Haematological and Immunological Response in Lambs Fed on Raw and Variously Processed Cottonseed Meal

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ABSTRACT : An experiment was conducted with twenty crossbred male lambs to assess the effect of cotton (*Gossypium*) seed meal (CSM) on blood constituents and immunity. Lambs were randomly assigned to a reference diet (30% deoiled peanut meal, DPNM) and four test diets containing 40% of either raw, 45 minutes cooked, 1% $Ca(OH)_2$ and iron (1 free gossypol, FG : 0.3 Fe) treated CSM (replacing approximately 50%, reference concentrate mixture). These isonitrogenous and isocaloric concentrate mixtures were fed to meet 80% of protein requirements (NRC, 1985) along with *ad lib* maize hay for 180 days. Blood was collected at 60, 120 and 180 days post feeding. The lambs were sensitized with *Brucella abortus* S_{99} antigen after 140 days and were subjected to ELISA and delayed type hypersensitivity. Blood haemoglobin, erythrocyte count, leucocyte count, total protein, total albumin, total globulin, urea, creatinine concentration and aspartate aminotransferase activity in lambs fed on raw or processed CSM were comparable to the values of reference lambs. The higher ($p<0.01$) blood glucose levels observed in CSM fed lambs at 60 days of feeding was latter reduced to the levels comparable with those on reference diet at 120 and 180 days of feeding. The alanine amino transferase activity was lower in lambs fed raw and cooked CSM containing diets at 120 and 180 days of feeding. A marginal increase in serum iron and alkaline posphatase activity was observed in iron treated group and raw CSM fed lambs, respectively. The humoral immune response and DTH reactivity was lower ($p<0.05$) in lambs fed raw CSM (consuming 302.83 mg FG/day). Cooking, $Ca(OH)_2$ and iron treatment of raw CSM showed a positive response in alleviating the suppression of immune response owing to the reduced consumption of FG by 40.19, 17.40% and 26.73%, respectively in these diets. The present study thus indicated that consumption of 40% raw CSM (302.83 mg FG/day) though did not affect majority of the haematological and blood biochemical parameters, but markedly suppressed the immune mechanism of lambs. (*Asian-Aust. J. Anim. Sci. 2001. Vol. 14, No. 1 : 21-29*)

Key Words : Gossypol, Lambs, Blood, Immunity

INTRODUCTION

Inadequate availability of energy and protein rich ingredients is the major constraint for improving the productivity of livestock in developing countries. Nutritionists are continually searching for alternative unconventional agro-industrial by-products to be included in the livestock rations. One among them is cotton (*Gossypium*) seed meal (CSM), a by-product of cotton oil and cotton fibre industry. India ranks third in cottonseed production (FAO, 1997) with an annual production of 5430 million tonnes. Crushing of all seeds leads to an availability of 4300 metric tonnes of undecorticated CSM, containing about 25-30% CP. However, the use of CSM in non-ruminants (Clawson et al., 1961; Haschek et al., 1989; Suryawanshi et al., 1993), small ruminants (Morgan et al., 1988; East et al., 1994), young dairy calves (Rogers et al., 1975) and in some instances in mature ruminants (Lindsey et al., 1980) resulted in growth depression, mortality and adverse physiological effect mainly due to yellow polyphenolic pigment, gossypol. Hemoglobin (Hb) and hematocrit were found to be inversely related to gossypol content of the ration (Braham et al., 1967). Similarly, Lindsey et al. (1980) observed depressed Hb and elevated total plasma protein by 9th week in lactating cows fed solvent extracted CSM incorporated rations supplying 3.6 to 24.2 g free gossypol (FG) /day. Also a sharp reduction in immuno-biological reactivity was reported by Rogozhin et al. (1986) in pigs fed rations containing 20% CSM.

