Dietary Protein Restriction on Growth and Immuno-biochemical Response of Crossbred Calves during Post-ruminant Phase of Life

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ABSTRACT : Sixteen crossbred (*Bos indicus*×*Bos taurus*) calves were randomly distributed in two groups (NP and LP) of eight calves each to study the effect of restricted (75%) protein supply on growth and immuno-biochemical response as an indicator of production and health of under-nourished animals during 3 to 9 months of age. The normal requirement of protein was provided to group NP and a less of 25% to group LP through calculated amount of concentrate and roughage in their daily ration. Assessment was made for weekly change in live weight, periodic alteration in blood metabolites and immunological status at six months of age in calves. An initial (during 3 to 6 months of age) depression ($p<0.05$) in growth was seen in low protein fed group (LP) compared to NP, which became non-significant in the later period of life (6 to 9 months of age). There was no significant effect on haemoglobin, total protein, albumin and globulin concentration except that of urea, which was decreased significantly ($p<0.05$) in animals fed on low protein diet (19.83±1.25 vs 25.93±1.29 mg/dl). The treatment effect that was seen in different periods of life was not uniform for other parameters except for urea, which showed a regular depression in LP compared to NP. The assessment of immunological status by indirect haemagglutination (IHA) test against *Pasteurella multocida* (P52 strain) was considerably ($p<0.05$) reduced in animals on LP ration compared to those on NP. It is thus argued that with poor nutrition (low protein) and state of compromised immunological response the production and health of the animals will be adversely affected. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 8 : 1121-1127)***

Key Words : Crossbred Calves, Protein Restriction, Growth, Blood Profile, Immunity

INTRODUCTION

Domestic livestock in tropics, especially in developing countries are undernourished due to chronic shortage of protein and energy rich feeds, which is further aggravated because of diversion of most of these ingredients to meet the requirements of ever increasing human population. As per the oil seed production estimate (USDA, 2002) there was a shortage of 55% digestible crude protein for livestock and poultry as against a demand of 35.82 million metric tons (MMT) in the year 2000-01, which may be narrowed down to 50% as per the projection by Feb. 2002. But ruminants to some extent fulfill their protein requirements through ruminal synthesis of microbial protein from other non-conventional nitrogen sources. In view of the feed resource crunch there is every possibility that the production and health of the animal will be affected adversely in a state of compromised immunological response. This is because of the fact that protein energy malnutrition (PEM) is invariably accompanied by infections; hence, the protein metabolic response to chronic protein and energy undernutrition is complicated by the concurrent response elicited by the infective stress (Jahoor et al., 1999). Epidemiological observations have established the aggravation of infections and many instances of vaccine failure in malnourished patients (Chandra, 1999). Sheffy and Williams (1982) examined the observations from India of the impact of seasonal starvation and refeeding of cattle on the frequency of foot and mouth disease outbreaks, and found a state of dysfunction in any one of the protective mechanisms resulting in some deficit in the inflamatory response and in specific immunity. Prolonged PEM in adult cattle was reported to influence both humoral and cell mediated immunity (Fiske and Adams, 1985). Protein deficiency results in decreased cytokine release and consequently affects regulation of the immune response (Klassing, 1988). A protein deficient diet in young growing animals may affect immunoglobulin synthesis (Reddy and Frey, 1993) and thus immunity in terms of humoral immune response is found to be an important criterion in assessing the intensity of invading microbial infection. Investigations were, thus carried out to see the immunological and biochemical response in three months old crossbred calves fed diets deficient by 25% of NRC (2001) protein requirements for 180 days.

MATERIALS AND METHODS

Animals and feeding

Sixteen three months old crossbred (*Bos indicus*×*Bos taurus*) calves were assigned at random in equal number to two isocaloric concentrate mixtures, one containing normal protein (NP) as per NRC (2001) and the other having 75% of NRC protein requirements (low protein, LP). The calves were fed measured quantities of respective concentrate

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mixtures (table 1) along with oat (*Avena sativa*) hay for initial 13 weeks and thereafter with wheat (*Triticum vulgare*) straw for the next 13 weeks at ad libitum level (120% of the calculated amount) to meet the stipulated requirements of growing animals as per NRC (2001). The animals were fed individually and the feed offered and residue left were recorded daily to monitor protein intake and so also to assess dry matter (DM) and nutrient intake during the above period.

Growth and protein intake

Weekly assessment of protein intake was made as per the increasing live weight they attained during the entire experimental period to regulate protein restriction in LP compared to NP. The live weight change was also recorded in the morning prior to feeding and watering to assess growth response in calves.

