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*Original Article*

# Effect of protein supplement on growth and blood parameters of native lamb

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Lamb, protein supplement, growth performance, blood parameters

**A B S T R A C T**

The study was conducted to investigate the effects of protein supplement (soybean meal and wheat bran) on the growth performance and blood parameters of native lamb. A total number of twelve indigenous lambs aged 10 months were randomly distributed into four dietary groups having 3 replications. First group ($T_1$) was fed control diet, second group ($T_2$) was provided 50 g protein supplement + green grass, third group ($T_3$) was provided 75 g protein supplement + green grass and fourth group ($T_4$) was fed 100 g protein supplement + green grass. The results showed that body weight gain was similarly significantly higher ($P<0.01$) in $T_3$ and $T_4$ though $T_3$ animals were provided lower amount protein supplement than $T_4$ animals. Significantly improved body length ($P<0.01$) was found in $T_4$. Heart girth value was significantly ($P<0.05$) higher in $T_3$ group. Glucose level of blood was found non-significant. Total protein and albumin were observed significantly ($P<0.01$) higher in $T_4$ group and lower in $T_1$ group. Results reveal that blood urea and blood nitrogen were statistically higher in $T_4$ and lower in $T_1$. Blood urea and blood nitrogen were found to increase gradually among the treatment groups starting from $T_1$ to $T_4$. It can be concluded that the supplementation of protein along with grazing in the diet had positive effects on growth performance and blood profile status. Protein supplement to the diet in the amount of 75g can be very effective to better growth performance of lamb by remaining the blood urea nitrogen (7-20 mg/dL) and other blood parameters in their normal range.



## Introduction

Bangladesh is a vast country of great animal wealth and diversified climatic conditions. It is considered amongst those countries having a great agricultural potential. Small ruminant raising is very important and for the livelihood of those who are inhibiting in the regions, where cattle production and cultivation of crops is not suitable (Daskiran *et al.*, 2006). In rural population small ruminants make very valuable contribution. The socio-economic importance of small ruminants is extensively recognized (Devandera, 2001). During the last ten years sheep population increased 5.91 lakhs. There are about 34.68 lakhs sheep in Bangladesh (DLS, 2018). Most of the sheep are indigenous, with few crossbreds (Bhuiyan, 2006) and are capable of bi-annual lambing and multiple births.

As an important meat animal, the sheep is now emerging as an alternative and attractive source of meat in other parts of the world (Devendra and Mcleory, 1990). Sheep represent the most important species of livestock in the traditional production systems due to their superior ability to thrive under drastic environment and their inherent qualities of early maturity (Devendra and Mcleroy, 1987). Native sheep are small (18-25 kg), highly prolific (2-3 lambs per lambing and two lambing per year) and meat producing (7-10 kg) animals (Dymundsson and Lee, 1972). Their inquisitive feeding habits enable them to extend their feed preferences and also perform well in situations where other ruminants may not be able to survive. Sheep rearing is directly involved with poverty alleviation, employment generation and nutrient supply. Native sheep are extremely resistant to infectious diseases including PPR. Sheep are multipurpose animals providing meat and wool. Sheep represent 58.8% (96.7% goat alone) of total livestock population and yielding 119 thousand metric tons (97.5% goat meat) of meat annually, which accounts

for 28.7% of total livestock meat (FAO, 1997). The production of meat from sheep, play an important role to supply animal protein and acceptable to people of all castes and religion of our country.

Some previous studies (Kochapakdee *et al.,* 1994) have reflected the importance of supplemental feed on growth and productivity sheep. These authors also reported that grazing alone may not be sufficient for optimizing live weight gain and wool production. If scavenging type of rearing can be supplemented with minimum amount of protein rich concentrate then the level of production may be increased at the minimum cost. Protein is one of the major determinants of ruminant feed that influence performance of the growing and fattening lambs. Provision of the quality of protein in the lamb's diet does not only improve the animal performance but also ensures profitable animal production. However, in developing countries ruminants are mainly fed on crop residues generally receiving only 62% of their crude protein (CP) requirements (Sarwar *et al*., 2002). Protein is an expensive but essential nutrient for animal growth (Dabiri and Thonney, 2004). Different protein sources have varying effect on ruminant's performance and their serum biochemistry (Jørgensen *et al*., 1984). This varied response in performance may be due to changes in rumen ecology and their different amino acid profiles (Hall and Huntington, 2008) that result in altered nutrient metabolism. Protein sources differ in their chemistry as far as amino acid profile and availability of CP in rumen and post-ruminal level (Gleghorn *et al*., 2004). Different protein sources in lamb diets like canola meal, cotton seed meal, soybean meal etc. provide the condensed nutrients that may be efficiently utilized at ruminal level (Solomon *et al*., 2008).

