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Research article

Effect of supplementation of berseem hay meal as replacer of mustard cake protein on nutrient intake, utilization and growth performance in *Jalauni* lambs

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Abstract

Berseem (*Trifolium alexandrinum*) hay meal (BHM) as a replacement for mustard cake (MSC) in the ration of growing *Jalauni* lambs was studied. About 15 *Jalauni* male lambs (23.25 ± 1.2 kg) were randomly distributed into three groups of five each in an experiment based on a randomized block design and were randomly assigned to one of the three dietary treatments. The animals were fed control concentrate mixture (GI) or concentrate mixtures in which 60% N of mustard cake was replaced with BHM containing 0.75% NPN (GII) or 1.0% NPN (GIII) making them iso-nitrogenous and iso-caloric. Gram straw (GS) was offered *ad libitum* to all the animals. Total dry matter intake (DMI) was comparable among the groups. Digestibility of nutrients, namely DM, organic matter (OM), and neutral detergent fiber (NDF) were non-significantly different among the groups. However, crude protein (CP) digestibility was higher in GI or GII than GIII. Rumen fermentation was not affected due to the incorporation of BHM as a replacer of mustard cake in the diet. N balance was also comparable. However, N retention as % of N absorbed was significantly (p <0.05) lower in GIII as compared to GI or GII. Nutrient content in terms of TDN or ME was similar among the experimental diets. However, DCP% was higher in GI or GII than GIII. Cost per kg live weight gain was significantly (p <0.05) lower in BHM-incorporated diets (GII or GIII) than in the control diet (GI). It was concluded that BHM can be incorporated into the diet of growing *Jalauni* lambs as a replacer of mustard cake for economical livestock production.

Keywords: Berseem hay meal, Growth performance, Lamb, Mustard cake, Nutrient utilization, Rumen fermentation

Introduction

In India, livestock contributes 16% to the income of small farm households as against an average of 14% for all rural households. Livestock provides livelihood to two-thirds of rural communities. It also provides employment to about 8.8% of the population in India. Seasonal variations in weather and other environmental constraints often reduce the availability of quality herbage for the grazing of small ruminants and consequently affect the production of animals (Shinde and Mahanta, 2020; Kumar *et al.*, 2023). The production requirement is often met through specific protein supplements like groundnut cake, mustard cake or cottonseed cake and very seldom from compounded concentrate mixture. However, the cost of concentrates and oilcakes limits their widespread use. Consequently, farmers have problems in supplying their livestock with good quality feed while keeping the feed cost at manageable levels. The increase in the cost of conventional feedstuffs with their irregular supply poses a great threat to the future livestock industry, including small ruminants. Accordingly, nowadays, there is an observed increasing demand for feedstuffs characterized by low price and availability, which can be utilized in livestock feed without any adverse effect on animal health and productivity.

Berseem (*Trifolium alexandrinum*) is a green leguminous fodder grown in northern and central India, producing nutritive, succulent and palatable forages in 4 to 6 cut during the winter season (Pradeep Behari *et al.*, 2015). The crude protein content of berseem ranges from 11 to 21%, and the gross energy content is 17.2 kJ/g (Chauhan *et al.*, 1992). Berseem is highly palatable and fairly digestible. Direct replacement of concentrate feed with legumes might result in reduced productivity (Khalili and Varvikko, 1992) because leguminous forages do not adequately replace the energy of concentrate feed. Therefore, berseem hay meal (BHM) was incorporated as a substitute of mustard cake in iso-caloric diets and there

was seemingly no report concerning such replacement of mustard cake in iso-caloric diets. Thus the effects were investigated for substituting BHM for mustard cake as a protein source in iso-nitrogenous and iso-caloric diet on feed intake, nutrient utilization and growth performance of *Jalauni* lambs.

Materials and Methods

Feeding trial: *Jalauni* lambs weighing around 23.25 ± 1.20 kg were fed control concentrate mixture (GI) or concentrate mixtures in which 60% N of mustard cake was replaced with BHM containing 0.75% NPN (GII) or 1.0% NPN (GIII) making them iso-nitrogenous and iso-energetic (Table 1). Gram straw was offered as basal roughage *ad libitum* to all the groups for a period of three months. Weighed quantities of feeds were offered in separate troughs to individual animals once daily at 10.00 hours for the entire experimental period. Leftovers were quantified daily. Clean drinking water was provided twice daily (11.00 and 16.00 hours). All the animals were weighed at fortnightly intervals for two consecutive days before feed and water were given. A metabolism trial of 7-day duration was conducted around the middle of the experimental feeding period. Standard procedures were adopted for the collection and aliquoting of the representative samples of feeds offered, residues, feces, and urine (Schneider and Flatt, 1975).

