



Chemical composition and anti nutritional factors in karanja (*Pongamia pinnata*) seed kernels and its *in vitro* evaluation

D DINESH KUMAR¹, S B N RAO², S JASH³, A V ELANGO VAN⁴ and S HEMALATHA⁵

National Institute of Animal Nutrition and Physiology, Adugodi, Bengaluru 560 030 India

Received: 22 September 2010; Accepted: 27 December 2010

ABSTRACT

Karanja (*Pongamia pinnata*) seeds collected from different places of Karnataka were analyzed for morphological measurements, chemical analysis and anti-nutritional factors like karanjin, pongamol, trypsin inhibitors. Variation were observed in seeds size (1.18–1.68 g), CP (14.46–23.09%), fat (30.76–39.99%), and ANF's like karanjin (0.57 to 1.75%), pongamol (0.25 –1.27%) and trypsin inhibitor activity (790–1278 µg / g seed meal). Further, karanja seed meal (KSM) and Karanja defatted meal (KDM) were incorporated in concentrate mixtures at graded levels (25, 50, 75 and 100%) to replace standard soybean meal. To this effect, 9 iso-nitrogenous concentrate mixtures were prepared using (C1 to C9) with 4 levels of replacement (25, 50, 75 and 100% on nitrogen basis) with KSM (C2 to C5) KDM (C6 to C8) and C1 as control. IVDMD, IVOMD (%) followed a decreasing trend when KSM was added to concentrate mixtures whereas complete removal of oil (KDM) resulted in similar trend as that of control. However, both KSM and KDM did not exert any effects on rumen fermentation parameters. Results obtained in the study indicated variation in chemical constituents, ANF's in the seed samples collected across Karnataka and complete removal of oil from karanj seeds caused beneficial effects on nutrient digestibility *in vitro*.

Key words: Karanja, Karanjin, *In vitro* dry matter digestibility, Pongamol, Trypsin inhibitors

Karanja (*Pongamia pinnata*) is a forest tree grown in all parts of India, particularly in Tamil Nadu, Andhra Pradesh and Karnataka, for its ecological advantages (Vinay and Sindhu Kanya 2008). The seed contains 27–39% oil, 20–30% protein and a group of furano-flavonoids that constitutes 5–6% by weight of the oil (Bringi 1987). The estimated production of seed is nearly 0.11 million tonnes per annum in India (National Oilseed and Vegetable Oils Development Board 1995). Gupta *et al.* (1981) reported that complete de-oiling of the cake by solvent extraction removes bitter taste and pungent odour. Vinay and Sindhu kanya (2008) reported that deoiled cake of *P. Pinnata* seeds obtained after solvent extraction contains up to 30% protein. While it has the potential to be used as sustainable animal feed supplement, its anti-nutritional factors restrict its usage as a feed /food. Nutritional quality of karanja seed proteins improved after reducing the anti nutrients in the meal. However, significant reduction in the feed intake, growth, and nutrient balance was found in the animals (Natanam *et al.* 1989) and Srivastava *et al.* (1990) observed reduced protein digestibility in growing

goat kids, fed at higher levels of solvent-extracted cake. In the present investigation, karanj seed samples collected from different regions of Karnataka were analyzed for different proximate principles and anti-nutritional factors like karanjin, pongamol and trypsin inhibitors to find out the regional variation. Further, graded levels of Karanja whole seed meal and Karanja defatted meal were incorporated in the concentrate mixtures to find out its effect on *in vitro* digestibility and rumen fermentation parameters to get an idea whether complete removal of oil will have beneficial effects for animal feeding point of view.

MATERIALS AND METHODS

Collection of karanj seeds: Karnaja seeds were collected from different regions of Karnataka with the help of non-governmental organization and Karnataka forest department officials in the year 2008 and 2009. The seeds were supplied to laboratory in cloth bags clearly marked with name of the region.

Formulation of concentrate mixtures: A control concentrate mixture was formulated with conventional concentrate feed ingredients such as maize (*Zea mays*) grain, deoiled soybean (*Glycine max*) cake, wheat (*Triticum aestivum*) bran (C 1). Four replacement levels, viz. 25, 50,

Present address: ¹Junior Research Fellow, ^{2,4}Principal Scientist (e mail²: bnrao@gmail.com), ³ Scientist (SS).

