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Effect of Dietary Protein Levels on Performance, Nutrient Balance, Plasma Enzyme and Hormone Profiles of Growing Sahiwal Calves

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ABSTRACT

The present study was carried out to observe the effect on performance, nutrient utilization, plasma enzymes and hormones in growing Sahiwal calves fed with three levels of proteins in the form of total mixed rations (TMRs). Fifteen male Sahiwal calves (12-18 month age) were distributed into three equal groups (147.35 ± 5.46 kg) and were fed on isocaloric diets containing different protein levels of 15% (T_1), 13.5% (T_2) and 16.5% (T_3) for 90 days. Nutrient intake and their digestibility except CP digestibility did not differ significantly irrespective of treatment groups. CP digestibility was higher in T_3 as compared to T_2, though T_3 did not differ from T_1. DCP (g/100 kg BW/day) intake was significantly higher ($P<0.05$) in T_3 than T_2 but comparable to T_1. TDN (kg/100 kg BW/day) intake was significantly higher ($P<0.05$) in T_2 than T_1 and T_3, but was comparable between T_1 and T_3. The intake and retention of nitrogen were similar between the treatments. The average daily gain was highest in T_2 followed by T_3 and T_1. The plasma enzymes alanine amiontransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) along with hormones (insulin and growth hormone) were comparable among all groups and were within normal physiological range. From the results, it was concluded that the performances of Sahiwal calves in terms of growth rate and feed conversion ratio was satisfactory even with low dietary protein level of 13.5%. Variation in dietary protein levels also did not affect the blood enzyme and hormone profiles of Sahiwal calves.

Keywords: Hormones, nutrient balance, plasma enzymes, protein levels, Sahiwal calves

In developing countries, more than 70% of the expenditure in dairy farming is incurred on the feeding of animals. Undernutrition is one of the most limiting factors in livestock production in rural India, mainly causing poor growth rate, delayed sexual maturity, anoestrus and repeat breeding in dairy animals along with other factors. Nitrogen (N) is one of the key elements in formulating rations for ruminants because of typical fermentative action on feed nutrients in rumen. Feeding excess dietary protein may be detrimental by increasing nitrogen excretion from ruminants in the form of ammonia into the environment (Klemesrud *et al*. 2000), whereas underfeeding dietary CP may result in decreased average daily gain, DM intake and gain to feed ratio (Galyean 1996). Accurate definition of protein requirement for dairy calves is vital to ensure an appropriate supply of the assortments of amino acids essential for rapid structural growth while at the same time minimizing cost and, more importantly, reducing wasteful excretion of excess N by circumventing protein overfeeding. For optimum production of microbial protein, synchronized availability of energy and protein to rumen microbes is required and feeding of rations in form of complete feed blocks and TMR has proved to be beneficial under most of the situations. Enzymes serve a number of functions in the body and any alteration in the constituents of the blood indirectly reflects the functional status of the system. Diseases of the newborn and neonatal mortality are a major cause of economic loss in livestock production. Thus, specific plasma enzymes and hormone reference ranges and their possible deviations in relation to age of the animals, plane of nutrition etc. could give indications of abnormal body conditions.

Thus the aim of the present study was to investigate the effect of dietary protein levels on Sahiwal calves with respect to their feed intake, growth parameters, nutrient utilization, plasma enzymes and hormone levels.

MATERIALS AND METHODS

The experimental design and plan of the present study strictly followed the norms of Institutional Animal Ethics Committee (IAEC) of National dairy Research Institute (NDRI), Karnal, Haryana, India.

Experimental animals and diet formulation

Fifteen male Sahiwal calves (12-18 months of age and average body weight of 147.35±5.46 kg) were distributed randomly into three treatment groups of 5 animals each. Three types of isocaloric TMRs (Table 1) were prepared by mixing concentrate mixture, berseem fodder and wheat straw in the ratio of 40: 40: 20 (on DMB) and offered *ad libitum* to the animals in respective groups for a period of 90 days.

