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Effect of Dietary Protein Levels on the Performance, Nutrient Balances, Metabolic Profile and Thyroid Hormones of Crossbred Calves

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ABSTRACT : An experiment was conducted to study the effect of different dietary protein levels on the performance, nutrient balances, blood biochemical parameters and thyroid hormones of crossbred calves. Thirty crossbred (*Bos taurus*×*Bos indicus*) calves aged 3-5 months were divided into 3 equal groups of 10 each and fed graded levels of crude protein, namely 100 (NP), 75 (LP) and 125 (HP) percent of the Kearl recommendations for 105 d. The calves had access to *ad libitum* oat hay as the basal roughage. A metabolism trial of 6 d duration was conducted at 90 d of the study. Blood collection and its analysis for various hematological and biochemical parameters as well as thyroid hormones was done both during the pre- and post-experimental periods. The fortnightly body weight changes and the net gain did not differ significantly due to dietary variation. The average daily gain was 367±21.6, 347±22.9 and 337±26.4 g in calves fed NP, LP and HP diets, respectively. Averaged across the feeding trial, oat hay intake was higher ($p<0.05$) in NP animals than HP or LP fed groups. The dry matter (DM) intake showed no significant difference between the 3 groups but the DM digestibility was higher ($p<0.05$) in the HP fed animals. The digestibility of crude protein, organic matter, crude fiber and nitrogen-free extract was significantly higher ($p<0.05$) on HP diets compared to LP or NP diets. The calves on all 3 diets were in positive nitrogen (N) balance, however the N retention was higher ($p<0.05$) in HP than in LP fed calves. The intake and retention of calcium and phosphorus were similar between the treatments. The blood biochemical profile revealed no significant influence of the dietary treatments on hemoglobin, packed cell volume as well as serum levels of glucose, total protein, albumin, globulin, Ca, P, and alkaline phosphatase. Serum levels of the circulating thyroid hormones (T_3 and T_4) tended to be lower ($p>0.05$) on feeding of the LP diet besides showing an increasing trend with the advancement of age. Considering the similar performance and metabolic profile, it could be concluded that crossbred calves can be satisfactorily reared on 25% lower protein level as recommended by Kearl for developing countries, which would not only economize the cost of production but also help to reduce environmental pollution attributable to livestock production.
(**Key Words :** Protein, Calves, Growth, Nutrient Balances, Blood, Thyroid Hormones)

INTRODUCTION

In developing countries, more than 70% of the expenditure in dairy farming is incurred on the feeding of animals (Singh et al., 2003). Protein is the most expensive component in the ration. Nitrogen is one of the key elements in formulating rations for ruminants, and because of increasing environmental concern of phosphorus and nitrogen, much more attention is being paid to their excretion by ruminants (Islam, 2002). Nutrients supply is a component of any management system that needs to be carefully evaluated. Excess dietary protein not needed by the ruminants is usually broken down into ammonia. 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 nitrogen by circumventing protein overfeeding. Animal growth and nutrition interact with one another in the sense that each can influence the other; the growth pattern of an animal determines its nutrient requirements (McDonald et al., 1995). Hence in these changing perspectives of livestock production scenario, there is an absolute need to redefine the protein requirement of animals keeping in mind their growth potential.

Feeding standards for livestock varies between countries. Because of this, the same feed is often valued in a different manner. Even the underlying assumptions of the basal diet for dairy calves differ amongst countries which can further

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Table 1. Ingredient and chemical composition of feeds and fodder

	Concentrate supplements[a]			Oat hay
	NP	LP	HP	
Ingredients composition (% as fed basis)				
Maize	47	50	42	-
Soybean meal	25	15	38	-
Wheat bran	25	32	17	-
Mineral mixture[b]	2	2	2	-
Salt	1	1	1	-
Calculated composition				
CP (%)	20.50	16.80	25.33	-
ME (Mcal/kg)	2.77	2.74	2.79	-
Analysed composition[c] (%on DM basis)				
OM	92.09	91.65	91.77	91.41
CP	19.46	16.22	24.81	8.28
EE	2.88	2.55	2.27	1.06
Ash	7.91	8.35	8.23	8.59
CF	6.48	7.09	7.07	40.06
NFE	63.27	64.98	57.62	40.28
Ca	1.10	1.44	1.28	0.69
P	1.24	1.34	1.22	0.57

NP: normal protein; LP: low protein; HP: high protein.