Chemical analysis: Dry matter in feed and feces was determined by oven drying at 100°C overnight. Rumen liquor was collected from each animal through a stomach tube at two hours after offering a feed. pH was measured immediately, and samples for NH_3-N and volatile fatty acids (VFA) analysis were stored frozen after acidification with concentrated sulphuric acid. NH_3-N in rumen fluid was estimated according to the micro-diffusion technique of Conway (1962). Total VFA (TVFA) concentration in rumen fluid was measured by Markham's distillation. For chemical analysis, pooled samples of feed offered, refusals, and feces were dried at 60°C and ground to pass through a 1-mm sieve. Wet feces and urine samples, preserved in diluted and concentrated sulphuric acid, respectively, were analyzed for N by the standard micro-Kjeldhal method. Feed and feces samples were analyzed for crude protein (CP), EE and total ash contents (AOAC, 1995) and fiber fractions (Van Soest *et al*., 1991).

Economics of live weight gain: Costs of each kg of concentrate mixture (including cakes and/or BHM) were Rs. 17.81, 14.46, and 14.71 for lambs of GI, GII and GIII groups, respectively. These costs were derived by multiplying feed ingredient contents of concentrate mixtures of each kg with the current prices of Rs. 20.00, 13.25, 13.25, 19.00, 9.00, 6.00, 6.00 and 110.00 per kg, respectively, of mustard cake, maize, barley, wheat flour, BHM, urea, salt, and mineral mixture. The cost of the gram straw was Rs. 5.00 per kg.

Statistical analysis: The data were subjected to analysis of variance for statistical significance among the treatment means (Snedecor and Cochran, 1994).

Table 1. Ingredient composition of concentrate mixtures (CM) and chemical composition (%DM basis) of experimental feeds

Ingredient	**CM-I**	**CM-II**	**CM-III**
Mustard cake	40	16	16
Barley	50	-	-
Maize	7	26	21
Berseem meal	_	48	48
Wheat flour	_	6.25	11.0
Urea	_	0.75	1.0
Salt	1	1	1
Mineral mixture	2	2	2
Cost/quintal (Rs)	1781	1446	1471

Chemical composition (% DM)					
	CM I	**CM II**	**CM III**	**GS**	**BHM**
DM	95.58	95.36	94.66	93.72	91.44
CP	19.90	19.86	20.16	6.45	15.82
NDF	39.59	43.75	44.26	69.39	52.76
ADF	17.49	23.16	24.08	50.75	38.64
Ash	9.51	11.97	12.37	8.92	13.76

Results and Discussion

Chemical composition of feedstuffs: BHM had lower CP content (Table 1) than mustard cake (MSC), requiring the incorporation of more BHM *vs* MSC dry matter (DM) for iso-nitrogenous replacement. The CP values of BHM were in agreement with earlier findings of Das *et al*. (2013) and Mandal and Banerjee (2009). However, Mohamed *et al*. (2020) and Misra *et al*. (2018) reported lower CP values for berseem hay compared to the present findings, which might be due to the loss of leaves during the preparation and processing of hay. The chemical composition of GS was similar to the earlier report of Bampidis and Christodoulou (2011). Dotaniya *et al*. (2022), however, reported lower CP values, which might be due to different chickpea varieties, leaf to stem ratio, and growing conditions (geographic, seasonal variations, climatic conditions, and soil characteristics).

Nutrient intake and utilization: The dry matter intakes were comparable among the treatment groups (Table 2). Thang *et al*. (2008) and Das *et al*. (2023) reported that there were no significant differences in total feed intakes in growing and milch cattle with increasing levels of replacement with mixed legume foliage. Sivaramkrishna *et al*. (2021) also concluded that supplementation of lucern hay did not increase total DM and forage intake in Nellore lambs. However, Das and Singh (2005) reported increased feed intake in growing crossbred male calves where groundnut cake was replaced with green berseem in a wheat straw-based diet. Similarly, Mekuriaw and Asmare (2018) reported higher DM intake in washera lambs with increased supplementation of ficus leaves as a replacer of concentrate mixture in the diet.

