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Effect of Feeding Cottonseed Meal on Nutrient Utilization, Blood Biochemical Profile, Cell Mediated Immune Response and Growth Performance of Buffalo Calves

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ABSTRACT

Background: Due to increased price of soybean meal, dairy farmers are seeking suitable and viable alternate protein supplement to soybean meal for economic ration formulation. Thus, a study was conducted to evaluate the effect of inclusion of cottonseed meal (CSM) as an alternate protein source in growing buffalo calves.

Methods: A total of 12 buffalo calves aged approximately 6-12 months with 149.88±9.93 kg average body weight were randomly assigned to three treatment groups with four calves each, designated as control (C), T1 and T2 groups. Soybean meal (SBM) in concentrate mixture fed to C group was replaced by cottonseed meal (CSM) at 75 and 100% level on w/w basis in T1 and T2 groups, respectively. The duration of study was 120 days.

Result: The digestibility of nutrients in T1 and T2 groups was similar to of C group. The total nitrogen intake, N outgo and N balance varied non-significantly among C, T1 and T2 groups. The blood metabolites and cell mediated immune response in T1 and T2 groups were similar to that of C group. The average daily gain in T2 group was numerically higher than other groups. It was concluded that CSM could replace SBM upto 100% on w/w basis in the concentrate mixture without any adverse effect on growth, nutrient utilization, blood biochemical profile, cell mediated immune response and growth of calves.

Key words: Blood metabolites, Cottonseed meal, Digestibility, Growth, Immune response.

INTRODUCTION

India is the first country in Asia for scientific and technological development in buffalo nutrition, production, reproduction, biotechnologies and genetic improvement. The buffalo forms the backbone of India's dairy industry and is rightly considered as the 'bearer cheque' of the rural farmers. Buffaloes are better converter of poor-quality fibrous feeds into milk and meat. However, buffalo calf nutrition and feeding management is the most neglected area in both buffalo husbandry and research. Cottonseed is one of the richest sources of oilseeds available in many temperate and tropical countries, mostly processed to extract oil that is used as edible fat. Cotton is grown mainly for the fiber, but cottonseed meal and cottonseed cake secured in the production of oil from cottonseed can serve as feeds for livestock. The commonly used methods of lipid extraction from oil seeds are pressing and extraction with organic solvents (cold or hot). Cottonseed meal (CSM) is a co-product of the cottonseed oil processing industry. With processing, typical yields from cottonseed are 50% meal, 22% hulls, 16% oil and 7% linters, with a 5% loss (Hinze *et al*. 2015). Increase in cottonseed production in India has resulted in a greater availability of CSM which can be used as the common protein source for livestock in cotton producing areas.

CSM is rich in tryptophan and methionine. Nutritional value of cottonseed meal depends up on method of extraction, proportion of husk, lint and degree of decortication. Due to increased price of soybean meal, dairy farmers are seeking suitable and viable alternate protein supplement to soybean meal. Therefore, keeping in view the above points, this study was proposed to determine the influence of dietary inclusion of CSM on buffalo calves in terms of nutrient digestibility, nitrogen retention, blood metabolites and growth performance.

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MATERIALS AND METHODS

Animals and management

The study was carried out at the Department of Animal Nutrition (latitude: 30°31′ 37″ and longitude: 74°43′ 9″), Guru Angad Dev Veterinary and Animal Science University,

Ludhiana Punjab (India) after obtaining the necessary approval from the Institutional Animal Ethics Committee. Twelve buffalo calves clinically healthy aged approximately 6-12 months and 149.88±9.93 kg average body weight were randomly assigned to three groups of four animals each (designated as C, T1 and T2) for 4 months experimental period. The calves were fed as per ICAR (2013) feeding standard

Formulation of diets

The ingredient composition and chemical analysis of experimental diets are displayed in Table 1. SBM based conventional concentrate mixture was prepared for C group and SBM in the conventional concentrate mixture was replaced by cottonseed meal (CSM) at 75 and 100% level on w/w basis. The animals in control group (C) were fed with basal diet consisting of fodder (berseem, oats, mustard mix) and SBM based conventional concentrate mixture. The animals in T1 and T2 groups were fed green fodder, wheat straw and concentrate mixtures having SBM replaced with CSM at 75 and 100% levels, respectively. The inclusion level of CSM was 11.25 and 15% in the concentrate mixtures fed to T1 and T2 groups, respectively. The daily record of feed intake and orts was maintained. The animals were weighed for 3 consecutive days at every fortnight interval and the feeding schedule was revised accordingly.