The T_1 group received dietary protein level of 15% as per requirements of NRC (2001), while groups T_2 and T_3 were offered 90% and 110% of the requirements, respectively. The TMRs differed only in the composition of their respective concentrate mixtures.

Table 1. Ingredient composition (% DM) of experimental diets

Ingredient composition (%)	Total mixed rations (TMRs)		
	T_1	T_2	T_3
GNC	4.8	2	8
MOC	4.4	2.4	6
Maize grain	14.4	20	10
DORB	7.6	7.6	5.6
Wheat bran	7.6	6.8	9.2
Mineral mixture	0.8	0.8	0.8
Salt	0.4	0.4	0.4
Wheat straw	20	20	20
Berseem green	40	40	40
Total	100	100	100

DM: Dry matter; GNC: Groundnut cake; MOC: Mustard oilcake; DORB: Deoiled rice bran

Table 2. Chemical composition (% DM) of experimental diets

Nutrient	Berseem green	Wheat straw	Total mixed rations (TMRs)		
			T_1	T_2	T_3
DM	17.05	91.52	31.03	31.14	30.72
OM	86.64	88.51	86.90	88.13	87.17
CP	17.90	3.39	14.98	13.46	16.48
EE	2.83	1.04	3.52	3.57	3.54
Total ash	13.36	11.49	13.10	11.88	12.40
NDF	43.28	79.48	53.97	53.05	52.81
ADF	22.22	50.99	35.64	34.10	35.74
ME (MJ/kg)	8.51	4.65	9.73	9.69	9.72

OM: Organic matter; CP: Crude protein; EE: Ether extract; NDF: Neutral detergent fiber; ADF: Acid detergent fiber; ME: Metabolizable energy; MJ: Mega Joule.

Table 3. Intake and digestibility of nutrients as affected by dietary treatments

Parameter	Treatment		
	T_1	T_2	T_3
Intake of nutrients			
Body weight (kg)	196.70±13.52	205.94±12.77	202.79±15.50
DMI/day (kg)	5.04±0.57	5.57±0.28	4.82±0.29
DMI/100 kg BW (kg/day)	2.54±0.14	2.74±0.18	2.40±0.09
OM intake/day (kg)	4.40±0.50	4.93±0.25	4.25±0.26
OM intake/100 kg BW (kg/day)	2.21±0.13	2.42±0.16	2.11±0.08
CP intake/day (g)	765.18±85.85	759.68±37.73	810.62±49.29
CP intake/100 kg BW (g/day)	385.33±21.59	373.24±25.03	402.81±15.30
EE intake/day (g)	191.66±20.61	202.16±9.18	193.96±11.67
Nutrient digestibility (%)			
DM	61.17±0.81	61.29±1.03	61.93±0.76
OM	61.66±1.02	62.65±1.43	62.80±1.35
CP	60.11^{ab}±2.19	57.18^{a}±1.34	63.56^{b}±1.75
EE	71.22±2.37	74.03±1.17	76.03±0.59
NDF	56.26±1.69	59.92±1.04	57.85±1.20
ADF	50.62±2.33	54.02±1.25	50.69±1.34

Digestible nutrients (%)			
DCP	9.01^{b}±0.33	7.72^{a}±0.39	10.48^{c}±0.29
TDN	58.65±1.04	58.81±1.30	59.23±0.55

$^{a, b, c}$ Means bearing different superscripts in a row differ significantly (*P<0.05). DCP: Digestible CP; TDN: Total digestible nutrients.

Animal trial and chemical analyses

The metabolism trial was carried out at the animal experiment premises of National Dairy Research Institute, Karnal, Haryana, India. All the calves were housed in clean and well ventilated individual pens with individual feeding and watering arrangements.

