



Original Research Article

Biochemical and histopathological changes in sheep fed different detoxified karanj (*Pongamia glabra*) seed cake as partial protein supplementsNira M. Soren^{a, b, *}, Anil K. Sharma^c, Vadali R.B. Sastry^a^a Division of Animal Nutrition, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly 243 122, Uttar Pradesh, India^b Animal Nutrition Division, ICAR-National Institute of Animal Nutrition and Physiology, Adugodi, Bangalore 560030, Karnataka, India^c Division of Pathology, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly 243 122, Uttar Pradesh, India

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ABSTRACT

The study investigated the long-term effect of feeding processed solvent extracted karanj (*Pongamia glabra*) cake (SKC) on gross pathology and histopathological changes in some vital organs, and on the activities of serum enzymes in Jalauni lambs. Twenty-four male lambs were divided into 4 groups and allotted randomly to a soybean meal (SBM) based control (CON) and 3 treatment groups receiving concentrate mixtures, containing water washed (WW), 2.5% lime (LM) and 0.4% binder (BN) treated SKC replacing 50% nitrogen of SBM to meet the protein requirements. Blood was collected after 150 days from all the lambs and serum activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) were assayed. All lambs were sacrificed after 196 days, and vital organs screened for gross pathological lesions. The representative tissues of liver, intestine, parathyroid gland, testis, and epididymis were sampled, preserved in 10% formalin and processed to examine histopathological changes by staining with haematoxylin and eosin (HE). The serum enzyme activities of AST and ALT were similar in the treatments, but the activity of LDH was higher ($P < 0.01$) in processed SKC-fed groups than the control. The weight and size of the liver were decreased in BN group, while those of testes were decreased in both LM and BN groups. The histological sections of the testes and epididymis of lambs from LM group showed hypoplastic seminiferous tubules and empty ductules, respectively. The histological sections of the parathyroid gland in the same diet revealed smaller and non-secretory chief cells. The small intestine of lambs from BN group showed infiltration of mononuclear cells (MNC) in lamina propria with mild fibroplasia in intervillous areas. The histological section of liver from this group showed MNC infiltration in portal areas. The inclusion of water washed SKC in the concentrate mixture of lambs did not show gross pathological and histological alterations in the tissues in the vital organs; however, the activity of LDH was significantly ($P = 0.001$) elevated in processed SKC-fed groups than the control. Thus, feeding of water washed SKC in the concentrate at 225 g/kg for a longer period do not cause any adverse effect in lambs. This is supported by normal activities of serum enzymes and intact histological features in the tissues of liver, intestine, parathyroid gland and testis.

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1. Introduction

The paucity of protein-rich feedstuffs for animals coupled with increasing cost of conventional feedstuffs in developing countries like India has compelled animal nutritionists to look for alternatives proteinaceous feeds, namely, agro-forest based industrial by-products as protein sources. In recent years, the search for alternate fuel like biodiesel has been extensively carried out in different countries to find alternatives to petroleum products. In India karanj (*Pongamia* sp.), jatropha (*Jatropha curcas*) and many other non-

edible oils have been evaluated for diesel fuel extender (Raheman and Phadatare, 2003). Briefly, the procedure involved in biodiesel production from the oilseed includes extraction of oil from the seeds. The oil, in turn, is converted into methyl esters by the transesterification process and the methyl ester thus formed during the process is known as biodiesel. Biodiesel is a renewable source of energy, which can be manufactured locally by our farmers by growing oilseed producing plants on their wastelands and barren land, which are also eco-friendly. Karanj (*Pongamia* sp.) belongs to the family Leguminosae which is a prominent species having non-edible oilseed and grows easily in existing wastelands. In India, about 0.93×10^6 ha of wastelands are covered with *Pongamia* trees in 8 states (Doshi and Srivastava, 2013). The yield of oilseed per tree is between 8 and 24 kg. The typical oilseed contains 30% to 33% oil (Padhi and Singh, 2011). Karanj (*Pongamia glabra*) seed cake is one such by-product available in large quantity after extraction of karanj oil from its seeds. Despite rich in protein, it cannot be completely incorporated into the diet of animals due to the presence of an anti-nutritional factor, karanjin. However, processing can, to some extent, reduce its toxic level (Soren et al., 2007).

