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Effect of Supplementation of Sugarcane Press Mud on Haematological, Blood Biochemical Parameters and Immune Response of Crossbred Calves

Lalit Kumar Maurya[1], S.K. Saha[1], Bornalee Handique[1]

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ABSTRACT

Background: Sugarcane press mud (SPM) is one such agro-industrial by-product sufficiently available in sugar factories which contains more protein and soluble calcium. The aim of the study is to evaluate the effect of supplementation of sugarcane press mud on haematological, blood biochemical and immune response of crossbred calves.

Methods: A total of 18 male crossbred calves (68.51±4.40 kg body weight) were divided into 3 groups of each based on complete randomized design. Calves of T_1, T_2 and T_3 groups were fed concentrate mixture with wheat bran, concentrate mixture with 20% SPM replacing wheat bran and concentrate mixture without 20% SPM/wheat bran, respectively. The experiment was conducted for 150 days. Blood was collected from each calf on 0, 75 and 150 days of experimental periods.

Result: Concentration of Hb, PCV, WBC and RBC were non-significant in different groups. Non-significant difference was also observed in serum biochemical and enzyme profile of crossbred calves. Humoral immune response was significantly ($P<0.05$) higher in T_2 and T_3 groups. Study concluded that supplementation of sugarcane press mud (20%) improved humoral immune response without affecting the haematological and biochemical profile in crossbred calves.

Key words: Haemoglobin, Immunity, Red blood cells, Serum enzyme, Serum glucose.

INTRODUCTION

Socio-economic development and national economy of our country is largely dependent on livestock and fisheries sector. There is deficit of 44% concentrate feeds, 35.6% green fodder and 10.95% dry crop residues (IGFRI, 2013). The requirement of dry fodder, green fodder and concentrate is 550, 1000 and 105 MT, respectively (Vision 2050, NIANP). The country is facing severe shortage of feed and fodder, to feed the present livestock population. In order to meet the gap between availability and requirement of feed, the new alternative feed sources are needed to be included in the diet of ruminants to overcome the shortage of feeds to some extent.

Sugarcane press mud (SPM) is one of the agro-industrial by-products sufficiently available in sugar factories. It is a soft, spongy, amorphous dark brown material (Suresh *et al*., 2006; Ghulam *et al*., 2010 and Bhosale *et al*., 2012) containing 10-17% CP and high amount of sugar, besides having good quality minerals. Press mud can be used in the formulation of livestock feed because CP content is higher than molasses and contains more soluble calcium, which is an important constituent of animal feed (Benerjee, 1993). During the processing of sugarcane, cane juice contains a large number of impurities which are in the form of precipitates and these impurities are separated using filtration process. Both types of filtration processes *i.e.* batch type filtration process or rotary vacuum filtration process produces cake. Amil *et al.* (2008) reported that SPM is a potential source of protein and other nutrients including major minerals Ca 2.4%, P 1.27%, K 1.81%, mg 1.28%, S 2.62% as well as trace elements Cu 22.6 ppm, Fe 2042 ppm, Zn 36.5 ppm and Mn 228 ppm. Sugarcane press mud is cheaply available from the sugarcane factories and no deleterious effect has been reported so far due to its feeding in animals, however its use as animal feed is not popular yet due to lack of detail scientific information regarding its feeding value. Therefore, the present experiment was conducted to determine the effect of suger cane press mud on growth, nutrient utilization and humoral immune response of crossbred calves.

[1]Division of Animal Nutrition, ICAR-Indian Veterinary Research Institute, Bareilly-243 122, Uttar Pradesh, India.

Corresponding Author: Lalit Kumar Maurya, Division of Animal Nutrition, ICAR-Indian Veterinary Research Institute, Bareilly-243 122, Uttar Pradesh, India. Email: vetlalitmaurya@gmail.com

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MATERIALS AND METHODS

This study was conducted at experimental animal shed of Animal Nutrition Division, ICAR-IVRI, Izatnagar in Uttar Pradesh of India. The experiment was approved by

Institutional Animal Ethics Committee (IAEC) and conducted under guidelines prescribed by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Animal housing and experimental design

Eighteen male crossbred calves (68.51±4.40 kg body weight) were distributed randomly into 3 groups of 6 each based on complete randomized design. Experimental calves were housed in a well-ventilated barn having provision for individual feeding and watering. They were reared under uniform conditions throughout the experimental period of 150 days.

