments. No difference in nutrient digestion was observed by Bohlen et al., (2015) that conducted a study consisting of a control, no supplement and four different supplemented treatments providing 100 mg of N/kg BW containing different levels of pongamia seedcake. Animals had karanjin infusion from 1.1 to 3.2 mg·kg BW^{-1} $\cdot d^{-1}$ and pongamol from 0.6 to 2.2 mg·kg BW^{-1} $\cdot d^{-1}$. Results reported by Bohlen (2015) show that digestion is not affected when steers consume up to 3.1 and 2.2 mg·kg BW^{-1} $\cdot d^{-1}$ karanjin and pongamol, respectively. Moreover, karanijn and pongamol could be fed up to 6.0 and 3.6 mg·kg BW^{-1} $\cdot d^{-1}$,respectively without affecting nutrient digestion. Similarly, Soren and Sastry (2009); Soren et al. (2009) reported comparable levels of digestion between lambs consuming the control supplement and those consuming at up to 4.4 mg·kg BW^{-1} $\cdot d^{-1}$. Rao and Kumar (2015) reported that lambs consuming between 0 and

53 mg·kg BW^{-1} $\cdot d^{-1}$ karanjin had a lower OM CP and ADF digestion compared to controls. Organic matter digestion decreased from 69.7 to 66.7%, CP digestion decreased from 79.3 to 68.9% and ADF from 47.7 to 37.6%.

In contrast to our study, which ruminally dosed karanjin and pongamol, (Ravi et al., 2000; Singh et al., 2006; Rao and Kumar, 2015) fed pongamia seedcake, which besides karanjin and pongamol, contains other anti-nutritional factors, present in EKC and SKC, such as, tannins (Natanam, 1989; Panda et al., 2006; Vinay and Kanya, 2008) and trypsin inhibitors (Natanam, 1989; Rattansi and Dikshit, 1997). Depression in CP, NDF and ADF digestion may largely be attributed to the other factors present in pongamia seedcake.

Intake and extent of digestion have an inverse relationship, because as intake decreases, passage rate typically decreases as well (Riewe and Lippke, 1970; Tyrrell and Moe, 1975; Van Soest, 1975). Decrease passage rate allows digesta to reside in the rumen longer; therefore, increasing extent of digestion. Failure to observe an effect of karankin and pongamol infusion on digestion in our study and Soren and Sastry (2009); Bohlen (2015) may resulted from lower intake and a concomitant increase in retention time.

In this study, ruminal pH was similar between all treatments. This is in agreement with Singh et al., (2006), Soren et al. (2010) and Bohlen (2015) who reports no difference in ruminal pH. Ravi et al., (2001) reported that feeding both expeller pressed and solvent extracted karanj cake had no adverse effects on concentration of total volatile fatty acids in the rumen and similar report was made by Soren et al., (2010) when lambs were fed from 0 to 4.4 mg·kg BW^{-1} $\cdot d^{-1}$karanjin. Ravi et al., (2001) reported that feeding both EKC and SKC had no adverse effects on volatile fatty acid concentration in the rumen.

Conclusion

Pongamia seedcake, when used as a feed ingredient for beef cattle, must be analyzed for content of karanjin and pongamol due to high variability of anti-nutritional factors content in pongamia seedcake. Our study demonstrates that lower intakes are also not totally associated with palatability issues of karanjin and pongamol present in the seedcake. However, nutrient digestion in steers fed a growing diet is not affected by infusion up to 6.0 and 3.6 mg/kg BW karanjin and pongamol, respectively. Karanjin and pongamol possibly have metabolic factors that depress intake of growing steers, beyond the often discussed low palatability of the seed cake. Further research is needed to understand possible metabolic factors, regarding to central nervous system and vital organs to better understand the impact of anti-nutrients present in pongamia seedcake.

CHAPTER III

EFFECTS OF ANTI-NUTRITIONAL COMPOUNDS PRESENT IN PONGAMIA SEEDCAKE ON INTAKE, DIGESTION, AND RUMINAL FERMENTATION OF BEEF CATTLE GRAZING BERMUDA HAY

Synopsis

Pongamia seedcake is a potential source of livestock feed. However, anti-nutritional compounds present in the seedcake such as karanjin and pongamol make the inclusion of this ingredient in cattle diets a challenge. Thus, it is necessary to determine their effects on intake, digestion, and ruminal fermentation in beef cattle grass fed.

