

Blood biochemical profile, immune response and rumen fermentation pattern in growing lambs fed processed *karanj* (*Pongamia glabra*) cake based diets

N M SOREN¹, V R B SASTRY², T K GOSWAMI³ and S K SAHA⁴

Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243 122 India

Received: 28 January 2009; Accepted: 25 August 2009

ABSTRACT

Effect of feeding of detoxified *karanj* (*Pongamia glabra*) cake was studied on rumen fermentation, blood biochemical and immune response in lambs. Male lambs (n=24) of body weight (12.9±0.15 kg) were divided into 4 groups and treatment groups were fed on concentrates, containing solvent extracted *karanj* cake (SKC) washed with water (WW), treated with 2.5% lime (LT) and 0.4% binder (BT) to replace 50% CP of soybean meal of control diet for a period of 196 days. Blood was collected at 150 d of feeding. Humoral immune (HI) response was studied by sensitizing lambs with *Brucella abortus* Strain-19 antigen and antibody (Ab) titre was determined at 0 and 21 d. Cell-mediated immune (CMI) response was assessed by intra dermal inoculation of phytohaemagglutinin-P and changes in skin thickness (mm) was measured. Intake of DM, CP and TDN was lower in treatment diets than in control. Body weight was lower in LT and BT than in CON and WW groups. Concentration of haemoglobin, glucose, urea nitrogen, albumin and globulin was similar in all diets but total protein was lower in LT, while cholesterol was higher in BT group. Ab titre was lower in BT group after 21 d. CMI response at 0, 24, 48, 72 h was similar. In rumen liquor drawn on 180 days of experimental feeding pH, total N and ammonia N was identical among the groups but TVFA and TCA-ppt nitrogen were lower in BT group. Thus, water washing and lime treatment of SKC were better than its treatment with binder.

Key words: Blood profile, Immune response, *Karanj* cake, Lambs, Rumen fermentation

Karanj (*Pongamia glabra*) cake is an unconventional feed available in sizeable quantity in the South East Asia. Two varieties of the cake are available depending upon the process of oil extraction, expeller pressed (EKC) and solvent extracted (SKC) *karanj* cake. Its inclusion (EKC) in the diet is restricted due to presence of a furanoflavonoid, karanjin, which is reported to cause harmful effects like loss of appetite, live weight, strong colored micturation, swelling in intermaxillary space and facial muscle in buffalo calves (Gupta *et al.* 1981) when fed as such. However, processing of the cake like physical, chemical and microbiological treatments can remove *karanj* in, the bitter principles associated with poor palatability (Soren *et al.* 2007).

Numerous performance studies are reported in different livestock species, namely, pig, sheep, cattle, goat and poultry

Present address: ¹Scientist, Division of Animal Nutrition, Central Sheep and Wool Research Institute, Avikanagar, Rajasthan 304 501, (E mail: nmsoren@rediffmail.com).

²Formerly Principal Scientist, H.No. 30–276/14/22/1 (Plot no. 22), Beside Mercy Model School., Poolapalli Balayya Road, Safi Nagar, Old Safil Guda, RK Puram Post, Secunderabad, Andhra Pradesh 500 056.

³Senior Scientist, Immunology Section, ⁴Senior Scientist, Centre of Advanced Studies in Animal Nutrition.

(Ravi *et al.* 2000, Prabhu 2002, Soren *et al.* 2009). Feeding of processed *karanj* cake to growing lambs at 225 g/kg in the concentrate did not affect microbial protein synthesis (Soren and Sastry 2009). Scanty reports are available concerning as such feeding of *karanj* cake on rumen fermentation (Ravi *et al.* 2001, Prabhu 2002) and long term feeding of such diet may affect the immunity of the animals rendering them vulnerable to infections. Feeding of protein and energy deficient diet to growing animals (calves) is reported to affect humoral immune response (Griebel *et al.* 1987). Feeding of unconventional feed as protein supplement on immune responses are available mainly on poultry (Panda *et al.* 2004, Panda *et al.* 2005) with few reports in sheep.

Therefore, in present experiment effect of feeding solvent extracted *karanj* cake, treated with different means on rumen fermentation, blood biochemical and immune response in lambs was studied.