The use of supplemental protein or high calorie rations for grazing lambs can enhance the provision of their daily nutrient requirements and increase growth rates during periods of insufficient forage availability (Canton and Dhuyvetter, 1997; Hess *et al.,* 2008). As such, fat supplements ostensibly offered as protein supplements have been used to increase average daily gain by increasing the energy density of high forage diets that are fed to ruminants (Palmquist, 1994), including lambs. The development of inexpensive non-commercial supplements which effectively synchronize protein and energy nutrient availability and enhance lamb growth performance for high forage fed lambs offers small scale producers increased opportunity to become profitable (Hersom, 2008).

Incorporation of protein in diets is recommended to increase growth rate and nitrogen retention in sheep. Increase digestibility of protein can be attributed to the increase in serum total protein and its fraction. Lower rumen degradability results higher concentrations of protein in abomasum and small intestine and higher absorption of dietary amino acids which lead to high level of plasma protein. Decreasing the absorbed ammonia via the ruminal wall is converted into urea in liver. So, the decreased level of ammonia in rumen of sheep fed supplemental protein reflects lower level of urea in their blood and lower blood urea nitrogen level in blood serum of lambs fed supplemental protein indicates the higher utilization of dietary protein. Considering the above facts and circumstances, the present study was conducted to determine the growth performance of native lamb raised under semi-intensive condition as well as to observe the changes of blood parameters after supplementation of dietary protein.

## Materials and methods

### Experimental location and duration

The whole working process of this experiment was divided into two effective steps-Feeding trial of animals and laboratory analysis. The Feeding trial of animal was done in the Goat and Sheep Farm, Bangladesh Agricultural University, Mymensingh and the laboratory analysis for blood parameter analysis was done in the laboratory of the Department of Animal Nutrition, Bangladesh Agricultural University, Mymensingh. This experiment was carried out for a period of 75 days (from August 8 to October 23, 2018).

### Animals and their Management

A total number of Twelve (12) indigenous growing lamb having initial average body weight 10 kg (age around 10 months) were selected for this experiment. They were purchased from the local market of Mymensingh district. They were dewormed at a time according to the clinical report of faeces test in veterinary clinic of BAU, Mymensingh. That was done fourteen days prior to the trial. To get identity of each animal, they were tagged with individual number hanged on their neck. Animals were grazed for a specific period (6 hours daily) during day and were kept overnight in a platform system housing with well ventilation. This platform was divided into four parts and three animals in each part were kept having separate manger and water trough to avoid mixing up feeds with water, urine and faeces. A skilled shepherd was engaged to rear the animals throughout the experiment. Identical housing, health care and sanitary measures were provided to all the lamb.

### Diet and method of feeding

Roughage and concentrate mix were fed separately. Concentrate mixture was consisted of wheat bran, soybean meal and common salt. Each treatment group was provided with an estimated amount of protein in $T_1$-14 g, $T_2$-34 g, $T_3$-43 g and $T_4$-53 g groups respectively. Animals of each treatment group were fed with protein supplemented feed along with grazing twice in a day at 9.00 AM and 5.00 PM. The increment of supplemental diet was made based on live weight gain and feed consumption. Fresh clean drinking water was supplied ad-libitum. The feeding trial was continued for 75 days.

### Measurement of growth changes

Growth was determined by measuring height, weight changes and body length of animal. At the beginning of the experiment, animals were weighted for two consecutive days and average weight was taken as the initial weight of the animal. Thereafter animals were weighted throughout the experimental period every 15 days interval. They were weighed before feeding in the morning at a fixed time. The live weight was taken with the help of an animal weighing balance. The live weight gain was estimated by dividing the total weight gain by total number of days. Final height and body length were taken to determine the growth changes at the expiration of the experiment.

### Blood collection and separation of blood serum

At the onset (0 day) of the experiment 5 ml of blood per lamb was drawn aseptically from Jugular vein, of which 3 ml of blood was transferred to a sterile vial containing disodium EDTA (1mg/ml of blood) in order to estimate routine blood parameters testing . Remaining 2 ml of blood sample was transferred to vacutainer tube for serum separation. Serum

samples obtained by centrifugation were used to determine total protein, albumin, glucose, urea and blood urea nitrogen using automatic biochemical analyzer.

