ABSTRACT: Immunobiochemical status of sheep exposed to periods of experimental protein deficit and realimentation was studied in 12 sheep (15 mo) randomly distributed into 2 equal groups and fed individually 2 different concentrate supplements along with wheat straw to provide 100% (normal protein, NP) or 50% (low protein, LP) of CP requirements. The study was comprised of 3 periods; during period 1 (0 to 13 wk) and 2 (14 to 26 wk) animals in the 2 groups were fed NP and LP diets, respectively; during period 3 (27 to 44 wk), animals in the LP group were switched over to NP diet to allow realimentation, whereas animals in the NP group remained on the same NP diet. Blood was collected from all groups at end of each period, and serum glucose, total protein, albumin (sAlb), globulin (sGlb), urea (sU), creatinine (sCr), cholesterol, triiodothyronine, and thyroxine concentrations were determined. During the same periods, the cell-mediated immune (CMI) response was measured by a delayed type hypersensitivity (DTH) assay and in vitro nitrite production by lymphocytes. At the end of periods 2 and 3, humoral immune response (HIR) was measured by sensitizing the sheep with Brucella abortus S99 antigen and measuring antibody titers on 0, 7, 14, 21, and 28 d postinoculation by ELISA. Feed intake decreased with prolonged protein deficit and showed recovery during period 3. Blood chemistry revealed reduced sAlb concentration in the LP group resulting in narrow sAlb:sGlb ratio, increased sCr concentrations ($P = 0.008$) accompanying a decreased ($P = 0.004$) sU:sCr ratio, and decreased glucose concentrations ($P = 0.05$). Other variables did not change significantly between the NP and LP groups. The DTH response at the end of period 1 and 2 showed marked ($P = 0.008$) effect of protein restriction on CMI. Nitrite production, basal and after lipopolysaccharide stimulation, was greater ($P = 0.04$) in the NP group. The HIR was less ($P = 0.04$) in the LP group during period 3. Realimentation of protein in the LP group during period 3 showed recovery in CMI and HIR. In conclusion, protein deprivation induced a decline in CMI and HIR in sheep accompanying alterations in related metabolic profile. However, a marked recovery was observed after realimentation.

Key words: immunity, metabolic profile, nutrition, protein deficit, sheep

INTRODUCTION

The effects of nutrition on immunological functions have been the focus of clinical and experimental studies. Field studies in developing countries have consistently linked severe malnutrition with immunologic crippling and increased susceptibility to infection (Good, 1981). Immune functions have traditionally been regarded as part of maintenance requirements, but an increasing body of evidence suggests sensitivity of immunity to nutrient supply (Galyean et al., 1999). Domestic livestock in tropics are undernourished due to chronic shortage of protein and energy-rich feeds. Sheep and goats are often raised on grazing with little or no supplementation. Unavailability of forage is the main reason of underfeeding, and protein is the most critical macronutrient. We have established that deficits in protein and reduced immune status adversely affect production of animals (Sahoo et al., 2002). Protein deficiency decreases cytokine release and consequently affects regulation of immune response (Klasing, 1988) and immunoglobulin synthesis (Reddy and Frey, 1993). Protein malnutrition is often accompanied by infections, and metabolic response to chronic protein and energy malnutrition is complicated by the concurrent response elicited by the stress due to infection (Jahoor et al., 1999). The diversion of nutrients from growth to immune-related processes is a major cause behind fail-
ure to achieve optimal growth in the face of an immune challenge (Spurlock, 1997). Epidemiological observations have established the aggravation of infections and many instances of vaccine failure in malnourished patients (Chandra, 1999). Sheffy and Williams (1982) reported the impact of seasonal starvation and refeeding of cattle on frequency of foot and mouth disease outbreaks in India. They suggested that dysfunction in any protective mechanism results in inadequate inflammatory response and specific immunity. Periodic starvation or malnutrition with a protein-deficit occurs in many developing countries (Devendra and Sevilla, 2002). Our study was carried out to assess immunological and biochemical responses in sheep fed a low protein (LP) diet and after realimentation with a normal protein (NP) diet.

MATERIALS AND METHODS

The protocol for the current study was approved by the Animal Ethics Committee of the Indian Veterinary Research Institute.

The experiment was conducted in 3 periods. Periods 1 (0 to 13 wk) and 2 (14 to 26 wk) were to assess the effects of protein deficit on a short-term and long-term basis, respectively, whereas period 3 (27 to 44 wk) was aimed at ascertaining the effects of realimentation of the deficit group, when switched over to normal level of protein feeding. The study was carried out using 12 apparently healthy 15-mo-old Muzzafarnagari rams as experimental animals. The animals were procured from the sheep farm of Central Institute for Research on Goats (Makhdoom, India). Before the beginning of the experiment, all the animals were fed a standard diet consisting of concentrate and wheat straw for a period of 30 d to ensure similar nutritional and metabolic status. The animals were housed in a well-ventilated shed with facilities for exercise in the adjacent paddock and had ad libitum access to fresh water.