Feeds and feeding

The experimental calves were offered concentrate mixture and wheat straw to meet their nutrient requirement as per ICAR (2013) feeding standards. The T_1 group was fed standard concentrate mixture, T_2 group was fed concentrate mixture having 20% sugarcane press mud (SPM) replacing wheat bran, while T_3 group was fed 20% less concentrate mixture as compared to other two experimental groups (restriction of 20% both SPM and wheat bran) (Table 1). Available green fodder was given to experimental calves to meet out the Vitamin A or carotene requirement. The daily feed intake was recorded throughout the trial period. The weight of concentrate mixture, roughage offered and residue left after 24 hours was measured to find out the feed intake.

Blood sample collection and analysis

Blood samples was collected on 0, 75 and 150 days of the experimental period from each calve were collected through jugular vein puncture early in the morning before feeding and watering. Blood was collected in the test tube with EDTA for haematological parameters and the remaining was taken in well cleaned, dry, sterilized test tubes and allowed for clotting. After clotting, the tubes were centrifuged to collect sera. The collected sera samples were stored in deep freeze (20°C) for further analysis. Serum biochemical parameters wre estimated by using commercial kits (Coral Clinical Systems-Tulip Diagnostics India).

Humoral immune response

At the end of experimental feeding all calves from each group were vaccinated with *Brucella abortus* strain 19 (killed) vaccine. Vaccine was administered 3 ml subcutaneously. Blood collection was done at 0, 7, 14, 21 and 28 days and serum was separated and stored at -20°C to assess the humoral immune response.

Data processing and analysis

The experimental data generated was analyzed by using SPSS computer package (SPSS version 20.0, SPSS Inc., Chicago, USA). The data were stastically analyzed by using ANOVA with Duncan's post hoc testing to compare experimental groups. For all stastical analysis, probability values less than 0.05 were considered as significant.

RESULTS AND DISCUSSION

The chemical composition of sugarcane press mud, concentrate mixture and wheat straw are presented in Table 2. The crude protein content of sugarcane press mud (15.08%) was slightly higher than the earlier values reported by Suresh and Reddy (2011) and Suma *et al*. (2007) *i.e.*11.8 and 12.76%, respectively but lower than that reported by Ankita, (2013) and Ranjan Kumar (2015) where the values ranges from 15.55 to 18.1%. The total ash (TA) content of sugarcane press mud (19.67%) was lower than values reported by Suresh and Reddy (2011) and Suma *et al*. (2007). The neutral detergent fibre (63.56%) and acid detergent fibre content (32.8%) of sugarcane press mud was lower than the values reported by Gupta and Ahuja (1998). From the proximate analysis, it is evident that the composition of sugarcane

Table 1: Ingredient composition of concentrate ration.

Ingredients	T_1	T_2	T_3
Maize	38	38	38
Wheat bran	39	19	19
Soyabean meal	20	20	20
Sugarcane press mud	-	20	-
Mineral mixture	2	2	2
Common salt	1	1	1

Table 2: Chemical composition (% DM) of sugarcane press mud concentrate mixture and wheat straw.

Attributes	Sugarcane press mud	T_1	T_2	T_3	Wheat straw
Crude protein	15.08	18.8	18.7	19.8	3.16
Ether extract	6.04	2.43	2.81	2.59	0.91
Total ash	19.68	6.43	8.87	7.40	5.44
Organic matter	80.19	93.57	91.13	92.6	94.56
Crude fibre	15.39	4.13	5.87	4.12	45.91
Nitrogen free extract	43.10	66.22	61.35	66.26	45.64
Neutral detergent fibre	63.56	28.96	33.85	28.41	81.69
Acid detergent fibre	32.80	9.68	11.44	8.69	55.38
Calcium	4.73	1.15	1.84	1.16	0.61
Phosphorus	1.25	0.98	1.18	0.84	0.13

press mud (SPM) is similar with that of certain cereal by-products like wheat bran as far as protein and crude fibre contents are concerned. Variations in the proximate composition of sugarcane press mud can be attributed to quality of sugarcane and composition of soil in which it was grown.

The overall dry matter through concentrate mixture was significantly (P<0.05) lower in T_3 (1179.3) group as compared to T_1 (1504.6) and T_2 (1504.5) groups, whereas wheat straw was statically similar in all the groups (Table 3), which was in accordance with results of Helal *et al.* (2011), who also reported reduced dry matter intake in calves fed 70 and 85% restricted diets of the concentrate mixture as compared to fed ad libitum TMR. The overall daily dry matter intake did not differ significantly among all the groups. The results of present study are in agreement with Ankita (2013) who reported comparable intake of concentrate, roughage and total dry matter in kids fed SPM @ 0, 10 and 20% SPM in concentrate mixture. The observations of present study suggest that inclusion of sugarcane press mud (SPM) in the concentrate mixture up to 20% level did not affect its palatability as there was no adverse effect on the dry matter intake of experimental calves.