[a] Added with vitamin mix at 20 g per 100 kg, containing 50,000 IU of vitamin A and 5,000 IU of vitamin D_3 per g.

[b] Moisture (maximum): 70 g kg^{-1}; calcium (minimum): 280 g kg^{-1}; phosphorus (minimum): 120 g kg^{-1}; iron: 5 g kg^{-1}; iodine: 0.26 g kg^{-1}; copper: 0.77 g kg^{-1}; cobalt: 0.13 g kg^{-1} (all minimum levels) and fluorine (maximum): 0.40 g kg^{-1}.

[c] Each analysis was performed in triplicate.

influence the feed value of a particular feedstuff or nutrient contained therein. The main feeding standards having widespread acceptance for various categories of livestock are National Research Council (NRC) recommendations of the United States and Agricultural Research Council (ARC) recommendations of the United Kingdom, which have been modified for local conditions in many countries such as for India (Sen et al., 1978; Ranjhan, 1991; ICAR, 1997) and for developing countries (Kearl, 1982).

In most of the current feeding systems, CP recommendations (on DM basis) for calves less than 250 kg BW exceed 16% with concentrations of 17 to 18% being broadly recommended for rapidly growing animals (Kertz et al., 1987). However, under tropical situations like in India, where calves are reared on a crop residue-based feeding system with limited concentrate supplementation, it is unclear about how much CP is actually effective in realizing the growth potential of crossbred calves. Brosh et al. (2000) have noted higher weight gain in male Holstein Friesian calves fed high protein diet (14.6%) in comparison to feeding of medium (12.6%) or low (10.6%) protein diets. On the contrary, Gonzalez et al. (1990) did not find any effect on live weight gain of grazing calves when fed at 0, 13, 17 or 21% levels of dietary CP; they suggested that the supplement with 9% CP provided adequate digestible protein to the intestine for the gains attained and there was no response in performance or in economics on increasing the protein intake beyond this level. In fact, earlier studies by various workers lack unanimity with respect to the dietary CP levels required for optimum growth performance of crossbred calves (Sengar et al., 1985; Kakkar et al., 1991; Sampath et al., 1991).

In light of the above backdrop, an attempt has been made in the present study to evaluate the impact of graded levels of dietary CP on growth performance, nutrients utilization, metabolic profile and thyroid hormones of crossbred calves during the early post-weaning phase of their lives.

MATERIALS AND METHODS

Animals, treatments, feeds and feeding

Thirty 3-5 months old male crossbred (*Bos indicus*×*Bos taurus*) calves were procured from the Livestock Production and Research (C&B) unit of Indian Veterinary Research Institute. The animals were fed a standard concentrate supplement on an oat hay based diet for a period of 15 d so as to adapt them to the new feeding and management conditions. Subsequently, the calves were randomly divided into 3 groups comprising of 10 animals each on the BW basis and were fed different levels of protein, on an oat hay based diet namely, 100 (normal; NP), 75 (lower; LP) and 125 (higher; HP) percent of the CP requirements suggested by Kearl (1982). The varying levels of protein were ensured by adjusting the CP content of 3 different concentrate mixtures which were otherwise isocaloric in nature. The ingredient composition of concentrate supplement fed to different groups is given in Table 1. The animals were maintained on the above feeding schedule for 105 d.

All the calves were housed individually in a well ventilated and cement floored shed having individual feeding and watering arrangements. Clean drinking water was provided to all the calves once each in morning and afternoon. The calves were let loose in adjoining paddock for two hours during morning hours for exercise. They were dewormed at the start by using broad-spectrum anthelmentics and were vaccinated against prevalent contagious diseases.