Accordingly, twelve cannulated Angus × Hereford steers (483 ± 14.5 kg of BW) were used in completely randomized block design to determine the effects of dosing 4 levels of karanjin (0, 1.5, 3.0 and 4.5 mg·kg BW^{-1} ·d^{-1}) combined with pongamol (0, 1.8, 2.7 and 3.6 mg·kg BW^{-1} ·d^{-1}). Steers were provided *ad libitum* access to Bermudagrass hay, 0.62 Mcal NEg /kg (NRC, 2016). The experimental period was 14 d with 8 d for steers to adapt to treatment, 5 days to determine intake and digestion, and 1 day to quantify ruminal fermentation.

Total dry matter intake, OM intake, total digestible OM intake, NDF intake, digestible NDF intake and ADF intake decreased quadratically ($P < 0.05$). Total DM decreased from 8.89 kg/d for 0 mg·kg BW^{-1} ·d^{-1} to 7.11 kg/d for 2.4 mg·kg BW^{-1} ·d^{-1} and increased slightly to 7.62 kg/d and 7.48 kg/d for 4.8 and 7.2 mg·kg BW^{-1} ·d^{-1}. Similarly, OMI decreased from 8.50 kg/d for 0 mg·kg BW^{-1} ·d^{-1} to 6.68 kg/d for 2.4 mg·kg BW^{-1}

$\cdot d^{-1}$ and increased slightly to 7.26 kg/d and 7.07 kg/d for 4.8 and 7.2 mg·kg $BW^{-1} \cdot d^{-1}$. The same response was observed for total digestible OMI, which decreased from 5.74 kg/d for 0 mg·kg $BW^{-1} \cdot d^{-1}$ to 4.32 kg/d for 2.4 mg·kg $BW^{-1} \cdot d^{-1}$ and increased slightly to 4.99 kg/d and 4.56 kg/d for 4.8 and 7.2 mg·kg $BW^{-1} \cdot d^{-1}$. However, no significant difference ($P \geq 0.22$) was observed between treatments for any measure of digestion. Organic matter digestion ranged from 64.2 to 67.5% for 2.4 mg·kg $BW^{-1} \cdot d^{-1}$ and 4.8 mg·kg $BW^{-1} \cdot d^{-1}$, respectively. No significant effects ($P \geq 0.46$) on ruminal fermentation were observed.

Our results indicate that increasing levels of karanjin and pongamol produced a quadratic intake response with lower intake at the lowest level of dosing, followed by an increase at higher dosing levels; however, digestion and ruminal fermentation were not affected.

Introduction

Estimates showed that biodiesel could represent as much as 20-22% of all on-road diesel used in Brazil, Europe, China, and India by the year 2020. The demand for biodiesel is increasing significantly (Kumar and Sharma, 2011). High demand for renewable sources of energy created either by regionally limited supplies of fossil fuels or regulatory mandate has caused significant growth in the biofuel industry. In parallel, the volume of co-products generated from the biofuel production process has also increased (Abbeddou and Makkar, 2012).

Recently, interest in the tree Pongamia (*Pongamia pinnata*) has mainly been focused on the use of oil from its seed for biodiesel, which is environmentally safe, nontoxic, and biodegradable (Kaushik et al., 2015). Following oil extraction of the pongamia seed, approximately two thirds by weight of the original seed is left as a residual meal or cake known as pongamia seedcake.