Several detoxifying procedures like extraction with various solvents, lysine supplementation, addition of calcium and iron salts, soaking, cooking etc were tried in the past to remove FG in CSM. Solvents like acetone, hexane, isopropanol were sucessful in reducing the gossypol of CSM to quite low levels, but the recovery of these solvents was difficult and not economical. A reduction of about 44% of FG was observed when CSM was cooked for 10 minutes in boiling water before mixing in swine diets (Jarquin et al., 1966). Metabolic ions of calcium and iron were also found effective in gossypol detoxification. Addition of 0.5% calcium hydroxide ($Ca(OH)_2$) to 42% CSM containing rations reduced the FG content by 25% (Braham et al., 1967) and a reduction of 54% of FG was reported by Shah et al. (1986) when CSM

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was treated with 1% $Ca(OH)_2$. Addition of 1, 1-2 and 4 parts of iron for each part of FG in the diets was found effective in alleviating the suppressing effect in swine (Tanksley and Knabe, 1981), broilers and layers (Waldroup, 1981), respectively. The present investigations were therefore conducted to evaluate the effect of feeding raw and variously processed CSM on haematological, blood biochemical constituents and the immune status of lambs.

MATERIALS AND METHODS

Processing of CSM

The CSM used in the present study contained 28.74% CP, 26.94% CF, 0.57% total gossypol (TG) and 0.27% FG. The three processing methods tried for detoxification of CSM were cooking for 45 min, 1.0% $Ca(OH)_2$ and ferrous sulfate (1 FG: 0.3 Fe) treatments. Cooked CSM was obtained after cooking the meal for 45 minutes in boiling water (100°C) in the ratio of 1:1.5 (w/v). The $Ca(OH)_2$ treated CSM was prepared by soaking the meal for 24 hours in water (w/v; 1:1) having $Ca(OH)_2$ @ 1% (w/w of meal). The iron treated CSM was prepared by soaking the meal in water (1:1, w/v) in which calculated amount of $FeSO_4$ $7H_2O$ was dissolved for 30 minutes so as to contain 0.3 parts of iron for each part of FG present in CSM. These variously processed CSM were then Sun dried and ground. The FG content was reduced to 0.20, 0.19 and 0.21%, respectively after cooking, $Ca(OH)_2$ and iron treatments.

Animals and management

Twenty healthy, crossbred male lambs between 3 and 4 months of age, were blocked to 5 groups in a completely randomised design. All the lambs were vaccinated against peste de petis in ruminants after keeping them in quarantine for 15 days.

The lambs were sheltered in a well-ventilated stall having facilities for individual feeding as well as watering and were raised under strict hygienic and uniform managerial conditions. They were vaccinated against sheep pox and haemorrahagic septicaemia before the onset of rainy season and were dewormed with an antihelmintic, fenbendazole (5 mg/kg body weight) at monthly intervals till the end of experimental period of 180 days.

Feeds and feeding

The lambs were fed for maintenance and an expected daily gain of 100 g as per the requirements of NRC (1985). Each group of lambs was fed one of the five isocaloric and isonitrogenous concentrate mixtures once in morning so as to meet 80% of total protein requirements, rest of the requirements was met by feeding *ad libitum* maize hay. The reference diet constituted 30% deoiled peanut meal (DPNM), while the test diets contained 40% of either raw (RCSM), cooked (CoCSM), calcium hydroxide (CaCSM) or iron treated (FeCSM) CSM to replace approximately 50% nitrogen moiety of reference concentrate mixture (table 1). Lambs had free access to water. The daily DM and FG intake of lambs on different diets are shown

Table 1. Dietary ingredients and chemical composition (%) of concentrate mixtures* and maize hay

Attribute	DPNM	Cottonseed meal				Maize hay
		Raw	Cooked	$Ca(OH)_2$ treated	Iron treated	
Ingredient (% air dry feed)						
Maize	30.0	30.0	34.0	34.0	32.0	
Deoiled peanut meal	30.0	15.0	15.5	14.0	15.5	
Raw CSM	-	40.0	-	-	-	
Cooked CSM	-	-	40.0	-	-	
Calcium hydroxide treated CSM	-	-	-	40.0	-	
Iron treated CSM	-	-	-	-	40.0	
Wheat bran	37.0	12.0	7.5	9.0	9.5	
Mineral mixture	2.0	2.0	2.0	2.0	2.0	
Common salt	1.0	1.0	1.0	1.0	1.0	
Chemical composition (% DM basis)						
Crude protein	22.66	23.60	23.29	22.06	23.74	7.57
Ether extract	2.18	1.64	1.99	1.26	1.55	1.26
Crude fibre	6.63	13.77	13.88	11.80	13.84	28.10
Total ash	7.67	7.52	7.62	8.29	7.75	10.04
Gross energy (kcal/kg)	4.73	4.66	4.63	4.50	4.73	4.60
TDN[a]	65.70	65.58	65.63	65.71	65.70	-