Results of haematological parameters in different groups of calves were presented in Table 4. Non-significant difference was observed in concentration of Hb (g/dl), PCV (%) and count of WBC and RBC levels of experimental calves irrespective of dietary treatments and periods. Results were supported by Malapure (2015) who reported comparable serum Hb and PCV levels in calves fed SPM at 0, 10 and 20% level in concentrate mixture. Similar findings were also reported by Ankita (2013) in kids fed SPM at 0, 10 and 20% level in the concentrate mixture. The result reveals that feeding of SPM up to 20% level in concentrate mixture has no adverse effect on haematological parameters of crossbred calves.

Table 5 shows the serum biochemical parameters in different groups. Serum glucose, total proteins, albumin, globulin and A: G ratio were comparable among various dietary treatment groups. Blood glucose levels of all the experimental calves were within the normal physiological range (Kaneko *et al*., 2008). The inclusion of SPM at 20% level in the concentrate mixture did not affect the blood glucose level, which indicated that the calves consuming the diets with SPM were in a normal energy status. Findings of present study was corroborated with that of Malapure (2015), who also reported comparable serum glucose level

Table 3: Feed intake of crossbred calves in different groups.

Attributes	T_1	T_2	T_3	SEM	P value
Concentrate (g/d)	1504.60[a]	1504.50[a]	1179.30[b]	54.80	<0.01
Wheat straw (g/d)	1533.30	1525.20	1489.30	85.3	0.98
Total (g/d)	3037.9	3029.70	2668.60	130.4	0.44

[ab]Means in the same row bearing different superscripts differ significantly (P<0.05).

Table 4: Haematological parameters of crossbred calves in different groups.

Attributes	Periods			Mean	P value		
	0 d	75 d	150 d		T	P	T*P
Haemoglobin (g/dl)							
T_1	11.56	12.03	11.85	11.82			
T_2	11.53	11.85	11.76	11.72	0.809	0.826	1.000
T_3	10.86	11.60	11.43	11.30			
Mean	11.32	11.82	11.68				
Packed cell volume (%)							
T_1	30.50	29.33	31.16	30.33	0.833	0.981	0.909
T_2	28.33	31.50	28.00	29.28			
T_3	29.16	28.50	29.00	28.89			
Mean	29.33	29.77	29.38				
WBC (× 10^3/µl)							
T_1	7.24	7.64	8.53	7.14			
T_2	7.62	8.05	9.26	7.64	0.312	0.255	0.810
T_3	7.84	8.85	9.69	8.13			
Mean	7.57	8.18	9.16				
RBC (× 10^6/µl)							
T_1	5.59	6.65	7.44	6.56			
T_2	5.70	6.84	7.25	6.60	0.130	0.210	0.117
T_3	5.65	6.76	7.83	6.75			
Mean	5.65	6.75	7.51	6.64			

in calves among the treatment groups fed 0, 10 and 20% SPM in the concentrate mixture. Concentrations of serum total protein, albumin, globulin and A:G ratio are in agreement with Ranjan Kumar (2015), Ankita (2013) and Sahu (2014) who reported statistically similar levels of these parameters in lambs, kids and crossbred pigs fed SPM up to 20% level in respective concentrate mixtures. The comparable levels of serum total protein and its fractions between control and SPM fed groups are suggestive of adequacy of the protein available to the animals. The serum urea concentration was in normal physiological range (Kaneko *et al*., 2008) in all experimental calves. Results are in agreement with Malapure (2015) who reported no change in serum urea concentration of experimental calves fed SPM at 10 and 20% level in concentrate mixture when compared to control group (0% SPM in the concentrate mixture). Similar results were also reported in lambs by Ranjan Kumar (2015) and in kids by Ankita (2013).

The serum enzyme concentration was presented in Table 6. The alkaline phosphatase (ALP) in experimental calves was statistically similar irrespective of dietary treatments (T_1, T_2 and T_3). However, mean serum ALP concentration (IU/L) at 75 and 150 d was significantly (P<0.05) higher as compared to 0 d. The mean serum concentrations of Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) of all the experimental calves under different dietary treatments were statistically similar irrespective of different dietary treatments and periods. The activity of ALT and AST was within normal physiological range (Kaneko *et al*., 2008) which indicates normal liver function in all the crossbred calves. The observations regarding comparable activity of serum enzymes (ALP, AST and ALT) clearly indicate that feeding of sugarcane press mud (SPM) to experimental calves upto 20% level in growing stage has no harmful effect on liver, heart, skeletal muscles and other organs of the body.