⁵Associate, VWR Lab products Pvt. Ltd., Coimbatore, Tamil Nadu.

75 and 100% on iso-nitrogen basis were formulated. C2, C3, C4 and C5 are concentrate mixtures replaced with karanja crushed seed meal where as C6, C7, C8 and C9 are concentrate mixtures replaced with karanja deoiled residue, which was obtained by refluxing with petroleum ether in rapid fat analyzer (soxhtherm). Urea was added at low levels at high levels of replacement to account for low CP in karanja (16.84% in KSM and 28.31% KDM compared to soya) (Table 1).

Table 1. Physical measurements, chemical composition and anti-nutritional factors of Karanja seeds

Parameters	No. of samples	Average $\pm$ SE	Range
<i>Physical measurements</i>			
Length (mm)	16	22.76 $\pm$ 0.52	17.68–24.27
Width (mm)	16	15.72 $\pm$ 0.35	14.00–17.83
Weight (g)	16	1.40 $\pm$ 0.05	1.18–1.68
<i>Chemical composition (%)</i>			
Dry matter	25	95.44 $\pm$ 0.45	90.70–98.11
Crude protein	25	18.94 $\pm$ 0.52	14.46–23.09
Crude fat	25	34.91 $\pm$ 0.42	30.76–39.99
Total ash	25	2.74 $\pm$ 0.03	2.37–3.10
Crude fiber	25	4.48 $\pm$ 0.21	3.05–6.88
Nitrogen-free-extract	25	38.90 $\pm$ 0.60	32.91–43.45
Acid insoluble ash	13	0.05 $\pm$ 0.01	0.01–0.15
Neutral detergent fiber	13	19.10 $\pm$ 1.33	12.02–27.75
Acid detergent fiber	13	11.09 $\pm$ 0.98	6.37–18.62
<i>Anti-nutritional factors (%)</i>			
Karanjin	13	1.30 $\pm$ 0.08	0.57–1.75
Pongamol	13	0.52 $\pm$ 0.08	0.25–1.27
Trypsin inhibitor activity (μ g / g protein)	13	1080.08 $\pm$ 46.91	790–1278

Physical analysis: The seeds were subjected to physical measurements like length, width and weight of seed with help of Vernier Calipers and chemical balance.

Chemical analysis

Proximate analysis: The CP, ether extract, crude fiber and ash of karanja seed and concentrate mixtures were determined by methods of AOAC (1995).

Mineral analysis: All the karanja seed and concentrate mixtures samples were analyzed for minerals in atomic absorption spectrophotometer. Copper and zinc in karanj seed and concentrate mixtures samples were analyzed either directly from acid extract without any further dilution or diluted with deionized water, if required (Pal *et al.* 2009).

Anti nutritional factors

Karanjin: Karanjin in the methanol-extracted feed sample was estimated by HPTLC against standard using toluene: ethyl acetate (70:30) solvent system and linomat 5 sample applicator. Scanning and detection was done by D² lamp at

260 nm (absorbance mode) using high performance thin layer chromatography (HPTLC) (Ravikanth *et al.* 2009).

Pongamol: Dried and powdered feed samples were extracted in methanol at 70°C and estimated using HPTLC against pongamol standard prepared in chloroform. Solvent system used was hexane: ethyl acetate (85:15) and scanning and detection done by D² lamp at 320nm (absorbance mode) using HPTLC.

Trypsin inhibitor activity: Trypsin activity was measured by using N- $\square$ -benzoyl DL-arginine p-nitroanilide (BAPNA) essentially according to the method described by (Erlanger *et al.* 1961) Feed samples were triturated in distilled and resultant extract was used for determination of trypsin inhibitors activity by colorimetric analysis. Benzoyl-DL-arginine paranitroanilide (BAPNA) was used as the substrate for the trypsin activity, which after incubation at 37°C for 35 min with appropriate control, was estimated at 410 nm. One unit of activity corresponds to that amount of trypsin inhibitor in μ g protein which gives 50% inhibitor of enzyme activity is expressed as trypsin inhibitor units (TIU) per g sample.