MATERIALS AND METHODS

Processing of karanj cake and experimental diet formulation: Ground solvent extracted *karanj* cake (SKC) was detoxified by water washing (1:2; w/v; 24 h), lime (calcium hydroxide; 2.5%, w/w; 24 h) and binder (hydrated sodium calcium alumino silicate; HSCAS; 0.4%; w/w; 24

h) treatment as per Soren *et al.* (2009). The processed cakes were sun-dried, ground and incorporated in the iso-nitrogenous concentrate mixtures, replacing 50% CP of soybean meal (SBM) of control ration.

Feeding management and growth: Non-descript male lambs (24) of uniform body weight (12.9 ± 0.15 kg) were randomly divided into 4 groups of 6 each in a completely randomized design. The lambs were housed in a well ventilated and hygienic barn having facilities for individual feeding and watering and fed for an experimental period of 196 days. They were vaccinated against Peste des petits ruminants (PPR), haemorrhagic septicaemia, sheep pox and enterotoxaemia and were dewormed and drenched with coccidiostat at regular intervals.

The lambs were fed on respective concentrate mixtures between 9:30 and 10:00 h daily to meet their protein requirements (NRC 1985) for maintenance and a daily gain of 100 g. DM requirements were met through *ad lib.* feeding of oat (*Avena sativa*) straw, which was offered after 2 to 3 h of offering concentrate to ensure its maximum consumption. The ration schedule was adjusted individually at fortnightly intervals, depending on their live weight. Fresh drinking water was provided *ad lib.* twice daily. DM intake was assessed based on DM determined in feed offered and refusals at weekly intervals.

Haematological and biochemical profile: Blood was collected in the morning 2 h before feeding at 150 d from all the lambs by puncturing the jugular vein and blood serum was separated by centrifugation. Haemoglobin was assayed by cynmethaemoglobin method (Cook 1985). Serum glucose was determined by GOD (glucose oxidase)/POD (peroxidase) method (Trinder 1969). Total serum protein and albumin were determined as per Doumas (1975) and Doumas *et al.* (1971), respectively. Serum globulin was obtained by subtracting of albumin from total serum protein. Serum urea was determined by diacetyl monoxime (DAM) method (Wybenga *et al.* 1971). Cholesterol in the serum was determined as per Wybenga *et al.* (1970).

Immunological studies

Humoral immune (HI) response: After 150 d of experimental feeding, 5 representative lambs from each group were sensitized subcutaneously with 3 ml of *Brucella abortus* Strain 19 antigen. Prior to sensitization, lambs were screened for brucellosis by using Rose Bengal plate test (Alton *et al.* 1975). Blood (5 ml) was drawn from each sensitized lambs on 0 and 21 d of post sensitization in sterilized glass tubes. The serum samples were stored in clean sterilized micro centrifuge tube at -20°C until they were subjected to serological studies. Humoral immune (HI) response was assessed by indirect enzyme linked immunosorbent assay (ELISA) as per Nielson *et al.* (1987) and absorbance was read at 492 nm.

Cell-mediated immune (CMI) response: At 170 d of

experimental feeding, CMI response of lambs was assessed by delayed-type hypersensitivity (DTH) reaction to intra-dermal inoculation of phytohaemagglutinin-P (PHA-P) as per the procedure of Sevi *et al.* (2001). A stock solution of PHA-P, prepared in phosphate buffer saline at a concentration of 500 $\mu\text{g/ml}$, filtered through membrane filter and suitable volume (0.1 ml) was injected so as to contain 10 $\mu\text{g/site}$ of injection. The skin of the neck region (both sides) with palpable loose skin was shaved 24 h before the injection of mitogen to facilitate subsiding of inflammation due to abrasion, if any. About 1 cm^2 area was encircled with a marker and thickness of the skin was measured with the help of vernier calipers before injection (0 h) 24, 48 and 72 h after injection. Phosphate buffer saline was injected at other side of the neck which served as control. The increase in skin thickness was compared against the control or among the different treatments for comparing the CMI response.

Rumen fermentation pattern: Samples of rumen liquor were collected from 3 representative lambs from each group for 3 consecutive days at 4 h post feeding at 180 d with the help of stomach tube attached to a suction pump. The pH of rumen liquor was recorded immediately after collection. The rumen liquor was filtered through four-layers of muslin cloth to obtain strained rumen liquor (SRL). Each SRL (5 ml) sample was analyzed for total volatile fatty acids (TVFA) (Barnett and Reid 1957), total nitrogen and ammonia nitrogen (Pearson and Smith 1943). Total nitrogen and TCA-precipitable-N fractions were estimated by micro-kjeldahl method.