The calves were individually offered weighed quantities of the respective concentrate mixtures at 09:00 h daily. The concentrate requirement was adjusted based on individual BW changes every fortnight. The quantity of roughage, offered at 11:00 h daily, was adjusted at about 120% of the previous day’s intake. The residue, if any, was weighed the following day, before offering the concentrate mixture in order to determine the daily voluntary intake. The body weight (BW) of individual calves were recorded at fortnightly intervals in the morning before feeding and watering in order to assess the change in BW and average daily gain (ADG).

Table 2. Effect of dietary protein levels on BW changes and feed intake of calves during the feeding trial

Attributes	Treatments		
	NP	LP	HP
BW changes			
Initial (kg)	53.81±2.86	53.60±2.57	52.47±2.64
Final (kg)	92.38±4.73	90.01±4.67	87.89±4.98
Net gain (kg)	38.56±2.27	36.41±2.40	35.41±2.77
ADG (g)	367±21.62	347±22.87	337±26.42
Mean daily feed (DM) intake			
Conc. (g/d)	1,387.98±53.27	1,277.32±43.26	1,379.98±56.21
(g/kg $W^{0.75}$/d)	55.64±0.47^B	51.92±0.26^C	57.01±0.46^A
Hay (g/d)	891.24±27.09^a	780.63±37.60^b	777.56±32.11^b
(g/kg $W^{0.75}$/d)	35.91±1.14	31.92±1.82	32.27±1.19
Total (g/d)	2,279.22±74.90	2,053.66±65.53	2,157.54±64.98
(g/kg $W^{0.75}$/d)	91.56±1.43^A	83.66±1.98^B	89.29±1.49^A
Conc:roughage	1.56	1.66	1.78
Feed conversion ratio	6.29±0.24	6.08±0.36	6.55±0.34

Means bearing different superscripts (capital: $p<0.01$; small: $p<0.05$) in a row differ significantly.

Metabolism trial

A metabolism trial of 6 d duration was conducted on all the experimental calves following 90 d of feeding trial to study the digestibility, plane of nutrition and kinetics of nutrients. The trial involved daily recording of feeds offered and residues left besides total excretion of faeces and urine after allowing for proper acclimatization of calves in metabolic cages. Representative samples of feeds offered and residues left were dried overnight at 100±5°C in hot air oven to determine daily dry matter (DM) intake. The samples were pooled over the 6 d of collection and then ground to pass through 2 mm sieve, and stored in airtight containers for further analysis.

The total quantity of faeces voided by individual calves during the preceding 24 h was collected and weighed quantitatively. Following thorough and uniform mixing in a clean plastic trough, representative samples were taken to the laboratory for further aliquoting. A suitable aliquot of the daily faecal excretion was then dried in hot air oven at 100±2°C for determining the DM content. The dried samples of faeces of individual calves was subsequently pooled, ground and secured for further analysis as in case of feeds. For nitrogen (N) estimation, an appropriate aliquot of the wet (fresh) faeces was weighed, mixed with few ml of 1:4 H_2SO_4 and pooled into a previously weighed air tight container. At the end of the collection period, the pooled aliquot of the wet faeces was weighed and mixed well, and used for N estimation.

The daily urinary output by individual calves was collected quantitatively in plastic bottles and measured. Suitable aliquots, in duplicate, were measured daily into kjeldahl flasks containing concentrated H_2SO_4 for N estimation. Another appropriate aliquot was pipetted into separate plastic containers and the pooled samples were kept under refrigeration for analysis of calcium and phosphorus.

The proximate analysis of feed, feces, residues, and N, Ca, P content of urine was performed as per AOAC (1990).

Blood collection and analysis

Blood samples were collected from all animals in each group at the beginning and end of the experimental feeding from jugular vein into two tubes - one with, and the other without anticoagulant. The anticoagulated blood was used for estimation of hemoglobin (Hb) by Sahli's method (Benjamin, 1985) and packed cell volume (PCV) by micro-haematocrit method (Jain, 1986). The non-anticoagulated blood samples were centrifuged at 3,000 rpm for 15 min at room temperature to separate the serum. The serum samples were stored at -20°C until further analysis for glucose by o-toluidine method (Hultman, 1959), total protein (Biuret method), albumin (bromocresol green method) and globulin (by difference) as per Webster (1977), urea by diacetyl monoxime (DAM) method (Rahmatullah and Boyde, 1980), calcium by orthocresophthalein complexone method (Baginski et al., 1973), inorganic phosphorus by molybdate method (Daly and Ertingshausen, 1972) and alkaline phosphatase by potassium ferricyanide method of Kind and King (1954). The thyroid hormones tri-iodothyronine (T_3) and thyroxine (T_4) were also estimated in the serum samples adopting radio-immuno assay technique using kits supplied by Bhabha Atomic Research Center, Mumbai, India. The inter- and intra-assay variability was 12.2 and 8.3%, respectively.