Animal and Experimental Design

The details of experimental design and distribution of sheep in different groups are presented in Figure 1. The animals were distributed randomly in 2 groups, NP-fed group (22.8 ± 2.0 kg) and LP-fed group (23.0 ± 1.6 kg), of 6 animals each. The NP group was fed a diet with 13% CP (on as-fed basis) consisting of concentrate mixture (Table 1) and wheat straw. The CP content of the diet in LP group was reduced to 50% of that in NP (i.e., CP 6.5% on as-fed basis) by reducing the level of protein meal in the concentrate mixture, but the energy content was similar. The ratio of concentrate and roughage was regulated once every 2 wk during the course of experimental feeding to meet the minimum growth potential (20 to 40 g of BW gain/d) as per NRC (1985) with minor modifications recommended by ICAR (1998) to account for the characteristically lesser BW and growth rate of crossbred sheep in India. The sheep were fed individually with required concentrate mixture and wheat straw (ad libitum with 10 to 15% refusal) offered separately in 1 feeding, and daily feed weigh backs were performed to assess the level of feed intake. Samples of the offered feeds were collected every 2 wk to determine DM content and nutrient composition, whereas samples of refusal were collected and weighed daily from each pen to determine DMI. The pooled samples of feeds and refusals were dried in a forced-air oven (50°C), ground in a laboratory mill (SM100, Retsch GmbH, Haan, Germany) through a 1-mm screen, and stored for laboratory analysis. The restriction of CP to 50% level was imposed gradually starting with 75%, then 60% of NP, with each period lasting 3 d. The study was comprised of 3 periods; during periods 1 (0 to 13 wk) and 2 (14 to 26 wk), animals under NP and LP groups were fed the respective NP and LP diets. However, immediately after period 2, starting from 27 wk, LP group animals were switched over to the NP diet, as a measure of dietary protein realimentation, whereas the NP group was allowed to be fed on the normal protein diet (period 3), extending up to 44 wk. Thus, the NP animals were fed the normal protein diet for the whole duration of the study (i.e., 44 wk), whereas LP animals were fed a protein-deficient diet for first 26 wk, which was followed by feeding a normal protein diet for the last 18 wk of the experiment. Period 3 was designed to assess recuperative changes after alimentation to a normal protein diet from a state of deficit protein nutrition.

BW Recording

The animals were weighed at regular intervals (once every 2 wk) in the morning before offering feed and water to record pattern of BW change during the 3 periods.
Blood Collection and Sampling

Blood samples were collected by jugular venipuncture at the end of each period to monitor the periodic alterations in hematobiochemical attributes viz. hemoglobin (Hb), total serum protein (sTP), albumin (sAlb), globulins (sGlb), urea (sU), creatinine (sCr), glucose (sG), cholesterol (sCh), triiodothyronine (T₃), and thyroxine (T₄). Blood samples were collected in 10-mL tubes without anticoagulant, left for 30 min at room temperature, and centrifuged for 20 min at 1,000 × g at room temperature (25°C), and the serum was stored at –20°C for analysis.

Feed Analysis

Samples of concentrate mixture, wheat straw, and refusals were analyzed for DM, ash, N, and Soxhlet ether extract (methods 930.15, 942.05, 990.02, and 920.39, respectively; AOAC, 1990), and NDF and ADF (Van Soest et al., 1991). Neutral detergent fiber was determined with sodium sulfite and α-amylase in the NDF extract (methods 973.18, 990.03, 990.04, and 990.05), respectively; AOAC, 1990), and NDF and ADF (Van Soest et al., 1991). Neutral detergent fiber was determined with sodium sulfite and α-amylase in the NDF reagent. The values for NDF and ADF were expressed with residual ash.

Blood Biochemical Assay

The Hb concentration of blood was determined immediately after collection by cyanomethemoglobin method (Coles, 1980). Briefly, 20 μL of blood was treated with 5.0 mL of Drabkin’s solution [0.05 g of KCN, 0.20 g of K₃Fe(CN)₆, 1.00 g of NaHCO₃ in 1 L of H₂O], and the absorbance was read at 548 nm. The sTP concentration was measured by treating with biuret reagent, where 25 μL of blood serum was treated with 3.0 mL of buffered (pH 4.2) dye solution (sucinate buffer, pH 4.0; bromocresol green 0.6 mM; Brij-35 30% solution 0.06%) and the absorbance was read at 525 nm (Rahmatullah and Boyde, 1980). The sCr was measured spectrophotometrically by Jaffé’s reaction (Heinegard and Tiderstrom, 1973). The sG was determined by O-toluidine reduction method (Hultén, 1959), and sCh was determined through oxidation with ferric chloride under acidic condition and measuring the purple colored complex at 570 nm (Zlatkis et al., 1953). All the measurements were done using a UV-visible spectrophotometer (UV2800, Labomed Inc., Culver City, CA). A diagnostic kit supplied by Bhabha Atomic Research Centre, Mumbai was used for the RIA of T₃ and T₄ with counting in a Multi Crystal Gamma Counter (LB 2103, Berthold-Wallac, Wildbad, Germany).