Pongamia seedcake contains approximately 30% CP and has been used in previous ruminant nutrition research and production (Singh et al., 2006; Soren et al., 2009; Nagalakshmi et al., 2011; Rao and Kumar, 2015). However, pongamia seedcake contains anti-nutritional components in the oil remained after extraction (i.e., karanjin and pongamol), which have been attributed to decreased ruminant performance (Konwar et al., 1987; Konwar, 1987; Ravi et al., 2000; Singh et al., 2006; Rao and Kumar, 2015). Therefore, the present research aims to further investigate the use of pongamia seedcake as a protein ingredient in beef cattle diets by dosing increasing levels of the anti-nutrients karanjin and pongamol to determine effects on intake, nutrient utilization and ruminal fermentation in forage fed cattle. Results obtained in this study will enable the development of a response surface to pongamol and karanjin inclusion in a supplement. Ultimately, this data can be used to determine the inclusion level of pongamia seedcakes containing varying levels of anti-nutrients in the residual oil present in commercially available supplements.

Material & methods

The experimental protocol was approved by the Institutional Animal Care and Use Committee at Texas A&M University.

Twelve ruminally cannulated Angus steers (483 ± 14.5 kg of BW) were used in a complete randomized block design experiment with 4 treatments and 3 blocks to evaluate the effect of different levels of karanjin and pongamol intake on intake, digestion and ruminal fermentation to cattle consuming Bermudagrass hay (Table 9). Steers were blocked in 3 groups according to the level of intake measured prior to the beginning of the trial. Blocks consisted of high, medium and low intake. Steers were housed in individual stalls in an enclosed barn and given *ad libitum* access to Bermuda grass hay and water.

Table 9. Nutrient composition of Bermuda grass hay

Items	Bermudagrass hay
Dry matter, %	90.80
	% DM
Organic matter	93.26
NDF	70.45
ADF	39.12
CP	8.24
ME[1]	2.04
NEm[1]	1.19
NEg[1]	0.62

[1]Values were calculated based on the ingredient values from the NRC (2016).

Treatments consisted of a control no karanjin and pongamol (CONTROL) and three levels of purified karanjin and pongamol (99 + % karanjin and 99 + % pongamol; INDOFINE Chemical Company, Inc., Hillsborough, NJ). A ratio of 1.0:0.6 karanjin to pongamol (Mandal et al., 1985; Kumar, 2011) and the level of dosing were determined from previous research in our laboratory and data collected from other published sources

(Mandal et al., 1986; Punj and Devendra, 1988; Prabhu et al., 2002; Panda et al., 2004; Panda et al., 2006; Soren et al., 2007; Soren et al., 2008; Vinay and Kanya, 2008; Soren and Sastry, 2009; Soren et al., 2009; Eipeson et al., 2010; Soren et al., 2010; Nagalakshmi et al., 2011; Susarla et al., 2012; Dineshkumar et al., 2013; Rao and Kumar, 2015). Karanjin and pongamol at a ratio of 1.0:0.6 were provided at 2.4, 4.8, and 7.2 mg·kg BW^{-1} $\cdot d^{-1}$ such that steers received 1.5, 3.0, and 4.5 mg·kg BW^{-1} $\cdot d^{-1}$ of karanjin and 0.9, 1.8, and 2.7 mg·kg BW^{-1} $\cdot d^{-1}$ of pongamol.

Karanjin and pongamol powder were weighed into gel capsules individually by steer according to individual weight and treatment amount (mg/kg BW). Gel capsules were dosed directly into the rumen through ruminal cannula daily, just prior to feeding for the duration of the project.

One 14-d period was conducted, 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 adaptation and throughout the collection period. Calculations of intake and digestion were made from observations on d 9 through 13. Feed refusals were collected and weighed prior to feeding 0600 h and a 200g sample was retained for analysis. Diet samples were composited across days on an equal weight basis. Ort samples were composited within steer across days. Diet samples were collected d 9 through 12 to correspond with fecal samples collected d 10 through d 13. Acid detergent insoluble ash (ADIA) was used as an internal marker to estimate fecal production. On d 10 through d 13 fecal samples were collected every 8 h, with sample timing 2 h prior the day before so that 12 samples were obtained over a 4 d collection period. Fecal samples collected were composited and frozen at -20°C until analysis. Prior to analysis, each sample was allowed to thaw, was thoroughly mixed, and a representative subsample was collected for analysis.