The data generated during the study were analyzed using one-way analysis of variance. The means were subjected to test of significance by Duncan's multiple range test as described by Snedecor and Cochran (1989) using Statistical Package for Social Science (SPSS 10.0). All statements of significance are based on a probability of

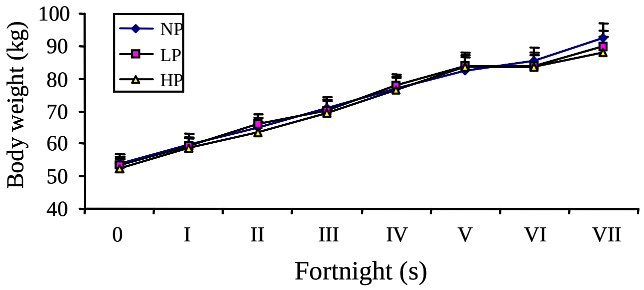

Figure 1. Fortnightly body weight change of calves fed normal (NP), low (LP) or high (HP) protein diets.

p<0.05 or p<0.01 unless otherwise indicated.

RESULTS AND DISCUSSION

Chemical composition of feeds and fodders

The chemical composition of the three concentrate mixtures fed to different groups is presented in Table 1. The analyzed CP content in the three formulated concentrated mixtures was almost identical with the estimated values. The organic matter, ether extract, total ash, nitrogen free extract and Ca and P contents were comparable among the concentrate mixtures.

Live weight changes and growth

The fortnightly change in BW of the calves is depicted in Figure 1 and the net gain and ADG are presented in Table 2. There was no significant difference among the groups with respect to growth during 105 d of experimental feeding. Similar to our findings, no significant difference in the BW gain was also noted by Gonzalez et al. (1990), Kakkar et al. (1991), Andersen et al. (1994) and Sengar et al. (1985) 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. (1994) and Verma (1998), and it was suggested that the growth rate increases with an increased availability in protein and energy levels in diets. Likewise, Brosh et al. (2000) have 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. 12.6, 14.3 and 17.8%

Table 3. Effect of dietary protein levels on intake and digestibility of nutrients and the nutritive value of the diets

Attributes	Treatments		
	NP	LP	HP
Digestibility of nutrients			
Dry matter			
Intake (g/d)	2,845.75±118.12	2,515.50±140.05	2,657.50±112.39
Digested (g/d)	1,944.87±80.01	1,741.50±103.12	1,930.12±70.33
Digestibility (%)	68.44±0.84^{b}	69.23±1.11^{b}	72.81±1.25^{a}
Organic matter			
Intake (g/d)	2,605.25±108.09	2,298.13±126.90	2,438.50±106.50
Digested (g/d)	1,829.14±74.91	1,634.85±94.06	1,824.56±69.80
Digestibility (%)	70.26±0.85^{b}	71.17±1.07^{b}	75.01±1.24^{a}
Crude protein			
Intake (g/d)	405.90±17.84^{B}	314.84±15.63^{C}	473.32±24.97^{A}
Digested (g/d)	230.20±11.55^{B}	117.09±7.73^{C}	293.77±18.49^{A}
Digestibility (%)	56.72±1.25^{b}	56.41±0.77^{b}	61.95±1.63^{a}
Ether extract			
Intake (g/d)	90.41±3.91	82.77±4.08	77.82±4.08
Digested (g/d)	52.17±2.47	49.59±2.47	46.46±2.99
Digestibility (%)	57.84±1.89	60.17±2.15	59.18±1.68
Crude fiber			
Intake (g/d)	601.13±46.94	428.97±42.25	553.65±19.82
Digested (g/d)	405.61±22.59	361.29±32.79	402.96±30.47
Digestibility (%)	66.95±1.33^{b}	67.77±1.11^{b}	72.94±1.64^{a}
Nitrogen-free-extract			
Intake (g/d)	1,517.77±62.95	1,379.07±71.50	1,348.63±60.65
Digested (g/d)	1,232.80±48.77	1,123.93±62.99	1,160.01±46.17
Digestibility (%)	81.32±1.25^{b}	81.57±1.80^{b}	86.19±1.08^{a}
Nutritive value of the diets			
CP (%)	14.27±0.22^{B}	12.59±0.32^{C}	17.76±0.28^{A}
DCP (%)	8.09±0.19^{B}	7.10±0.21^{C}	11.01±0.35^{A}
ME (Mcal/kg)	2.31±0.03^{B}	2.33±0.04^{B}	2.47±0.04^{A}