Growth studies conducted so far with the inclusion of raw oil seed cake in the concentrate mixtures in different species of live-stock have not proven to be fruitful due to poor palatability of the karanj cake or meal. Feeding of raw karanj cake to buffaloes even at 4% level led to the development of toxic symptoms like loss of appetite and weight, frequent and strong coloured micturition, swelling of intermaxillary spaces and facial muscles, discolouration of skin and loss of hair, watery to thick sticky lacrimation and gangrene of the tail followed by sloughing (Gupta et al., 1981). However, feeding of processed solvent extracted karanj cake (SKC) to lambs showed no effect on growth and nutrient digestibility (Soren et al., 2009; Soren and Sastry, 2009). Karanj balance study (Soren and Sastry, 2009) in growing lambs demonstrated that residual karanjin is retained inside the body in small quantity even after its excretion from the body through faeces and urine. Thus, the presence of residual karanjin in the body may affect some vital organs and alter their morphology. Few pathomorphological studies are available on karanj cake feeding to sheep (Dineshkumar et al., 2013; Krishnamoorthy et al., 2014). In the present experiment, different detoxification methods were adopted to remove the residual toxins present in the SKC, and these cakes were incorporated in the concentrate to replace 50% of the soybean meal nitrogen. The study evaluated the effect of feeding detoxified SKC on the activities of serum enzymes and the gross and histopathological changes in the vital organs of lambs due to residual toxins, if any, left back after detoxification.

2. Material and methods

2.1. Feed processing and diet formulation

Solvent extracted karanj cake was detoxified by adopting 3 processing methods, namely water washing and treatments with lime [$\text{Ca}(\text{OH})_2$] and binder (hydroxyl sodium calcium aluminosilicate [HSCAS]). For water washing treatment, SKC was soaked in water (1:2; wt/vol) in a plastic tub. The supernatant from water soaked and the intermittently stirred cakes were siphoned off after 24 h, followed by washing with the same quantity of water twice, and they were sun dried on a polythene sheet. Lime and binder treatments involved soaking of SKC in water (1:1, wt/vol) containing 2.5% $\text{Ca}(\text{OH})_2$ (wt/wt) and 0.4% HSCAS (wt/wt). The homogeneously mixed cakes were kept in air-tight plastic tub and sun dried after 24 h on a polythene sheet.

Four isonitrogenous and isocaloric concentrate mixtures were formulated (Table 1). The SBM served as the sole source of protein

in the concentrate mixture of control diet (CON), while the test concentrate mixtures contained different processed SKC, namely water washed SKC (WW), and 2.5% lime [$\text{Ca}(\text{OH})_2$] treated SKC (LM) and 0.4% binder (HSCAS) treated SKC (BN) replacing 50% nitrogen moiety of SBM present in control concentrate mixture. The chemical composition and fibre fractions of different concentrate mixtures CON, WW, LM and BN and oats straw are presented in Table 1. The crude protein content of the concentrates was similar and ranged from 215.7 to 228.2 g/kg DM. Other chemical constituents of the respective concentrate mixtures were also comparable.

2.2. Experimental animals and housing

Twenty-four male lambs of the indigenous Jalauni breed of uniform body weight (12.9 ± 0.15 kg) and age (4 months) were randomly divided into 4 groups with 6 lambs in each group in a completely randomized design, and each lamb served as the experimental unit. The lambs were kept in a well-ventilated shed with facilities for individual housing, feeding and watering in separate troughs. All the lambs were reared under hygienic and uniform managerial conditions throughout the 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 a coccidiostat at regular intervals. The Animal Ethical Committee of Indian Veterinary Research Institute approved the experiment protocol.

2.3. Feeding regimen

All the lambs were fed individually with measured quantities of their respective concentrate mixtures between 09:30 and 10:00 to

Table 1

Ingredients (g/kg as fed basis) and chemical composition (g/kg on DM basis) of concentrate mixtures¹ and oats straw.

Item	CON	WW	LM	BN	Oats straw
Ingredients					
Maize	350	350	350	350	
Soybean meal	270	135	135	135	
Karanj cake (processed)	0	225	225	225	
Wheat bran	50	260	267	260	
Rice bran	300	0	0	0	
Mineral mixture ²	20	20	13	20	
NaCl	10	10	10	10	
Added Supplevit-M ³ (250 g/100 kg)					
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
Neutral detergent fibre	442.2	429.4	418.7	409.1	824.0
Acid detergent fibre	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
Acid detergent lignin	28.1	24.7	24.2	24.9	48.1

¹ Concentrate mixtures contained: CON – (control) soybean meal, WW – water washed solvent extracted karanj cake (SKC), LM – 2.5% lime [$\text{Ca}(\text{OH})_2$] treated SKC, BN – 0.4% binder (hydroxyl sodium calcium aluminosilicate [HSCAS]) treated SKC.