Metabolism trial

Digestibility of feed nutrients was assessed by conducting a 7-day metabolic trial towards the end of growth experiment. During metabolism trial, the animals were kept in specially designed metabolic cages, where a metallic pipe led the excreted urine into a narrow mouth plastic container containing 40 ml of 20% H_2SO_4. Faeces were collected manually by trained persons, who were put on duty round the clock. However, the collection of urine was automatic. For nitrogen estimation, 30 grams fresh faeces was preserved in previously tarred wide mouth plastic bottles daily, containing 25 ml of 20% sulphuric acid solution added on the first day of collection. An aliquot of urine equal to one by tenth part of the total urine voided was preserved in narrow mouth glass bottles (1000 ml capacity) daily, which were kept in a refrigerator till analyzed for nitrogen content.

The diet samples, residue, fecal samples were taken daily during the collection period. At the end of the collection period, samples were mixed and ground through 1 mm screen for chemical analysis. The chemical analysis of feed, residuals and faeces was carried out using the procedure of Association of the Official Analytical Chemists (AOAC 2005). Neutral detergent fibre, acid detergent fibre (Van Soest *et al*. 1991) and cellulose (Crampton and Maynard, 1938) were determined.

Blood sampling

Blood samples were drawn from jugular vein puncture in the morning before feeding. Vials were then centrifuged at 3000 rpm for 10 min and plasma was separated. Plasma was stored at -20°C until analyzed for blood biochemical parameters (glucose, total protein, AST, ALT, Albumin, cholesterol, triglycerides, BUN and GGT) were analyzed using commercial test kits (Transasia Biomedicals Ltd., India).

Immune response

In vivo delayed type of hypersensitivity reaction against phytohemagglutinin-P (PHA-P) was adopted to study the cell-mediated immune (CMI) response (Abbas *et al*., 2014). The 150 µg of PHA-P in 200 ìl of phosphate buffer saline solution (pH 7.4) was injected intra-dermally at the marked area on neck region. To avoid inflammatory reaction due to abrasion, the skin area of neck region was cleaned and shaved prior to 24 h. The skin thickness measured by a digital vernier caliper at 0 h represented the basal value. After intradermal injection of PHA-P, the skin thickness was measured up to 96 h at 24 h interval.

Statistical analysis

Statistical analysis was done according to the general linear model in SAS (2001). Differences among groups for feed intake, nutrient digestibility, blood parameters, immunity status, daily gain were evaluated by one way analysis of variance. The Duncan multiple range test was used to test the effect of treatments. The level of significance was set at $P<0.05$.

RESULTS AND DISCUSSION

DM intake and nutrient digestibility

The results in Table 2 indicate that the DM intake during metabolic trial was 5.40, 5.02 and 5.12 kg/d in control (0% CSM), T1 (75% CSM) and T2 (100% CSM) groups, respectively (Table 2). The DM intake in T1 and T2 groups was similar to C group. The treatment groups (T1 and T2) had similar DM intake to that of control indicating that CSM inclusion in the diet of buffalo calves had no adverse effect on palatability of the diet. However, Tripathi *et al*. (2014) reported higher DM intake in lambs fed Bt-CSM based diet replacing groundnut oil meal. The digestibility of DM, OM, CP, NDF ADF and cellulose in the T1 and T2 groups was similar to C group. This indicates that nutrient digestibility was not affected ($P>0.05$) by replacing SBM with CSM at graded levels in the diet. The results of the current study are in agreement with the earlier results of Fadel and Ashmawy (2015) who reported no significant difference in DM and OM digestibility of goats fed untreated CSM and CSM treated with tannin. Tripathi *et al*. (2014) also reported non-significant difference in CP digestibility in lambs fed Bt-CSM and CSM replacing groundnut oil meal.