### Immunological Variables

The cell-mediated immune (CMI) response of the experimental animals was assessed at the end of each period. Delayed type hypersensitivity (DTH) was assessed from increase in skin thickness after intradermal inoculation of phytohaemagglutinin-P (PHA-P) antigen at 24, 48, 72, and 96 h. In vitro nitrite production, basal and after simulation with lipopolysaccharides (LPS) by lymphocytes in the culture medium as a measure of CMI, was determined by Griess’s reaction method (Green et al., 1982). Mononuclear cells from the peripheral blood were separated by density gradient centrifugation (Boyum, 1968). Briefly, whole blood (5 mL) was collected into heparin-coated tubes (Hindustan Syringes and Medical Devices Limited, New Delhi, India) by jugular venipuncture from each animal. The blood was diluted 1:1 with Hank’s balanced salt solution (HBSS) and 7 mL of the blood/HBSS mixture was layered over 4 mL of histopaque (Sigma, St. Louis, MO). Samples were centrifuged at 400 × g for 30 min at 25°C. After centrifugation, the interface cells were removed and washed twice with sterile HBSS. Finally, cells were dispensed in RPMI-1640 medium (phenol free) having 10% fetal calf serum, 100 IU of penicillin, 100 μg of streptomycin, and supplemented with 5 mM of L-arginine to achieve a cell concentration of 5 × 10⁶ cell/mL. Ninety-six well flat-bottomed tissue culture plates were seeded with 5 × 10⁶ cells in 100 μL of above medium containing Brucella antigen (10 μg/mL) followed by incubation at 37°C under 5% CO₂ for 48 h. For the estimation of nitrite, culture superna-

### Table 1. Physical composition (% as-fed basis) of concentrate supplement fed to sheep during the experiment

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Concentrate with normal protein</th>
<th>Concentrate with low protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground maize</td>
<td>12.5</td>
<td>26.0</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>18.0</td>
<td>69.0</td>
</tr>
<tr>
<td>Soybean meal, solvent extracted</td>
<td>68.0</td>
<td>33</td>
</tr>
<tr>
<td>Mineral mixture¹</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Salt</td>
<td>0.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

¹Contained per kilogram: 280 g of Ca; 120 g of P; 5 g of Fe; 0.26 g of I; 0.77 g of Cu; 0.13 g of Co.

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**Table 1.** Physical composition (% as-fed basis) of concentrate supplement fed to sheep during the experiment.
tant (50 μL) was mixed with an equal volume of Griess reagent [0.1% N-(1-naphthyl) ethylenediamine dihydrochloride, 1% sulfanilamide, and 2.5% H₃PO₄], and absorbance was recorded at 570 nm using a microplate reader (Microscan-MS5605A, Electronic Corporation of India Limited, Hyderabad, India). Nitrite level was determined by comparison with sodium nitrite standard curve. Similar observations were also recorded toward the end of reallimentation period (44 wk). Before that, humoral immune response (HIR) of the animals was assessed in both the phases by sensitizing the sheep with *Brucella abortus* S99 antigen and measuring the antibody titer at 0, 7, 14, 21, and 28 d postinoculation by ELISA. A constant dilution of serum on different days (1:1,000 in PBS with Tween-20) for each test serum was used for antigen-antibody reaction, and the color development was measured at 492 nm. Greater absorbance indicated greater antibody titer in the sample, thus indicating qualitative difference between the treatments.

### Statistical Analysis

A mixed model with repeated measures was used for the analysis of data. The data were subjected to ANOVA and Student’s *t*-test (Snedecor and Cochran, 1989). The statistical difference between periods 1, 2, and 3 was assessed separately. Analysis of covariance was employed to ascertain the postchallenge periodic difference in immunological parameters between the treatments. The statistical software SPSS (SPSS Inc., Chicago, IL) was used for the analysis of data.

### RESULTS

#### Nutrient Composition and Intake

The basal roughage of wheat straw contained 39 g of CP/kg of DM, whereas it was 339 and 141 g/kg of DM in concentrate mixtures for NP and LP, respectively (Table 2). Animals in LP group did not show any sign of gastrointestinal disorder during the course of protein restriction. The feed DMI in both the groups was statistically similar (P = 0.91) during period 1, but the CP intake was greater in NP (101 g/d) than LP (53 g/d) as it was experimentally reduced in the later group (Table 3). Prolonged protein deficit (period 2) reduced (P = 0.007) both DMI (62.7 ± 2.59 vs. 52.0 ± 1.85 g/kg of BW⁰.⁷⁵) and CP intake of animals in the LP group compared with animals in the NP group. Animals in the LP group showed increased (P = 0.03) intake of feed DM and CP during the reallimentation period (period 3) and became similar (P = 0.22) to the animals in NP group.

#### Pattern of BW Change

The BW at 13, 26, and 44 wk of experimental feeding (Figure 2) was not different (P > 0.05) between the groups. However, there was a decrease in net BW gain of animals in the LP group compared with animals in the NP group during periods 1 (1.4 ± 0.5 vs. 3.6 ± 0.5 kg; P = 0.02) and 2 (0.8 ± 0.3 vs. 2.8 ± 0.5 kg; P = 0.009). During period 3, animals in the LP group showed a comparative increase (P < 0.001) in BW gain (3.6 ± 0.5 kg) relative to the animals in the NP group (2.4 ± 0.4 kg).