Chemical analysis: Concentrates and oat straw were analyzed for proximate principles (AOAC 1995) and fibre fractions (Van Soest *et al.* 1991). The total carbohydrate (TCHO) was derived by subtracting the values of CP and EE from organic matter (Sniffen 1988). Calcium and phosphorus were estimated as per Talapatra *et al.* (1940) and AOAC (1995), respectively. The extraction and HPLC determination of anti-nutritional factor, karanjin in the processed cakes and concentrate mixtures was done as per Soren *et al.* (2007).

Statistical analysis: Data on body weight change, nutritional plane, serum biochemical profile, immune response and rumen fermentation were subjected to one-way analysis of variance by a mathematical model of Harvey (1975) as $Y_{ij} = \mu + T_j + e_{ij}$, where, Y_{ij} is the i th observation of the j th treatment, μ is the population mean, T_j the effect j th treatment ($j=1,2,3$, or 4) and e_{ij} the random error. Differences among the means were tested by Duncan's multiple range test (Duncan 1955).

RESULTS AND DISCUSSION

Chemical composition of the diets: The CP, EE and total carbohydrates (TCHO) content of the experimental diets were identical, and higher total ash and P contents in control than other diets was due to inclusion of rice bran (Table 1). An

Table 1. Ingredients (g/kg on as feed basis) and chemical composition (g/kg DM basis) of concentrate mixtures and oats straw

	Diets [‡]				Oats straw
	CON	WW	LT	BT	
Ingredients					
Maize	350	350	350	350	
Soybean meal (solvent extracted)	270	135	135	135	
<i>Karanj</i> cake (processed)	0	225	225	225	
Wheat bran	50	260	267	260	
Rice bran (de-oiled)	300	0	0	0	
Mineral mixture [§]	17.5	17.5	10.5	17.5	
Salt	10	10	10	10	
Commercial mixture	2.5	2.5	2.5	2.5	
Chemical composition					
Organic matter	888.4	917.2	908.4	909.8	930.5
Crude protein	228.2	221.5	223.9	215.7	34.7
Ether extract	12.4	12.5	12.4	12.0	11.3
Total carbohydrates	647.8	683.2	672.1	682.1	884.5
Total ash	111.6	82.8	91.6	90.2	69.5
Calcium	8.2	9.3	10.0	11.0	4.1
Phosphorus	11.5	7.0	6.4	7.3	1.4
NDF	442.2	429.4	418.7	409.1	824.0
ADF	144.0	124.8	119.5	118.6	538.0
Cellulose	93.8	88.7	83.8	83.4	456.6
Hemicellulose	298.2	304.6	299.2	290.5	285.9
Lignin	28.1	24.7	24.2	24.9	48.1
<i>Karanjin</i>	0	0.221	0.214	0.234	-

[‡]Diets: CON, soybean meal (control); WW, water washed SKC; LT, 2.5% lime treated SKC; BT, 0.4% binder treated SKC incorporated concentrate mixtures.

[§]Mineral mixture contained (g/kg): Ca, 280; P, 120; I (as KI) 0.001; Cu, 0.0013.

[†]Commercial mixture contained (per 250 g): Vitamin A- 500,000 IU, vitamin D₃-100,000 IU, vitamin B₂-0.2 g, vitamin E-75 Units, vitamin K-0.1 g, calcium pantothenate- 0.25 g, nicotinamide-1.0 g, vitamin B₃-0.6 mg, choline chloride-15 g, Ca-2.75 g, Mn-2.75 g, I-0.1 g, Fe-0.75 g, Zn- 1.5 g, Cu-0.2 g, Co-0.045 g.

increase in Ca content in LT and BT diets was due to the Ca content of lime and binder. Higher P content in CON diet was due to rice bran inclusion. Other diets contained wheat bran having equal P content.