Table 4. Plane of nutrition and nitrogen balance in different dietary treatments

Parameter	Treatment		
	T_1	T_2	T_3
Plane of nutrition			
DCP intake/day (g)	464.16±64.10	433.95±21.54	517.75±41.93
DCP intake/100 kg BW (g/day)	232.35^{b}±18.18	213.97^{a}±16.83	250.63^{b}±14.83
TDN intake/day (kg)	2.94±0.30	3.27±0.16	2.86±0.19
TDN intake/100 kg BW (kg/day)	1.50^{b}±0.05	1.61^{a}±0.13	1.42^{b}±0.06
Nitrogen balance			
N intake (g/day)	122.43±13.74	121.55±6.04	129.70±7.89
N voided in faeces (g/day)	48.40^{ab}±2.99	52.04^{b}±3.59	47.27^{a}±1.89
N voided in urine (g/day)	49.82^{b}±2.74	47.39^{a}±1.58	55.03^{c}±3.67
Total N outgo (g/day)	98.23^{a}±2.00	99.43^{a}±3.15	102.30^{b}±3.51
N balance (g/day)	24.20±3.99	22.12±2.96	27.40±3.76
Absorbed N as % of intake	60.47±5.14	57.18±2.62	63.55±1.75
Retained N as % of intake	19.76±1.02	18.20±1.03	21.12±1.46
Retained N as % of absorbed	32.69±3.35	31.82±1.59	33.24±2.94

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

Clean drinking water was provided free of choice once each in morning and afternoon. Feed intake was recorded daily while body weights were recorded at fortnightly intervals in the morning hours before offering any feed or water. After an adaptation period of about 8 weeks in pens, calves were shifted to metabolism shed for a six day metabolism trial. Total amount of TMR offered, residues, faeces and urine voided by each animal were recorded throughout the period of metabolism trial. Samples of all the above were collected daily and then pooled at the end for further analysis. Chemical as well as fiber analysis of the samples were carried out using standard procedures of AOAC (2005) and Van Soest *et al*. (1991), respectively. Metabolisable energy content of concentrate and TMRs was estimated by using *in vitro* gas production technique (Menke *et al*. 1979) along with respective blank in triplicate. The blood samples from the calves were collected at fortnightly intervals in heparinised vaccutainer. Samples were centrifuged at 3000 rpm for 15 minutes to separate the plasma and plasma samples were stored at -20^{o}C for the estimation of plasma enzymes like AST, ALT and LDH by kits supplied by Span Diagnostics Ltd., India. Insulin and GH levels were determined by 'Bovine Insulin ELISA Test kit' and 'Bovine GH ELISA Test Kit', respectively from Endocrine Technologies, 35325, Fircrest Street, Newyork, USA.

Statistical analysis

The obtained results were subjected to one way analysis of variance (ANOVA) by SPSS version 16.0 (2010) for Windows and means were compared by one way analysis of variance (ANOVA) test at 5% level of significance (P< 0.05).

RESULTS AND DISCUSSIONS

Chemical composition of experimental feeds and TMRs

The chemical compositions of concentrate mixture, berseem fodder, wheat straw and TMRs fed to different groups are presented in Table 2. The concentrate mixtures fed to groups T_1, T_2 and T_3 had CP content of 19.13, 15.21 and 22.03%, respectively while that of TMRs were 14.98, 13.46 and 16.48%, respectively. The estimated CP values

of TMRs were pretty close to the predicted values. The ME contents of the concentrate mixtures were 13.21, 12.58 and 12.79 MJ/kg, respectively. The TMRs were isocaloric as they contained similar ME of 9.73, 9.69 and 9.72 MJ/kg, respectively.