² Mineral mixture contained (per kg): calcium, 280 g; phosphorus, 120 g; iodine (as potassium iodide), 0.001 g; copper, 0.0013 g.

³ Supplevit-M 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; calcium, 2.75 g; manganese, 2.75 g; iodine, 0.1 g; iron, 0.75 g; zinc, 1.5 g; copper, 0.2 g; cobalt, 0.045 g.

meet total protein requirements for maintenance and an expected daily gain of 100 g as per NRC (1985). The rest of the requirements were met through *ad libitum* feeding of oat (*Avena sativa*) straw, which was provided after 2 to 3 h of offering concentrate in order to ensure its maximum consumption. The rationing schedule was adjusted individually based upon the body weights and growth rates recorded at fortnightly intervals. Fresh water was provided *ad libitum*.

2.4. Chemical analysis

Proximate principles viz. crude protein (code 984.13), ether extracts (code 920.39); crude fibre (code 962.09) and ash (code 942.05) of the concentrate mixtures and oats straw were determined by the methods of AOAC (1995). Fibre fractions (neutral detergent fibre, acid detergent fibre and acid detergent lignin) in feed samples were determined as per Van Soest et al. (1991). Calcium in the concentrate mixtures and oat straw was determined as per the method of Talapatra et al. (1940).

2.5. Serum enzyme profile

Blood was collected after 150 days of experimental feeding from all the lambs by puncturing the jugular vein with the help of a clean sterilized needle into 10 mL sterilized clean glass test tubes, which were kept in slanted position at room temperature for approximately 30 min. Later on, the clot was gently removed from the sides of the tube by trimming it with a thin glass rod. The collected serum was then centrifuged at $832 \times g$ for 10 min and transferred to sterile microcentrifuge tubes, which were deep frozen (-20°C) for estimation of serum enzymes later on.

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were estimated (Reitman and Frankel, 1957) and expressed as IU/L. Serum lactate dehydrogenase (LDH) was determined as per the method of Keiding et al. (1974).

2.6. Pathomorphology

After the end of 196 days of feeding, all the lambs ($n = 24$) from each group were slaughtered by the halal method and details of carcass sampling, and measurement was done as per Soren et al. (2008). The vital organs, including liver, heart, lungs, spleen, kidney, testes and intestines were subjected to the examination for gross pathological lesions. The representative tissues ($n = 6$) of these vital organs were fixed in 10% formal saline and later washed thoroughly under running tap water for several hours, dehydrated in ascending grades of alcohol, cleared in turpentine oil and embedded in paraffin (at 60°C) blocks (Iyer, 1984). Sections of 4 to 5 μm thickness was prepared from tissue blocks with the help of microtome and stained with haematoxylin and eosin (Culling, 1963). The stained sections were examined for histopathological lesions, if any, due to the feeding of processed SKC and compared with the control. For histopathological studies, 2 technical replicates from each tissue were examined microscopically.

2.7. Statistical analysis

The data on serum enzymatic profile was subjected to one-way analysis of variance by using SPSS (2008) software. The statistical model of Harvey (1975) was followed: $Y_{ijk} = \mu + T_i + B_k + e_{ijk}$, where Y_{ijk} is the dependent variable (metabolic body weight, intake of DM and CP, AST, ALT and LDH, organ weight of liver, heart, kidney, testis, spleen), μ is the overall mean, T_i the effect of i th treatment ($i = 1, 2, 3, 4$) and j th observation, B_k the random effect

(body weight), e_{ijk} is the random error. Treatment means were separated using Duncan's multiple range test (Duncan, 1955). A P -value of less than 0.05 was accepted to indicate statistical significance.

3. Results

3.1. Live weight and dry matter intake

The metabolic BW was significantly ($P = 0.001$) lower in LM and BN groups than the control, and WW group after 196 days of experimental feeding (Table 2). The dry matter and crude protein intake of lambs were also lower ($P = 0.001, 0.003$) in processed SKC fed groups than the control.