### Table 2. Chemical composition (% of DM) of the concentrate supplement and wheat straw fed to sheep during the experiment

<table>
<thead>
<tr>
<th>Item</th>
<th>Concentrate supplement¹</th>
<th>Wheat straw</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NP</td>
<td>LP</td>
</tr>
<tr>
<td>OM</td>
<td>90.7</td>
<td>93.0</td>
</tr>
<tr>
<td>CP</td>
<td>33.9</td>
<td>14.1</td>
</tr>
<tr>
<td>Ether extract</td>
<td>3.0</td>
<td>2.8</td>
</tr>
<tr>
<td>Total carbohydrate²</td>
<td>53.7</td>
<td>76.1</td>
</tr>
<tr>
<td>NDF</td>
<td>38.8</td>
<td>43.7</td>
</tr>
<tr>
<td>ADF</td>
<td>9.7</td>
<td>15.5</td>
</tr>
</tbody>
</table>

¹NP = normal protein; LP = low protein.
²Total carbohydrate = OM − CP − ether extract.

### Table 3. Pattern of DM and CP intake in sheep exposed to protein restriction and reallimentation<br>³

<table>
<thead>
<tr>
<th>Item²</th>
<th>Period¹</th>
<th>Overall (0 to 44 wk)</th>
<th>P-value⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMI, g/(kg of BW⁰.⁷⁵·d)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NP</td>
<td>48.6 ± 4.24⁵</td>
<td>62.7 ± 2.59⁵</td>
<td>68.1 ± 2.32⁵</td>
</tr>
<tr>
<td>LP</td>
<td>53.6 ± 3.03⁵</td>
<td>52.0 ± 1.85⁵</td>
<td>64.4 ± 2.58⁵</td>
</tr>
<tr>
<td>CP intake, g/(kg of BW⁰.⁷⁵·d)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NP</td>
<td>8.0 ± 0.108⁵</td>
<td>8.1 ± 0.077⁵</td>
<td>8.1 ± 0.098</td>
</tr>
<tr>
<td>LP</td>
<td>4.2 ± 0.071⁵</td>
<td>4.4 ± 0.052⁵</td>
<td>8.0 ± 0.087⁵</td>
</tr>
</tbody>
</table>

⁵Within a row, means without a common superscript letter differ (P < 0.05).
⁶Means in a column (between treatment groups NP and LP) differ (P < 0.05).
¹The data are expressed as mean ± SEM from n = 6 observations.
²NP = normal protein diet, provided CP for maintenance as per NRC (1985) and ICAR (1998); LP = low protein diet, provided 50% CP of NP (LP diet was replaced with NP during period 3).
³Period 1 and 2 refer to short- and long-term protein restriction, respectively; period 3 refers to period of reallimentation.
⁴P-values for period effects.
Blood Variables

The Hb concentration (g/dL) showed no difference (P = 0.09) between the NP and LP groups in period 1, but declined (P = 0.04) later in period 2, thereby revealing variation (P = 0.007) between the 2 groups (9.0 vs. 12.0 g/dL; Table 4). In period 3, there was recuperation and the values became comparable between LP and NP. Concentration of sTP in the LP group declined (P = 0.04) in period 2 (5.4 g/dL) and was less (P = 0.05) compared with the NP group (6.6 g/dL). There was also decrease in sAlb concentration in the LP group (2.3 g/dL) showing apparent difference (P = 0.05) from the NP group (3.2 g/dL) during period 2, which recovered partially during period 3 (2.5 g/dL), resulting in an overall decreased (P = 0.06) value (2.5 g/dL) compared with the NP group (3.0 g/dL). No alteration (P = 0.24) in sGlb concentration was observed in LP during period 1 to 3, and there was no difference between the groups (P = 0.95). The sAlb:sGlb ratio revealed similar treatment effect and treatment × period interaction as that observed for sGlb. Prolonged feeding of LP also reflected in decreased (P = 0.05) sCr concentration (18.6 ± 2.4 mg/dL during period 2), which showed recovery during the realimentation period (21.9 ± 1.85 mg/dL). There were similar (P = 0.57) sU values in the NP and LP groups during period 1, but it declined (P = 0.05) in the LP group during period 2 and showed recovery during period 3. However, there was no difference between the treatment groups (P = 0.81). Concentration of sCh (mg/dL) did not differ between the treatments or the periods showing an average value of 116.8 mg/dL in NP and 111.1 mg/dL in LP groups. The concentration of T3 was comparatively less in LP than NP during period 1 (P = 0.10) but not during period 2 (P = 0.47) or 3. However, serum concentration of T4 did not differ (P = 0.56) between the NP and LP groups during any period of observation.

Immunological Response

The CMI response of sheep as assessed through DTH reaction at the end of periods 1, 2, and 3 revealed increased (P < 0.001) skin thickness after 24 and 48 h of intradermal inoculation of PHA-P antigen, which reduced (P < 0.001) subsequently at 72 and 96 h (Figure 3). There was a difference (P = 0.007) in the rate of DTH reaction between the NP and LP groups in periods 1 and 2. After realimentation (period 3), the gap was narrowed down with no difference (P > 0.05) between normal and deficient groups. Similarly, the CMI response assessed through in vitro nitrite production revealed greater (P = 0.04) values for basal and post-LPS stimulation in the animals of LP group during period 2 (Figure 4). The protein deficient animals in LP group, however, showed recovery with similar (P > 0.05) in vitro nitrite production when shifted to NP diet during period 3.