In vitro evaluation

Tilley and Terry 1-stage *in vitro* assay (Goering and Van Soest 1970) was followed. For studying, fermentative characteristics, incubation was stopped at the end of 24 h. Incubations were performed in a shaking water bath at 39°C in quadruplicate. On the day of incubation, rumen liquor was collected from fistulated crossbred cattle at experimental livestock unit of the institute and strained with muslin cloth into in CO₂ flushed conical flask and brought to the laboratory in thermos flask containing lukewarm water (39°C) immediately. 40 ml Mc Dougall's buffer and 10 ml strained rumen liquor was incubated with 0.5 g of substrate in for 24 h in shaking water bath at 39°C. The substrate (0.5 g) of the substrate was weighed in triplicate in the conical flasks fitted with rubber corks having Bunsen valve like arrangement for releasing excess gas in the *in vitro* flasks. At the end of incubation period of 24 h the contents of the flask were filtered through sintered crucibles and the filtrate was collected in to 50 ml centrifuge tubes. The filtrate was acidified with 2 or 3 drops of conc. H₂SO₄ and preserved in refrigerator till analysis. The residue was thoroughly washed with hot water followed by acetone. The residue was dried in the hot air oven at 100°C and IVDMD was calculated. The residue containing crucibles are weighed and kept in microwave muffle furnace at 600° for 2 h. After ashing, the crucibles are further weighed to determine the IVOMD.

In vitro rumen fermentation parameters

The ammonia nitrogen in the incubation medium was measured using Conway micro diffusion technique (Conway 1957). The TVFA was measured using Markham's apparatus (Barnett and Reid 1957). Total nitrogen and TCA precipitable nitrogen are analyzed further for nitrogen contents (AOAC

1995). The nitrogen degradability was estimated by subtracting residual nitrogen from the total nitrogen present in substrate.

Statistical analysis

Analysis of variance (One-way) was done (Snedecor and Cochran 1994) to find the significance of parameters and Post-hoc Tukey was done to find the pair-wise significance between treatments. The statistical software SPSS 8.0 was used for analysis of data and Microsoft word and excel were used to generated data. Results were expressed as mean $\pm$ SE. The results were considered statistically significant if the P values were 0.05 or less.

RESULTS AND DISCUSSION

Analysis of karanj seeds: The data regarding physical measurements and chemical analysis of karanj seeds was presented in Table 1. The seeds represented different regions of Karnataka and 1 sample was collected from Palakkad, Kerala. The seeds were of different sizes. There appears to be variations in CP content of the seed kernels collected from different regions. We can expect a CP % 22.22 to 35.47 on fat free basis which is a point of interest for animal feeding. The variation in CP and EE content of seeds as the sampling was done from different parts of Karnataka and also from different trees. Karanjin (%) ranged from 0.57 to 1.75 in different seed kernels. Very recently, Vismaya *et al.* (2010) reported 1.90% of karanjin in whole seeds and it reduced to 0.05% upon hexane extraction of *ghani*-pressed cake. The karanjin (%) in expeller seed cake samples contained 0.324 (Panda *et al.* 2006) and 0.19 (Prabhu *et al.* 2002). More quantities of karanjin in seed kernels are primarily due to high oil content in kernels (34.91%). The pongamol (%) was

ranging from 0.25–1.27 in different samples and whereas TIU activity was ranging from 790–1278 μ g / g seed meal in different seed samples. It is evident that these ANF tend to vary in different seed samples, hence, point of interest to animal nutritionists. However, while processing all these ANF have to be completely removed. TIA contents in different cultivars of soybean revealed that there exist a genotype $\times$ cultivar interaction among different varieties of soybean (Vineet Kumar *et al.* 2003 and Manjaya *et al.* 2007).

Chemical composition of concentrate mixtures: The details of the chemical composition are presented in Table 2. The CP% is almost 20 in all the concentrate mixtures (19.965 to 20.053) as all the concentrate mixtures were formulated to be iso-nitrogenous. The fat % ranged from 7.54 to 24.36% in karanj seed meal (KSM) replaced rations (C2 to C5) and 1.48 to 1.83% in karanja defatted seed meal KDM rations (C6 to C9). Higher percentage of EE in concentrate mixtures (C2 to C5) was basically due to more EE in KSM (35.78). The total ash % was ranging from 5.16 to 7.16 KSM (C2 to C5) in KDM (C6 to C9) 6.04 to 7.04. Lower ash content in KSM replaced rations (75%, 100%) was due to low ash content KSM. The Cu and Zn (ppm) was ranging from 5.92 to 13.6 and 72 to 89 respectively. KDM used in the study had low CP compared to literature values (Ravi *et al.* 2000) that resulted in higher levels of inclusion in the concentrate mixtures.