3.2. Serum enzymatic profile

No significant difference in AST activity was observed among lambs fed different diets (Table 2). Serum ALT activity in the serum was also similar across the dietary treatments. The activity of LDH varied significantly ($P < 0.01$) among the lambs fed different diets. The LDH activity ranged from 332.24 to 467.13 IU/L and was higher ($P < 0.01$) in the BN group and the lowest for the control group (Table 2).

3.3. Gross pathology

No gross pathological lesions were seen in any of the vital organs of the lambs fed SBM-based control diet and processed SKC based test diets. However, the weight of liver of lambs fed BN treated SKC based test diet was significantly ($P = 0.017$) lower than control and other groups. The weight of the testis was lower ($P = 0.029$) in LM and BN groups than the CON and WW groups. However, the weight of heart, spleen, and kidney was similar across the groups (Table 2).

3.4. Histopathology

3.4.1. Control group

The sections from liver, lung, heart, brain, spleen, adrenal, pancreas, thyroid, skeletal muscles, intestine, and lymph nodes revealed organ-specific normal histological features. Histological section of the testes revealed morphologically normal with orderly arranged spermatogonial cells and adequate spermatogenesis as an evident from the presence of spermatids and spermatozoa in a good number. The epididymal ductules were dilated, and their lumina contained a large number of spermatozoa (Fig. 1). The section of the parathyroid gland revealed regular histological features with the chief cells showing adequate activities (Fig. 2). The intestinal section also revealed normal villi and intact superficial epithelium. In the same group, the histological section of the liver showed normal hepatocytes and distinct central vein with radiating hepatic cords.

3.4.2. Water washed solvent extracted karanj cake fed group

Histologically, liver, brain, heart, spleen, pancreas, parathyroid, intestine, testis and epididymis did not show any pathological alterations, and the histological structures were comparable to those of control.

3.4.3. Lime treated solvent extracted karanj cake fed group

Histologically, in this group, no significant alterations were seen in lung, heart, spleen, adrenal, thyroid, pancreas, skeletal muscle, intestine and lymph nodes. In 5 out of 6 animals from this group, seminiferous tubules revealed a variable degree of degeneration

Table 2
Body weight, serum enzymes activity and organ weight of lambs.¹

Item	CON	WW	LM	BN	SEM	P-value
Metabolic BW, kgW ^{0.75}	8.85 ^a	8.57 ^a	7.68 ^b	7.60 ^b	0.159	0.001
DM intake, g/kgW ^{0.75}	62.17 ^a	49.18 ^b	51.74 ^b	49.85 ^b	1.536	0.001
CP intake, g/kgW ^{0.75}	9.18 ^a	7.64 ^b	7.76 ^b	6.35 ^b	0.301	0.003
Serum enzymes						
AST, IU/L	61.37	60.20	63.05	63.74	0.94	0.567
ALT, IU/L	36.82	35.78	36.57	38.30	2.812	0.934
LDH, IU/L	332.24 ^c	396.75 ^b	410.55 ^b	467.13 ^a	18.491	0.001
Organ weight, g						
Liver	281.5 ^a	256.3 ^a	266 ^a	216.8 ^b	8.01	0.017
Heart	74.5	70.0	67.2	60.5	2.25	0.189
Kidney	68.0	66.8	59.6	54.3	2.10	0.064
Testis	135.5 ^a	127.0 ^{ab}	78.4 ^{bc}	63.0 ^c	10.87	0.029
Spleen	31.8	31.5	23.6	24.0	1.77	0.180

AST = aspartate amino transferase; ALT = alanine amino transferase; LDH = lactate dehydrogenase.

^{a,b,c} Means with different superscripts in a row differ significantly ($P < 0.05$).

¹ CON – (control) soybean meal, WW – water washed solvent extracted karanj cake (SKC), LM – 2.5% lime [Ca(OH)₂] treated SKC, BN – 0.4% binder (hydroxyl sodium calcium aluminosilicate [HSCAS]) treated SKC.