The HIR by ELISA showed a reduced (P = 0.04) antibody titer (OD492) against Brucella abortus S99 antigen in the animals on the LP diet (Figure 5). The titer showed an increasing trend until 21 d postinoculation of antigen and continued to remain at peak concentration until 28 d and declined thereafter. There was no difference (P = 0.52) in antibody titer between the NP and LP groups during realimentation period, indicating a recovery in HIR.

DISCUSSION

Crude protein intake by animals in the NP group was within the range recommended by NRC (1985) and ICAR (1998) for the maintenance requirement of sheep. However, the CP intake in the LP group was reduced by nearly 50% of that in the NP group. Paul et al. (2003) in a comprehensive study recommended a reduced protein requirement (6.98 g of CP and 4.49 g of digestible CP per kg of BW0.75) for the maintenance of growing Indian sheep (BW range > 15 kg) under the tropical condition, compared with the recommendations of NRC and ICAR. Thus, when compared with the recommendations suggested by Paul et al. (2003), CP intake by the LP animals in the present study fell short by nearly 35%.
The decrease in feed and nutrient intake with the long-term protein deficit might be attributed to a complex set of metabolic alterations related to ruminal digestion and fermentative activities (Michalet-Doreau and Doreau, 2001; Doreau et al., 2003). During the realimentation phase (period 3), animals in group LP did not show hyperphagy, which may be attributed to the roughage component of the diet providing an intrinsic limitation to voluntary feed intake (Weston, 1996; Baumont et al., 2000; Fisher, 2002). A decreased BW gain in LP during periods 1 and 2 was attributed to less total feed and nutrient intake. However, a greater BW gain during period 3 by LP group animals compared with NP animals, without any apparent variation in DMI, may be attributable to metabolic readjustment to prolonged LP and reduced feed intake, thereby economizing the available nutrient resources for a greater rate of gain in compensation.

The Hb level decreased during period 2 as an effect of prolonged protein deficit. A severe protein deficiency has been suggested to interfere with Hb production (Whitehair, 1958). In domestic ruminants, sTP and sAlb concentrations are used for the assessment of nutritional status (Kaneko, 1989), and reduced concentrations are a measure of protein deficiency and malnutrition. The sAlb has a long half-life, but inadequate dietary protein intake leads to a slow and gradual decrease in the albumin concentration when enough AA are not supplied to the liver cells for its synthesis, and thus, its concentration provides a long-term indicator of protein intake in ruminants.