Table 5. FCR and plasma enzyme-hormone profiles in different treatment groups

Parameter	Treatment		
	T_1	T_2	T_3
Feed conversion ratio (Growth trial)			
Initial body weight (kg)	147.27±10.04	148.34±8.66	146.46±11.72
Final body weight (kg)	207.59±15.39	221.36±13.67	217.89±15.67
Weight gain (kg)	60.32±5.50	73.02±5.15	71.43±1.02
Daily weight gain (kg)	0.670±0.06	0.811±0.06	0.793±0.03
Avg. daily DMI (kg)	5.24±0.88	5.52±0.66	5.10±0.54
Feed conversion ratio	7.82	6.81	6.43
Plasma enzyme-hormone profiles			
AST (IU/L)	108.14±3.75	106.50±5.20	105.95±5.55
ALT (IU/L)	24.45±0.38	24.32±0.85	25.26±1.00
LDH (IU/L)	372.23±14.92	356.83±16.39	358.37±17.08
Insulin (ng/ml)	1.51±0.19	1.67±0.22	1.56±0.13
GH (ng/ml)	25.27±1.59	22.93±2.42	20.00±2.46

[a, b, c] Means bearing different superscripts in a row differ significantly ([*]P<0.05)

Intake and digestibility of nutrients

The intake of nutrients by calves (Table 3) was almost similar among groups though higher DMI (g/d and % of BW) was recorded in T_2 followed by T_1 and T_3. Similar trend was observed with respect to OM and EE intake by treatment groups. Average CP intake in T_1 (765.18), T_2 (759.68) and T_3 (810.62) was also statistically insignificant among groups. Results clearly indicated that feeding of various CP levels to calves did not influence their DMI or other nutrients intake significantly. Wadhwa and Bakshi (2006) reported slightly lower DMI (4.7 kg/day) by buffalo calves (181 kg BW) offered with TMR containing concentrate and wheat straw in the ratio of 40:60 than present results. Dietary CP levels ranging from 11 to 14% in finishing steers were not found to have any significant influence on DMI (Bailey *et al*. 2008). Verma *et al*. (2009) fed growing Murrah bulls on isocaloric diets containing different levels of protein (100, 90 and 80%) requirements as per Kearl (1982). Intake was similar in G_1 and G_2 but significantly (P<0.05) higher than G_3. Present report differed from the above in that the group offered lower dietary CP (T_2) recorded higher DMI.

Nutrient digestibility results (Table 3) showed that CP digestibility was significantly higher (P<0.05) in T_3 as compared to T_2, though did not differ from T_1. It might be due to lower CP intake in T_2 group, which corroborated with the findings of Mehra *et al*. (2006) that CP digestibility might increase with the increase CP level in the diets. However, Malic *et al*. (1998) reported that the digestibility of CP was not influenced even when CP intake was 40% more than the recommended levels of NRC (1989) in crossbred heifers. Similar to our observations, Sampath *et al*. (1991) also reported that protein supply had no effect on average daily feed intake. Digestibility of DM and OM did not differ significantly among the treatment groups. Fardin (2005) reported similar digestibility of DM, OM and CP in crossbred calves on feeding TMR containing wheat straw and concentrate in the ratio of 60:40. Wadhwa and Bakshi (2006) reported lower, but comparable DM and OM digestibility in buffalo calves with present results, but CP digestibility was reported higher. DCP contents of the experimental diets in three groups were significantly different, however TDN values of the TMRs in the treatment groups were similar.

Plane of nutrition and nitrogen balance

Plane of nutrition and nitrogen balance in treatment groups are presented in Table 4. DCP intake (g/100kg BW) was significantly higher (P<0.05) in T_1 and T_3 as compared to T_2 however, TDN intake (g/100kg BW) was significantly (P<0.05) higher in T_2 as compared to T_1 and T_3. Lohakare *et al*. (2006) reported lower CP and DCP intake in 3-5 month old male crossbred calves offered different dietary protein levels. Similar observations were recorded by Singh *et al*. (2009) in 10-13 month old Bhadawari buffalo. Both reports corroborated with the findings of this study that higher the protein intake, higher was the protein digestibility and the

DCP intake. Contrary to present findings, Girdhar *et al*. (2006) reported higher DMI and TDN intake in Frieswal bull calves fed on different planes of nutrition.