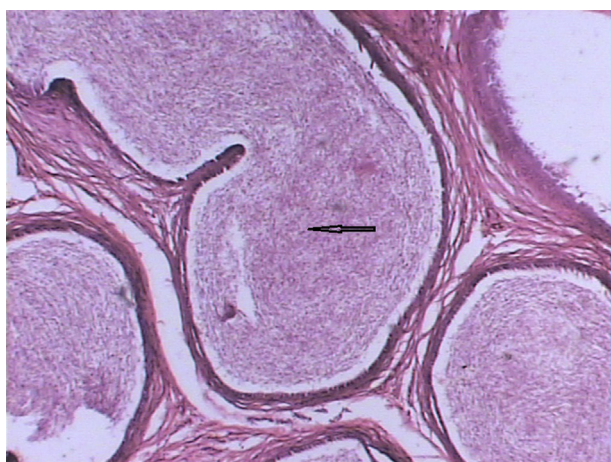


Fig. 1. Normal epididymis showing dilated tubules with a mass of spermatozoa in the lumen on the control diet. Haematoxylin and eosin ($\times 100$).

and disappearance of spermatogonial cells with little or no spermatogenesis (Fig. 3). The epididymal ductules in 2 cases showed the presence of a comparatively low number of spermatozoa and in 3 cases, no spermatozoa (Fig. 4). The chief cells of parathyroid in 2

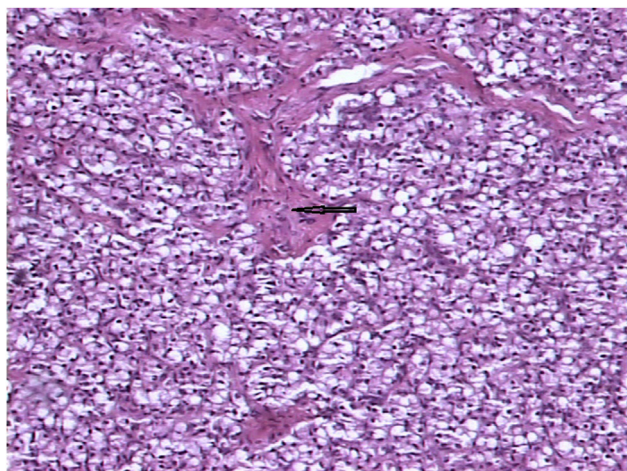


Fig. 2. Normal chief cells of parathyroid showing secretory activity on the control diet. Haematoxylin and eosin ($\times 100$).

cases appeared smaller and non-secretory when compared with that of the reference group (Fig. 5).

3.4.4. Binder treated solvent extracted karanj cake fed group

No appreciable changes were noted in brain, spleen, adrenal, pancreas, thyroid, skeletal muscles. However, few isolated cases revealed minor histological changes in kidney, lung, heart and mesenteric lymph nodes. The significant histological changes were recorded in liver, intestine and testis and epididymis. Four out of 6 cases revealed mild to moderate mononuclear cell (MNC) infiltration in portal areas. Blood vessels (central vein and portal vein) were engorged (Fig. 6). The hepatocytes variably degenerated. Five cases from this diet showed testicular degeneration with little or no spermatogenesis. Histological features of the testes in this group showed fewer spermatogonial cells, which were seen isolated in the lumen of the tubules. Epididymal ductules were empty due to the absence of spermatozoa. Lining epithelium was normal. Variable degrees of periductular fibrosis were seen.

Four out of 5 cases showed moderate to severe infiltration of MNC in the intestinal villi which comprised of mainly of lymphocytes and plasma cells in lamina propria with mild fibroplasia in intervillous areas (Fig. 7). The superficial mucosa also showed desquamation.

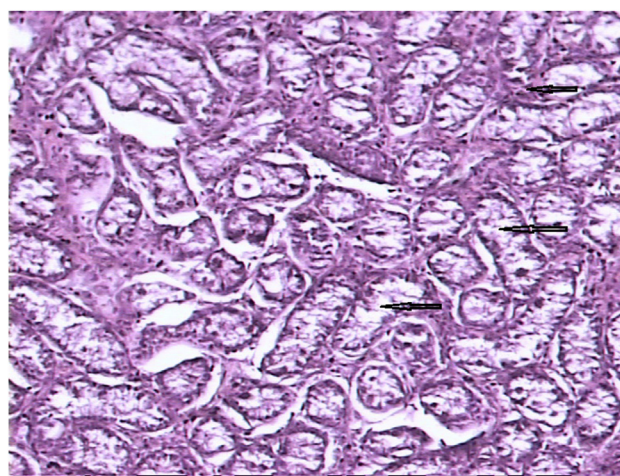


Fig. 3. Testis showing small collapsed hypoplastic seminiferous tubules with the presence of spermatogonial cell on Ca(OH)₂ treated solvent extracted karanj cake (SKC) diet. Haematoxylin and eosin ($\times 100$).