### Table 4. Effects of protein restriction and recuperation on blood variables of sheep

<table>
<thead>
<tr>
<th>Attribute (^2)</th>
<th>1 (0 to 13 wk)</th>
<th>2 (14 to 26 wk)</th>
<th>3 (27 to 44 wk)</th>
<th>Overall (0 to 44 wk)</th>
<th>(P)-value(^4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin, g/dL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NP</td>
<td>12.4 ± 0.39</td>
<td>12.0 ± 0.33(^x)</td>
<td>12.7 ± 0.43</td>
<td>12.4 ± 0.38</td>
<td>0.86</td>
</tr>
<tr>
<td>LP</td>
<td>11.3 ± 0.57(^x)</td>
<td>9.0 ± 0.41(^b)</td>
<td>11.2 ± 0.29(^a)</td>
<td>10.5 ± 0.40</td>
<td>0.04</td>
</tr>
<tr>
<td>Total protein, g/dL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NP</td>
<td>6.8 ± 0.43</td>
<td>6.6 ± 0.35(^x)</td>
<td>6.5 ± 0.28</td>
<td>6.6 ± 0.21</td>
<td>0.92</td>
</tr>
<tr>
<td>LP</td>
<td>6.6 ± 0.27(^x)</td>
<td>5.4 ± 0.33(^b)</td>
<td>6.0 ± 0.25(^ab)</td>
<td>6.0 ± 0.18</td>
<td>0.04</td>
</tr>
<tr>
<td>Albumin, g/dL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NP</td>
<td>3.2 ± 0.17</td>
<td>3.2 ± 0.21(^x)</td>
<td>3.0 ± 0.21(^x)</td>
<td>3.0 ± 0.18(^x)</td>
<td>0.98</td>
</tr>
<tr>
<td>LP</td>
<td>2.9 ± 0.18(^x)</td>
<td>2.3 ± 0.16(^b)</td>
<td>2.5 ± 0.11(^b)</td>
<td>2.5 ± 0.12</td>
<td>0.04</td>
</tr>
<tr>
<td>Globulin, g/dL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NP</td>
<td>3.8 ± 0.26</td>
<td>3.6 ± 0.21</td>
<td>3.5 ± 0.35</td>
<td>3.6 ± 0.21</td>
<td>0.93</td>
</tr>
<tr>
<td>LP</td>
<td>3.7 ± 0.25</td>
<td>3.2 ± 0.19</td>
<td>3.5 ± 0.39</td>
<td>3.5 ± 0.21</td>
<td>0.24</td>
</tr>
<tr>
<td>Albumin:globulin ratio</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>NP</td>
<td>0.81 ± 0.047</td>
<td>0.85 ± 0.051(^a)</td>
<td>0.85 ± 0.057</td>
<td>0.84 ± 0.039(^x)</td>
<td>0.93</td>
</tr>
<tr>
<td>LP</td>
<td>0.77 ± 0.039</td>
<td>0.70 ± 0.037</td>
<td>0.71 ± 0.044</td>
<td>0.73 ± 0.031</td>
<td>0.44</td>
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<tr>
<td>Urea, mg/dL</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>NP</td>
<td>22.8 ± 2.65</td>
<td>24.4 ± 3.16</td>
<td>24.2 ± 2.80</td>
<td>23.8 ± 2.59</td>
<td>0.86</td>
</tr>
<tr>
<td>LP</td>
<td>25.7 ± 1.58(^x)</td>
<td>18.6 ± 2.36(^b)</td>
<td>21.9 ± 1.85(^ab)</td>
<td>22.1 ± 2.17</td>
<td>0.05</td>
</tr>
<tr>
<td>Creatinine, mg/dL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NP</td>
<td>0.9 ± 0.081</td>
<td>0.8 ± 0.069</td>
<td>0.9 ± 0.092</td>
<td>0.9 ± 0.064</td>
<td>0.42</td>
</tr>
<tr>
<td>LP</td>
<td>1.2 ± 0.108</td>
<td>1.4 ± 0.137(^x)</td>
<td>1.1 ± 0.101</td>
<td>1.2 ± 0.088(^x)</td>
<td>0.33</td>
</tr>
<tr>
<td>Urea:creatinine ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NP</td>
<td>23.9 ± 2.59</td>
<td>30.8 ± 2.43(^x)</td>
<td>26.6 ± 2.38</td>
<td>27.6 ± 1.97(^x)</td>
<td>0.08</td>
</tr>
<tr>
<td>LP</td>
<td>21.4 ± 2.01(^a)</td>
<td>13.6 ± 1.25(^b)</td>
<td>18.9 ± 1.67(^ab)</td>
<td>18.0 ± 1.51</td>
<td>0.02</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NP</td>
<td>50.9 ± 2.58</td>
<td>52.9 ± 2.33(^b)</td>
<td>55.8 ± 1.03</td>
<td>53.2 ± 1.45</td>
<td>0.24</td>
</tr>
<tr>
<td>LP</td>
<td>48.6 ± 2.13</td>
<td>47.0 ± 1.70</td>
<td>51.2 ± 2.47</td>
<td>48.9 ± 1.35</td>
<td>0.39</td>
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<td>Cholesterol, mg/dL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NP</td>
<td>123.0 ± 11.31</td>
<td>109.7 ± 5.90</td>
<td>117.6 ± 9.44</td>
<td>116.8 ± 5.06</td>
<td>0.56</td>
</tr>
<tr>
<td>LP</td>
<td>112.8 ± 9.41</td>
<td>107.7 ± 7.62</td>
<td>112.7 ± 6.57</td>
<td>111.1 ± 4.20</td>
<td>0.76</td>
</tr>
<tr>
<td>Triiodothyronine, ng/dL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NP</td>
<td>2.0 ± 0.22</td>
<td>2.0 ± 0.27</td>
<td>2.6 ± 0.28</td>
<td>2.2 ± 0.19</td>
<td>0.08</td>
</tr>
<tr>
<td>LP</td>
<td>1.5 ± 0.18(^b)</td>
<td>1.7 ± 0.25(^b)</td>
<td>2.6 ± 0.21(^a)</td>
<td>1.9 ± 0.15</td>
<td>0.03</td>
</tr>
<tr>
<td>Thyroxine, ng/dL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NP</td>
<td>30.2 ± 2.71</td>
<td>30.5 ± 5.11</td>
<td>28.6 ± 5.79</td>
<td>29.7 ± 2.48</td>
<td>0.96</td>
</tr>
<tr>
<td>LP</td>
<td>28.0 ± 3.97</td>
<td>28.1 ± 3.69</td>
<td>25.5 ± 4.44</td>
<td>27.2 ± 2.14</td>
<td>0.93</td>
</tr>
</tbody>
</table>

\(^{a,b}\)Within a row, means without a common superscript letter differ (\(P \leq 0.05\)).

\(^x\)Means in a column (between treatment groups NP and LP) differ (\(P \leq 0.05\)).

\(^1\)The data are expressed as mean ± SEM from \(n = 6\) observations.

\(^2\)NP, normal protein diet, provided CP for maintenance as per NRC (1985) and ICAR (1998); LP, low protein diet, provided 50% CP of NP (LP diet was replaced with NP during period 3).

\(^3\)Period 1 and 2 refer to short- and long-term protein restriction, respectively; period 3 refers to period of realimentation.

