Effect of Feeding Different Levels of Dietary Protein with High or Low Rumen Degradable: Undegradable Dietary Nitrogen on Awassi Lambs Performance 3-Selected Biochemical Parameters

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Abstract : Twenty four individual Awassi lambs (weighing 26.5 kg ± 1.1 and 5 months) were used to study biochemical changes in rumen parameters as affected by feeding diets containing different levels of dietary crude protein (low, medium and high- CP) formulated with high and low rumen degradable to undegradable dietary nitrogen ratios(RDN:UDN). Blood samples were withdrawn from lambs before feeding (0 times), 3 and 6 hrs post feeding. Results revealed that blood glucose concentration (BG) and blood total protein (BTP) were significantly increased (P<0.05) due to feeding low RDN:UDN ratio but not due to increasing level of dietary protein. However, blood urea nitrogen (BUN) concentration was significantly increased (P<0.01) due to both factors. Result of interaction showed that better blood parameters were detected in blood samples withdrawn from lambs fed low RDN: UDN ratio. Higher BG concentrations were detected when low and medium levels of CP. Higher BTP concentrations were detected when medium and high levels of CP were offered with this ratio. In conclusion high and low ratios of RDN:UDN improved the utilization of medium level of dietary protein in comparison to high level as evidenced by blood urea nitrogen concentrations.
Keywords: Protein, degradability, blood parameters, lambs

INTRODUCTION

Protein is an important limiting nutrient in ruminants. It contains two fractions: rumen degradable nitrogen (RDN) and undegradable dietary nitrogen (UDN). The rumen microbes breakdown RDN to small peptide, amino acids (AA) and ammonia. These, in turn, can be used for synthesis of microbial protein. Therefore, sufficient supply with RDN and UDN or AA is important to satisfy animal's requirements (Ali et. al. 2009). Blood plays an important role in carrying end products of metabolism processes out of body tissues and supplying these tissues with different nutrients that may need. These nutrients differ in concentrations according to differences in diets and ingredients or diet components or treatments may apply on diets via their interaction in metabolic pathways naturally occurring in different body tissues (Almalah, 2007). Gleghorn (2003) reported that blood urea nitrogen concentrations increased with increasing CP level, but effects of CP source were variable across time. However, Shamoon et al (2009) demonstrated that both the level of protein and degradability (formaldehyde treatment) had no significant effect on blood glucose, total protein and urea concentrations. The effects of protein level and degradability on productive parameters and rumen characteristics were reported by Hassan and Saeed (2011; 2012).

The objectives of this study were to evaluate the effects of protein level and degradability on blood parameters in male lambs allowed to consume restricted amount of concentrate and barley straw on *ad libitum*

basis. We hypothesized that minimizing wasted N by optimizing RDN: UDN ratio may reflected better performance which is not exclusively achieved with higher level of CP, consequently, lower level may beneficially utilized by growing animals if they supplied with a proper fractions of RDN and UDN.

MATERIALS and METHODS

Twenty four Awassi male lambs weighing 26.5±1.1 kg and 5 months of age were used to investigate biochemical changes in blood parameters due to feeding three levels of dietary CP (11.5, 13.5 and 15.5%), and two RDN:UDN ratios within each level, high (70:30) achieved by incorporating intact soybean meal (SBM), and low (60:40) achieved by substituting SBM with formaldehyde-treated SBM (FTSBM). Concentrate diets were offered to lambs at rate of 3% of live BW in addition to free choice of barley straw. The FTSBM was prepared by spraying 4% formaldehyde (HCHO) solution into the meal at a rate of 10 ml /100 g SBM DM, equivalent to 1 g HCHO per 100 g of air dry SBM CP (Hassan et al., 1990). The treated SBM was then mixed well and packed into polyethylene sacs which were tightly closed and left at room temperature (25C°) for 3 days and were shaken occasionally, then all sacs were opened and the treated SBM was exposed to air to remove the excess HCHO 24 hrs before mixing with the other ingredients in concentrate diets. Chemical analysis of ingredients and barley straw and formulation and chemical composition of concentrate diets are presented in Tables 1 and 2, respectively.