Means bearing different superscripts (capital: $p<0.01$; small: $p<0.05$) in a row differ significantly.

Table 4. Effect of dietary protein levels on balances of nitrogen, calcium and phosphorus in calves

Attributes	Treatments		
	NP	LP	HP
Nitrogen balance			
Intake (g/d)	64.95±2.85[B]	50.37±2.50[C]	75.73±4.00[A]
(g/kg $W^{0.75}$)	2.31±0.06[B]	1.86±0.04[C]	2.76±0.07[A]
Fecal outgo (g/d)	28.11±1.44[a]	22.05±1.35[b]	28.72±1.73[a]
Urinary outgo (g/d)	14.51±1.50[B]	11.10±0.70[B]	23.60±1.43[A]
Retention			
(g/d)	22.33±1.85[ab]	17.23±1.31[b]	23.42±2.03[a]
(g/kg $W^{0.75}$)	0.86±0.07[a]	0.63±0.04[b]	0.85±0.06[a]
(as % intake)	34.55±2.77	34.04±1.81	30.67±1.54
(as % absorbed)	60.52±3.99[a]	60.36±3.08[a]	49.48±2.01[b]
Calcium balance			
Intake (g/d)	25.29±1.20	27.48±1.34	27.03±1.53
Fecal outgo (g/d)	14.57±1.04	14.00±0.75	14.07±0.80
Urinary outgo (g/d)	0.39±0.08	0.26±0.03	0.29±0.03
Retention (g/d)	10.33±0.84	13.22±1.14	12.66±1.22
(as % intake)	40.90±2.79	47.77±2.36	46.29±3.01
Phosphorus balance			
Intake (g/d)	26.51±1.14	25.15±1.24	25.06±1.29
Fecal outgo (g/d)	11.32±1.06	10.23±0.59	9.46±0.06
Urinary outgo (g/d)	4.52±0.74	3.47±0.58	3.86±0.20
Retention (g/d)	10.67±0.62	11.44±0.76	11.75±0.83
(as % intake)	40.93±2.96	45.31±1.41	46.71±1.90

Means bearing different superscripts (capital: $p<0.01$; small: $p<0.05$) in a row differ significantly.

for the LP, NP and HP groups, respectively (Table 3). With no apparent effects of dietary CP levels, it could be concluded that even the LP diet with 12.6% CP was apparently able to provide adequate digestible protein to the intestine for the gains attained and there was no response in performance on increasing the protein intake beyond this level. Hence it can be inferred that the crossbred calves could be satisfactorily reared on 25% lower CP requirements than the level suggested by Kearl (1982).