**Determination of plasma glucose level**

Plasma glucose level was estimated by Enzymatic Colorimetric Test (CHOD-PAP method). Readings for glucose was taken spectrophotometric by tracking at corresponding wavelengths according to previously published methods. At first, Wavelength 505nm (500-510) temperature 37°C and conversion factor were adjusted. Then, adjusted the instrument to zero with distilled water and pipette into clean dry test tubes labeled as Blank (B), Standard (S) and sample. Sample was mixed well and incubated at 37° C for 10 minutes. Measured the absorbance of the standard and test sample against blank and after incubation the color was stable between 15-30 minutes.

**Determination of serum albumin**

Blood serum albumin concentration was determined using quantitative colorimetric kit albumin by Bromcresol green method (Vivia Biotech S.L. Ctra. Santa Coloma, Spain). The intra and inter assay CV were respectively 0.42% and 6.20% for albumin.

At first adjustment of automated chemistry analyzer was done according to reagent guideline. In that, wavelength was 630 nm and temperature was adjusted at 15-25 °C. Then blank, standard and blood serum sample were prepared in 3 inch test tube using the following guideline.

**Table 1. Blank, standard and blood serum sample preparation for determination of serum albumin.**

| Reagent (R) | Blank test tube | Standard test tube | Sample test tube |
|---|---|---|---|
| | 1.0 mL | 1.0 mL | 1.0 mL |
| **Standard** | ----- | 5 µL | ----- |
| **Serum sample** | ----- | ----- | 5 µL |

Then contents of each test tube was mixed properly and incubated for 10 minutes at 25 °C. After incubation, blank, standard and serum sample test tubes were placed under probe chronologically and start button was then pressed. The instrument was then automatically performed measurement according to the preset item parameter. Finally, result was printed out following each measurement and expressed in g/dL.

**Determination of serum urea**

Blood serum urea concentration was determined using quantitative colorimetric kit urea-Liquor by urease-GLDH kinetic liquid method (CHEMELEX, S.A. Pol. Ind. Can Castells, Barcelona, Spain). The intra and inter assay CV for urea-LQ were 2.79% and 2.65%, respectively.

At first adjustment of automated chemistry analyzer was done according to reagent guideline. In that, wavelength was 340 nm and temperature at $25^0$C was adjusted. Then blank, standard and blood serum sample were prepared in respective test tube (3 inch) using the following guideline.

**Table 2. Blank, standard and blood serum sample preparation for determining serum urea.**

| Working reagent | Blank test tube | Standard test tube | Sample test tube |
|---|---|---|---|
| | 1.0 mL | 1.0 mL | 1.0 mL |
| **Standard** | --- | 10 µL | --- |
| **Serum sample** | --- | --- | 10 µL |

Working reagent was prepared by mixing of 800 µL $R_1$ buffer with 200 µL of $R_2$ enzymes.

Then contents of each test tube were mixed properly. Then blank, standard and serum sample test tubes were placed under probe chronologically and start button was then pressed. The instrument was then automatically recorded absorbance after 30 second and 90 second according to the preset item parameter. Finally, result was printed out following each measurement and expressed in mg/dL.

**Determination of blood urea nitrogen level**

Urea is hydrolyzed by urease to form $CO_2$ and ammonia. The ammonia formed then reacts with α-ketoglutarate and NADH in the presence of glutamate dehydrogenase (GLDH) to yield glutamate and NAD+. The decrease in absorbance due to consumption of NADH is measured kinetically.

**Determination of Total protein**

Total protein was determined by following the principle that $Cu^{2+}$ can be reduced to $Cu^{+}$ by protein in alkaline condition. $Cu^{+}$ can combine with BCA reagent and form purple complex, which has a maximum absorption peak at 562 nm. The absorbance value is proportional to the protein concentration. Therefore, the protein concentration can be calculated according to the OD (Optical Density) value.

**Statistical analysis**

This experiment was laid out at completely randomized block design (CRD). Data were represented as the mean ± SD (standard deviation). At first the raw data were organized using computer Excel program and then analyzed using SPSS (20) statistical program for one-way analysis of variance (ANOVA). The significance of difference among means was determined using Tukey's HSD test (1953) and differences at $P<0.05$, $P<0.01$ were considered statistically significant.