In vitro nutrient digestibility: The data regarding IVDMD, IVOMD and N-degradability are presented in Table 3. The IVDMD after 24 h incubation showed a decreasing trend ($P < 0.05$) when KSM was added as a replacement of soybean cake (C2 to C5) especially at higher replacement levels (from 83.17% in control to 69.25% in 100% KSM concentrate mixture). There is no reduction of IVDMD when KDM was

Table 2. Ingredient and chemical composition (%) of concentrate mixtures

Ingredient	C1	C2	C3	C4	C5	C6	C7	C8	C9
Maize	41.00	23.75	23.20	22.15	11.91	30.00	31.00	29.46	28.87
Soybean cake	26.00	19.50	13.00	6.50	0	19.50	13.00	6.50	0
Wheat bran	30.00	37.00	27.00	17.00	17.37	37.50	33.10	31.0	28.10
KSM*	-	16.50	33.00	50.00	66.00	-	-	-	-
KDM**	-	-	-	-	-	10.00	19.70	29.70	39.50
Urea	-	0.25	0.80	1.35	1.72	-	0.20	0.34	0.53
Mineral mixture	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Salt	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Dry matter	88.04	88.14	89.06	89.99	90.39	88.21	88.96	88.21	88.96
Organic matter	92.82	92.84	93.73	94.4	94.84	92.92	93.24	92.96	93.96
Total ash	7.09	7.16	6.27	5.60	5.16	7.00	6.76	7.04	6.04
Crude fat	2.01	7.54	13.16	18.96	24.36	1.83	1.72	1.60	1.48
Crude fiber	4.24	5.19	5.04	4.91	5.41	4.96	4.90	5.01	5.05
Crude protein	19.997	20.016	19.999	19.970	20.003	20.053	20.006	19.965	19.985
NFE	66.58	59.96	55.54	50.56	45.07	66.08	66.61	66.38	67.44
Cu (ppm)	13.3	12.9	10.4	5.92	6.59	10.9	13.6	12.3	11.7
Zn (ppm)	89	83	76	83	72	83	76	81	78

*KSM, karanja seed meal; **KDM, karanja defatted meal.

Table 3. *In vitro* nutrient digestibility and rumen fermentation characteristics of incubation medium

Concentrate	IVDMD (%)	IVOMD (%)	N- degradability (%)
C 1	83.17 ^{cd} ±1.54	87.98 ^d ±0.80	90.13 ^d ±0.72
C 2	78.87 ^{bc} ±0.82	80.60 ^c ±0.26	83.75 ^b ±0.48
C 3	74.66 ^{abc} ±1.76	75.59 ^b ±1.61	89.50 ^d ±0.50
C 4	70.62 ^{ab} ±0.51	70.48 ^a ±0.07	72.25 ^a ±0.85
C 5	69.25 ^a ±2.39	72.53 ^{ab} ±1.14	85.25 ^{bc} ±0.85
C 6	90.41 ^d ±2.70	90.49 ^d ±0.43	91.00 ^d ±0.41
C 7	81.98 ^{cd} ±4.00	89.74 ^d ±0.36	88.50 ^{cd} ±0.96
C 8	89.44 ^d ±0.29	90.42 ^d ±0.23	89.00 ^{cd} ±1.08
C 9	89.80 ^d ±1.22	91.48 ^d ±0.22	90.00 ^d ±0.91

Values bearing different superscripts in a column differ significantly ($P < 0.05$); each value is an average of 4 observations.

added to the rations (C6 to C9) indicating beneficial effect on digestibility due to removal of oil from seeds (from 83.17% in control to 89.80% in 100% KDM concentrate mixture).

The IVOMD (%) followed the same trend as that of IVDMD. The IVOMD was following a decreasing trend ($P < 0.05$) when KSM was added as a replacement of soybean cake (C2 to C5) especially at higher replacement levels (from 87.98% in control to 70.48% in 75% KSM concentrate mixture). There was no effect of reduction of IVOMD when KDM was added to the rations (C6 to C9) thus having a favorable effect on digestibility due to removal of oil from seeds (from 87.98% in control to 91.48% in 100% KDM concentrate mixture).