Fig. 4. Epididymis showing empty ductules with periductular fibrosis on Ca(OH)_2 treated solvent extracted karanj cake (SKC) diet. Haematoxylin and eosin ($\times 100$).

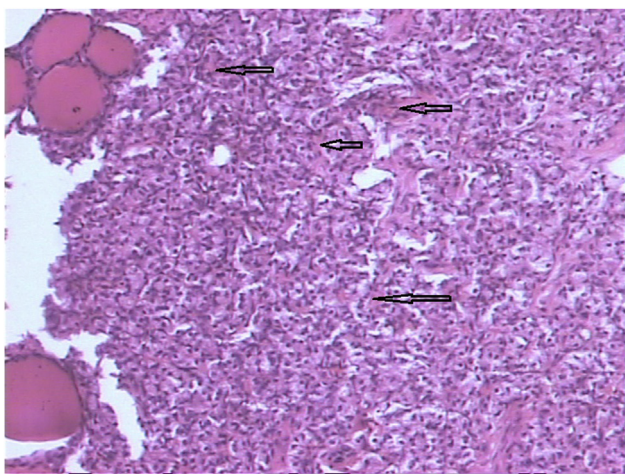


Fig. 5. Chief cells of parathyroid gland are small and inactive on Ca(OH)_2 treated solvent extracted karanj cake (SKC) diet. Haematoxylin and eosin ($\times 100$).

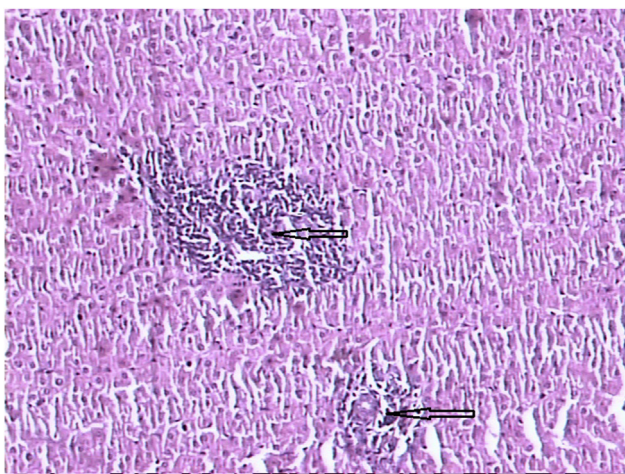


Fig. 6. Liver showing mononuclear cells (MNC) infiltration in portal areas on hydroxyl sodium calcium aluminosilicate (HSCAS) treated solvent extracted karanj cake (SKC) diet. Haematoxylin and eosin ($\times 100$).

4. Discussion

4.1. Dry matter intake and live weight

The dry matter intake (DMI) in processed SKC-fed group of lambs was lower than the SBM based control diet-fed group. The lower intake in these groups may be due to the bitter and pungent taste of residual karanjin, pongamol, glabrin, which may be present in lower concentration even after processing of the cake (Krishnamoorthy et al., 2014). Significant reductions in DMI have been reported earlier by replacing de-oiled groundnut cake with SKC at 265.3 g/kg in the ration of growing crossbred bulls (Konwar and Banerjee, 1987). Similar was the finding when detoxified karanj cake was included at a higher level (290 g/kg) in lambs (Krishnamoorthy et al., 2014). Contrary to our finding, Srivastava et al. (1990) did not notice any difference in DMI of kids when DGNC nitrogen was replaced by de-oiled karanj cake at 400 g/kg. Ravi et al. (2000) also reported similar observation in growing lambs when karanj cake was incorporated at a moderate level. The drop in DMI was the attributing factor for lower CP intake in all the processed karanj cake based diet fed groups. Lower body weight of lambs in LM and BN groups revealed the growth-depressing effect of karanj cake, which was mainly due to lower DMI and other nutrients, mainly the protein intake. Akin growth depression was demonstrated in lambs (Dineshkumar et al., 2013) when detoxified karanj cake replaced 75% of the soybean meal protein. Our study further indicated that simple water washing treatment of SKC at 1:2 ratio before incorporating in the concentrate would be a better means of processing the cake for feeding to animals as the body weight of lambs in WW and the control groups was similar.