On d 14 of each period, ruminal fermentation parameters were measured. A suction strainer (19 mm diameter, 1.5 mm mesh; Raun and Burroughs, 1962) was used to collect rumen fluid samples prior to feeding (0 h), and at 2, 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^{o}C for later determinations of VFA analysis. Prior to freezing, 8 ml of rumen fluid were combined with 2 ml of 25% *m*-phosphoric acid for VFA analysis.

Diet, ort and fecal 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 samples were composited on an equal weight basis across days. Ort and fecal samples were composited by steer across days within period. Diet, ort and fecal samples were ground with a Wiley mill to pass a 1-mm screen and analyzed for DM, OM, NDF, ADF and CP. Organic matter was determined as the loss in dry weight upon combustion for 8 h at 450^{o} F. 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. Hay, ort and fecal samples were sent to a commercial laboratory (SDK Labs, Hutchinson, KS) for CP analysis. Fecal production was calculated by difference of

ADIA concentration from the amount present on feed by the amount present on feces. ADIA quantity was obtained upon combustion of ADF samples of hay, orts and feces for 8 h at 450°F. Digestion was calculated by the following formula: [1-(output of nutrient/intake of nutrient)]×100.

Intake, digestion and ruminal fermentation parameters were analyzed using the MIXED procedure of SAS 9.3 (SAS Inst. Inc., Cary, NC) for completely randomized block design with animal as the experimental unit. Steers were blocked by intake measured prior the treatment application. Orthogonal polynomial contrasts were used to determine linear or quadratic responses and treatment means was calculated using LSMEANS option on intake, digestion and ruminal fermentation data collected throughout the experiment. Ruminal fermentation parameters were analyzed using repeated measures techniques, with hour as the repeated variable and steer as the subject.

Results

Total DM, OM, total digestible OM, NDF, digestible NDF and ADF intake decreased quadratically (Table 10; $P < 0.05$) in response to increasing karanjin and pongamol. However, CP intake was not significantly ($P = 0.14$) different between treatments, ranging from 0.54 to 0.69 kg/d. Total DM intake decreased quadratically from 8.89 kg/d for 0 mg·kg BW^{-1} $\cdot d^{-1}$ to 7.11 kg/d for 2.4 mg·kg BW^{-1} $\cdot d^{-1}$ and increased slightly to 7.62 kg/d and 7.48 kg/d for 4.8 and 7.2 mg·kg BW^{-1} $\cdot d^{-1}$. Similarly, OMI decreased from 8.50 for 0 mg·kg BW^{-1} $\cdot d^{-1}$ to 6.68 kg/d for 2.4 mg·kg BW^{-1} $\cdot d^{-1}$ with increasing dosing of karanjin and pongamol. Moreover, intake of NDF and ADF were also quadratically decreased from 8.06 to 6.92 and 3.42 to 2.81 kg/d, respectively.

Table 10. Effect of increasing karanjin and pongamol Bermuda grass hay intake in steers.

	Treatments[1]					*P* – value	
Item[2]	0mg/kg BW	2.4 mg/kg BW	4.8 mg/kg BW	7.2 mg/kg BW	SEM[3]	Linear	Quadratic
TDMI	8.89	7.11	7.62	7.48	0.40	0.04	0.04
OMI	8.50	6.68	7.26	7.07	0.36	0.03	0.03
TDOMI	5.74	4.32	4.99	4.56	0.34	0.01	0.03
NDFI	8.06	6.92	7.35	7.00	0.19	< 0.01	0.01
DNDFI	6.10	5.25	5.74	5.23	0.20	< 0.01	0.04
ADFI	3.42	2.81	2.96	2.94	0.15	0.06	0.04
CPI	0.69	0.54	0.61	0.59	0.04	0.25	0.14

[1] 0 mg/kg BW = control treatment were not dosed karanjin and pongamol, 2.4 mg/kg BW = dosed with 1.5 and 0.9 mg/kg BW of karanjin and pongamol, respectively, 4.8 mg/kg BW = dosed with 3.0 and 1.8 mg/kg BW of karanjin and pongamol, respectively, 7.2 mg/kg BW = dosed with 4.5 and 2.7 mg/kg BW of karanjin and pongamol, respectively.
[2] TDMI: Total dry matter intake; OMI: Organic matter intake; TDOMI: Total digestible organic matter intake; NDFI: Neutral detergent fiber intake; DNDFI: Digestible neutral detergent fiber; ADFI: Acid detergent fiber intake; CPI: Crude protein intake
[3] Standard error of the mean.