Similarly, Silva *et al*. (2009) observed no significant difference in CP digestibility in cows fed with graded levels of CSM replacing SBM. However, the findings of the current study are contrary to those of Nomeary *et al*. (2021), who reported significantly higher ($P<0.05$) CP digestibility in lambs fed CSM than those fed black cumin seed meal and sesame seed meal-based diets. The NDF digestibility are in accordance with the results previously observed by

Tripathi *et al*. (2014), who found similar NDF digestibility in lambs fed Bt-CSM and CSM replacing groundnut oil meal. Silva *et al*. (2009) also reported no significant difference in NDF digestibility in cows fed CSM at graded levels replacing SBM. Solomon *et al*. (2008) noted no difference in apparent digestibility of ADF among control and CSM supplemented goats. However, Tripathi *et al*. (2014) reported significantly higher (P<0.05) ADF digestibility in lambs fed CSM based diet than Bt cottonseed meal diet and control (groundnut oil meal) diet.

Nitrogen balance

The total nitrogen (N) intake varied non-significantly among the groups indicating no effect of CSM inclusion on N intake (Table 2 and Fig 1). These results agree with the findings previously observed by Tripathi *et al*. (2012) and Lorena-

Table 1: Ingredient and chemical composition of concentrate mixtures, fodder and wheat straw.

Items	C	T1	T2	Fodder	Wheat straw
Ingredient composition (%)					
Yellow corn	34	34	34	-	-
Soybean meal	15	3.75	0	-	-
Cottonseed meal	0	11.25	15	-	-
Mustard cake	15	15	15	-	-
Wheat bran	10	10	10	-	-
Deoiled rice bran	17	17	17	-	-
Rice polish	6	6	6	-	-
Mineral mixture	2	2	2	-	-
Salt	1	1	1	-	-
Chemical composition (% DM basis)					
Dry matter (DM)	91.00	91.00	93.00	17.30	86.80
Organic matter (OM)	90.45	90.85	90.55	88.53	92.40
Crude protein (CP)	20.07	20.52	20.86	16.5	4.40
Ether extract (EE)	5.36	5.36	5.64	3.35	1.14
Total ash	9.55	9.15	9.45	11.47	7.60
Neutral detergent fibre (NDF)	31.40	30.46	30.73	48.30	84.90
Acid detergent fibre (ADF)	16.40	16.05	14.75	31.90	58.65
Cellulose	9.40	8.10	7.70	20.60	46.90
Hemicellulose	15.00	14.41	15.98	16.4	26.25

Table 2: Effect of dietary level of CSM on nutrient utilization and growth performance in buffalo calves.

Parameters	C	T1	T2	SEM
DM intake, kg/d	5.40	5.02	5.12	0.15
		Nutrient digestibility (%)		
DM	63.38	61.24	63.17	0.83
OM	66.64	64.80	66.42	0.76
CP	75.43	76.29	76.06	0.55
NDF	53.45	51.80	51.94	1.05
ADF	49.34	46.48	48.53	1.19
Cellulose	60.50	56.06	57.72	1.02
		N balance (g/d)		
Total nitrogen intake	126.06	121.75	124.87	3.50
Urinary N	29.18	32.91	29.36	1.00
Faecal N	30.27	28.74	29.76	0.86
Total N outgo	59.45	61.64	59.12	1.43
N balance	66.61	60.10	65.74	2.73
		Body weight changes		
Initial body weight (kg)	149.29	150.08	150.25	9.93
Final body weight (kg)	238.25	244.83	248.83	13.76
BW gain (kg, 120 days)	88.96	94.75	98.58	4.54
Average daily gain (g)	741.32	789.58	821.53	37.82

DM-Dry matter; OM-Organic matter; CP-Crude protein; NDF-Neutral detergent fibre; ADF-Acid detergent fibre.

Rezende *et al*. (2012) in the lambs and barrows fed CSM, respectively. Urinary-N, fecal-N and total N-outgo in T1 and T2 groups fed CSM based diets were as similar to that of C group in current study. Similar to our findings, Li *et al*. (2012) reported no significant difference in N excretion from urine of pigs fed CSM diets and control (corn and soybean meal) diet.