N-degradability (%) too followed a similar trend as that IVDMD and IVOMD. It was following a decreasing trend in KSM replaced concentrate mixtures (from 90.13% in control to 70.48% in 75% KSM concentrate mixture). In KDM, the N degradability (%) did not record a decreasing trend (90.13%) in control as compared to 90.00% in 75% KDM concentrate mixture. It is evident from this nitrogen present is KDM highly soluble as that soybean meal under in vitro systems.

In vitro rumen fermentation parameters: The data regarding pH, $\text{NH}_3\text{-N}$, TVFA, TCA-ppt N are presented in Table 4. There was no effect of feeding different concentrate mixtures on pH of the incubation medium (7.07 to 7.28). The $\text{NH}_3\text{-N}$ (mg / 100 incubation medium) was significantly increased from (4.63 to 11.15) in KSM replaced concentrate mixtures. Probably, higher urea levels used in concentrate mixtures (0.25 to 1.72%) might have caused elevated ammonia levels. The rise of ammonia level in KDM replaced concentrate mixtures was not evident (2.40 to 6.00) as levels of urea used was not high (0.20 to 0.53%). On the whole the TVFA concentrations (meq/100 ml incubation medium) ranged from 4.64 in case of C6 to 6.48 in case of C8 indicating

Table 4. *In vitro* nutrient digestibility and rumen fermentation characteristics of incubation medium

Concentrate	pH	$\text{NH}_3\text{-N}$ (mg/100 ml)	TVFA (meq/100 ml)	TCA ppt-N (mg/100 ml)
C 1	7.17±0.04	3.66 ^{ab} ±0.28	4.68 ^a ±0.50	16.46±1.78
C 2	7.14±0.07	4.63 ^{ab} ±0.49	6.40 ^{bc} ±0.10	22.30±0.99
C 3	7.18±0.06	5.83 ^{ab} ±1.58	4.84 ^{abc} ±0.15	15.44±2.21
C 4	7.28±0.04	7.86 ^{bc} ±1.18	5.06 ^{abc} ±0.36	19.11±3.42
C 5	7.21±0.08	11.15 ^c ±1.68	4.73 ^{ab} ±0.09	20.03±1.76
C 6	7.03±0.03	5.49 ^{ab} ±1.31	4.64 ^a ±0.20	24.35±1.72
C 7	7.26±0.06	2.40 ^a ±0.59	5.95 ^{abc} ±0.11	22.64±1.98
C 8	7.21±0.05	3.69 ^{ab} ±0.64	6.46 ^c ±0.21	24.19±1.68
C 9	7.07±0.08	6.00 ^{ab} ±0.89	5.74 ^{abc} ±0.14	24.71±0.60

Values bearing different superscripts in a column differ significantly ($P < 0.05$); each value is an average of 4 observations.

karanja seed meal as well as defatted karanja at all levels of replacement did not exert adverse effect on rumen fermentation compared to control.

The TCA ppt-N is was ranging from (15.44 to 24.71 mg per 100 ml incubation medium) and was found to be same ($P < 0.05$) in all the groups. TCA-ppt-N is a relevant index of net microbial protein synthesis in the ruminant animal is not being affected by different concentrate mixtures. The present study was conducted even though a lot of literature is being published on feeding of raw, expeller and solvent extracted karanj cake (Mandal and Banerjee 1974 a,b; Gupta *et al.* 1981, Konwar *et al.* 1984, 1987, Natanam *et al.* 1989, Srivatsava *et al.* 1990, Ravi *et al.* 2000, Singh *et al.* 2006, Soren *et al.* 2007). The regional variations in proximate composition and ANF contents are being reported first time for this part of country. As such karanja oil is being used in medicinal preparations, hence, it is not appropriate to use expeller karanja cake (having at least 8–10% oil), hence, we have attempted to use KDM after complete removal of oil and a comparison with a KSM having full oil. Literature suggested that karanjin can be a good source of bioactive molecule which has many biological attributes like pesticidal and insecticidal properties (Parmar and Gulati 1969). Tamrarkar *et al.* (2008) proved that compounds 1 and 2 isolated from the chloroform-soluble fraction of the ethanolic extract of *Pongamia pinnata* fruits possesses beneficial effects on the treatment and prevention of hyperglycemia in diabetes mellitus, and that these effects are, at least in part, mediated via the inhibition of PTPase-1B, which is a major mediator of insulin signaling and insulin-resistance