4.2. Serum enzymatic profile

The serum AST activity present in both cytoplasmic and mitochondrial enzyme is released by even mild degenerative changes that occur in acute and occasionally in chronic liver diseases, but remarkably higher values have been recorded in muscle damage (Pensent, 1983; Petrie, 1987). On the other hand, ALT activity is a specific indicator of liver damages in primates, dogs, cats, rabbits and rats, but in the tissues of pig, horse, cattle, sheep or goat, it will be too low to be of diagnostic value (Evans, 1988). Increased ALT activity has been reported by feeding of unconventional feeds (solvent extracted mahua cake; *Madhuca longifolia*) in sheep and

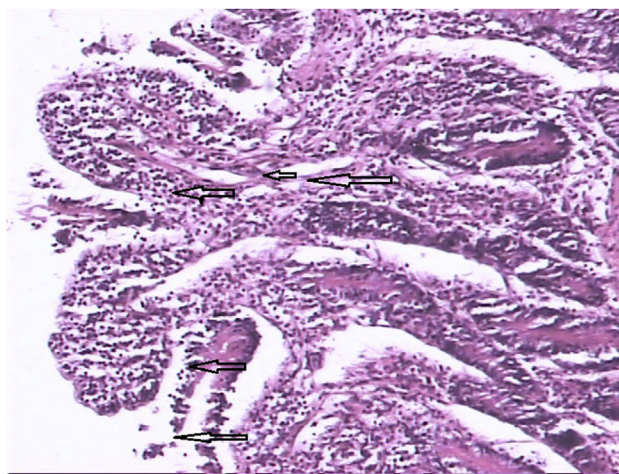


Fig. 7. Intense infiltration of mononuclear cells (MNC) at the tip of intestinal villi with the loss of superficial epithelium on hydroxyl sodium calcium aluminosilicate (HSCAS) treated solvent extracted karanj cake (SKC) diet. Haematoxylin and eosin ($\times 100$).

deoled sal (*Shorea robusta*) seed meal in lambs (Singh, 1987; Garg, 1989). However, the activity of both AST and ALT in the current study was found to be similar in the lambs fed different diets, indicating that level (225 g/kg) of incorporation in the concentrate mixture after processing of SKC was safe and did not induce any tissue damage. The result of the present study confirmed the earlier findings in sheep and lambs (Ravi et al., 2000; Prabhu, 2002). Singh et al. (2006) also reported a significant increase in both AST and ALT activities in the serum of sheep fed either with 200 g/kg SKC or 240 g/kg expeller pressed karanj cake (EKC) in the concentrate mixture for 280 days. However, the LDH activity was significantly higher in processed SKC fed groups, especially in BN group, which was beyond the normal physiological range indicating the interference of normal functioning of the liver due to residual karanjin. Enhanced LDH activity in the serum of binder treated SKC incorporated diet fed group can be correlated with significant histological changes in the liver tissue caused by mild to moderate MNC infiltration in the portal areas in the present study.

In contrast to the above findings, lower LDH activity in lambs fed concentrate containing either EKC or SKC, which replaced 50% of groundnut cake (GNC) nitrogen was reported by Sastry et al. (2000). Elevated LDH activities are generally encountered in the liver disease like acute hepatitis and cirrhosis, renal disease and muscular dystrophy. Elevated LDH activity in the serum of BN group of lambs can be related to histopathological changes in the liver in the same group where moderate infiltration of MNC was observed in portal areas of liver section (Fig. 6).

4.3. Gross pathology

While most of the organs (heart, kidney, and spleen) did not exhibit gross pathological lesions, but significantly lower liver weight in BN group is suggestive of marked hepatic changes for this group. This was clearly demonstrated by the altered histological structure of the liver in this group. The liver is the main organ for the detoxification of anti-nutritive compound present in the feed. The presence of residual karanjin in the binder treated karanj cake affected the liver tissues and caused moderate MNC infiltration in portal areas with engorged blood vessels. Lower testes weight of lambs in LM and BN groups may have severe reproductive implication in these groups. The histopathological section of the testis in LM and BN groups revealed an alteration in the normal histological features of the testis. Changes like the degeneration of the seminiferous tubules with the presence of a fewer number of spermatogonial cells may affect the reproductive performance when such rams are used in the breeding purpose. Contrary to our finding, Dineshkumar et al. (2013) did not notice any changes in the testicular weight of rams when detoxified karanj cake (DKC) was included at a higher level (18% and 29%) in the concentrate. However, the spermatozoa concentration was reduced when DKC was included at 29% in the concentrate. However, the histological section of the testis of lambs fed 18% DKC in the concentrate mixture showed edema in intertubular space with moderate damage to the seminiferous tubular cells (Dineshkumar et al., 2013), while feeding of DKC at higher level (29%) in the same experiment showed severe damage to seminiferous tubular epithelial cells with loss of interstitial cells.