Body weight and intake of nutrients: Body weight of lambs fed on CON and WW diets was higher ($P<0.05$) than those on LT and BT diets (Table 2) due to growth depressing effect of residual *karanj* in and the observation was similar to the earlier report (Soren *et al.* 2009). Water washing might have removed major portion of *karanj* in and bitter taste (Soren *et al.* 2007) associated with it. DM intake through concentrate as well as oat straw feeding was lower ($P<0.01$) in experimental diets than in control, indicating dietary *karanj* in level as low as 0.214 to 0.234 g/kg reduced palatability of diet in lambs. The intake of oat straw as basal roughage was

Table 2. Intake of nutrients under different processed *karanj* cake incorporated diets

	Diets [‡]				SEM
	CON	WW	LT	BT	
Body weight* (kgW ^{0.75})	8.85 ^a	8.57 ^a	7.68 ^b	7.60 ^b	0.159
Intake (g/kgW ^{0.75} /d)					
Concentrate**	34.93 ^a	29.82 ^b	30.01 ^b	25.38 ^b	1.073
Oat straw**	27.23 ^a	19.36 ^c	21.73 ^{bc}	24.47 ^{ab}	0.983
Total dry matter**	62.16 ^a	49.18 ^b	51.74 ^b	49.85 ^b	1.536
Crude protein**	9.18 ^a	7.64 ^b	7.76 ^b	6.35 ^c	0.301
Digestible crude protein	5.35	4.06	4.13	3.17	0.308
Total digestible nutrients**	32.25 ^a	24.67 ^b	23.81 ^b	23.46 ^b	1.008

[‡]Diets: CON, soybean meal (control); WW, water washed SKC; LT, 2.5% lime treated SKC; BT, 0.4% binder treated SKC incorporated concentrate mixtures.

^{a,b,c} Means bearing different superscripts in a row differ significantly: * $P<0.05$; ** $P<0.01$

also lowered in SKC based diets, thereby giving rise to lower DMI in these groups. Intake of CP (g/kgW^{0.75}/d) was lower ($P<0.01$) in processed SKC incorporated diets *versus* the control. However, digestible CP intake was similar in all the groups. Identical intake of DCP suggests that *karanj* cake inclusion does not affect CP digestibility (Soren *et al.* 2009). Total digestible nutrients (TDN) intake was lower ($P<0.01$) in WW, LT and BT diets in comparison to CON. Bitter taste is the major factor, responsible for lower intake of processed SKC included diets apart from residual *karanj* in. Present finding was in contrast to the report of Konwar *et al.* (1987) with bull calves, Srivastava *et al.* (1990) with kid; Ravi *et al.* (2000) and Prabhu (2002) in lambs, where palatability remained unaffected in their studies, when SKC, untreated or treated, was limited to 150 to 250 g/kg in the concentrate. The intake of DM, CP and TDN was lower in lambs as compared to the stipulated requirement of NRC (1985) for similar body weight and gain with moderate growth rate. This can be attributed to poor palatability of such diets in addition to poor genetic make up of experimental lambs.

Haematological and biochemical profile: Haemoglobin was similar among groups (Table 3) as reported in calves (Konwar and Banerjee 1987) and lambs (Prabhu 2002) on feeding SKC containing concentrate. A similar serum glucose level among groups following the feeding of processed SKC was also recorded (Ravi *et al.* 2001, Prabhu 2002). Serum total protein was lower ($P<0.05$) in lime-treated SKC group than in other groups. The concentration of serum albumin, globulin and albumin: globulin ratio and blood urea were also similar among groups, which is in agreement with the findings of Prabhu (2002). Feeding of processed SKC at 225 g/kg in concentrate to lambs did not cause deleterious effect on microbial protein synthesis (Soren and Sastry 2009). An increase in blood urea level may reflect an accelerated rate of protein catabolism (Kaneko 1980). While reduced level

Table 3. Haematological and serum biochemical profile

	Diets [‡]				SEM
	CON	WW	LT	BT	
Haemoglobin (g/100 ml)	11.24	11.49	10.99	11.09	0.252
Serum glucose (mg/100 ml)	40.29	38.82	37.52	37.88	0.524
Total protein (g/100 ml)*	5.43 ^{ab}	5.42 ^{ab}	5.12 ^b	5.64 ^a	0.067
Albumin (g/100 ml)	3.00	2.76	2.83	2.97	0.057
Globulin (g/100 ml)	2.43	2.66	2.29	2.68	0.068
Albumin to globulin ratio	1.24	1.06	1.26	1.13	0.046
Urea nitrogen (mg/100 ml)	20.51	19.05	19.94	18.10	0.95
Serum cholesterol (mg/100 ml)*	41.90 ^b	41.31 ^b	42.98 ^b	55.95 ^a	2.31

[‡]Diets: CON, soybean meal (control); WW, water washed SKC; LT, 2.5% lime treated SKC; BT, 0.4% binder treated SKC incorporated concentrate mixtures.