Tripathi *et al*. (2012) also noted similar urinary N excretion among the lambs fed CSM, Bt-CSM and control (groundnut oil meal) diet. Tripathi *et al*. (2012) reported similar faecal N excretion among the lambs fed CSM, Bt-CSM and control (groundnut oil meal) groups. Similar N-intake and total N-outgo in the present study was responsible for similar N balance among the groups. Similar N balance could be due to similar protein content of SBM and CSM used in the study and isonitrogenous concentrate mixtures fed to the animals. However, Lorena-Rezende *et al*. (2012) reported that the diets containing cottonseed meal (30% replacement with control diet with or without enzymes) resulted in higher (P<0.01) amount of retained nitrogen as compared to control (corn and soybean meal) diet in barrows. Present finding is corroborated by Tripathi *et al*. (2012) who reported no significant difference in N retention among lambs fed CSM and Bt-CSM diets replacing groundnut oil meal.

Growth performance

Results presented in Table 2 showed that there was no significant difference in the gain in body weight during the experimental period of 120 days among C, T1 and T2 groups. The average daily gain in T2 group was marginally higher than C and T1 groups, though the difference was not statistically significant (P>0.05). The results of the present study were in accordance with those of Tripathi *et al*. (2014) who found no significant difference in final BW, total BW gain and ADG among control, CSM and Bt-CSM fed lambs for 120 days. Ojewola *et al*. (2006) also reported no significant difference in birds mean daily weight gain and feed to gain ratio in which CSM was substituted by SBM at graded levels.

However, Nomeary *et al*. (2021) reported highest (P<0.05) daily BW gain in lambs fed CSM diet as compared to lambs fed black cumin seed meal, sesame seed meal and soybean meal diets, respectively. Hassanabadi *et al*. (2009) also reported improved (P<0.05) weight gain and FCR in lysine supplemented CSM with 5, 10, 15, 20% levels in broiler chicks as compared to control group fed corn and soybean meal-based diet.

Blood metabolites

Blood metabolites are shown in Table 3. In this study, there were no differences (P>0.05) in glucose, BUN, total protein, albumin, triglycerides, cholesterol, AST, ALT and GGT among groups. The results are also in agreement with those of Kannan *et al*. (2013) who reported non-significant difference in blood glucose (mg/dl) in lambs fed 40% raw CSM, 40% raw CSM supplemented with vitamin E and 40% CSM with 1.5% calcium hydroxide replacing soybean meal. Saijpaul *et al*. (2006) revealed no significant difference in blood glucose in lactating crossbred cows fed water soaked whole linted cottonseed replacing equal quantity of control concentrate mixture. Kannan *et al*. (2013) reported no significant difference in BUN in lambs fed 40% raw CSM, 40% vit E supplemented raw CSM and 40% calcium hydroxide treated CSM replacing SBM.

Kannan *et al*. (2013) noted no significant difference in serum albumin (g/dl) in lambs fed 40% raw CSM, 40% vit E supplemented raw CSM and 40% calcium hydroxide treated CSM replacing soybean meal. Similarly, Saijpaul *et al*. (2006) also reported no significant difference in cholesterol in lactating crossbred cows fed water soaked whole linted cottonseed replacing equal quantity of control concentrate mixture. Kannan *et al*. (2013) also reported no significant difference in AST and ALT in lambs fed 40% raw CSM, 40% vit E supplemented raw CSM, 40% calcium hydroxide treated CSM replacing SBM. The level of liver function enzymes (ALT and AST) in the current study was similar in treatment