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Table 1. Chemical Analysis of Concentrate Ingredients and Barley Straw and Effective Degradability of Their CP Content

Ingredients % of DM	DM %	% of DM										Effective degradability
		OM	CP	CF	EE	NFE	NDF	ADF	Cellulose	Hemicellulose	ADL	
Barley	92.3	90.3	8.4	6.2	3.2	72.5	25.2	5.8	1.0	4.8	19.4	80*
Yellow corn	91.2	92.6	8.5	3.9	4.6	75.5	13.7	6.3	1.8	4.5	7.5	60*
SBM	90.8	91.2	43.1	5.3	2.7	40.2	45.5	10.9	2.1	8.7	34.6	70**
FTSBM	90.8	91.2	43.1	5.3	2.7	40.2	45.5	10.9	2.1	8.7	34.6	30**
Wheat bran	90.2	92.0	13.8	9.6	5.0	63.6	50.5	13.2	3.0	10.2	37.3	67***
Barley straw	95.7	90.2	2.4	40.2	2.1	45.5	73.0	52.0	38.9	21.0	13.6	-

*(Humady, 1988) ** (Abdullah, 1988) *** (Paya et al., 2008)

Table 2. The Formulation and Chemical Composition of Experimental Concentrate Diets (%)

Level of CP %	11.50		13.50		15.50	
RDN:UDN ratio	70:30	60:40	70:30	60:40	70:30	60:40
Ingredients %						
Barley	40	40	40	40	40	40
Wheat bran	35	35	35	35	35	35
Yellow corn	18	18	13	13	8	8
SBM	5	0	10	0	15	0
FTSBM	0	5	0	10	0	15
Mineral and vitamin mixture	2	2	2	2	2	2
Chemical composition						
DM	93.71	93.92	93.89	93.76	94.16	93.99
OM	93.47	94.02	93.97	93.27	93.62	93.90
CP	11.38	11.42	13.36	13.23	15.38	15.28
CF	7.00	6.69	7.00	7.15	6.85	6.72
EE	2.89	2.44	3.01	2.06	3.10	2.57
NFE	72.20	73.47	70.60	70.83	68.29	69.33
NDF	36.02	37.10	34.41	41.43	35.20	39.76
ADF	8.56	7.68	7.33	8.34	8.38	9.54
Cellulose	6.30	5.50	5.08	6.45	5.87	7.38
Hemicellulose	27.46	29.42	27.08	33.09	26.82	30.22
ADL	2.26	2.18	2.25	1.89	2.51	2.16
RDN	1.27	1.10	1.50	1.27	1.72	1.46
UDN	0.54	0.73	0.64	0.84	0.73	0.97
* ME	12.13	12.15	12.10	11.92	12.02	11.99

* Metabolizable energy (ME) values are estimated according to equation of Kearl (1982).

ME (MJ/kg DM) = [- 0.45 + (0.04453×% TDN)] × 4.184

TDN is estimated according to the following equations

TDN for roughages (% of DM) = -17.2649+1.2120(%CP) +0.8352%NFE+2.4637% EE+0.4475 % CF

TDN for concentrate (% of DM) = 40.3227+0.5398 % CP+0.4448 % NFE+1.4218% EE–0.7007 % CF

Blood samples (10 ml) were withdrawn from lambs via jugular venipuncture into vacutainer plain tubes, which were immediately placed in refrigerator, before morning feeding (zero time) 3 and 6 hrs thereafter to determine blood glucose (BG), blood total protein (BTP), blood urea nitrogen (BUN), blood triglycerides (BTG) and blood cholesterol (BCH) concentrations. Blood samples were centrifuged and separated serum was collected and stored at -20 C^o until analysis was perfumed. Blood glucose was immediately measured using Accu-Chek electronic device as described by Al-Saady (2008) Where, BG was read 5 second following putting the sample in the device. Other blood parameters were measured spectrophotometerically. Methods of determination, corresponded commercial kits used and the manufactured companies were:

Blood total protein (BTP): Biuret colorimetric method, Linear Chemicals, Cromatest, 1153005. Barcelona- Spain. Blood urea nitrogen (BUN): Modified Urease-Berthelot method, Randox. UR 2316, Antrim, UK.Blood triglycerides (BTG): Fossati and Prencipe method associated with Trinder reaction. Biolabo SA, 80019, Maizy, France.

Blood cholesterol (BCH): Enzymatic end point method. Randox, CH 200, Antrim, UK.

Data obtained was statistically analyzed using 3×2 factorial experiment design using completely randomized design model (CRD) procedure by (SAS, 2001). Duncan's multiple range tests was used to determine the significance of differences between treatments means (Duncan, 1955).