^{a, b} Means bearing different superscripts in a row differ significantly: * P<0.05.

is observed in severe hepatic insufficiency and diminished protein intake, and it increases in renal tubular necrosis (Kaysen *et al.* 1985). Increased (P<0.05) serum cholesterol level on BT *vis-à-vis* control and other test diets might be due to stress caused by lower DM intake due to higher residual *karanj* in in BT diet as well as bitter taste (Soren *et al.* 2007) of the cake. Lower body weight and nutrient intake in this group substantiate above finding (Soren *et al.* 2009). The results revealed that cholesterol stored in the lipid droplets of the adipose tissue cells is released into plasma and is the chief source of the hypercholesterolemia observed during stress (Hem Lata *et al.* 2004). However, the values were within the normal physiological range.

Immune response: The seroreactivity in *B. abortus* S19 sensitized lambs at 0 and 21st days post sensitization is shown in Fig. 1. The antibody titre was similar in all the dietary

Table 4. Rumen metabolites in lambs fed different processed *karanj* cake based diets

	Diets [‡]				SEM
	CON	WW	LT	BT	
pH	6.46	6.32	6.48	6.39	0.114
Total volatile fatty acids (mmol/100 ml)*	7.69 ^a	7.52 ^a	6.71 ^{ab}	5.97 ^b	0.460
Total nitrogen (mg/100 ml)	130.66	128.33	129.50	120.17	6.793
Ammonia nitrogen (mg/100 ml)	18.67	17.83	17.73	16.57	1.196
TCA-ppt nitrogen** (mg/100 ml)	82.60 ^a	80.73 ^{ab}	78.40 ^b	71.40 ^c	1.299

[‡]Diets: CON, soybean meal (control); WW, water washed SKC; LT, 2.5% lime treated SKC; BT, 0.4% binder treated SKC incorporated concentrate mixtures.

^{a, b, c} Means bearing different superscripts in a row differ significantly: * P<0.05; ** P<0.01.

treatment groups at 0 d but it was lower (P<0.05) in BT group at 21st days as compared to the control and other groups. This indicated that residual *karanjin* in the processed cake might have induced nutritional stress as evidenced by lowered body weight and suppressed immune response. Similar low seroreactivity levels were also reported by Nagalakshmi *et al.* (2001) in lambs fed raw cotton seed meal (CSM), where gossypol of raw CSM depressed antibody titre.

Cell-mediated immune response (Fig. 2) assessed by increased skin thickness through DTH reaction to PHA-P sensitized lambs were similar at 0, 24, 48 and 72 h among the different dietary treatments. However, within a treatment maximum response (P<0.01) was obtained at 24 h but declined with increase in post sensitization interval. The present findings are in contrary to those of Anil Kumar and Rajan (1987) where CMI response was suppressed in kids

Fig.1. Seroreactivity in lambs sensitized against *Brucella abortus* strain-19 antigen on different processed *karanj* cake incorporated diets. ^{a, b} Means bearing different letters differ significantly, P<0.05.

Fig.2. Delayed-type hypersensitivity response in lambs sensitized by phytohaemagglutinin-P (PHA-P) on different processed *karanj* cake based diets.

by phytohaemagglutinin test at 7 d due to ingestion of aflatoxin at 0.25 mg/kg body weight. Suppression of cellular immunity was also noticed in guinea pigs (McLougulin *et al.* 1984) and bovines (Bodine *et al.* 1984) following aflatoxicosis similar to humoral response.

Rumen fermentation pattern: The pH, total nitrogen and $\text{NH}_3\text{-N}$ concentration in rumen liquor was identical among the groups (Table 4). However, the concentration of TVFA and TCA-ppt-N was lower ($P<0.05$) in LT and BT groups than the others, which was due to lower DM intake as reported earlier by Singh *et al.* (2006), on feeding SKC up to 200 g/kg to lambs in the concentrate mixture. Lower $\text{NH}_3\text{-N}$ concentration (11%) in BT group indicates slower rate of deamination (Ravi *et al.* 2001) of binder processed *karanj* cake in the compound feed. Similar concentration of rumen liquor N and $\text{NH}_3\text{-N}$ indicated that processed SKC provided better quality of protein. Similar TCA-precipitable (TCA-ppt.)-N in control and WW group indicating optimum microbial nitrogen synthesis on these diets as reported earlier under similar dietary regime (Soren and Sastry 2009). The inhibitory effect of residual *karanjin* on the rumen microbes due to feeding of binder treated SKC incorporated diet might have lowered the TCA-ppt. N.