There was no significant difference in the nitrogen intake (g/d) in the different groups, though high intake was recorded in T_3 than T_1 and T_2 which might be due to high protein content of the TMR offered to group T_3. Significant ($P<0.05$) difference was observed in case of nitrogen voided in the faeces and urine (g/d). Faecal N excretion was comparatively less ($P<0.05$) in groups T_1 and T_3 than group T_2 while a reverse trend was observed for urinary N excretion. Retention of N (g/d) was highest in T_3 than T_1 and T_2. The group received higher dietary protein ration showed higher N intake and N balance. Similar observations were made by Singh *et al*. (2009), who reported N balance of 22.70, 17.57 and 33.63 g/d in 10-13 month old Bhadawari buffalo calves with corresponding N intake of 59.98, 51.26 and 69.56 g/d. Lohakare *et al*. (2006) and Tauqir *et al*. (2011) reported same kind of trend in N intake and N balance in 3-5 month old male crossbred calves and 6-7 month old Nili Ravi buffalo calves, respectively. Diaz *et al*. (2001) and Blome *et al*. (2003) reported that increasing N intake while keeping energy intake constant did not increase N digestibility but increased N retention, which was in agreement with findings of this study. The N retention as % of N intake or % of N absorbed did not vary significantly due to dietary CP levels.

Feed conversion ratio and plasma enzyme-hormone profiles

There was no significant difference among the groups with respect to total and daily weight gain (kg) during 90 days of experimental feeding (Table 5). Similar to our findings, no significant difference in the BW gain was also noted by Gonzalez *et al*. (1990) and Kakkar *et al*. (1991) in growing calves receiving varied protein levels in their diets. Increased BW gain on normal and high than low protein diets was reported in buffalo calves by Singh *et al*. (2009) and Verma *et al*. (2009) which differed from present findings in that the BW gain was more in groups fed lower and higher protein diets than the recommended level, when all experimental diets were isocaloric. Likewise, Brosh *et al*. (2000) noted higher weight gain in male Holstein-Friesian calves fed high protein diet in comparison to feeding of medium or low protein diets. In the present study, however, no effect of dietary CP on BW gain was apparent even though the levels were much varied and even higher i.e. 15, 13.5 and 16.5% for the T_1, T_2 and T_3 groups, respectively. There were no significant differences in FCR among the groups, however, FCR tended to better in groups T_2 and T_3 than T_1. Tauqir *et al*. (2011) and Lohakare *et al*. (2006) reported almost similar FCR of 6.66, 6.10 and 6.83 in 6-7 month old Nili Ravi buffalo calves and 6.29, 6.08 and 6.55 in 3-5 month old crossbred calves, respectively which received three different dietary protein levels.

There were no significant differences among the treatments regarding plasma enzyme-hormone levels (Table 5). The results indicated that the animals given isocaloric TMR but varying in protein contents did not influence plasma ALT and AST activity in Sahiwal calves. Similar observations were made by Nagpal *et al*. (2011) regarding ALT and AST levels in camel calves fed isocaloric feed blocks with varying protein levels. The differences were small indicating the effective homeostatic mechanism in calves to maintain normal metabolic processes. Verma *et al*. (2009) reported higher AST and ALT (IU/L) values in Murrah bulls receiving 90% of protein requirements as per Kearl (1982) than those receiving 100 and 80% of the requirement. Interspecific differences in the activity of LDH has been reported by Avallone *et al*. (1996) which can be affected by physiological factors such as age, sex, and body condition of animal, *etc*. During the neonatal period, the changes that involve turbulent synthetic activities and growth are responsible for the progressive increase of the LDH levels in cattle calves. Mean insulin and GH concentration did not differ significantly among the treatment groups.

CONCLUSION

From these experiments, it could be concluded that even the low protein diet with 13.5% CP was apparently able to support for the gains attained by Sahiwal calves and there was no response in performance on increasing the protein intake beyond this level. Though growth rate and FCR tended to be better in the Sahiwal calves given 13.5 and 16.5% dietary protein but the differences were not significant. Values of different blood enzyme and hormones were similar and within normal physiological range in

all groups of Sahiwal calves indicating that variations in dietary protein levels did not affect the plasma enzyme-hormone profiles.

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