Voluntary feed intake and FCR

The DM intake through concentrate mixture (CM) and oat hay have not shown any specific trend during various fortnights, and accordingly not varied significantly among treatments. During the first and second fortnights, calves fed LP diets exhibited lower ($p<0.05$) DMI through CM compared to NP or HP groups. The data on mean daily DM intake averaged across the 105 d of experimental feeding (Table 2), revealed that the intake of CM (g/d) was statistically similar among the groups, but when expressed as intake per kg $W^{0.75}$, the level of CM intake increased ($p<0.01$) linearly as the CP level in diet increased. The DM intake through oat hay (g/d), was higher ($p<0.05$) in NP calves fed normal protein in comparison to the other two groups. However, when expressed as per kg $W^{0.75}$, no variation was observed among the diets. Overall, the study indicated no beneficial effects of increasing dietary CP levels beyond 12.6% (LP diet) on the voluntary consumption of basal (oat hay) feed. Similarly, no influence of increasing CP levels on intake of wheat straw was noted by Sengar et al. (1985) in their studies on male calves. However, Promkot and Wanapat (2005) reported that DM intake by lactating cows tended to increase linearly with increasing dietary CP levels in the diets. The feed conversion ratio showed by the calves under the 3 groups also did not differ due to dietary treatments similar to the findings of Dabiri and Thonney (2004) in lambs. The lowest, although non-significant, DM intake required for per kg gain was seen in LP fed groups. This implies that calves, during the early post-weaned phase of life, can be reared on 25% lower protein as that recommended by Kearl (1982), without any adverse effect on growth or feed efficiency.

Intake and digestibility of nutrients

The intake and digestibility of proximate constituents by calves is shown in Table 3. Feeding various levels of CP to calves did not influence the DM intake (g/d) significantly. Similar to our observations, Sampath et al. (1991), Anderson et al. (1994) and Dabiri and Thonney (2004) also reported that protein supply had no effect on average daily feed intake. But Patil and Kaur (1994) reported higher DM intake in pregnant goats fed high CP in their ration. The CP content of diets is often related positively to DM intake (Roffler et al., 1986; Allen, 2000); this is partly from increased RDP effects on digestibility of feeds (Oldham, 1985), and the mechanism presumably involves a reduction in distension as fiber and DM digestibility increase.

As in case of DM, no influence of dietary CP levels was apparent on the mean daily intakes of organic nutrients other than CP; the latter was obviously keeping in line with the dietary treatments used for the experiment. Intake of CP has also been reported to increase linearly with increasing dietary CP level (Promkot and Wanapat, 2005). The digestible protein intake also followed the same trend showing significant increase ($p<0.01$) in HP group followed by NP and LP groups. Higher CP and thus higher DCP intake because of high protein feeding was also observed by Singh et al. (1994) in buffalo calves when fed varied CP levels.

Percent digestibility of DM, although has not shown any particular trend with respect to varying levels of dietary CP, but it was significantly higher ($p<0.05$) in HP group with no difference observed between NP and LP groups. The digestibility of CP has been reported to increase linearly with increasing dietary CP level (Dabiri and Thonney, 2004; Promkot and Wanapat, 2005). However, no effect of dietary CP levels on DM digestibility was noticed in other similar studies (Sengar et al., 1985; Sampath et al., 1991; Kakkar et al., 1991; Andersen et al., 1994). Patil and Kaur (1994) observed that feeding of CP at varied proportions of NRC requirements to crossbred goats has not imparted any difference in its digestibility in spite of significant differences in the intake of both CP and DCP. Oldham (1985) reported that the marginal increase in diet DM digestibility per unit percentage increase in diet CP content decreased as the CP content of the diet exceeded 15%, but was still positive when diet CP content exceeded 20%. This could explain, at least partly, the present findings.

Significantly higher ($p<0.05$) digestibility of OM, CF and NFE was observed upon feeding of HP than LP or NP diets, reflecting the same trend as that of DM. There were no significant differences however in the digestibility of EE among 3 groups, similar to the findings of Kakkar et al. (1991).

Balances of nutrients

Nitrogen balance : Average daily N intake by calves under different dietary regimes varied significantly ($p<0.01$) matching the CP levels used in the experiment (Table 4). The N outgo in faeces was lower ($p<0.05$) in LP fed animals in comparison to NP or HP groups. A positive relationship between N intake and its excretion through faeces has been noted by several authors (Al Jassim et al., 1991; Pattanaik et al., 2003). In the present case, however, no such clear-cut pattern was evident except for the lower faeces N excretion upon feeding LP diet, similar to the findings of Patil and Kaur (1994) and Kakkar et al. (1991). Contrary to the case with faecal N, the N excretion through urine apparently showed a positive relationship with the intake as have been observed by Dabiri and Thonney (2004). It could be attributed to a possible increase in the endogenous N losses due to increase in dietary protein levels. The urinary N excretion was higher ($p<0.01$) in HP group than NP or LP groups. Singh et al. (1994) have also found significant effect of protein level on protein loss through faeces and urine in buffalo calves.