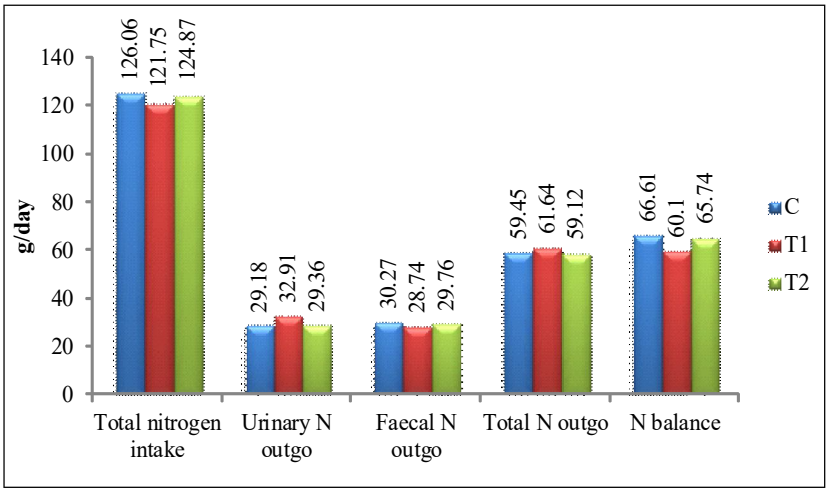

Fig 1: Effect of dietary level of CSM on nitrogen balance (g/d) in buffalo calves.

Table 3: Effect of dietary level of CSM on blood parameters in buffalo calves.

Parameters	C	T1	T2	SEM
Glucose, mg/dl	71.33	69.07	70.00	0.16
BUN, mg/dl	27.09	28.89	26.85	1.15
Total protein, g/dl	6.61	6.79	6.20	0.16
Albumin, g/dl	3.53	3.51	3.42	0.05
Triglycerides, mg/dl	25.31	29.12	27.75	1.30
Cholesterol, mg/dl	86.90	83.26	81.40	1.89
AST, U/L	98.94	101.51	96.11	1.05
ALT, U/L	29.12	28.28	29.87	1.32
GGT, U/L	10.97	12.90	13.20	0.85

BUN- Blood urea nitrogen, AST- Aspartate aminotransferase, ALT- Alanine aminotransferase, GGT- Gamma glutamyl transferase.

Table 4: Effect of dietary level of CSM on *in vivo* delayed type of hypersensitivity (DTH) response to PHA-P in buffalo calves.

Hours post-inoculation	C	T1	T2	SEM
0	100	100	100	0.00
24	148.24	147.80	128.81	4.93
48	127.48	120.72	114.70	4.82
72	115.57	110.27	106.14	3.33
96	103.35	100.82	100.72	0.72

PHA-P- Phyto haemagglutinin-P.

and control groups indicating no adverse effect of CSM feeding on liver function.

Cell mediated immune response

Treatment groups T1 and T2 exhibited similar CMI response to control group (Table 4). The CMI response was higher after 24 h of antigen administration in all the groups as compared to response after 48 h. Decrease in skin thickness *i.e.*, CMI response after 48 h, 72 h and 96 h was almost similar in all the groups. Nagalakshmi *et al.* (2001) reported that cooked, $Ca(OH)_2$ and iron treated raw CSM showed better cell mediated immunity (CMI) than raw CSM where the diets included 40% of either raw, 45 min cooked, 1% calcium hydroxide or iron treated CSM replacing deoiled groundnut cake in lambs. The similar immune response observed in CSM fed groups (T1 and T2) to that of control (C) group in the present study could be due to similar nutrient utilization which ultimately affects the cellular integrity and immune response of the animals._CSM inclusion the diet did not have any adverse effect on the immunity of animals.

CONCLUSION

The inclusion of CSM up to 15 per cent in the concentrate mixture of buffalo calves completely replacing SBM (100% on w/w basis) resulted in marginally higher average daily gain. In addition, dietary inclusion of CSM resulted in similar nutrient digestibility, blood biochemical profile and cell mediated immune response to that of SBM based control diet. Therefore, from the results of present study, it can be concluded that CSM can replace SBM upto 100% on w/w basis in the concentrate mixture of buffalo calves without any adverse effect on nutrient digestibility, blood biochemical profile, cell mediated immune response and growth of animals and feeding can be economized keeping in view the high cost of SBM.

Statement of animal right

All institutional animal ethics committee guidelines for care and use of animals were followed (GADVASU/IAEC/55/13).

Conflict of interest: None.

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