* Vitablend AD_3/100 kg was added to concentrate to provide 3000 IU vitamin A and 300 IU vitamin D_3 per kg.

[a] Calculated values.

Table 2. Intake of dry matter and free gossypol

Attribute	DPNM	Cottonseed meal				SEM
		Raw	Cooked	$Ca(OH)_2$ treated	Iron treated	
Concentrate (g/day)	244.06^c	280.53^b	302.96^a	281.05^b	236.41^c	5.372
Total drymatter (g/day)	449.74^c	543.39^b	601.63^a	524.34^b	448.80^c	12.585
Free gossypole (mg/day)	-	302.83	215.10	250.13	222.22	-

Means with different superscripts in a row differ significantly. $p<0.05$.

in table 2.

Blood sampling

Blood was collected thrice during the experimentation i.e. at 60, 120 and 180 days of feeding. Jugular blood was collected into two 5 ml bottles, one containing ethylene diamine tetra acetate for haematology and other having sodium fluoride for blood glucose estimation. Blood was also collected into sterilized glass tubes and the clotted blood was then centrifuged at 3000 rpm for 10 minutes. The resultant was utilized for estimation of various bio-chemical constituents, which included serum iron, total protein, total albumin, total globulin, blood urea, serum creatinine, alkaline phosphatase, alanine amino transferase and aspartate amino transferase.

Sensitization of lambs

The effect of gossypol on immune response of lambs was assessed using *Brucella abortus* S_{99} as an antigen. Lambs were screened for Brucellosis with help of Rose Bengal Plate Test (Alton et al., 1975) prior to sensitization. After 135 days of feeding lambs were sensitized with 2 mg protein of heat killed *B. abortus* S_{99} equally mixed with Freunds incomplete adjuvant. The antigen was administered subcutaneously. A booster was again given on day 4th by the same route.

Five ml of blood was collected from sensitized lambs on 0, 4, 8, 15, 22, 28, and 35th day of post sensitization and serum was separated to assess the seroreactivity by indirect ELISA.

Delayed type hypersensitivity (DTH)

DTH was performed in post-sensitized lambs at every 10 days interval, i.e. at 10, 20, 30, and 40th day to assess alterations in cell mediated immune (CMI) response as per the method of Chukwu (1986). Antigen at dose rate of 100 μg protein in phosphate buffer saline (PBS) was administered intradermally on skin fold of cervical region to sensitized lambs. Similarly a set with sterile PBS was also conducted as a negative control. Skin reaction was read at 24 and 48 hours of antigen inoculation in terms of diameter of skin induration in millimeters.

Analytical studies

The total gossypol (TG) and FG contents were estimated as per the procedure described by Botsoglou and Kufidis (1990) and Botsoglou (1991), respectively. Total erythrocyte count (TEC) was determined on the same day as per standard conventional procedure (Jain, 1986) and haemoglobin (Hb) as per the sahle's acid haematocrit method (Benjamin, 1985). Blood glucose was determined by Toludine method (Cooper and Daniel, 1970). Serum was analysed for alkaline phosphatase (Bergmeyer, 1974), aspartate amino transferase (AST) and alanine amino transminase (ALT) (Reitman and Frankel, 1957), urea nitrogen (Rahmatullah and Boyde, 1980), creatinine (Bones and Tausskey, 1945), iron by Ferozine method (Tietz, 1976), protein by Biuret method (Hiller et al., 1927) and serum globulin was analysed by first precipitation of albumin by ammonium sulphate and then determination of globulin in supernatant by Biuret method.