\(^4\)\(P\)-values for period effects.
The concentration of sGlb may increase in chronic infections or during decreased immunity, and a reduced sAlb:sGlb ratio may indicate an increase in sGlb concentration caused by chronic parasitism or compensation for the sAlb loss evident in protein malnutrition (Payne, 1987). Nonetheless, the ratio should always be interpreted with care by taking into account the concurrent changes in sTP concentration. The concentrations of sGlb were less affected by a low protein intake than sTP and sAlb, which could be partially attributable to a tendency to compensate for the decrease in sAlb. It is also likely that immunological functions are prioritized over productive function (Coop and Kyriazakis, 1999), and thus sGlb are better conserved during nutritional constraints, particularly that of protein leading to narrow sAlb:sGlb ratio. Interestingly, sAlb concentration remained depressed during realimentation in period 3 with sGlb remaining unaltered. Experimental and clinical hypoproteinaemia has been reported to result in liver atrophy (Oser, 1971) that affects albumin synthesis in protein-deficient diets (Charland et al., 1994). Usually, the sGlb concentration is the last to be affected in prolonged hypoproteinaemia, resulting in lowered immunity in animals (Tizzard, 1992). Infection, inflammation, and injury do not suppress the rate of synthesis of albumin; rather, such conditions may bring about an increase in catabolism or intravascular loss of albumin and total protein (Fleck et al., 1985). In support of this argument, the concentration of sCr (mg/dL) was elevated in the LP group, especially toward the end of protein deficit period 2.

Urea production and its concentration in blood respond to immediate changes in dietary protein intake, thereby reflecting short-term changes in protein metabolism and complementing the information provided by the analysis of sTP and sAlb concentrations (Payne, 1987). There was a fall in sU concentrations despite the fact that the body has an excellent regulatory mecha-

Figure 3. Delayed type hypersensitivity response of sheep subjected to protein restriction and realimentation. NP = normal protein diet for maintenance; LP = low protein diet (50% of NP). The LP group was realimented with NP diet during period 3. A treatment × time interaction revealed during period 1 and 2 at 48 h postinoculation; *P < 0.05.

Figure 4. Cell-mediated immunity (CMI) in sheep subjected to protein restriction and realimentation as assessed through in vitro nitrite production both at basal (unstimulated) level and after stimulation with lipopolysaccharide (LPS). NP = normal protein diet for maintenance; LP = low protein diet (50% of NP). The LP group was realimented with NP diet during period 3. The CMI response was greater in NP than LP for basal and after LPS stimulation during period 2; *P < 0.05; **P < 0.01.
nism of a decreasing urinary N excretion with a ma-
ior proportion of urea being recycled into the rumen
through saliva (Satter and Roffler, 1977). There ex-
ists a negative relationship between N intake and the
percentage of hepatic urea recycled from gut to lumen
(Harmeyer and Martens, 1980) consequential to a re-
duction in overall N flux or in its proportion. This may
result in decreased urinary N excretion due to relative
preservation of N over carbon during AA catabolism on
reduced protein intakes (Millward et al., 1991). The net
transfer of urea across portal drained viscera increases,
as a percentage of N intake, in underfed ewes (Nozière
et al., 2000), which contributes to sparing of N, to-
gether with decreased N uptake by the kidney (Cirio
and Boivin, 1990) and urinary loss (Harmeyer and
Martens, 1980; Nozière et al., 2000). The decreased sU
and increased sCr concentrations, with the consequent
reduction in the sU:sCr ratio recorded during period 2,
can be related to physiological adaptation of renal func-
tion to save protein through recycling of urea with low
dietary protein intake. The effect of protein intake on
the blood urea concentration was confounded when a
low protein intake was combined with a restricted feed
intake or by increased production of urea due to muscle
catabolism.

Nutrition-related changes in the sCr concentrations
include elevated concentrations during fasting, malnu-
trition, or food restriction, which have been related to
a reduction in filtration in the kidneys and increased
production due to muscle catabolism (Kaneko, 1989).

The possibilities of assessing the nutritional status of
deer based on plasma or serum urea and creatinine
concentrations have attracted considerable interest.
DelGiudice et al. (1992) observed an increase in sCr,
a decrease in sU and a reduction in the sU:sCr ratio
through autumn from above 50 to 10 mg/dL in free-
ranging white-tailed deer exposed to seasonal undernu-
trition. The increase in sCr concentration on a low pro-
tein diet might have resulted from the decrease in the
glomerular filtration rate and the decrease in the excre-
tion of creatinine into urine (Kaneko, 1989). The sCr
concentration has also been suggested to increase when
protein derived from muscle is used as an energy source
at times of nutritional deprivation. However, the feed/
energy intake in the present experiment was similar in
NP and LP animals, and thus, the possibility of muscle
catabolism can be overruled. The observed sU:sCr ratio
is principally affected by the plasma urea concentra-
tion, and its reduction reflects a shift in metabolism
toward conserving endogenous protein (Kaneko, 1989).