The results indicated that feeding of lime and binder processed *karanj* cake through concentrate mixture to lambs affected the growth, rumen fermentation and caused lowering of serum protein in the former group. Humoral immunity was lowered in lambs fed binder processed SKC incorporated concentrate mixture. However no such adverse effect was evident when water washed *karanj* cake was fed to lambs in the concentrate.

REFERENCES

- Alton G G, Jones L M and Pietz D E. 1975. *Laboratory Techniques in Brucellosis*. 2nd edn. WHO monograph series 55, WHO, Geneva.
- Anil Kumar T V and Rajan A. 1987. Immunopathological response of goats in aflatoxicosis. *Indian Journal of Animal Sciences* **57**: 817–22.
- AOAC. 1995. *Official Methods of Analysis*. 16th edn. Association of Official Analytical Chemists, Washington, DC.
- Barnett A J G and Reid R L. 1957. Studies on the production of volatile fatty acids from grass by rumen liquor in an artificial rumen. 1. The volatile acid production from fresh grass. *Journal of Agricultural Science* **48**: 315–21.
- Bodine A B, Fisher S F and Ganjee S. 1984. Effect of aflatoxin B₁ and major metabolites on phytohaemagglutinin stimulated lymphoblastogenesis of bovine lymphocytes. *Journal of Dairy Science* **67**: 110–14.
- Cook J D. 1985. *Measurement of Iron Status*. A report of the International Nutritional Anaemia Consultative Group. New York. Washington DC. p. 4.
- Doumas B T, Watson W A and Biggs H G. 1971. Albumin standards and the measurement of serum albumin with bromocresol green. *Clinica Chimica Acta* **31**: 87–97.
- Doumas B T. 1975. Standards for total serum protein assays- A collaborative study. *Clinical Chemistry* **21**: 1159–66.
- Duncan D B. 1955. Multiple range and multiple F tests. *Biometrics* **11**: 1–42.
- Griebel P J, Schoonderwoerd M and Babiuk L A. 1987. Ontogeny of the immune response: effect of protein energy malnutrition in neonatal calves. *Canadian Journal of Veterinary Research* **51**: 428–35.
- Gupta B S, Srivastava J P, Tripathi A K, Verma A K and Thakur S. 1981. Biological evaluation of *karanj* (*Pongamia glabra*) cake. *Indian Journal of Animal Health* **6**: 75–77.
- Harvey W R. 1975. *Least Square Analysis*. United States Department of Agriculture Research Services (ARS), Washington, DC, USA.
- Hem Lata, Ahuja G K, Narang A P S and Walia L. 2004. Effect of immobilization stress on lipid peroxidation and lipid profile in rabbits. *Indian Journal of Clinical Biochemistry* **19**: 1–4.
- Kaneko J J. 1980. *Clinical Biochemistry of Domestic Animals*. 3rd edn. Academic Press. Inc. Orlando, Florida USA.
- Kaysen G A, Pond S M and Roger M H. 1985. Combined hepatic and renal injury in alcohols during therapeutic use of acetaminophen. *Archives International Medicine* **145**: 2019–23.
- Konwar B K and Banerjee G C. 1987. Effect of feeding deoiled *karanj* cake on blood, urine and body composition of cross-bred calves. *Indian Journal of Animal Nutrition* **4**: 120–23.
- Konwar B K, Banerjee G C and Mandal L. 1987. Effect of feeding deoiled *karanj* (*P. glabra*) cake on growing calves. *Indian Veterinary Journal* **64**: 399–402.
- McLougulin M E, Pier A C and Thurston J R. 1984. Use of bacteria and yeast to identify T lymphocytes in peripheral blood and lymphoid tissue of healthy guinea pigs and guinea pigs fed aflatoxin. *American Journal of Veterinary Research* **45**: 1795–99.
- Nagalakshmi D, Sastry V R B, Agrawal D K and Katiyar R C. 2001. Haematological and immunological response in lambs fed on raw and variously processed cottonseed meal. *Asian Australasian Journal of Animal Sciences* **14**: 21–29.