Blood collection, sampling, and preservation

Blood samples were collected by jugular venepuncture at fortnightly intervals upto 22 weeks of age. The serum was harvested after clotting by centrifugation and stored at -20°C for the analysis of various biochemical constituents.

Table 1. The formula and chemical composition of concentrates and roughages fed to calves

Item	Period 1 (3 to 6 m)		Period 2 (6 to 9 m)	
	NP	LP	NP	LP
Ingredient composition (%)				
Crushed maize	70	75	45	45
Wheat bran	5	7	35	35
Deoiled groundnut cake	15	5	10	0
Molasses	7	10	7	17
Mineral mixture#	3	3	3	3
Chemical composition (% DM)				
Concentrate				
DM	87.9	86.1	87.2	85.4
CP	15.30	11.65	14.70	11.65
NDF	29.3	27.5	40.6	38.6
Roughage				
DM	88.5	88.5	89.7	89.7
CP	6.3	6.3	3.4	3.4
NDF	79	79	74	74
ME* (total diet. Mcal/kg)	2.40	2.25	2.37	2.28

NP, Normal protein fed group; LP, Low protein fed group.

* ME=TDN×3.6.

Dicalcium phosphate 48.65, Calcium carbonate 17.40, Sodium chloride 31.57, Ferrous sulphate 2.00, Manganese oxide 1.58, Zinc sulphate 0.75, Copper sulphate 0.24, Cobalt sulphate 0.042, Potassium iodide 0.029, Sodium fluoride 0.111.

For immunological assay seven calves from each group were sensitized with H.S. (*Pasteurella multocida* P52 strain) oil adjuvant vaccine after 13 weeks of experimental feeding. Blood samples were collected at 0 and 21 d post-sensitization and the sera samples were preserved after inactivation at 56°C under deep freezing condition (-20°C) for assessing humoral immune response.

Feed analysis

The chemical composition of feed and fodder samples was determined as per the method described in AOAC (1990) and the neutral detergent fiber (NDF) by applying the procedure described in Van Soest (1991).

Blood biochemical assay

The haemoglobin concentration of blood was determined immediately after collection by cyanmethaemoglobin method (Coles, 1980). Briefly, 20 µl of blood was treated with 5.0 ml of Drabkin's solution (0.05 g KCN, 0.20 g $K_3Fe(CN)_6$, 1.00 g $NaHCO_3$ in 1 l H_2O) and the absorbance was read at 548 nm. The serum samples were analysed for urea by treating the protein-free serum sample (0.20 ml) with acid-ferric solution containing diacetylmonoxime and thiosemicarbazide (3.0 ml) and the colour intensity was read at 525 nm (Rahmtullah and Boyde, 1980). The serum total protein concentration was measured by treating with biuret reagent, where the formation of blue peptide-Cu complex in alkaline solution was measured spectrophotometrically at 540 nm (Gornall et al., 1949). The albumin was measured by dye-binding method, where 25 µl of blood serum was treated with 3.0 ml buffered (pH 4.2) dye solution (succinate buffer, pH 4.0; bromocresol green 0.6 mM; Brij-35 30% solution 0.06%) and the absorbance was read at 630 nm (Doumas et al., 1971) and the globulin was calculated by difference (total protein minus albumin).

Immunological assay

The humoral immune response in pre (0 d) and post (21 d) sensitized calves was assayed by indirect haemagglutination (IHA) test. Serial two fold dilutions of the sera samples ranging from 1:5 to 1:1.280 were exposed to agglutination using glutaraldehyde treated sheep red blood cells (RBC) sensitized with ultrasonicated antigen of *P. multocida* following the procedure of Sawada et al. (1982).

Statistical analysis

The data were subjected to analysis of variance and Student's 't' test as described in Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

Status of crude protein intake and live weight gain

The dry matter intake from concentrates and roughages and their relative contribution to total availability of CP in experimental animals was presented in table 2. The trends of protein intake during 3 to 6 and 6 to 9 months of age are shown in figure 1 and 2, respectively. A significant ($p<0.05$) correlation between CP intake and live weight of animals was indicative of relative increase in CP requirement in response to growth during 3 to 9 months of age. Experimental restriction of CP intake by diluting the CP concentration in concentration mixture was observed to induce about 26% and 23% less supply of CP in group LP compared to NP during 3 to 6 and 6 to 9 months of age, respectively. The narrowing of gap in CP intake between group NP and LP during second phase (6 to 9 months) was attributable to CP accountability from increased consumption of wheat straw in LP (table 2), which in fact was not available to the animals. Further, the change in roughage source from oat hay with 6.3% CP to wheat straw with 3.4% CP may have induced some degree of protein starvation and the animals tried to compensate the deficit by increased consumption of wheat straw in the later phase.