Nitrogen balance, expressed either in absolute terms or in relation to kg $W^{0.75}$, showed a lower ($p<0.05$) retention in LP than HP group. Bunting et al. (1989) found maximum balance of N for high protein diets than for low protein diets. Contradictorily, no significant variation in N retention in beef calves fed different dietary protein levels was reported by Neville et al. (1977). The N retention, as percent of N intake, did not vary significantly due to dietary CP levels. However, the retention as percent of N absorbed was significantly ($p<0.05$) lower in HP group fed high protein diets in comparison to NP or LP groups. Although there was a significant difference in the intake of N in NP and LP groups, the retention as percent of N absorbed was almost similar in the two groups. On the other hand, significantly ($p<0.05$) lower retention of N as percent of absorbed N in HP group indicated a higher excretion through urine. This suggest that the intake was in excess of the animals capacity to utilize N efficiently, possibly because of a reduced energy availability. This was, in fact, more apparent when the ratios between dietary intake of ME and N were compared. The corresponding values of 101.0, 116.3 and 86.4 for the three groups, respectively, indicated a lower availability of ME per unit N for its effective utilization. Similar observations on N retention in relation to dietary energy (ME) intake have been observed earlier (Kishan et al., 1987). Further, while correlating between the N retention and ADG, a discrepancy was apparent in the sense that the LP group of calves exhibited similar ($p>0.05$) ADG in spite of lower ($p<0.05$) N retention. This could possibly be explained by a relatively greater availability of ME per unit N in this group (116.3) compared to the other two groups (101.0 and 86.4).

Calcium and phosphorus balance : The mean daily intake and excretion of calcium and phosphorus remained unaffected due to dietary treatments (Table 4). Although there were minor variations in the calcium content of the three concentrate mixtures, the net intake was, however, without any appreciable difference owing mainly to varying intakes of concentrate and oat hay by animals under the three groups. Although the quantity and quality of dietary protein could be expected to influence utilization of phosphorus in ruminants, the dietary levels tested in the present experiment appeared to have exerted no influence on its utilization. Similar to present findings, no difference in the calcium and phosphorus metabolism was also observed by Sengar et al. (1985) when different levels of

Table 5. Effect of dietary protein levels on the hematological, serum biochemical thyroid hormonal parameters[1] of calves

	Treatments		
	NP	LP	HP
Hb (g/L)	93.8±0.45	100.9±0.52	99.4±0.46
PCV (%)	27.31±1.18	28.13±0.85	30.50±1.36
Glucose (mg/L)	563.1±2.47	533.7±2.14	534.0±2.10
Total protein (g/L)	63.3±0.18	64.2±0.13	66.0±0.14
Albumin (g/L)	38.9±0.13	38.2±0.13	40.3±0.15
Globulin (g/L)	24.4±0.17	25.9±0.15	25.7±0.20
Urea (mg/L)	203.1±1.23	224.8±1.24	205.1±0.76
Calcium (mg/L)	95.2±0.27	101.5±0.25	101.2±0.27
Phosphorus (mg/L)	60.6±0.36	61.3±0.43	69.1±0.41
Alkaline phosphatase (U/L)	229.76±7.53	232.13±8.52	208.50±6.54
Tri-iodothyronine, T_3 (ng/ml)	1.32±0.18	1.16±0.13	1.38±0.19
Thyroxine, T_4 (ng/ml)	18.66±1.99	15.50±1.04	19.05±1.85

[1] Means of two collections.

protein were fed to male buffalo calves.

Metabolic profile and thyroid hormones

The data on blood biochemical profile along with that of the thyroid hormones are listed in Table 5. The blood levels of Hb and PCV did not vary significantly among the three groups and were in the suggested normal range (Singh et al., 1988; Radostits et al., 1994). The Hb and PCV are indicators of erythrocytic normality and general well being of the animal (Radostits et al., 1994). Absence of significant variations in these parameters, therefore, is suggestive of an optimum nutrient availability to the calves irrespective of dietary treatments.