- Nielson K, Wright P F, Cherwonogrodzky J, Duncan J R and Stemshorn B. 1987. Enzyme immunoassay for diagnosis of bovine brucellosis. *Annales de l'Institut Pasteur* **138**: 75–79.
- NRC. 1985. *Nutrient Requirements of Sheep*. National Academy Press. Washington, D.C.
- Panda A K, Sastry V R B and Mandal A B. 2005. Growth performance, humoral immune response and carcass characteristics of broiler chickens fed alkali processed *karanj* cake incorporated diet supplemented with methionine. *Asian Australasian Journal of Animal Sciences* **18**: 677–81.
- Panda A K, Sastry V R B, Mandal A B, Saha S K and Kumar A. 2004. Effect of dietary incorporation of raw and processed *karanj* cake (*Pongamia glabra*) on nutrient retention and immune competence of broiler chickens. *Animal Nutrition and Feed Technology* **4**: 121–34.
- Pearson R M and Smith J A B. 1943. The utilization of urea in the bovine rumen. 3. The synthesis and breakdown of protein in rumen digesta. *Biochemical Journal* **37**: 153–59.
- Prabhu T M. 2002. 'Clinico nutritional studies in lambs fed raw and detoxified *karanj* (*P. glabra* vent) meal as protein supplement.' Ph.D. Thesis. Indian Veterinary Research Institute, Izatnagar, India.
- 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.
- Ravi U, Singh P, Garg A K, Verma A K and Agrawal D K. 2001. Effect of feeding expeller pressed and solvent extracted *karanj* cake on blood and rumen parameters in growing lambs. *Indian Journal of Animal Sciences* **71**: 183–85.
- Sevi A, Annicchiarico G, Albenzio M, Taibi L, Muscio A and Dell'aquila S. 2001. Effect of solar radiation and feeding time on behavior, immune response and production of lactating ewes under high ambient temperature. *Journal of Dairy Science* **84**: 629–40.
- 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.
- Sniffen C J. 1988. *Proceedings of Application of Nutrition in Dairy Practice*. American Cyanamide, Wayne, New Zealand.
- Soren N M and Sastry V R B. 2009. Replacement of soybean meal with processed *karanj* (*Pongamia glabra*) cake on the balances of karanjin and nutrients, as well as microbial protein synthesis in growing lamb. *Animal Feed Science and Technology* **149**: 16–29.
- Soren N M, Sastry V R B, Saha S K and Kumar A. 2007. Quantification of karanjin in raw and variously processed expeller pressed *karanj* (*Pongamia glabra*) cake using high performance liquid chromatography. *Animal Nutrition and Feed Technology* **7**: 261–68.
- Soren N M, Sastry V R B, Saha S K, Wankhade U D, Lade M H and Kumar A. 2009. Performance of growing lambs fed processed *karanj* (*Pongamia glabra*) oil seed cake as partial protein supplement to soybean meal. *Journal of Animal Physiology and Animal Nutrition* **93**: 237–44.
- Srivastava J P, Gupta B S, Thakur S and Verma A K. 1990. Utilization of deoiled *karanj* (*Pongamia glabra*) cake in kid grower rations. *Indian Journal of Animal Nutrition* **7**: 15–20.
- Talapatra S K, Roy S C and Sen K C. 1940. Estimation of phosphorus, chlorine, calcium, magnesium, sodium and potassium in food stuffs. *Indian Journal of Veterinary Science and Animal Husbandry* **10**: 243–58.
- Trinder P. 1969. Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen. *Journal of Clinical Pathology* **22**: 158–61.
- Van Soest P J, Robertson J B and Lewis B A. 1991. Methods for dietary fibre, neutral detergent fibre and non starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* **74**: 3583–97.
- Wybenga D R, Giorgio J D and Pileggi V J. 1971. Manual and automated methods for urea nitrogen measurement in whole serum. *Clinical Chemistry* **17**: 891–95.
- Wybenga D R, Pileggi V J, Dirstine P H and Giorgio J D. 1970. Direct manual determination of serum total cholesterol with a single stable reagent. *Clinical Chemistry* **16**: 980–84.