ELISA was performed as per the procedure described by Perlman and Engvall (1971). The protein concentration of autoclaved *B. abortus* S_{99} antigen was determined by Lowry's method (Lowry et al., 1951). The dose of the above antigen was determined by checkerboard analysis. Accordingly, the protein concentration of antigen was fixed at 40 μg/ml and serum samples were diluted to 1:400. The optical density and hence the seroreactivity was measured at 450 nm in Anthos Labtec ELISA reader.

Statistical analysis

The data was subjected to the test of significance as per methods of Snedecor and Cochran (1967).

RESULTS

The data pertaining to haematological and biochemical constituents of blood from lambs on various diets at 60 (period I), 120 (period II) and 180 (period III) days of feeding are detailed in table 3 and 4, respectively. Incorporation of either processed or unprocessed CSM did not influence the Hb, TEC in blood, urea and creatinine in serum. The iron treated CSM feeding increased the serum iron levels

Table 3. Blood haematological profile

Constituent	Haemogobin (g/L)	Total erythrocyte count ($10^6/\mu$ l)	Total leucocyte count ($10^3/\mu$ l)
Diet			
DPNM	84.38±5.625	9.57±0.369	10.68±0.938
Raw CSM	82.08±1.893	9.73±0.448	10.10±0.770
Cooked CSM	78.46±1.854	9.28±0.530	11.48±1.444
$Ca(OH)_2$ CSM	80.21±2.688	8.91±0.441	10.40±0.926
Iron CSM	78.33±3.710	9.99±0.453	9.01±0.910
Critical difference	8.2280	1.5049	3.4962
Period			
I	74.95 ± 2.259^{b}	9.43±0.339	10.16±0.832
II	85.75 ± 3.355^{a}	9.45±0.416	10.29±0.760
III	81.38 ± 1.445^{ab}	9.59±0.293	10.54±0.617
Critical difference	6.7629**	1.0124	2.0324

Means with different superscripts in a sub column differ significantly. p<0.01.

Table 4. Blood haematological profile

Constituent	Serum Iron (μ mol/L)	Total protein (g/L)	Total albumin (g/L)	Total globulin (g/L)	Blood urea (m mol/L)	Serum creatinine (μ mol/L)
Diet						
DPNM	32.96±1.733	55.06±2.664	27.57±1.239	27.49±2.169	5.57±0.481	68.93±7.901
Raw CSM	33.07±1.551	53.99±2.321	27.95±1.457	26.04±1.469	6.11±0.657	67.90±8.134
Cooked CSM	32.98±2.424	55.87±1.825	30.88±1.131	24.97±1.831	5.80±0.514	63.49±5.772
$Ca(OH)_2$ CSM	33.24±2.985	55.82±1.581	31.52±1.249	24.29±1.541	5.86±0.465	59.88±6.316
Iron CSM	39.12±3.488	55.36±2.426	31.18±1.847	24.18±2.538	5.74±0.482	64.38±7.434
Critical difference	6.5262	8.8793	4.8786	7.8044	1.4553	19.0725
Period						
I	$37.07^{a}\pm0.990$	55.17±0.793	29.68±0.723	$25.49^{ab}\pm1.053$	$7.54^{a}\pm0.187$	$91.73^{a}\pm3.668$
II	$25.69^{b}\pm0.988$	58.21±0.906	29.58±0.752	$28.64^{a}\pm1.335$	$5.74^{b}\pm0.298$	$53.17^{b}\pm2.745$
III	$40.06^{a}\pm2.039$	52.27±2.470	30.21±1.672	$22.05^{b}\pm1.679$	$4.17^{c}\pm0.242$	$49.85^{b}\pm3.594$
Critical difference	3.8623**	4.4840	3.1349	3.0237**	0.6124**	6.8806**

Means with different superscripts in a sub column differ significantly. p<0.01.