Digestion was not significantly affected by treatments (Table 11; linear, ($P \geq 0.46$; quadratic $P \geq 0.22$). Dry matter digestion ranged between 66.3 and 62.0%. No difference was also observed in OMD, which had values ranging between 64.2 and 67.5%. Neutral detergent fiber digestion averaged was 76.0 % and ranged between 78.0 and 74.8%.

Table 11. Nutrient digestion in steers fed with Bermuda grass hay

	Treatments[1]					*P* – value	
Item[2]	0 mg/kg BW	2.4 mg/kg BW	4.8 mg/kg BW	7.2 mg/kg BW	SEM[3]	Linear	Quadratic
DMD	65.10	62.09	66.31	61.98	2.58	0.46	0.66
OMD	67.54	64.24	68.76	64.32	2.53	0.47	0.71
NDFD	75.70	75.93	77.95	74.75	1.57	0.89	0.22
ADFD	59.51	57.27	61.41	56.91	2.61	0.65	0.53
CPD	53.97	44.96	61.59	50.39	5.36	0.73	0.77

[1] 0 mg/kg BW = control treatment were not dosed karanjin and pongamol, 2.4 mg/kg BW = dosed with 1.5 and 0.9 mg/kg BW of karanjin and pongamol, respectively, 4.8 mg/kg BW = dosed with 3.0 and 1.8 mg/kg BW of karanjin and pongamol, respectively, 7.2 mg/kg BW = dosed with 4.5 and 2.7 mg/kg BW of karanjin and pongamol, respectively.

[2]DMD: Dry matter digestion; OMD: Organic matter digestion; NDFD: Neutral detergent fiber digestion; ADFD: Acid detergent fiber digestion; CPD: Crude protein digestion.

[3] Standard error of the mean.

There were no significant ($P \geq 0.09$) treatment × hour interactions for any of the ruminal fermentation responses measured (table 12). The effect of hour was significant ($P < 0.05$) for all of responses measured and was the result of offering feed once daily and the corresponding increases in ruminal fermentation that occurred within 2h of feeding, expect for acetate which increased 4h after feeding (figure 3) . In contrast, there was no significant linear ($P \geq 0.11$) and quadratic ($P \geq 0.29$) response for pH, acetate:propionate ratio and all VFA proportions.

Acetate, propionate, butyrate, isobutyrate, isovalerate, valerate molar concentration did not showed treatment effect ($P \geq 0.38$), as well as the proportion ($P \geq 0.46$).

Table 12. Effect of treatment on ruminal fermentation in steers fed Bermuda grass hay.

	Treatments[1]					*P* – value		
Item	0 mg/kg BW	2.4 mg/kg BW	4.8 mg/kg BW	7.2 mg/kg BW	SEM[2]	Treatment[3]	Hour[4]	Treat x hour[5]
pH	6.52	6.39	6.47	6.47	0.08	0.72	< 0.01	0.81
Total VFA, m*M*	107.44	113.64	109.16	117.98	5.92	0.59	< 0.01	0.74
VFA, mol/100 mol								
Acetate, %	74.80	74.58	74.65	74.47	0.51	0.98	< 0.01	0.51
Propionate, %	15.72	15.55	15.32	15.31	0.24	0.56	0.06	0.86
A:P	4.76	4.81	4.88	4.87	0.10	0.84	0.02	0.81
Butyrate, %	7.18	7.44	7.63	7.79	0.28	0.47	< 0.01	0.25
Isobutyrate, %	0.79	0.87	0.86	0.85	0.05	0.69	< 0.01	0.09
Isovalerate, %	0.82	0.90	0.90	0.88	0.05	0.67	< 0.01	0.69
Valerate, %	0.69	0.66	0.63	0.69	0.04	0.73	< 0.01	0.49

[1] 0 mg/kg BW = control treatment were not dosed karanjin and pongamol, 2.4 mg/kg BW = dosed with 1.5 and 0.9 mg/kg BW of karanjin and pongamol, respectively, 4.8 mg/kg BW = dosed with 3.0 and 1.8 mg/kg BW of karanjin and pongamol, respectively, 7.2 mg/kg BW = dosed with 4.5 and 2.7 mg/kg BW of karanjin and pongamol, respectively.