There was relatively decreased live weight gain in animals under LP compared to control (NP) and the difference was more acute (20% less gain) during the first phase (3 to 6 months of age). The tendency of growing animals to convert nutrients more efficiently is seen during early growing phase and that the restricted supply of protein during 3 to 6 months of age may thus be attributable to more acute response compared to that in latter phase. In addition, chronic dietary protein deficiency is associated with a reduction in whole body protein turnover, where the utilization of dietary amino acids by the muscle bed is reduced which in turn directed towards the maintenance of splanchnic protein synthesis (Hirschfield and Kern, 1969; Clowes et al., 1980). This redistribution serves the beneficial purpose of facilitating the transfer of amino acids from the peripheral tissues to the liver for the synthesis of essential proteins required in priority to tissue growth and thus affected growth in undernourished animals (Beisel.

Table 2. Status of protein intake and live weight gain in calves during 3 to 6 and 6 to 9 months of age

Attributes	Group NP	Group LP	'P' value
3 to 6 months			
Dry matter intake			
Concentrate (kg/d)	1.80±0.12	1.82±0.11	0.989
Roughage (kg/d)	0.40±0.04	0.39±0.04	0.983
Total (kg/d)	2.20±0.14	2.21±0.12	0.978
Total protein intake (g/d)	291±12	215±10	0.001**
Roughage% in the diet	18.1±2.7	17.8±2.1	0.931
Protein intake from oat hay (g/d)#	24.2±1.0 (8.28)	22.6±1.0 (11.02)	0.943
Live weight gain			
Initial body weight (kg)	42.4±1.8	42.9±2.8	0.883
Final body weight (kg)	80.9±4.8	73.4±4.3	0.264
Average daily gain (g)	423±31	335±24	0.041*
6 to 9 months			
Dry matter intake			
Concentrate (kg/d)	2.11±0.13	2.02±0.10	0.869
Roughage (kg/d)	0.56±0.07	0.60±0.04	0.908
Total (kg/d)	2.67±0.17	2.62±0.12	0.923
Total protein intake (g/d)	343±15	265±12	0.002**
Roughage% in the diet	20.7±2.0	22.9±2.0	0.434
Protein intake from straw (g/d)#	19.5±1.0 (5.68)	21.1±0.9 (7.96)	0.865
Live weight gain			
Initial body weight (kg)	80.9±4.8	73.4±4.3	0.264
Final body weight (kg)	118.0±5.6	105.2±6.0	0.121
Average daily gain (g)	415±29	350±21	0.095

NP, Normal protein fed group; LP, Low protein fed group.

* $p<0.05$; ** $p<0.01$.

Figures are expressed as Mean±SE.

Values in parentheses indicate CP intake from roughage as percent of total intake.

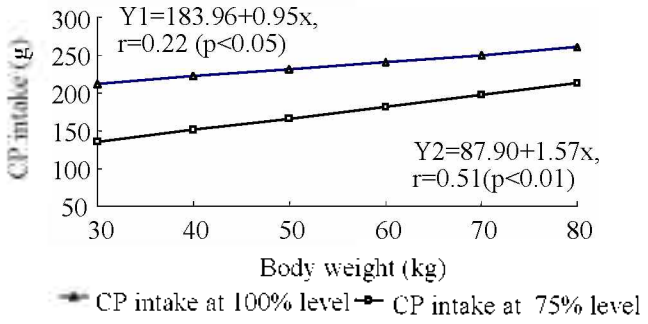

Figure 1. Regression of CP intake (g) on body weight (kg) during 3-6 months of age

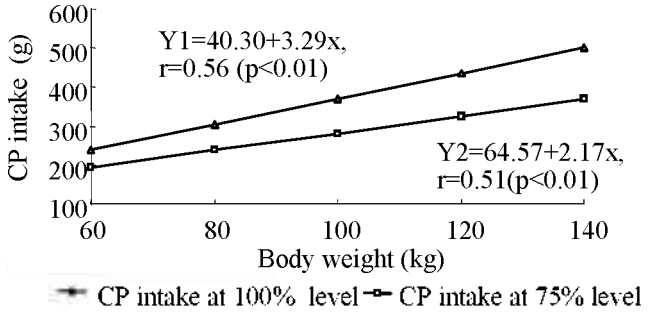

Figure 2. Regression of CP intake (g) on body weight (kg) during 6-9 months of age.