**Results**

**Growth performance of lamb supplemented with protein**

Protein supplement of four dietary groups of animals ($T_1$, $T_2$, $T_3$ and $T_{4)}$ were 0, 50, 75 and 100 gm/day respectively that differed significantly ($p<0.01$) among the groups of animals. The results indicated that feeding of protein supplemented diet resulted in significant differences among the treatment of animals. The initial live weight of the lambs did not differ significantly but the final live weight was higher for lambs fed protein supplement. The live weight changes of four dietary treatments ($T_1$, $T_2$, $T_3$ and $T_4$) are shown in Table 3. The average initial live weight before the commencement of the experiment was 11.33, 12.00, 11.33 and 11.3 kg respectively with no significant differences, while after the end of 75 days experiment, the animals reached 12.36, 14.60, 15.66 and 15.66 kg live weight respectively and significant differences ($p<0.01$) among the four dietary groups were observed.

At the end of experiment, the average live weight gain was 1.03, 2.60, 4.33, and 4.36 kg in $T_1$, $T_2$, $T_3$ and $T_4$ groups respectively. The results showed that there were significant differences ($p<0.01$) in live weight gain due to different dietary protein levels. Figure 1 showed that the average live weight gains were significantly increased with increased protein level. Group$T_2$, $T_3$, $T_4$ were highly significant than group $T_1$ but there were minimum differences between the mean value of group $T_3$ and $T_4$ with different amount protein supplemented feed. Again Group $T_2$ was more significant than group $T_1$ but group $T_2$ was less significant than group $T_3$ and $T_4$. So growth response of group $T_3$ animals with less protein supplement is better than group $T_4$ and any other treatment group.

**Table 3. Effect of protein supplement on body weight gain.**

| Treatments | Initial Weight (kg) | Final Weight (kg) | Gain (kg) | Mean±SD | *p* value | Significance Level |
|---|---|---|---|---|---|---|
| $T_1$ (control) | 12 | 13 | 1 | $1.03^{c}$±0.57 | 0.0006 | ** |
| | 11.5 | 12.6 | 1.1 | | | |
| | 10.5 | 11.5 | 1 | | | |
| $T_2$ (50 g SM) | 11 | 13.5 | 2.5 | $2.60^{b}$±0.10 | | |
| | 14 | 16.6 | 2.6 | | | |
| | 11 | 13.7 | 2.7 | | | |
| $T_3$ (75g SM) | 12.5 | 17 | 4.5 | $4.33^{a}$±0.28 | | |
| | 11 | 15 | 4 | | | |
| | 10.5 | 15 | 4.5 | | | |
| $T_4$ (100g SM) | 11.4 | 16 | 4.6 | $4.36^{a}$±0.28 | | |
| | 12 | 16 | 4 | | | |
| | 10.5 | 15 | 4.5 | | | |

The mean with different superscripts differ significantly at 0.01 level of significant. SM: Soybean meal; SD: Standard Deviation.

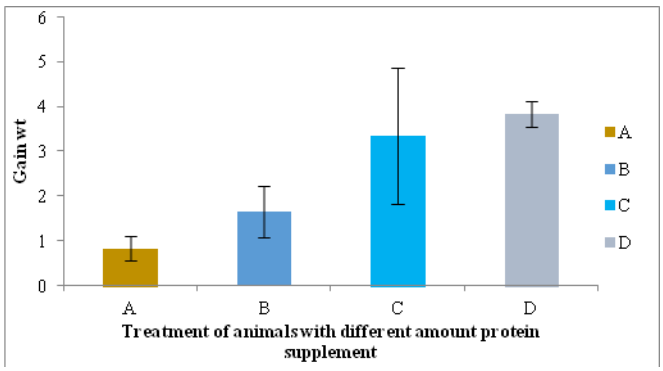


**Figure 1. Live weight gain at different treatment levels supplemented with protein.**

A= control feed (100 g wheat bran + 6 hours grazing), B= 50 g protein supplement (soybean meal) + control feed, C= 75 g protein supplement + control feed, D= 100 g protein supplement + control feed

Data in table 4 shows the effect of protein supplement on body length, Heart girth and height of animals as growth indicators of different treatment group. Body length of animal is significantly increased ($P<0.01$) with gradual increase of protein supplement. Group $T_4$ animals had higher body length increase compared with group $T_1$, $T_2$ and $T_3$ animals. Feeding of protein supplement also caused a significant increase of heart girth length of each animal of different treatment group. Heart girth length was significantly higher in group $T_3$ animals compared with other animals. Data presented in table illustrates that there was no significant difference ($P> 0.05$) in height with the increase of protein levels in the feed of the animals.