Table 5: Blood biochemical parameters of crossbred calvs in different groups.

Attributes	Periods			Mean	P value		
	0 d	75 d	150 d		T	P	T*P
Glucose (g/dl)							
T_1	65.96	66.13	65.69	65.93	0.99	0.99	1.000
T_2	65.87	66.49	65.25	65.87			
T_3	65.34	65.69	65.52	65.52			
Mean	65.72	66.10	65.49				
Total protein (g/dl)							
T_1	7.05	7.71	7.20	7.32			
T_2	7.18	7.53	7.18	7.30	0.92	0.77	0.96
T_3	7.20	7.07	7.17	7.15			
	7.14	7.44	7.18				
Albumin (g/dl)							
T_1	2.96	3.20	3.29	3.15			
T_2	3.11	3.13	3.17	3.14	0.92	0.68	0.92
T_3	3.10	3.01	3.14	3.09			
Mean	3.06	3.11	3.20				
Globulin (g/dl)							
T_1	4.09	4.52	3.90	4.17			
T_2	4.07	4.33	4.01	4.14	0.98	0.79	0.99
T_3	4.09	4.06	4.03	4.06			
Mean	4.08	4.30	3.98				
A:G							
T_1	0.80	0.77	1.21	0.93			
T_2	0.84	0.79	1.35	0.99	0.82	0.34	0.85
T_3	0.86	0.84	0.83	0.84			
Mean	0.83	0.80	1.13				
Urea (g/dl)							
T_1	37.57	40.37	41.67	39.86			
T_2	37.81	41.67	41.15	40.21	0.93	<0.01	0.92
T_3	37.95	41.32	40.76	40.01			
Mean	37.78[b]	41.11[a]	41.19[a]				

[ab]Means in the same row bearing different superscripts differ significantly (P<0.05).

Table 6: Serum enzyme profile of crossbred calves of different groups.

Attributes	Periods			Mean	P value		
	0 d	75 d	150 d		T	P	T*P
Alkaline phosphatase (IU/L)							
T_1	108.94	123.62	136.48	123.01			
T_2	114.60	121.56	138.01	124.72	0.74	<0.01	0.80
T_3	109.70	123.17	135.71	122.86			
Mean	110.80^{c}	124.72^{b}	136.73^{a}				
Aspertate aminotransferase (AST) (IU/L)							
T_1	86.46	86.71	87.97	87.05			
T_2	86.04	85.83	87.05	86.31	0.96	0.855	1.00
T_3	86.29	86.42	87.72	86.81			
Mean	86.26	86.32	87.58				
Alanine aminotransferase (ALT) (IU/L)							
T_1	23.12	25.14	25.98	24.75			
T_2	24.61	25.03	25.88	25.17	0.974	0.814	1.00
T_3	23.12	24.50	25.56	24.39			
Mean	23.62	24.89	25.81				

^{abc}Means in the same row bearing different superscripts differ significantly (P<0.05).

Table 7: Humoral immune response (SAT titre in log_2) of growing crossbred calves.

Treatment	Periods					Mean	P value		
	0 d	7 d	14 d	21 d	28 d		T	P	T*P
T_0	1.00	6.16	8.49	8.66	7.16	6.29	0.96	<0.01	1.00
T_1	1.00	6.16	8.32	8.60	7.16	6.26			
T_2	1.00	5.99	8.49	8.66	6.99	6.22			
Mean	1.00^{d}	6.10^{c}	8.43^{a}	8.66^{a}	7.10^{b}				

^{abcd}Means in the same column bearing different superscripts differ significantly (P<0.05).

The data pertaining to humoral immune response of crossbred calves against *Brucella abortus* strain-19 (killed) is presented in Table 7. The antibody titre (log2) against *Brucella abortus* strain-19 was statistically similar in all experimental calves over the entire duration in different groups (T_1, T_2 and T_3). Antibody titre was increased significantly (P<0.05) up to 21 d of post inoculation then started declining gradually in all the groups. Similarly, Malapure (2015) also reported that there is no significant difference in humoral immune response among different treatment groups of calves fed different level of SPM in concentrate mixture. On the contrary Ankita (2013) observed significantly (P<0.05) reduced humoral immune response in kids fed 20% SPM in the concentrate mixture than the kids offered no SPM in the concentrate mixture which is in contrary with the findings of present study.

CONCLUSION

Supplementation of sugarcane press mud improved humoral immune response without affecting the haematological and blood biochemical parameters of crossbred calves. The study recommended that 20% sugarcane pressmud can be incorporated in the concentrate mixture fed to crossbred calves without affecting the blood parameters.

Conflict of interest

All authors declare that they have no conflict of interest.

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