1984; Jahoor et al., 1999). This was the reason attributable to present set of findings in live weight gain between NP and LP groups during 3 to 6 and 6 to 9 months of age. However, the nutrient (ME) availability for the observed live weight gain in both NP and LP during the above period corresponded well with that delineated in NRC (2001).

Blood profile

Dietary protein reduction did not significantly affect the blood concentration of haemoglobin, total protein, albumin, globulin and albumin: globulin ratio (table 3). The treatment effect that was seen in different periods of life was not uniform for other parameters except for urea, which showed a regular depression in LP compared to NP. Ruminants, therefore, to some extent can tolerate the protein deficient diets by compensating the same through ruminal synthesis of microbial protein. A severe protein deficiency does interfere with haemoglobin production (Whitehair, 1958) but the 25% reduction in CP requirement for the calves in this study seemed not to be of severe nature and it did not also affect the serum protein concentration. The serum urea concentration was significantly ($p<0.05$) depressed in LP and thus indicative of a lower absorption of ammonia from the rumen due to restricted supply of protein compared to that in NP. This fall in serum urea level was despite the body regulatory mechanism of a decreased urinary nitrogen excretion with major proportion of urea being recycled in to the rumen through saliva (Satter and Roffler, 1977). There is either a fall in overall nitrogen flux or in its proportion resulting in decreased urinary nitrogen excretion (Millward et al., 1976). Further, Millward et al. (1991) suggested a relative preservation of nitrogen over carbon during amino acid catabolism on reduced protein intakes. Kidneys appear to play a significant role in regulating nitrogen metabolism during protein insufficiency through more salivary secretion with reduced urinary excretion (Bars, 1967).

In the present study, the non-significant effect on the concentration of protein, albumin and globulin with respect to experimental hypoproteinaemia (75% of normal) may have suggested that dietary protein reduction is better compensated by adaptive regulation of protein metabolism. Experimental and clinical hypoproteinaemia might result in liver atrophy (Oser, 1971), which affects albumin synthesis as shown in rats on protein deficient diets (Charland et al., 1994). According to Tizzard (1992) the globulin level was the last to be affected in prolonged hypoproteinaemia resulting in lowered immunity in animals. However, the endogenous protein synthesis would be well maintained throughout the period of protein deficiency by recycling the plasma protein pool (Jeejeebhoy et al., 1973; Golden et al., 1977). The stress of infection, inflammation and injury do not suppress the rate of synthesis of albumin, rather an increase in catabolism or intravascular loss of albumin and total protein may have induced a depressing effect (Fleck et al., 1985), which was not observed in the present experiment. The body metabolism shows an adaptive change to a chronic protein deficiency and maintains the plasma pool of protein and albumin unless and until it crosses the threshold level, which is determined by the balance between its rate of synthesis and catabolism or loss from the vascular compartment.

Immune response

There was significant depression in IHA titre (68.5±10.9 vs 32.0±6.0) in protein deficient animals compared to normal protein fed animals. Such effect with deprived protein nutrition was not uncommon (McGillivray, 1967; Tizzard, 1992; Reddy and Frey, 1993). This may be due to depression in antibody synthesis and antigen driven clonal expansion of lymphocytes (Pugliese, 1990). The general metabolic response to stress of infection, inflammation or trauma is characterized by an increased rate of whole body protein turnover and a redistribution of protein synthetic activity away from the synthesis of muscle and tissue proteins in support of immune related processes that are critical for survival (Jahoor et al., 1988; Fleck, 1989). However, in prolonged protein insufficiency there is a disjunctive effect on humoral and cell-mediated immunity

Table 3. Blood profile in calves exposed to experimental dilution of protein during 3 to 6 months period