Reduced dry matter intake (DMI) was also reported by Richards *et al*. (1994) when concentrate was replaced with *Gliricidia sepium* leaves. The variation in DMI due to the

Table 2. Nutrient intake and utilization in growing lambs fed experimental diets

Parameter	GI	GII	GIII	Pooled SE
Average body weight (kg)	26.4	27.0	26.5	2.68
DM intake (g/d)	1038.34	1032.81	1042.95	77.49
Plane of nutrition (per kg BW $^{0.75}$/d)				
DM (g)	89.68	87.78	90.06	2.26
DCP (g)	7.56	7.52	7.09	0.61
TDN (g)	58.46	56.88	57.71	1.44
ME (kcal)	211.36	205.64	208.64	5.23
Nutrient digestibility (%)				
DM	60.88	60.51	59.69	1.00
OM	63.43	62.85	61.79	0.96
CP	63.64^{b}	62.45^{b}	59.84^{a}	1.02
NDF	54.82	50.98	54.18	1.69
ADF	46.89	49.58	45.62	1.43
Nitrogen balance (g/d)				
Intake	21.57	21.86	21.79	0.64
Fecal loss	7.91	8.28	8.72	0.63
Urinary loss	1.79	2.33	2.21	0.28
Balance	11.87	11.24	10.85	0.50
Retention				
% of N intake	55.40	51.77	49.67	1.16
% of N absorbed	63.63^{b}	62.45^{b}	59.84^{a}	2.71
Nutritive value				
DCP (%)	8.44^{b}	8.60^{b}	7.87^{a}	0.97
TDN (%)	65.22	64.86	64.07	0.22
ME (kcal/g)	2.35	2.34	2.32	0.04

^{ab}Means with different superscripts in a row differed significantly (p <0.05)

replacement of concentrates with leguminous fodder could be due to the nature of the basal diet or the presence of anti-nutritional factors affecting the palatability of the diet. In the present study, berseem hay used, was highly palatable, and there is no report indicating the presence of an anti-nutritional factor in berseem, which might decrease intake. The mean total DM intake of sheep in the current study was 88.54 g DM kg^{-1} $W^{0.75}$, which was similar to the results reported earlier in sheep (Duguma *et al*., 2019). The total DM intake expressed as a percent of body weight obtained from the current study was in agreement with that of Yigzaw *et al.* (2019), where the values were within the range of the recommended dry matter intakes in sheep (ICAR, 2013).

DCP intake was comparable among the groups, as reported earlier by (Leketa *et al*., 2019). TDN intake in different groups was also similar and corroborated with earlier findings of Hassen *et al.* (2020), where concentrate mixture was replaced with legumes in the diet of goats. In contrast to the present finding, Mahanta *et al.* (2010) reported higher TDN intakes in sheep fed diet containing green berseem as a replacer of mustard cake, which might be due to a combination of poor-quality crop residues like sorghum stover and green fodder resulting better ruminal environment and ultimately higher nutrient intake and their utilization in animals.

Digestibility of nutrients, namely DM, organic matter (OM), neutral detergent fiber (NDF) and acid detergent fiber (ADF) was non significantly different among the groups. However, CP digestibility was higher in GI or GII than in GIII. No significant difference in the digestibility of nutrients was also observed earlier when oil seed meal was replaced with leucaena hay or cowpea hay in the diet of goats (Leketa *et al*., 2019; Amare and Girmay, 2020). Das and Singh (2005), however, reported higher OM, NDF and ADF digestibility in calves when GNC was substituted with berseem hay. On the contrary, Mekuriaw and Asmare (2018) reported lower NDF and ADF digestibility in washera lambs due to graded levels of substitution of concentrate with ficus leaves. This effect was probably associated with an inadequate intake of energy that limited the growth of cellulytic bacteria. In the present experiment, the diet offered to the animals was iso-energetic, so there was no inadequacy of energy for the growth of fibrolytic bacteria, resulting in similar fiber digestibility.

All the animals were in positive N balance, which did not differ among the treatment groups. Das *et al.* (2012) also recorded a non-significant difference in N balance in growing calves when the concentrate mixture was replaced with a stylo meal in the diet. Similarly, Ahmed *et al*. (2012) also observed similar N retention in sheep when cottonseed cake was replaced with *Grewia oppositifolia* leaves. Supplementation of BHM showed tendencies of non-significantly higher excretion of fecal and urinary nitrogen. The retention of nitrogen as a percentage of intake was similar among the groups. However, grams of absorbed N were significantly (p <0.05) different in GI and GII than in GIII. The findings of the present experiment indicated that dietary inclusion of BHM as a replacer of mustard cake had no adverse effects on N utilization, as observed earlier (Nurfeta *et al*., 2013; Mahanta *et al*.,1999**).**