RESULTS and DISCUSSION

Determination of blood parameters aimed to investigate the biochemical changes may occur during an experiment due to nutritional circumstances. This is mainly because metabolic processes can be reflected on these changes in addition to their correlation with ruminal fermentation characteristics. On this basis, Valkeners et al (2006) reported that diurnal variations in ruminal NH_3-N concentrations and blood urea concentrations were greatly influenced by the feeding patterns of the diet. Therefore, changes in some blood parameters may possibly help to explain the beneficial effect of additives in the diet (Miner et al., 1990). The following discussion will deal with mean effect of the factors involved in a current study, including the level of dietary protein, RDN:UDN ratio within each level and the interaction between them on the blood parameters including BG, BTP, BUN, BTG and BCH. Regarding the diurnal changes in these parameters in sampling time involved, illustration will be limited to figures to avoid unnecessary elaborateness.

The mean effect of level of protein on blood parameters during 24 hours

The mean effect of level of dietary CP on blood parameters during 24 hrs is presented in Table 3. Increasing level of dietary CP had no significant effect on BG, BTP and BTG. Similar results concerning BG were observed by many studies (Ali et al., 2005; Salih, 2007 and Shamoon et al., 2009). Whereas, Rusche et al., (1993) demonstrated that increasing CP level resulted in increased BG. Similar result concerning BTP was also reported (Shamoon et al., 2009) due to increasing level of dietary CP in lambs, While, Hoffman et al., (2001) observed a significant increase (P<0.001) in Holstein heifers. The little insignificant response of increasing dietary level of CP on BTP in a current study may be attributed to the fermentative activity of the rumen

(Bergen et al., 1973). Therefore, serum AA patterns are more closely related to the fate of AA in the rumen.

The BTG, similar to our results, Salih (2007) showed that there were no significant differences in BTG concentrations in samples withdrawn from lambs fed diets containing increased level of CP, whereas, a significant (P<0.01) differences were observed by Dosky (2007) with Karadi ewes. Although, BG, BTP and BTG concentrations were insignificantly affected by increasing level of dietary CP, results revealed that BUN concentration was significantly (P<0.01) increased. Similar result was reported by several investigations (Gleghorn, 2003; Dabiri and Thonney, 2004; Hristov et al., 2004 and Dosky, 2007). The BUN represents an indicator for protein metabolism and its utilization and it is positively and linearly correlated to ingested CP (Huntington et al., 2001). The NH_3 produced in the rumen due to extensive degradation of dietary CP when it accumulates and exceeds microbial requirement it is absorbed via rumen wall into the blood which carry it to liver where it is converted to urea. Urea is then carried back by blood to kidney to be excreted with urine, while, some is recycled to digestive tract through rumen wall or saliva (Church, 1993). Increased ruminal NH_3 decreases the permeability of the ruminal epithelium to urea (Huntington and Archibeque, 1999). However, the gradient in urea that established between the blood and the digestive tract depends largely on the ureolytic activity of bacteria associated with the luminal walls of the tract (Glenghorn, 2003). Hence, the increased BUN concentrations observed with increased dietary CP can be explained largely by increased absorption of ruminal ammonia, resulting in greater quantities of ammonia being detoxified in the liver to form urea. James et al., (1999) reported that urea is produced in the liver when microbial degradation of dietary CP in the rumen is not incorporated into microbial protein, but absorbed, causing an elevation of BUN.

Regarding BCH concentration, it was observed that increased dietary CP from low to medium level resulted in a significant increase, elevated cholesterol can be indicative of dietary lipid content or tissue catabolism (Miner et al., 1990). However, increasing dietary CP to high level resulted in a little non significant decrease in BCH as compared with low level. Diurnal changes in blood parameters as affected by increasing levels of dietary CP were presented in Figures 1, 2, 3, 4 and 5.

Table 3. Mean Effect of Level of Dietary Protein (A) On Blood Parameters During 24 Hrs.