Table 5. Blood glucose concentration (mmol/L)

Constituent	DPNM	Cottonseed meal			
		Raw	Cooked	$Ca(OH)_2$ treated	Iron treated
Period I	$3.52^{dy}\pm0.177$	$7.21^{bx}\pm0.516$	$5.38^{cx}\pm0.377$	$6.16^{bcx}\pm0.450$	$9.17^{ax}\pm0.236$
Period II	$3.22^{dy}\pm0.588$	$3.48^{dy}\pm0.386$	$3.21^{dy}\pm0.406$	$4.09^{dy}\pm0.116$	$4.08^{dy}\pm0.532$
Period III	$3.49^{dy}\pm0.425$	$3.99^{dy}\pm0.570$	$3.94^{dy}\pm0.250$	$3.75^{dy}\pm0.125$	$3.52^{dy}\pm0.296$

^{a,b,c,d} Means with different superscripts in a row differ significantly. p<0.01.

^{x,y,z} Means with different superscripts in a column differ significantly. p<0.01.

Diet × period. p<0.01.

marginally. The blood urea levels irrespective of diet, decreased with advancement of age. Similarly, serum creatinine concentration decreased (p<0.05) after 60 days of feeding. The total protein and globulin concentration in serum did not differ significantly between DPNM and CSM fed lambs. The serum protein remained constant throughout 180 days of feeding. The serum globulin levels decreased significantly (p<0.01) after 120 days of feeding. The CSM feeding significantly (p<0.01) increased the blood glucose levels in lambs at 60 days of feeding highest (p<0.05) being observed when iron treated CSM was incorporated in their diets (table 5). The significantly (p<0.05) higher blood glucose level at 60 days of

feeding declined in later stages (120 and 180 days) of feeding.

The alkaline phosphatase and AST was found comparable between lambs fed DPNM and CSM containing diets (table 6). An increase in alkaline phosphatase activity was observed at 180 days of feeding. Significant ($p<0.05$) interaction was noticed between diets and periods for ALT activity (table 7). This enzyme activity was lower ($p<0.01$) in raw and cooked CSM fed lambs in comparison to other group of lambs at 180 days of feeding.

The seroreactive antibody levels as assessed by indirect ELISA in *B. abortus* S_{99} sensitized lambs fed reference and test diets rose from 4th day onward till 22nd day (figure 1). The seroreactive levels at 0 day were comparable and ranged between 0.140 and 0.170. On day 8 and day 35, the seroreactivity against administered *Brucella* antigen in reference diet fed lambs were significantly ($p<0.05$) higher than in CSM fed lambs.

The maximum DTH response was attained by 20th day in lambs on various diets, which thereafter declined in CSM fed lambs (figure 2 and 3). The cell-mediated immune response was higher after 24 hours of antigen administration on all diets of testing as compared to the response after 48 hours. The DTH response was poor in CSM fed lambs than that fed reference diet. Among the CSM fed lambs, the CMI response was at times better among the lambs on either cooked or $Ca(OH)_2$ treated CSM than in raw CSM fed lambs.

Table 6. Serum enzymatic activities (IU/L)

Constituent	Alkaline phosphates	Serum aspartate amino transferase
Diet		
DPNM	179.76±25.43	100.81±5.401
Raw CSM	307.10±50.202	79.72±4.810
Cooked CSM	222.99±29.629	76.67±5.892
$Ca(OH)_2$ CSM	215.97±44.121	88.08±5.616
Iron CSM	134.10±23.536	89.08±7.738
Critical difference	128.9597	24.221
Period		
I	182.82 ± 18.922^{b}	88.15±6.956
II	173.08 ± 22.785^{b}	84.52±3.010
III	280.04 ± 39.261^{a}	87.95±3.916
Critical difference	58.2214*	14.3689

Means with different superscripts in a sub column differ significant ($p \leq 0.05$).