The absence of differences in concentration of sG and
sCh between animals in NP and LP groups was indi-

cative of similar energy status. The concentration of
T3 revealed some periodic alteration and was also in
response to a realimentation diet during period 3. The
treatment and period did not have significant effect on
T3, but T3 was numerically less (17 to 24%) in LP than
NP animals during protein deficit periods (1 and 2).
Less T3 with normal concentrations of T4 is observed
in nutritional (protein) limitation (Stockigt, 2004). To-
dini (2007) stated that circulating thyroid hormones
can be considered as indicators of the metabolic and
nutritional status of the animals, and the quantity and
quality of feed eaten is a major factor determining plas-
ma concentrations of T3. It is also thought that thyroid
hormones play an important role in activation and pro-
liferation of lymphoid (thymomimetic) organ and tissue
of the body (Bidey et al., 1999), which could be due to
a possible role in eliciting the production of factors such
as thymulin and IL-2, which affect the immune system
(Marsh, 1995). Singh et al. (2006) also observed a sig-
nificant depression in both cell-mediated and humoral
immune response in goitrous goats and attributed this
to a reduced thyroid hormone concentration that could
have impaired the functional status of lymphoid organ.
The sTP, sAlb, sU, and sCr concentrations and the
sU:sCr ratio especially showed significant variation that
could be related to physiological adaptation to deficit
protein nutrition and therefore appear as useful indica-
tors of the protein metabolism in sheep.

Cell-mediated and humoral immune responses are
critical to the host defense against intracellular bacte-
rial pathogens. A variety of in vitro and in vivo immune
function tests have been developed for their monitoring
(Cheville et al., 1993; Chiodini, 1996). The immuno-
logical variables evaluated in the present investigation
were aimed at providing an indication of the potential
effects of nutritional treatments on immune function in
dependent of any diseased state. There was a decline in

![Figure 5. Humoral immunity of sheep subjected to protein restric-
tion and realimentation as assessed through antibody titer (OD 492)
against Brucella abortus S99 antigen. NP = normal protein diet for
maintenance; LP = low protein diet (50% of NP). The LP group was
realimented with NP diet during period 3. A treatment × day interac-
tion revealed during period 2 at d 7, 14, and 21 postchallenge with
Brucella abortus antigen; **P < 0.01; ***P < 0.001.
]
CMI with a prolonged period of protein deficit. The attainment of peak DTH response and its continuity also declined during periods 1 and 2, which was indicative of decreased immunological response to an unknown antigen. Similarly, the nitrite production response, both basal and post-LPS stimulation, was less. However, when analyzed critically, the increment in nitrite production from basal to LPS stimulation was comparable. This shift in immunological response was a clear indication of prioritization of metabolic activation toward antigenic stimuli. Peripheral blood mononuclear cells (lymphocytes in the present study) produce nitric oxide (NO) in response to stimulation with antigens, and its determination in cell cultures may thus provide an indication of immunological response of the animal. A key component of this response is the clonal expansion of lymphocytes (Pugliese, 1990) and the elaboration of cytokines that activate macrophages for killing of pathogenic antigen located within the phagosomal compartment. The potent mediators for this are reactive N intermediates (e.g., NO) produced via the induction of inducible NO synthase (iNOS), often as a sequel to IFN-γ, TNF-α, or LPS stimulation (MacMicking et al., 1997; Kaufmann, 1999). When exposed to infectious and inflammatory agents, the host responds by initiating a wide range of metabolic adjustments mediated mainly by cytokines, in addition to the specific and nonspecific immune responses (Klasing, 1988).

Several in vitro or in vivo studies in the past have confirmed cytokine (alone or in combination)-mediated changes of nutritional importance (anorexia, fever, increased energy expenditure, and a marked increase in N excretion), demonstrating net protein catabolism (Klasing, 1987). Thus, 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). Therefore, among the macronutrients, protein seems to be critical to meeting the increased catabolic activity and initiating an immunological response against the invading pathogens or any antigenic stimuli. The reduced antibody titer (OD₉₀₀) against Brucella abortus S99 antigen, reflecting a decline in HIR in LP compared with NP animals was indicative of prolonged protein insufficiency and a disjunctive effect on immunological status of animals. In an earlier study (Sahoo et al., 2002), calves fed a reduced protein level (75% of normal) had significant depression in indirect hemagglutination titer against Pasteurella multocida (F52 strain). Further, the observation of a decrease in feed/nutrient intake with prolonged protein deficit, coupled with reduced energy consumption, might have overstated the effect.

The pleiotropic activities of the cytokines released as part of the immune response strongly indicate an altered nutrient requirement, the quantitative nature of which has not been well investigated. Realimentation of LP group with normal protein diet during period 3 showed partial recovery in the blood metabolic profile and in the cell-mediated and humoral immune response. However, the realimentation period of 18 wk seems to be inadequate, and it appears a longer intervention period, or an increased level of CP (greater than the normal requirement), or both would have brought about appreciable recuperative metabolic activation, which needs critical investigation. Because poor nutrition (low protein) was associated with a state of compromised immunological response (both humoral and cell-mediated) and had adverse effects on production (e.g., BW gain) and health of the animals, a detailed investigation involving variable degrees of protein deficit in relation to stage of production, diseased state, and normal functioning of animals along with concurrent field observations on various production variables and response to prophylactic regimes (disease prevention, vaccination, etc.) is essential for making definite recommendations.

**LITERATURE CITED**