Items	Level of dietary protein			Significance of effects
	Low	Medium	High	N = 72
BG mg/ 100 ml	70.92 ± 0.66	69.45 ± 0.65	68.37 ± 0.59	NS
BTP g/ 100 ml	6.64 ± 0.12	6.81 ± 0.12	6.96 ± 0.10	NS
BUN mg/ 100 ml	$40.97^b \pm 0.71$	$42.14^b \pm 0.95$	$45.73^a \pm 1.38$	**
BTG mg/ 100 ml	31.02 ± 0.78	32.23 ± 1.14	32.37 ± 0.78	NS
BCH mg/ 100 ml	$66.33^{ab} \pm 1.40$	$71.11^a \pm 1.62$	$64.71^b \pm 2.25$	*

Means in the same row with different superscripts are significantly different
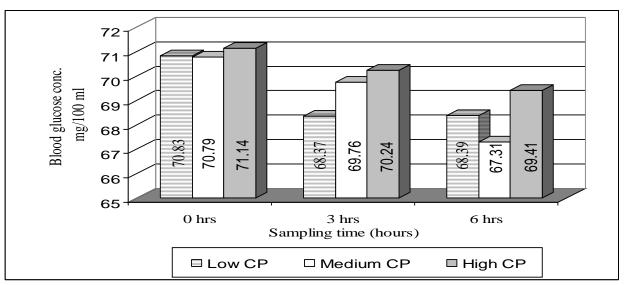* (P<0.05) ** (P<0.01) NS= Non significant

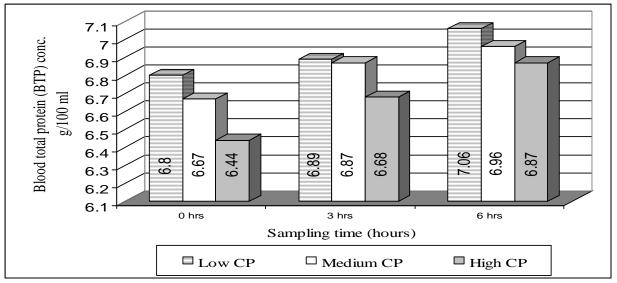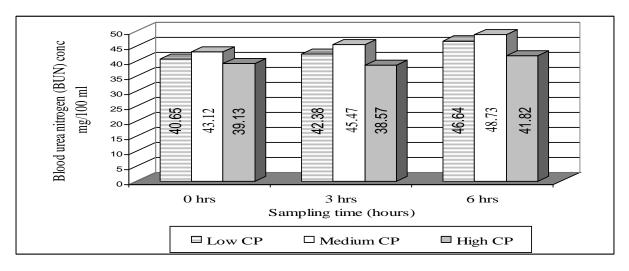Figure 1. Diurnal Pattern of Blood Glucose Concentration (BG) mg/100 ml as Affected by Increasing G Level of Protein

Figure 2. Diurnal Pattern of Blood Total Protein Concentration (BTP) g/100 ml as Affected by Increasing G Level of Protein

Figure 3. Diurnal Pattern of Blood Urea Nitrogen Concentration (BUN) mg/100 ml as Affected by Increasing G Level of Protein
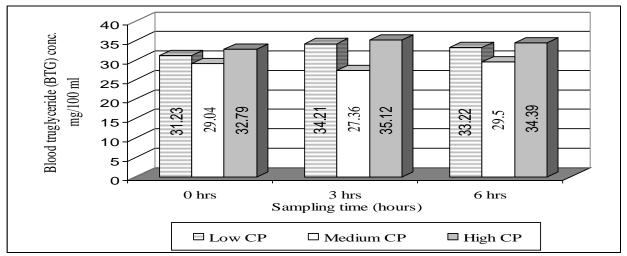

Figure 4. Diurnal Pattern of Blood Triglyceride Concentration (BTG) mg/100 ml as Affected by Increasing G Level of Protein
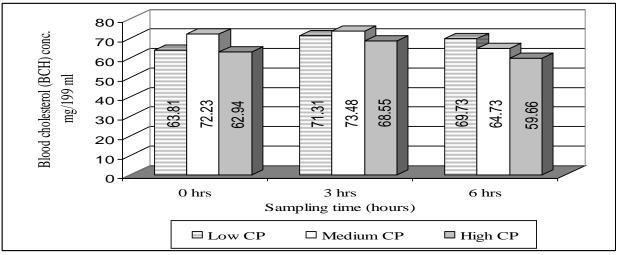

Figure 5. Diurnal Pattern of Blood Cholesterol Concentration (BCH) mg/100 ml as Affected by Increasing G Level of Protein

The mean effect of RDN:UDN ratio on blood parameters during 24 hrs.