[2] Standard error of the mean.

[3] Treatment effect

[4] Hour effect

[5] Treatment × hour effect

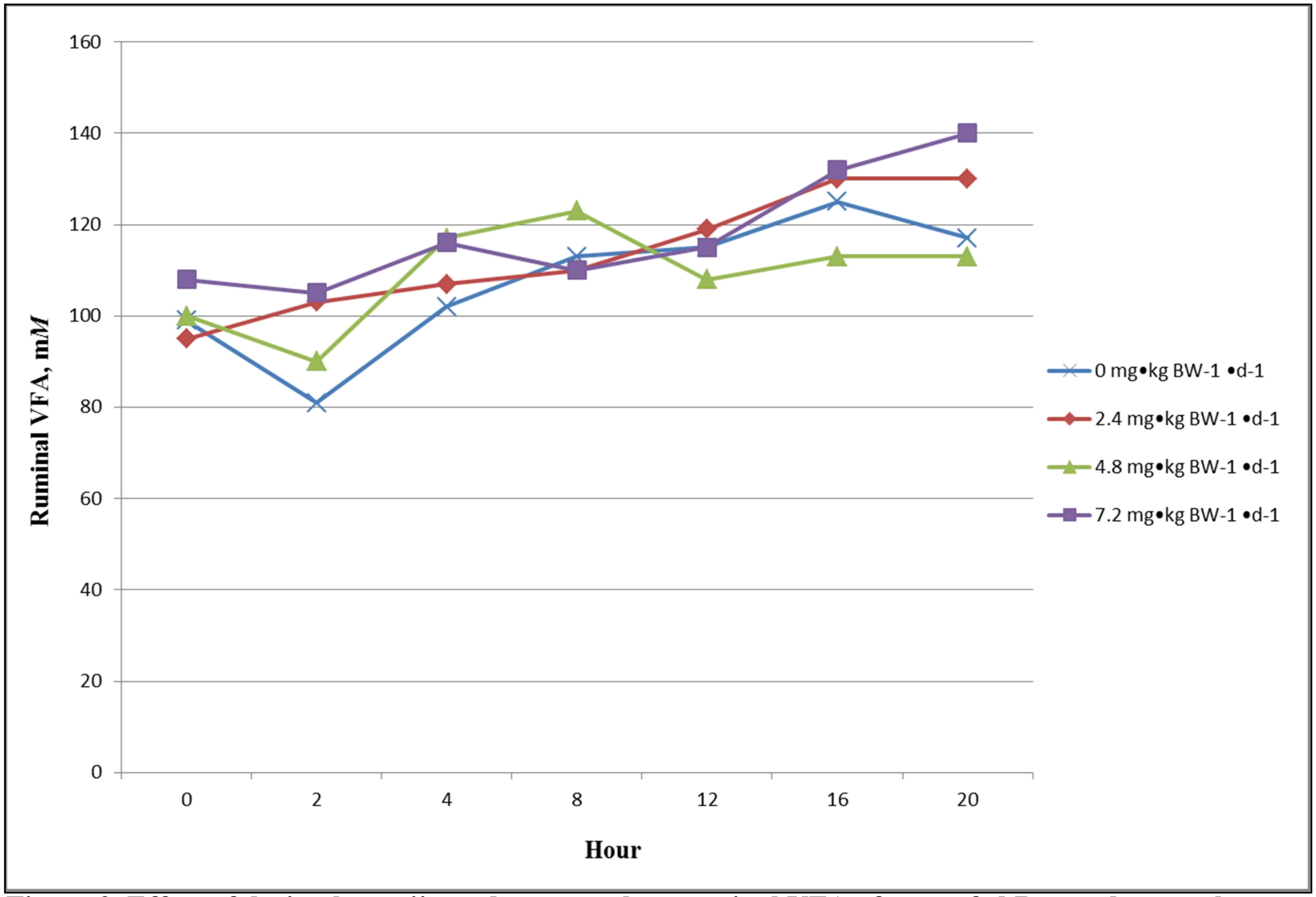

Figure 3. Effect of dosing karanjin and pongamol on ruminal VFA of steers fed Bermuda grass hay.
Treatment effect $P = 0.59$
Hour effect, $P < 0.01$
Treatment × hour interaction, $P = 0.74$