Feeding of expeller karanja cake has resulted in significant reduction of DMD over solvent extracted karanj cake (Srivastava *et al.* 1990 in kids and Ravi *et al.* 2000 in lambs) which implies that complete removal of karanjin would result in big value addition to karanja cake for animal feeding purposes. Further negative effects on CP digestibility were

observed in goat kids by Srivastava *et al.* (1990). Soren and Sastry (2008) did not find any detrimental effect on DMD upon feeding of solvent extracted karanja cake. We had also obtained similar results in under *in vitro* conditions when KSM and KDM are incorporated in concentrate mixtures.

We observed variations in proximate composition, seed measurements, ANF's (karanjin, pongamol and TIA) in the collected seed samples across Karnataka. Results of *in vitro* studies using graded levels of KSM and KDM indicated that IVDMD and IVOMD was following a declining trend at higher levels of KSM inclusion but not observed in KDM based concentrate mixtures. Results further confirmed that earlier animal studies involved with feeding of Karanja seed cake. Keeping in view that great value addition potential of karanjin it is recommended to remove oil completely before attempting detoxification and subsequent animal feeding.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the DBT for sanctioning a research project 'Detoxification and utilization of key agro-forest based non conventional oil cakes in the feeding of livestock (BT/PR9097/AAQ/01/328/2007 dated 13.02.2008), Dr K T Sampath, Director, NIANP for providing support and facilities, Mr Y K Krishnakumar, Deputy Conservator of Forests, Karnataka for providing precise Karanj seed samples, and Ayurved Research Foundation, Baddi, Himachal Pradesh, for estimation of anti nutritional factors in Karanja seed samples.