The mean effect of RDN:UDN ratio on blood parameters during 24 hrs is presented in Table 4. Higher (P<0.05) BG concentration was detected in samples withdrawn from lambs fed diets formulated with low RDN:UDN ratio. This increase may attributed to a decrease in degradability of these diets due to formaldehyde (HCHO) treatment as compared to untreated diets which characterized with high RDN:UDN ratio. The increased flow of AA to duodenum due to decreased CP degradability (Scholljegerdes et al., 2005) may provide higher amounts of substrates for gluconeogenesis processes. MacRae et al., (1985) reported that abomasal infusion of casein increased the efficiency of ME utilization in sheep, possibly by supplying more gluconeogenic precursors. However, our result disagreed with that

obtained by Hassan (2009), who revealed that lambs fed high level of UDN caused a significant (P<0.05) decrease in BG, in this case the inconsistency may attributed to lower concentrate ratio (60%) offered in this study as compared to 70% used in our study, in addition to breed of animals used in both studies.

Results also revealed that higher BTP concentration was detected in group of lambs fed low RDN; similar finding was reported by many studies (Huntington et al., 2001; Scholljegerdes et al., 2005). This increase in BTP observed in diets formulated with low RDN:UDN ratio may attributed to lower CP degradability in these diets due to HCHO treatment SBM. Ali et al (2005) reported an increase (P<0.05) in BTP (0.52) due to HCHO treatment of SBM exactly similar to that observed in a current study (0.51 g/100 ml). Degradability of dietary CP in the rumen affects amounts and proportions of AA available for absorption in the small intestine (Erasmus

et. al., 1994). Moreover Richardel (2004) concluded that feeding supplemental sources of low ruminal degradable. CP may correct the deficiency in quality of

the AA profile provided solely by microbial protein (MP).

Table 4. Mean Effect of RDN:UDN Ratio (B) on Blood Parameters During 24 Hrs.

Items	RDN:UDN ratio		Significance of effects
	High	Low	N = 72
BG mg/ 100 ml	$68.28^b \pm 0.46$	$70.88^a \pm 0.53$	*
BTP g/ 100 ml	$6.55^b \pm 0.08$	$7.06^a \pm 0.09$	*
BUN mg/ 100 ml	$44.73^a \pm 0.97$	$41.16^b \pm 0.74$	**
BTG mg/ 100 ml	32.28 ± 0.82	31.47 ± 0.67	NS
BCH mg/ 100 ml	67.21 ± 1.73	67.55 ± 1.28	NS

Means in the same row with different superscripts are significantly different
* ($P<0.05$)   ** ($P<0.01$)   NS= Non significant

Regarding BUN, statistical analysis of data showed that feeding diets formulated with high RDN:UDN ratio, as expected, resulted in higher ($P<0.01$) BUN concentration, similar result was observed by several investigations (Chumpawadee et al., 2006; Al-Mallah, 2007; Nisa et al., 2008; and Hassan and Muhamad, 2009). Hassan, (2009) fed Karadi lambs diets composed of 40% roughage: 60% concentrate and formulated with three levels of RDN (1.0, 1.3 and 1.6 g RDN / MJ of ME) or three levels of UDN (7 and 10 g UDN / kg DM), results indicated that BUN concentration was significantly increased due to increasing level of RDN and UDN respectively. The increased BUN with increased dietary RDN level can probably be explained by increased absorption of ruminal NH_3-N, resulting in greater quantities of NH_3-N being detoxified in the liver to form urea N, and subsequently higher renal reabsorption of urea (Nisa et al., 2008). Ruminal degradability affects the amount of ammonia available for absorption, and ruminal ammonia absorption has a direct relationship with hepatic urea synthesis (Huntington et.al., 1996). The high RDN fraction in the diet has been reported to be highly linked to increased BUN concentration and increased urinary N excretion (Reynal and Broderick, 2005; Kalscheur et al., 2006). Huntington et al (2001) reported that increased RDN increased ($P<0.05$) endogenous production of urea, as evidenced by higher concentrations of urea in blood and urinary N excretion. The BUN concentration, as an indicator of kidney function, was significantly higher in buffaloes fed low UDN diet than those fed medium UDN and high UDN diets (Nisa et al., 2008).