Discussion

We evaluated the effects of increased dosing of karanjin and pongamol on intake, nutrient digestion, and ruminal fermentation in steers fed Bermudagrass hay. Ultimately, our goal was to describe the response to dosing karanjin and pongamol on nutrient utilization and ultimately cattle performance. Clearly describing the effects of karanjin and pongamol would allow recommendations to be created for formulating supplements that contain pongamia seedcake. In the current study, inclusion of increasing levels of karanjin and pongamol quadratically ($P < 0.05$) decreased all measures of intake. Specifically, the first increment of karanjin and pongamol, 2.4 $mg \cdot kg\ BW^{-1} \cdot d^{-1}$, dosed ruminally resulted in a 25% reduction in TDOMI; however, 4.8 and 7.2 $mg \cdot kg\ BW^{-1} \cdot d^{-1}$ resulted in 13 and 21% reductions, respectively, but did not significantly differ between them. In another study using ruminal infusion, Bohlen (2015) observed a 20% decrease in TDOMI when a protein supplement containing 20% expeller pressed pongamia seedcake isonitrogenous replaced a protein supplement containing 0% expeller pressed pongamia seedcake. The reduction they observed effectively erased the increase in TDOMI created by the protein supplement containing 0% expeller pressed pongamia seedcake. Furthermore, the 20% expeller press pongamia seedcake supplement provided 3.1 $mg \cdot kg\ BW^{-1} \cdot d^{-1}$ of karanjin and pongamol per day. In the same study by Bohlen, provision of a supplement containing 40% solvent extracted pongamia seedcake and providing 1.7 $mg \cdot kg\ BW^{-1} \cdot d^{-1}$ karanjin and pongamol per day decreased TDOMI by 18% compared to the protein supplemented control (0% pongamia seedcake). Our project and Bohlen's work are unique in that the animals were ruminally dosed with either karanjin and pongamol (our study) or dosed with a supplement containing pongamia seedcake (Bohlen's work). These data suggest that the decreased intake observed by Bohlen (2015) and Rao and Kumar (2015) of 37 and 25%, respectively when pongamia seedcake was supplemented to cattle consuming a forage diet are explained by broader effects than just taste.

Rao and Kumar (2015) fed lambs pongamia seedcake at 0, 25, 50 and 75 levels of the concentrate mixture with the remaining being soybean meal on an isonitrogenous basis. Karanjin intake amounts corresponded to 0, 11, 19 and 27 $mg \cdot kg\ BW^{-1} \cdot d^{-1}$, respectively. Authors reported through regression analysis ($R^2 = 0.99$) that DM intake linearly decreased from 0.68 to 0.51 kg/d as the level of pongamia seedcake increased in the diets. It was further opined by these authors that the intake decrease was mainly caused by anti-nutritional substances like karanjin, trypsin inhibitors, pongamol and pongapin present in the residual oil portion of pongamia seedcake; however, among the compounds contained in pongamia seedcake, the present study dosed only karanjin and pongamol, which demonstrates that these two anti-nutritional compounds are potent enough to lower feed consumption of cattle.