DISCUSSION

Incorporation of raw and processed CSM in diets

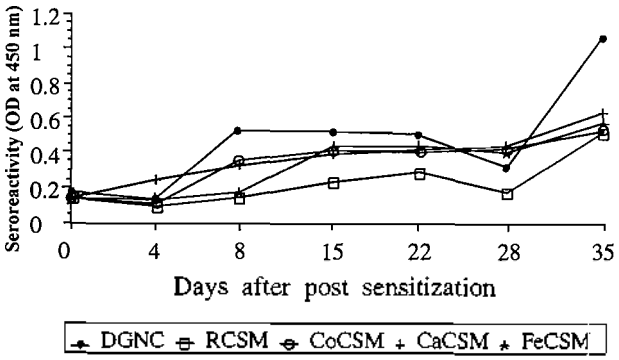

Figure 1. Seroreactivity by ELISA in *B. abortus* sensitized lambs at different days of post sensitization

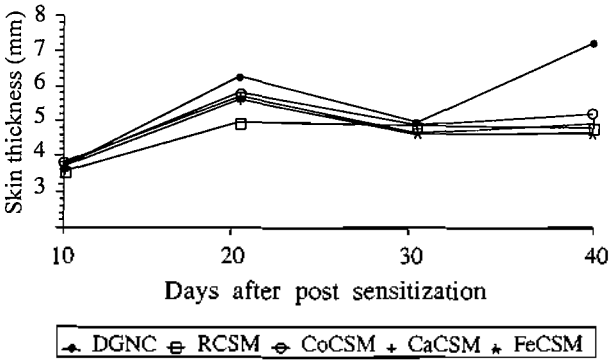

Figure 2. DTH reactivity after 24 hours in *B. abortus* sensitized lambs at different days of post sensitization

Table 7. Serum alanine amino transferase (ALT/SGPT) activity (IU/L)

Constituent	DPNM	Cottonseed meal			
		Raw	Cooked	$Ca(OH)_2$ treated	Iron treated
Period I	$45.12^{ax}\pm1.983$	$36.12^{abx}\pm6.814$	$32.62^{abx}\pm2.004$	$28.25^{bxy}\pm2.287$	$33.75^{abx}\pm6.273$
Period II	$46.12^{ax}\pm4.557$	$27.15^{bcxy}\pm6.843$	$32.18^{bcx}\pm1.957$	$22.54^{cy}\pm3.465$	$37.62^{abx}\pm6.965$
Period III	$44.75^{ax}\pm3.449$	$18.00^{by}\pm1.472$	$20.75^{bx}\pm1.371$	$35.88^{ax}\pm3.880$	$39.50^{ax}\pm4.364$

^{a,b,c,d} Means with different superscripts in a row differ significantly. $p<0.05$.

^{x,y} Means with different superscripts in a column differ significantly. $p<0.05$.

Diet × Period. $p<0.05$.

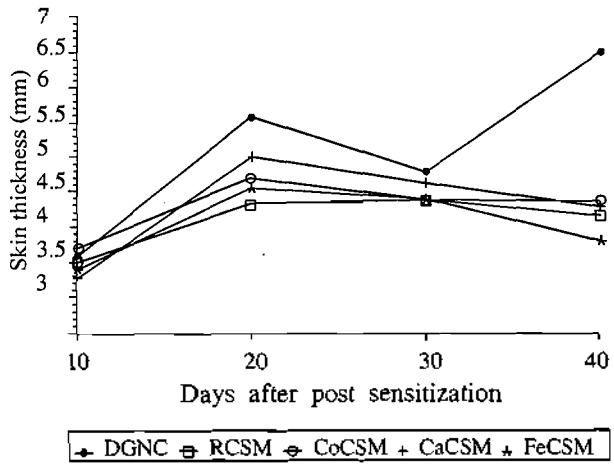

Figure 3. DTH reactivity after 48 h in *B. abortus* sensitized lambs at different days of post sensitization