With respect to BTG and cholesterol BCH concentrations, statistical analysis showed that both were not significantly affected by RDN:UDN ratio. Whereas, in other studies, it was found that BTG (Dosky, 2007; Salih, 2007; Shamoon et al., 2009) and BCH concentrations (Ali et al., 2005) were significantly increased by lower CP degradability due to HCHO treatment. The differed responses due to this treatment between these studies and ours with respect to BTG and

BCH concentrations may be attributed to higher extent of the HCHO treatment applied in those studies. Where, it limited to SBM in a current study, while, two or more concentrate components such as barley and wheat bran, were included in formaldehyde treatment applied in the mentioned studies. Diurnal changes in rumen fermentation parameters as affected by RDN:UDN ratio were presented in Figures 6, 7, 8, 9 and 10.

Mean effect of the interaction between levels of dietary protein × RDN:UDN ratios (A×B) on blood parameters
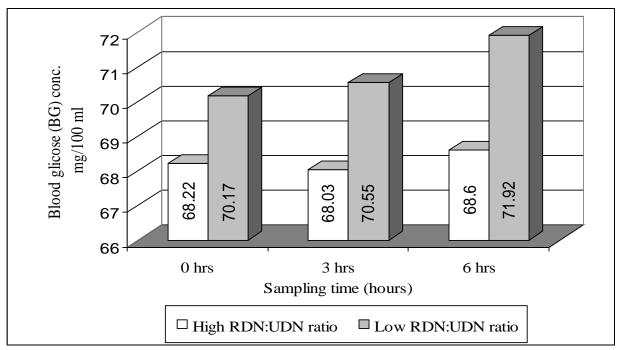
The mean effect of interaction between level of dietary protein × RDN:UDN ratio on blood parameters during 24 hrs is presented in Table 5. The BG was significantly ($P<0.05$) affected by this interaction. It is clear that this is due to the effect of RDN:UDN ratio, where, BG concentrations were significantly ($P<0.05$) higher when lambs fed diets formulated with low RDN:UDN ratio, within each level of dietary CP, whereas, there was no effect due to increasing this level. This, as discussed earlier may attributed to increase the flow of AA which is an important substrate for gluconeogenesis (Miner et al., 1990). Higher BG concentration was detected in blood samples withdrawn from lambs fed diets containing medium level of CP formulated with low RDN:UDN ratio. BTP was also significantly ($P<0.05$) affected by the mentioned interaction. Higher BTP values were observed due to formulating concentrate diets with low RDN:UDN ratio, However, the difference was significant ($P<0.05$) only in blood sample withdrawn from lambs fed low and medium levels of dietary CP. The positive effect of increasing UDN or inclusively decreasing RDN fractions on BTP has been well documented (Huntington et al., 2001; Scholljegerdes et al., 2005). This increase in BTP detected in diets formulated with low RDN:UDN ratio may attributed to lower CP degradability of these diets, Degradability of dietary CP is conversely correlated to AA flow into small intestine (Erasmus et al., 1994).

Table 5. Mean Effect of the Interaction Between Levels of Dietary Protein × RDN:UDN Ratio (A×B) on Blood Parameters During 24 Hrs.

			Interactions				Significance of effect
	A_1B_1	A_1B_2	A_2B_1	A_2B_2	A_3B_1	A_3B_2	n = 24
BG mg/ 100 ml	69.5[bcd]± 0.76	72.4[a] ± 0.93	68.3[cd] ± 0.69	70.7[ab] ±1.02	67.1[d] ± 0.88	69.6[bc] ± 0.65	*
BTP g/ 100 ml	6.3[c] ± 0.16	7.0[ab] ± 0.15	6.6[bc] ± 0.12	7.1[a] ± 0.19	6.8[ab] ± 0.11	7.1[a] ± 0.16	*
BUN mg/ 100 ml	42.2[b]± 0.92	39.7[b] ± 1.00	43.6[b] ± 1.44	40.7[b] ± 1.13	48.4[a] ± 2.08	43.1[b] ± 1.57	**
BTG mg/ 100 ml	30.1 ± 0.76	32.0± 1.35	34.2 ± 1.92	30.3 ± 1.06	32.6 ± 1.20	32.1 ± 1.06	NS
BCH m	66.2[b] ± 2.19	66.5[b] ± 1.84	73.6[a] ± 2.45	68.6[ab] ± 1.97	61.9[b] ± 3.38	67.5[ab]± 2.88	*

Means in the same row with different superscripts are significantly different
* (P<0.05)   ** (P<0.01)   NS= Non significant
A_1, A_2 and A_3 represent low, medium and high level of CP respectively, B_1 and B_2 represent high and low RDN:UDN ratio respectively.