**Table 4. Effect of protein supplement on body length, height and heart girth length difference of animal.**

| Parameter | $T_1$ | $T_2$ | $T_3$ | $T_4$ | *p* value | Significance level |
|---|---|---|---|---|---|---|
| Length (inch) | $0.83^{b}$± 0.28 | $1.66^{ab}$± 0.57 | $3.33^{a}$± 1.52 | $3.83^{a}$± 0.28 | 0.008 | ** |
| Heart girth (inch) | $1.00^{b}$± 0.00 | $2.50^{ab}$± 0.50 | $5.33^{a}$± 3.05 | $4.00^{ab}$± 1.00 | 0.053 | * |
| Height (inch) | $0.83^{a}$± 0.28 | $1.66^{a}$± 0.28 | $3.50^{a}$± 2.29 | $2.66^{a}$± 1.04 | 0.134 | NS |

Mean with different superscripts differ significantly
**= 1% level of significance, *= 5% level of significance, NS= Non-significant

**Glucose, Total Protein and Albumin**

Table 5 shows the glucose, total protein and albumin levels in blood of the treatment groups. No significant difference observed in case of glucose level among the treatments ($P>0.05$). But a slight increase of glucose level was seen with the increase of protein supplementation in some of the replications. In case of total protein and albumin with the increase of protein supplementation both levels increased significantly ($P<0.01$).

**Table 5. Glucose level (mmol/L), Total Protein (g/dL) and Albumin (g/dL) in blood.**

| Parameters | $T_1$ | $T_2$ | $T_3$ | $T_4$ | *p* Value | Level of Significance |
|---|---|---|---|---|---|---|
| Glucose (mmol/ L) | $2.09^{a}$± 0.07 | $2.08^{a}$± 0.04 | $2.28^{a}$ ± 0.17 | $2.28^{a}$± 0.07 | 0.052297 743 | NS |
| Total Protein (g/dL) | $6.06^{a}$± 0.05 | $6.24^{b}$± 0.04 | $6.52^{c}$ ± 0.13 | $6.85^{d}$ ± 0.05 | 6.37109E -06 | ** |
| Albumin (g/dL) | $4.01^{a}$± 0.11 | $4.23^{b}$± 0.03 | $4.75^{c}$ ± 0.10 | $4.95^{d}$ ± 0.05 | 1.61854E -06 | ** |

Mean with different superscripts differ significantly
**= 1% level of significance, *= 5% level of significance, NS= Non-significant

The difference is observed among all treatments starting from $T_1$ to $T_4$. A gradual increase of albumin and total protein is shown in table 5 with the increase of protein supplementation. Normal range of total protein in blood serum of sheep is 6.0-6.5 g/dL. Highest values for total protein found in $T_4$ treatment group and lowest value was found in $T_1$ treatment group.

**Blood Urea and Blood Urea Nitrogen (BUN)**

Table 6 shows the blood urea and blood urea nitrogen (BUN) levels of the treatment groups. In both cases with the increase of protein supplementation the levels of urea and BUN increased gradually among the treatment groups starting from $T_1$ to $T_4$. And the increase of blood urea and BUN were found statistically significant among the groups ($P<0.01$).

**Table 6. Urea (mg/dL) and BUN (mg/dL) in blood.**

| Parameters | $T_1$ | $T_2$ | $T_3$ | $T_4$ | *p* Value | Level of Significance |
|---|---|---|---|---|---|---|
| Urea (mg/dL) | $20.33^{a}$± 0.76 | $25.67^{b}$± 1.53 | $35^{c}$±1 | $45^{d}$±2 | 1.15E-07 | ** |
| BUN (mg/dL) | $9.65^{a}$± 0.14 | $11.99^{b}$± 0.72 | $16.35^{c}$± 0.47 | $21.03^{d}$± 0.94 | 9.76E-08 | ** |

Mean with different superscripts differ significantly
**= 1% level of significance, *= 5% level of significance, NS= Non-significant

The difference is also observed in each treatment groups which shows that with the increase of protein level, urea and BUN increased in every steps of protein increase in feed. In sheep normal range of blood urea is 25-35 mg/dL and BUN is 7-20 mg/dL. Highest values for blood urea and BUN found in $T_4$ treatment group and lowest values found in $T_1$ group i.e. control group.