of lambs did not impart any depressing effect on blood Hb concentration and TEC and were within the normal range (Ullrey et al., 1965) (table 3). Decreased Hb and TEC are symptomatic of gossypol toxicity. Such effects were reported by various workers in lactating cows (Lindsey et al., 1980), pigs (Jarquin et al., 1966; Braham et al., 1967) and wethers (Warren et al., 1988) fed 45% solvent extracted CSM, 42% raw CSM and 50% whole cottonseed (WCS) incorporated diets, respectively. Similar to the present findings Nikokyris et al. (1991) could not observe any effect on Hb in lambs when fed diets incorporated with CSM up to 30%. The lambs fed raw CSM diet consumed 302.8 mg FG daily (table 2), whereas the FG intake was reduced to 215.10, 250.13 and 222.22 mg/day when the CSM processed with cooking, $Ca(OH)_2$ and iron treatment were included in their rations, respectively. Lactating cows in the studies of Lindsey et al. (1980) consumed 42.7 mg FG/kg BW/day when fed 45% solvent extracted CSM, which was much higher than the consumption by the lambs in present study, fed raw and processed CSM (ranging between 12.89 to 20.29 mg FG/kg b.wt./day). The lower levels of FG consumed by these lambs in the present study might have been insufficient to exert such depressing effect on Hb and TEC.

Dietary gosspol reduces iron absorption and retention (Herman and Smith, 1973) and there is a possibility that gossypol in CSM containing rations may precipitate iron deficiency anaemia by binding with dietary iron. But such effect of FG present in raw and processed CSM was not observed in present study, as indicated by serum iron levels (table 4) which were within the normal physiological limits of 29.7 to 39.7 μmol/l as suggested by Kaneko (1980). Inspite of much higher intakes of iron by lambs fed diets incorporated with iron treated CSM (containing 889 ppm Fe), the serum iron levels in this group did not attain statistical significance because the iron in excess of its requirement was deposited as ferritin and haemosedrin in liver, spleen, kidneys and intestines as indicated in their histopathological sections in studies of Nagalakshmi et al. (2000).

The dietary variation did not significantly influence the concentration of serum total proteins in lambs, which ranged from 53.99 to 55.87 g/L (table 4). Similar to these findings, the lamb diets containing 30% CSM did not adversely affect the total protein concentration in plasma which were 42.6, 55.3 and 52.79 g/L at 0, 30 and 60 days of feeding, respectively (Nikokyris et al., 1991). Such alterations were also not observed in pigs fed raw CSM based diets or even after addition of 1% $Ca(OH)_2$ or 0.1% $FeSO_4$ $7H_2O$ to these diets up to 12 weeks (Jarquin et al., 1966). On the other hand, an increase ($p<0.05$) in total protein concentration in plasma of lactating cows fed 45% solvent extracted CSM over that of soybean meal fed cows was noted during 9th week but returned to normal by 13th week (Lindsey et al., 1980). Like total proteins, serum globulin levels were found comparable among lambs fed reference and test diets. Thus, the comparable serum proteins and globulin on different feeding regimes in the present study indicates normal balance between anabolism and catabolism of body proteins.

The blood urea levels did not differ significantly between lambs on the reference and experimental diets corroborating with the findings of Barraza et al. (1991) in calves, Braham et al., (1967) in pigs, Nikokyris et al. (1991) and Keery et al. (1991) in lambs fed 50% whole cottonseed, 42% CSM, 30% CSM and 21% CSM or ensiled whole cottonseed, respectively. Whereas, the levels were found to be increased in pigs fed 42% CSM both at 6th and 12th weeks of feeding but attained normal levels by addition of 1% $Ca(OH)_2$ and 0.1% $FeSO_4$ $7H_2O$ (Jarquin et al., 1966).

Muscle damage was observed in wethers due to feeding of 50% WCS at 127 days of feeding (Warren et al., 1988) as indicated by higher ($p<0.01$) creatinine and creatinine kinase activity. But such alteration in serum creatinine activity was not observed in present study due to incorporation of either raw or processed CSM in lamb rations (table 4).

Higher ($p<0.05$) range of blood glucose levels in experimental lambs at 60 days of feeding (3.52 to 9.17 mmol/L) was latter reduced to the levels comparable with those on reference diet at 120 and 180 days of feeding (table 5). Similar increased blood glucose levels were also observed in Deboulliet ewes (Peterson et al., 1992) and in crossbred lambs (Sainz et al., 1994) due to feeding of diets supplemented with CSM. On the other hand, in the studies of

Nikokyris et al. (1991) though the blood glucose decreased in mid trial due to feeding of 30% CSM incorporated diets, it attained the pre trial levels by the end of the experiment.