The serum levels of glucose, total protein as well as albumin and globulin did not vary among the groups and were within the reported normal range (Kolbikava, 1978; Melvin, 1982). Likewise, Hallford et al. (1982) found no difference in serum total protein between ewes receiving protein deficient or adequate diets. Albumin serves as the major amino acid pool (Kaneko et al., 1997); the catabolism of albumin provides protein precursors needed for growth or other physiological needs. Higher protein intake has been reported to increase serum albumin by Skyes and Field (1973), Hallford et al. (1982) and Shetaewi and Ross (1991). Total protein and albumin thus reflect availability of protein and their concentration decline in the face of protein deficiency. Albumin has a relatively short half-life and can reflect protein deficiency problems over a period of a month or two. However, the similarity in the concentration of serum proteins after three-and-a-half months of feeding is suggestive of adequacy of the protein even on the LP diet.

The serum levels of urea were within normal range of 200-300 mg/L suggested by Kaneko et al. (1997) and did not vary significantly among diets. Hallford et al. (1982) have also noticed no effect of dietary protein levels on the serum urea concentrations. In fact, it was observed that high protein diets seldom cause increase in blood urea nitrogen in clinically healthy animals (Duncan and Prasse, 1986). However, Moss and Murray (1992) found higher plasma urea concentration due to supplementary protein intake on dairy calves. Similarly, Sun and Christopherson (2005) and Promkot and Wanapat (2005) have observed a positive relationship between blood/plasma urea-nitrogen and dietary CP in ruminants. Serum urea concentrations are influenced by a wide variety of interrelated parameters including: dietary protein intake and rumen degradability; dietary amino acid composition; protein intake relative to requirement; liver and kidney function; muscle tissue breakdown; and dietary carbohydrate amount and effective rumen degradable protein intake (Eicher et al., 1999; Murphy, 1999). But the lack of any variation in serum urea concentration in the present study indicated similar availability of dietary energy and protein across the three treatments.

The serum levels of calcium and inorganic phosphorus did not show any variation due to dietary treatments and the values exhibited by different groups were within the normal range reported by Radostits et al. (1994) and Kaneko et al. (1997). Shetaewi and Ross (1991) have also recorded similar levels of calcium and inorganic phosphorus in the serum of cows fed protein restricted or adequate diets. No treatment related effects were observed in the serum levels of alkaline phosphatase either. There used to be an increased secretion of ALP from the liver to the blood when the animals are in active stage of bone growth (Singh and Swarup, 1994). Hence the non-significant variation in the serum ALP could be the reflection of the similar ($p>0.05$) growth rate observed in the three groups (Table 2).

The dietary protein levels exerted no significant effects on the serum T_3 and T_4 levels, although animals fed LP diet tended to exhibit comparatively lower values for both the hormones. Pattanaik et al. (2002) have observed reduced levels of circulatory thyroid hormones in sheep and goats, when fed on protein-restricted diets. In the present study, a trend towards reduced serum thyroid hormones in calves fed lower dietary protein levels further points to an apparently positive relationship between protein nutrition and thyroid status. During the initial stage of growth (pre-experimental), lower serum levels of both T_3 and T_4 were observed which increased significantly (p<0.01) during the subsequent post-experimental period. This shows that as the age advances, the T_3 level in serum also increases. As the animals were in active growing stage, the thyroid gland must have been activated to secrete more T_3 and T_4 leading to their increased levels in the blood (Radostits et al., 1994). Similar findings were earlier reported by Shukla et al. (1994) in crossbred calves. The range of T_4 observed in the present study however, is much lower than the values reported for cows (Khang and Wiktorsson, 2005).

Based on the growth performance and metabolic profile data, it may be concluded that the crossbred calves can be satisfactorily reared on 25% lower protein level than the recommendations of Kearl (1982) in Indian conditions, thereby reducing cost of production and at the same time contributing towards reduced environmental pollution.

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