## Discussion

### Growth performance of lamb

The results showed that feeding of protein supplemented diet resulted in faster growth rate than non-protein nitrogen supplemented diet. There was significant (P<0.01) difference among the dietary treatments for growth. However, there was a trend to increase growth rate with increasing levels of protein supplementation. Huston *et al.* (1988) reported that feed intake in goat and sheep was increased when an increased amount of supplemental protein was fed. Previous studies with goats (Lu and Potchoiba, 1990; Shahjalal *et al.*, 1992) have also shown increased growth performance with increasing protein concentration in diet. Shahjalal *et al.* (1997) reported that growth rate of grazing Black Bengal goats can be slightly improved under conditions of increased protein supplementation which is similar to the present study.

Similarly lamb given high protein diet gained significantly (P<0.05 to P<0.01) higher live weight in all growth intervals from 0-75 days of experiment compared to those received the low protein diet. Mazumder *et al.* (1998) reported that the local sheep grazing natural grasses can only grow 15.7g/d and on grazing +300g concentrate can grow 40.5 g/d which supports the present study. Taie (1998) found that there was a strict correlation between dietary protein and average daily gain in sheep. Sufficient supply of protein especially the most essential amino acids is a very crucial factor for proper growth. The effect of protein levels on the performance of lamb was reported in this study. Wiese *et al*. (2003) found that increasing the dietary level of methionine by using Smartamine to Merino lambs did not lead to any increase in growth rate, daily feed intake, feed conversion or final body weight which completely disagreed with the findings of the present work. Atti *et al*. (2004) reported that the optimum crude protein level in growing goats' concentrate (DM = 89.8%) for maximum performance is approximately 130 g/kg BW and that any increase above this level did not improve performance.

In a different study conducted by Shahjalal *et al*. (2000), studying effect of diets with 16.9 and 20.35 CP in black Bengal goats indicated a higher live body weight gain with increasing dietary protein (20.3%), which agreed with our findings. This agreement may result from breed, feed type, stage of growth and environmental factors (Negesse *et al*., 2001).

For lambs, Zundt *et al*. (2002) indicated a positive effect of protein level (12, 16, 20 and 24%) on average daily gain which similar with our results. But Nuno *et al*. (1997) reported that the protein levels in the diet (14, 16 and 18%) had little or no effect on the performance of Dorper or Pelibuey lambs during fattening which is in disagreement with our results.

For the feed intake, the result was inconsistent with Prieto *et al*. (2000) and Chobtang *et al*. (2009) who found that there was no significant effect of different levels of protein in diet on the feed intake of Thai indigenous male goats, Spanish and Boer-Spanish crossbred kids. Moreover, Zundt *et al*. (2002) detected significant effect of increasing dietary crude protein on dry matter intake by growing lambs which agreed with our results. According to our findings, it is clear that feeding growing lamb 75 g protein supplement is quite adequate to cover their protein requirements.

### Glucose, Total Protein and Albumin

Results of blood glucose level shows that no significant difference (P>0.05) found with the increase of protein levels in feed of the lambs. But a slight increase of blood glucose level was found in some cases which is not statistically significant. All the results were within the reported normal range (Kolbikava, 1978; Melvin, 1982). The results of glucose levels were similar to Lohakare *et al.* (2006). In case of total protein and albumin significant difference (P<0.01) were found among the groups. A gradual increase of total protein and albumin levels were observed with the increase of protein levels. The obtained result was agreed with Mahmoud, (2011) and (2013). In general, plasma proteins concentration can be used as indicator to evaluate the ruminant nutrition (Kumar *et al.*, 1980). Total proteins of all experimental groups are nearly within the normal range being; 6-8 g/dl (Kancko, 1989). 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.

### Blood Urea and Blood Urea Nitrogen (BUN)

Blood urea and BUN significantly increased among the treatment groups (P<0.01) with the increase of protein intake in feed. Urea concentration in blood plasma was very high in high protein ration being 45 g/dl compared with other rations. Similar results were reported by Mahmoud (2013) in Rahmani lambs and Ahmed (1995), who found that feeding Zaraibi goats gave similar range of urea level being; 39.33 to 55.33 mg/dl. 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).

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