A rise in AST and ALT activity observed in toxic conditions indicates acute and chronic hepatic injury (Kaneko, 1980). The AST activity in the present study was similar among all the groups through out the experiment (table 6). This clearly indicated that feeding of CSM incorporated diets, which resulted in a daily FG consumption of 302.83 mg on raw and 215.1 to 250 mg on processed CSM, did not exert any toxic effect on liver as was seen in Merino wethers and pigs fed 50% WCS (Warren et al., 1988) and 42% CSM (Braham et al., 1967), respectively. Similar to the present findings, AST activity remained unaffected in lactating cows fed 45% screw pressed or solvent extracted CSM resulting in the daily total gossypol consumption of 251 to 273 mg/kg/day (Lindsey et al., 1980). Though the ALT activity was lower ($p<0.05$) in raw and cooked CSM fed lambs in comparison to other test and reference group lambs at the end (180) of feeding trial, a specific trend could not be noticed (table 7).

ELISA, when employed to study the seroreactive levels against *Brucella* as an antigen, indicated a rise in seroreactivity from 8th day until 22nd day of post sensitization in lambs on all diets (figure 1). In a normal unprimed animal, after the injection of antigen, a short lag phase of 12 days would exist before the antibody level begins to rise (Tizzard, 1995). But this lag phase was shortened on all the diets in the present study as indicated by the increase in seroreactive levels on day 8 itself. This might be the result of booster given on day 4, thus reducing the lag phase. The seroreactive levels were significantly ($p<0.05$) depressed in lambs fed CSM based diets on day 8th (0.194 to 0.309) and day 35th (0.598 to 0.701) in comparison to those fed reference diet (0.504 and 1.062, respectively) indicating the depressing effect of gossypol on immune response of the animals. Similar depression in immunobiological reactivity and destruction of immunity in vaccinated pigs was observed by Rogozhin et al. (1986) due to long term feeding of 20% CSM, which was later increased to 30-40% of diets. The antibody titres were found to be reduced in chickens due to ingestion of other feed ingredients containing toxic principle like *Phaseolus vulgaris* (Marzo et al., 1991) and aflatoxin (Boulten et al., 1982). In general, feeding of processed meal to lambs improved ($p>0.05$) the seroreactive levels against antigen to only some extent. This was apparently due to reduction of FG content to a certain extent as a result of processing. But no particular advantage of one processing method over the other was observed in counteracting the depressing effect on seroreactivity.

The DTH response indicating the cell mediated immune response was consistently seen up to 40th day of post sensitization in all groups of lambs, but the maximum response was noticed by 20th day on various CSM diets. Similar to the antibody response, the CMI response was significantly ($p<0.05$) depressed in raw CSM fed lambs [(5.00 and 4.32 mm of skin induration) on 20th day of post sensitization in comparison to reference group fed lambs (6.28 and 5.58 mm skin induration) after 24 and 48 hours of local administration of antigen, respectively)] (figure 2 and 3). Whereas, the CMI response was in general better in cooked and calcium hydroxide treated CSM fed lambs. Similar results of suppression of cellular immunity were noticed in guinea pigs (McLougulin et al., 1984), bovines (Bodine et al., 1984) and kids (Anil Kumar and Rajan, 1987) due to ingestion of aflatoxin.

Processing of CSM with cooking, calcium hydroxide and iron treatment and their inclusion in lamb diets reduced the consumption of FG by 40.19 17.40 and 26.73%, respectively in comparison to those fed diets containing raw CSM. The above studies indicated that consumption of 302.83 mg FG/day (20.29 mg FG/kg b.wt./d) did not affect much of the haematological and biochemical constituents in blood, but sufficiently suppressed the immune mechanism in lambs. Processing of CSM by above methods was though effective in reducing the suppression of immune mechanism, but could not completely alleviate it.

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