REFERENCES

- AOAC.1995. Animal Feeds. *Official Methods of Analysis of AOAC International*. 16th edn. Vol.1, Virginia, USA, pp.1–18.
- Barnett A J G and Reid R L. 1957. Studies on volatile fatty acids from grass by rumen liquor in artificial rumen. *Journal of Agriculture Science* **48**: 315.
- Bringi N V.1987. *Non-traditional Oilseeds and Oils in India*. pp. 143–166. Oxford and IBH Publishing Co. Pvt. Ltd. New Delhi.
- Conway E J. 1957. *Micro diffusion Analysis and Volumetric Error*. 4th edn, Cross by Lockwood and sons, London.
- Erlanger B F, Kokowsky N and Cohen W. 1961. The preparation and properties of two new chromogenic substrates of trypsin. *Archives of Biochemistry and Biophysics* **95**: 271.
- Goering H K, Vansoest P J. 1970. Forage fiber analyses (Apparatus, reagents, procedures and some applications). *Agriculture Handbook No.379*. Agriculture Research Service, United State Department of Agriculture.
- Gupta B S, Srivastava J P, Tripathi A K, Verma A K and Thakur S.1981. Biological valuation of karanja (*P. glabra*) cake. *Indian Journal of Animal Health* **6**: 75–77.
- Manjaya J G, Gopalakrishna T, Pawar S E and Bapat V A. 2007. Genetic variability for trypsin inhibitor content in soybean [*Glycine max* (L.) Merrill.] and its correlation with oil and protein. *Indian Journal of Genetics* **67** (1): 51–55.
- Konwar B K, Banerjee G C and Mandal L. 1984. Nutritive value of deoiled karanja cake (*Pongamia glabra* Vent.) in adult cattle. *Indian Journal of Animal Sciences* **54** (5): 489–90.
- Konwar B K, Banerjee G C and Mandal L. 1987. Effect of feeding deoiled karanja (*Pongamia glabra*. Vent.) cake on growing calves. *Indian Veterinary Journal* **64** (5): 399–402.
- Mandal L and Banerjee G C. 1974. Studies on the utilization of karanja (*Pongamia glabra*) oil cake in poultry rations. *Indian Journal of Poultry Science* **9**: 141–47.
- Natanam R, Kadirvel R and Chandrasekeran D. 1989. Chemical composition of karanja (*Pongamia glabra* Vent [*P.Pinnata*]) kernel and cake as animal feed. *Indian Journal of Animal Nutrition* **6**: 270–73.
- National Oilseed and Vegetable Oils Development Board. 1995. *Workshop on Strategies for development of tree-borne oilseeds and niger in tribal areas*. (fide 12th Annual Report 1995–96.)
- Pal D T, Gowda N K S, Prasad C S, Amarnath R, Bharadwaj U, SureshBabu G and Sampath K T. 2010. Effect of copper- and zinc-methionine supplementation on bioavailability, mineral status and tissue concentrations of copper and zinc in ewes. *Journal of Trace Elements in Medicinal and Biology* **24**: 89–94.
- Panda A K, Sastry V R B, Mandal A B, Saha S K and Kumar A. 2006. Quantification of karanjin, tannin and trypsin inhibitors in raw and detoxified expeller and solvent extracted karanj (*Pongamia glabra*) cake. *Asian-Australasian Journal of Animal Sciences* **19**: 1776–83.
- Parmar B S, Gulati K C. 1969. Synergists for pyrethrins (II)-karanjin. *Indian Journal of Entomology* **31**: 239–43.
- Prabhu T M, Deva Kumar C, Sastry V R B and Agarwal D K. 2002. Quantification of karanjin using high performance liquid chromatography in raw and detoxified karanj (*Pongamia glabra* vent) seed cake. *Asian-Australasian Journal of Animal Sciences* **15**: 416–240.
- Ravi U, Singh P, Garg A K and Agrawal D K. 2000. Performance of lambs fed expeller pressed and solvent extracted karanj (*Pongamia pinnata*) oil cake. *Animal Feed Science and Technology* **88**: 121–28.
- Ravikanth K, Thakur M, Singh B and Saxena M. 2009. TLC based method for standardization of *Pongamia pinnata* (Karanj) using karanjin as marker. *Chromatographia* **69**: 597–99.
- Singh P, Sastry V R B, Garg A K, Sharma A K and Agrawal D K. 2006. Effect of long term feeding of expeller pressed and solvent extracted karanj (*Pongamia pinnata*) seed cake on the performance of lambs. *Animal Feed Science and Technology* **126**: 157–68.
- Snedecor G W and Cochran W G.1994. *Statistical Methods*. 8th edn.Iowa State University Press, Ames, Iowa, USA 50010.
- Soren N M, Sastry V R B, Saha S K and Kumar A. 2007. Quantification of karanjin in raw and variously processed karanja (*Pongamia glabra*) cake using high performance liquid chromatography. *Animal Nutrition and Feed Technology* **7**: 261–68.
- Soren N M and Sastry V R B. 2008. Replacement of soybean meal with processed karanj (*Pongamia glabra*) cake on the balances of karanjin and nutrients, as well as microbial protein synthesis in growing lamb. *Animal Feed Science and Technology* **149**: 16–29.
- Srivastava J P, Gupta B S, Thakur S and Verma A K. 1990. Utilisation of deoiled karanj (*Pongamia glabra*) cake in kid grower rations. *Indian Journal of Animal Nutrition* **7**:15–20.
- Tamrakar A, Yadav K, Tiwari P, Priti, Maurya R and Srivastava A K. 2008 Identification of pongamol and karanjin as lead

- compounds with antihyperglycemic activity from *Pongamia pinnata* fruits. *Journal of Ethnopharmacology* **118**: 435–39.
- Vinay B J and Sindhu Kanya T C. 2008 Effect of detoxification on the functional and nutritional quality of proteins of karanja seed meal. *Food Chemistry* **106**: 77–84.
- Vineet K, Anita R, Chanda T and Mukti J. 2003. Lipooxygenase isozymes and trypsin inhibitor activities in soybean as influenced by growing location. *Food Chemistry* **83**: 79–83.
- Vismaya W, Sapna E, Manjunatha J R, Srinivas P, Sindhu Kanya T C. 2010. Extraction and recovery of karanjin: A value addition to karanja (*Pongamia pinnata*) seed oil. *Industrial Crops and Products* **32**: 118–22.

ICAR