Groups	Age of calves (weeks)					Group mean
	14	16	18	20	22	
Haemoglobin (g/dl)						
NP	9.57±0.46	10.28±0.62	11.25±0.38	11.19±0.58	11.80±0.77	10.82±0.28
LP	9.18±0.59	10.27±0.63	11.28±0.38	11.76±0.60	11.64±0.39	10.83±0.27
Periodic mean	9.38±0.36^{n}	10.27±0.43^{n}	11.26±0.26^{m}	11.72±0.42^{m}	11.48±0.42^{m}	10.82±0.19
Urea (mg/dl)						
NP	29.13±3.72^{a}	27.20±3.04^{a}	25.15±2.32	21.49±3.32	26.70±2.23	25.93±1.29^{a}
LP	21.27±3.20^{b}	18.86±3.00^{b}	21.53±3.18	17.36±1.89	20.34±3.01	19.83±1.25^{b}
Periodic mean	25.20±2.46	22.93±2.34	23.34±1.96	19.43±1.92	23.52±1.99	22.88±0.96
Total protein (g/dl)						
NP	6.34±0.22	6.82±0.26	7.26±0.29	6.85±0.29	7.05±0.13^{a}	6.86±0.12
LP	6.50±0.15	6.86±0.14	6.91±0.23	7.31±0.22	6.57±0.18^{b}	6.83±0.09
Periodic mean	6.42±0.13^{n}	6.84±0.14^{mn}	7.09±0.19^{m}	7.08±0.19^{m}	6.81±0.11^{mn}	6.85±0.07
Albumin (g/dl)						
NP	3.39±0.20	3.71±0.07	4.17±0.20	3.68±0.22	4.03±0.10	3.80±0.09
LP	3.46±0.15	3.82±0.11	3.67±0.14	4.09±0.19	3.54±0.10	3.72±0.07
Periodic mean	3.42±0.12^{n}	3.77±0.12^{m}	3.92±0.14^{m}	3.89±0.15^{m}	3.79±0.09^{m}	3.76±0.06
Globulins (g/dl)						
NP	2.96±0.06	3.01±0.07	3.02±0.10	3.03±0.10	3.01±0.09	3.00±0.04
LP	2.98±0.05	2.95±0.07	3.09±0.08	3.08±0.07	2.93±0.07	3.01±0.02
Periodic mean	2.97±0.04	2.98±0.05	3.05±0.06	3.06±0.06	2.97±0.06	3.01±0.06
Albumin: globulins ratio						
NP	1.15±0.06	1.23±0.07	1.35±0.04^{a}	1.23±0.07	1.35±0.04^{a}	1.26±0.03
LP	1.14±0.05	1.30±0.05	1.19±0.04^{b}	1.28±0.05	1.22±0.05^{b}	1.24±0.02
Periodic mean	1.15±0.04^{n}	1.27±0.04^{m}	1.29±0.04^{m}	1.27±0.05^{m}	1.26±0.03^{m}	1.25±0.02

NP, Normal protein fed group; LP, Low protein fed group.
Figures are expressed as Mean±SE.
Group means/ periodic means bearing different superscripts in a column/ row differ significantly ($p<0.05$).

Table 4. Indirect haemagglutination titres in the sera samples of crossbred calves immunized with H.S. oil adjuvant vaccine

Protein status	Days post-sensitization	Observation on animals							Mean±SE
		1	2	3	4	5	6	7	
100% CP	0	0	0	0	0	0	0	0	0.0±0.0
	21	64	32	64	128	64	64	64	68.5±10.9^{a}
		(6)	(5)	(6)	(7)	(6)	(6)	(6)	(6.00±0.22^{a})
75% CP	0	0	0	0	0	0	0	0	0.0±0.0
	21	64	32	16	32	32	16	32	32.0±6.0^{b}
		(6)	(5)	(4)	(5)	(5)	(4)	(5)	(4.86±0.26^{b})

Means bearing different superscripts differ significantly ($p<0.05$).
Values in parentheses represents $\log_2$ values.

(Cooper et al., 1974). Further, in growing animals, a low dietary protein would have effected depression in antigenic response compared to adults where rumen microbes are capable of producing amino acids from carbohydrates and non-protein nitrogen so long the calorie intake is adequate (Satter and Roffler, 1977). On the other hand, low protein intake seemed to exaggerate the effect of low energy consumption (Woodard et al., 1980). In the present study, the energy density of total diet was observed to be similar in both NP and LP due to non-significant difference in roughage intake and a fixed level of concentrate, which was made iso-caloric but with desired difference in protein level (100% and 75% of NRC, 2001).

CONCLUSION

Although the blood level of cellular (albumin and globulin) integrity was not disturbed a significant depression in growth and humoral immunity might be adverse to future ruminant productivity. Level of immunocompetence was thus observed to be a sensitive indicator of the adequacy of nutritional regimens and should serve as diagnostic in addition to metabolic profiles in their evaluation. Further, it warrants thorough investigation involving large number of animals with wider variation of protein restriction, which should go concurrent with field observations on various production parameters before any recommendation is being made.

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