In our study, nutrient digestion was not significantly different between treatments. Similar to our study which no difference on nutrient digestion was observed, Soren and Sastry (2009); Soren et al. (2009) reported comparable DM, OM, NDF, ADF digestion in lambs consuming supplement containing karanjin between 3.6 and 4.4 mg/kg BW fed with SKC treated with lime and binder replacing 50% nitrogen of soybean meal on diets of lambs fed *ad libitum* chaffed oats, indicating that digestion was also not affected by the presence of karanjin and pongamol in the rumen. The no difference in nutrient digestion was also observed by Bohlen et al., (2015) that conducted a study consisting of karanjin infusion from 1.1 to 3.2 mg·kg $BW^{-1}\cdot d^{-1}$ and pongamol from 0.6 to 2.2 mg·kg $BW^{-1}\cdot d^{-1}$. Organic matter and NDF digestion were comparable between treatments, remaining from 58.1 and 61.3% and 57.2 and 58.4%, respectively. Results provided by Bohlen (2015) indicated that digestion is not affected when steers intake up to 3.1 and 2.2 mg/kg BW/d of karanjin and pongamol, respectively. In our study, karanijn and pongamol could be fed up to 4.5 and 2.7 mg·kg $BW^{-1}\cdot d^{-1}$ of karanjin and pongamol, respectively, without affecting nutrient digestion.

Research conducted by (Ravi et al., 2000; Singh et al., 2006; Rao and Kumar, 2015) fed animals pongamia seedcake, which besides karanjin and pongamol, also contain other anti-nutritional factors that are present in EKC and SKC, such as, tannins (Natanam, 1989; Panda et al., 2006; Vinay and Kanya, 2008) and trypsin inhibitors (Natanam, 1989; Rattansi and Dikshit, 1997). Depression in CP, NDF and ADF digestion may largely be attributed to the cumulative effects of all the anti-nutritional factors present in pongamia seedcake. Therefore, inaccurate conclusion could be attributed to karanjin and pongamol being responsible for decreasing nutrient digestion.

Previous research which reported no difference in digestion is similar to ours, in that intake decreased (Soren and Sastry, 2009; Bohlen, 2015). Intake and extent of digestion have an inverse relationship, because as intake decreases, passage rate typically decreases as well (Riewe and Lippke, 1970; Tyrrell and Moe, 1975; Van Soest, 1975). Decrease passage rate allows digesta to reside in the rumen longer; therefore, increasing extent of digestion. Failure to observe an effect of karankin and pongamol infusion on digestion in our study and Soren and Sastry (2009); Bohlen (2015) may resulted from lower intake and a concomitant increase in retention time.

In this study, ruminal pH was similar between all treatments. This is in agreement with Singh et al., (2006) who reports no difference in ruminal pH of lambs when fed a control groundnut cake supplement, EKC supplement, and a SKC supplement. Similarly, Soren et al. (2010) observed no difference on ruminal fluid pH when 3 treatments containing different methods of pongamia oil extraction were fed to sheep. Bohlen et al., (2015) also reports no difference in rumen pH between treatments, which agree with this study. Soren et al., (2010) conducted study in sheep fed *ad libitum* oat straw which had karanjin intake ranging from 3.6 to 4.4 mg/kg BW and showed no difference on ruminal pH. Therefore, results presented by our study, in accordance with Singh et al. (2006); Soren et al. (2010); Bohlen (2015) indicates that karanjin and pongamol do not alter ruminal pH. Ravi et al., (2001) reported that feeding both EKC and SKC had no adverse effects on volatile fatty acid concentration in the rumen. Similar to our study, Bohlen (2015) reported no significant difference in molar concentration of acetate, propionate, butyrate, isobutyrate, or valerate between steers in all treatments. Besides Bohlen (2015), none of the Pongamia studies previously conducted measured molar concentration of different VFA. Karanjin and pongamol dosing seems to not affect VFA concentrations.

CHAPTER IV

CONCLUSION

Pongamia seedcake, when used as a feed ingredient for beef cattle, must be analyzed for content of karanjin and pongamol due to high variability of anti-nutritional factors content in pongamia seedcake. Our study demonstrates that lower intakes are also not totally associated with palatability issues of karanjin and pongamol present in the seedcake. However, nutrient digestions in steers fed Bermudagrass hay are not affected by infusion up to 4.5 and 2.7 mg/kg BW karanjin and pongamol, respectively. However, karanjin and pongamol possibly have metabolic factors that depress intake of growing steers, beyond the often discussed low palatability of the seed cake. Further research is needed to understand possible metabolic factors, regarding to central nervous system and vital organs to better understand the impact of anti-nutrients present in pongamia seedcake.

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