Among the processed SKC fed group, the lambs fed water washed SKC in the concentrate revealed no such altered histological features in any of the lambs from this group. This indicated that water washing of SKC could remove most of the incriminating factors present in the SKC. However, the excretion of residual karanjin from the body of sheep was also reported earlier and was mainly through the feces and to a smaller extent through the urine (Soren and Sastry, 2009), which indicated that small quantity of

residual karanjin present in the body may incite damage to different tissues of the body, mainly the liver and testis.

4.4. Histopathology

Feeding of expeller pressed karanj cake has been reported to depress feed intake, cause histopathological changes within the tissues of vital organs and toxicity in different livestock species. The concentrate mixture containing 4% EKC was found to be unpalatable to buffalo calves and the animals developed toxic symptoms like loss of appetite and weight, frequent and strong coloured micturition, swelling in intermaxillary space and facial muscles, discolouration of skin and loss of hair, watery to sticky lacrimation and gangrene of the tail followed by its sloughing (Gupta et al., 1981).

Pathomorphological studies involving karanj cake feeding are available for sheep as well as for poultry. A long-term study of 280 days involving karanj cake feeding revealed no gross pathological lesion in the vital organs of sheep (Annual Report, 2000). However, karanj cake feeding interfered with normal spermatogenesis due to testicular degeneration in the same study. Similar histopathological changes were reported in the testis of sheep (Singh et al., 2006) when karanj cake was incorporated up to 240 g/kg in the concentrate mixture. In their study, karanj cake feeding (either EKC or SKC) in the concentrate interfered with normal spermatogenesis and resulted in testicular degeneration caused by altered histological architecture. However, in our study, similar features were observed only in sheep of LM and BN groups but absent in WW group of sheep. This was due to lower residual karanjin content in water washed SKC containing concentrate mixture.

In contrast to the above findings, Prabhu (2002) did not observe any gross or histopathological lesions in the tissues of vital organs of lambs fed a diet containing SKC replacing nitrogen moiety of SBM at either 25% or 50% in the concentrate mixture for 180 days. However, in the same study, the group fed 8.25% SKC, and 16.50% processed SKC showed lesions in the muscular tissue like loss of striations, thin and increased intermyofibrillar space due to edema in the tissue.

Feeding of EKC to poultry showed histopathological lesions in kidney, liver, and spleen of broiler birds fed 25% of raw or processed EKC (Panda et al., 2008). In their study, histological section of the liver showed hepatic degeneration with distortion, kidney revealed tubular degeneration with necrotic lesions, spleen showed degeneration with necrotic foci and depletion of lymphocytes. While birds fed processed SKC showed degenerative changes of testicular follicles and vacuolation indicating the effect on spermatogenesis. In another experiment feeding of raw and processed SKC (with or without methionine supplementation) based diets to broiler chicken did not reveal any significant changes in lungs, spleen, kidney, pancreas, skeletal muscle and brain, and the histological architecture was comparable to histology of the respective organs of birds of reference diet (Panda, 2004). However, in the same study liver revealed mild degeneration of hepatocytes, which appeared to be swollen with granular cytoplasm in the birds fed diet incorporated with 40% alkali treated SKC without methionine supplementation. Singh et al. (2006) in their study reported mild degenerative changes with fatty infiltration in the liver of sheep with either EKC or SKC incorporation in the concentrate mixture.

5. Conclusions

The use of lime for detoxification and its effective level should be determined as it decreases the activity of chief cells in the parathyroid in the present study. Further, lesions in the testis and epididymis in BN and LM groups suggests that such lambs might

not be suitable for the breeding purpose. Among the various processing methods tried for detoxification of SKC, water washing was found to be promising as its incorporation in the concentrate mixture replacing up to 50% of SBM nitrogen had no adverse effect on the gross and histopathological architecture of vital organs, with normal activities of AST, ALT, and LDH.

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