Rumen fermentation: The pH, NH_3-N and VFA concentration are the main internal environmental indicators of rumen fermentation (Table 3). pH of rumen fluid was not significantly different among groups. However, rumen pH in all the diets was well above the cellulytic threshold value of 6.2 as suggested by Mould and Orskov (1983). The rumen pH was within the range of 6.2 to 7.0, which indicated that the ecological environment of rumen microorganisms was relatively stable, which could ensure normal rumen fermentation (Zhang *et al*., 2013). The rumen pH values obtained in the present study were consistent with previous studies (Chen *et al*., 2015). The rate of nitrogen decomposition and ammonia absorption and utilization by rumen microorganisms mainly determines the ruminal NH_3-N concentration. In the present study, comparable NH_3-N concentrations in different groups indicated that utilization of ammonia was similar both for berseem and mustard cake in the rumen, as reported earlier by Mahanta *et al*. (2010). However, in contrast to the present study, Das and Singh (2005) reported gradual replacement of groundnut cake with berseem resulted in the gradual reduction of NH_3-N concentration in rumen fluid. TVFA concentration (mmol/L) in GI was non-significantly higher than GII or GIII. Similarly, Liu *et al*. (2019) also did not observe any significant impact on TVFA production with the gradual replacement of concentrate with oat hay in the diet of Tibetan sheep.

Growth performance and economics of feeding: Average daily gain was non-significantly higher in GI and GII than GIII (Table 4). The similarity in weight gain among the treatment groups could be due to the similarity in the DM intake and digestibility. Leketa *et al*. (2019) did not find a significant difference in daily weight gain in goats when oil cake was substituted with *Leucaena* hay in the diet. Nurfeta *et al*. (2013) also recorded a non-significant difference in daily weight gain in sheep due to the substitution of pigeon pea leaves for noug seed cake. However, Mekuriaw and Asmare (2018) observed

Table 3. Rumen metabolites of experimental animals 2 hours post-feeding

Parameter	G1	GII	GIII	Pooled SE
pH	6.76	6.70	6.83	0.03
NH_3-N (mg/dl)	30.14	29.49	28.28	1.54
TVFA (mmol/L)	154.2	151.2	146.1	3.52
Total N (mg/dl)	96.13	91.9	88.40	6.53

Table 4. Effect of dietary treatment on growth and economics of feeding in growing *Jalauni* lambs fed different experimental diets

Parameter	G1	GII	GIII	Pooled SE
Initial body weight (kg)	23.35	23.48	22.92	1.20
Final body weight (kg)	29.60	29.45	28.78	1.51
Gain (kg)	6.26	5.98	5.86	0.13
Average daily gain (g/d)	69.53	66.50	65.13	1.77
Feed conversion ratio	10.89	11.32	12.25	0.16
Economics of feeding				
Concentrate intake (kg)	36.51	35.32	34.92	0.21
Gram bhusa intake (kg)	31.56	32.83	34.24	0.48
Feed cost (Rs)	808.09[b]	674.84[a]	684.87[a]	19.01
Cost per kg weight gain (Rs)	129.35[b]	114.16[a]	118.95[ab]	2.02

[ab]Means with different superscripts in a row differed significantly (p <0.05).

that the replacement of the concentrate mixture with ficus leaves significantly reduced the growth rate, which might be due to the reduced availability of digestible protein and energy. Feed conversion efficiency in the present experiment was comparable among the groups as reported earlier by Mengistu *et al*. (2020), where noug seed cake was replaced with dried mulberry (*Morus indica*) and *Vernonia amygdalina m*ixed leaves in the diet of Bonga sheep. Leketa *et al*. (2019) also did not observe any difference in feed conversion ratio in goats when the concentrate mixture was replaced with *Leucaena* hay in iso-nitrogenous and iso-caloric diets. Hence, dietary inclusion of BHM in iso-nitrogenous and iso-caloric diets did not adversely affect the growth rate of animals.

The feed cost per kg live weight gain (FCWG) depends on feed intake, cost of feed and efficiency of feed utilization. The FCWG (Rs. per kg) was significantly (p >0.05) lower for diets with BHM (GII and GIII) than for diets containing MSC (GI). This was due to comparatively lower concentrate intake by GII and GIII lambs along with the lower cost of concentrate mixtures with BHM. Similarly, Shinde *et al*. (2019) reported that mulberry leaves could be incorporated in the diet of Osmanabadi kids up to 50% replacement of DCP from concentrate mixture to reduce the cost of feeding significantly without affecting live weight gain. Gwiriri *et al*. (2016) also reported velvet bean and lablab-based diets had significantly higher dietary net income and lower production costs per liter of milk produced than a commercial diet.

Conclusion

From the above study, it was concluded that berseem hay meal supplementation did not influence DM, OM, and fiber digestibility. Similarly, rumen fermentation, N utilization and growth performance was also not affected when mustard cake was replaced gradually with berseem hay meal. Although DCP content of the diet was reduced significantly (p <0.05) in the treated groups, however, DCP intake was similar among the groups. Therefore, BHM could be used safely and economically as a source of protein for the replacement of mustard cake in the iso-nitrogenous and iso-caloric diet of growing *Jalauni* lambs for economical livestock production in small holders' farming system without affecting the nutrient intake, utilization, and growth performance.

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