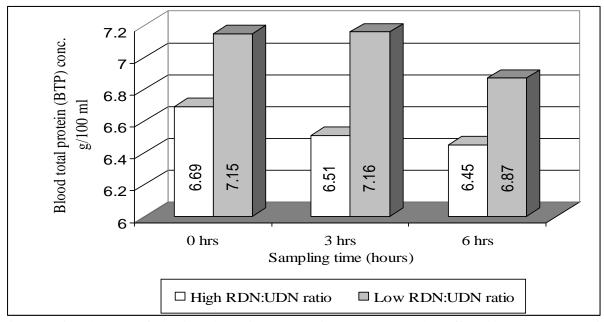Figure 6. Diurnal Pattern of Blood Glucose Concentration (BG) mg/100 ml as Affected by RDN: UDN Ratio

Figure 7. Diurnal Pattern of Blood Total Protein Concentration (BTP) g/100 ml as Affected by RDN: UDN Ratio
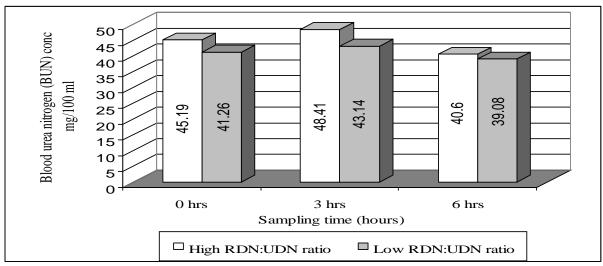
Figure 8. Diurnal Pattern of Blood Urea Nitrogen Concentration (BUN) mg/100 ml as Affected by RDN: UDN ratio
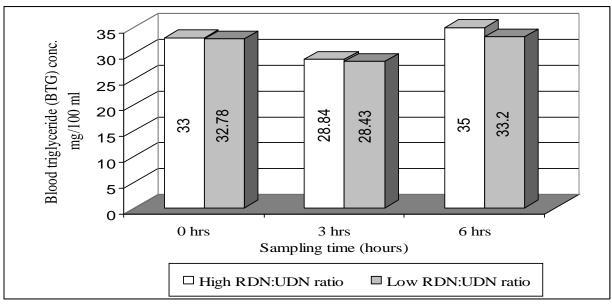

Figure 9. Diurnal Pattern of Blood Triglyceride Concentration (BTG) mg/100 ml as Affected by RDN: UDN Ratio
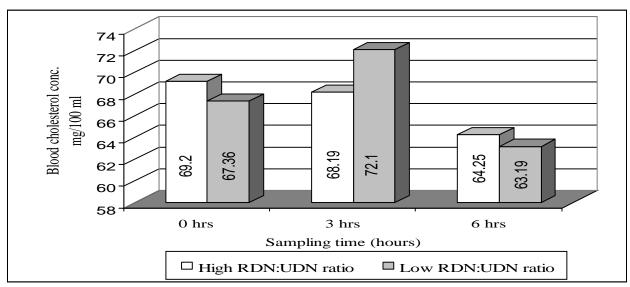

Figure 10. Diurnal Pattern of Blood Cholesterol Concentration (BCH) Mg/100 Ml as Affected By RDN: UDN Ratio

Regarding BUN statistical analysis revealed that it was significantly (P<0.01) affected by the interaction between level of CP and RDN:UDN ratio, Higher (P<0.01) value (48.35 mg/ 100 ml) was observed due to feeding diets containing high level of CP, but only when these diets were formulated with high RDN:UDN ratio. The accompaniment of both factors, level of CP and RDN:UDN ratio, in their effect on BUN is not coincidence, because both have, as previously shown, a proved (Tables, 4 and 5, respectively) and documented effect on BUN. Accumulation of ruminal NH_3-N directly affects BUN according to the close relationship between them (Huntington et al., 2001), consequently, higher level of CP may result in high BUN concentrations (Gleghorn 2003; Hristov et al., 2004 and Dosky, 2007). High BUN concentration was also found by many studies (Chumpawadee et al., 2006; Al-Mallah, 2007 and Nisa et al., 2008) to be caused by extensive degradation of dietary CP and to a subsequent detoxification in the liver of absorbed ammonia into blood stream (Huntington et al., 1996).

Results have also revealed that BTG was not significantly affected by the above interaction, this seems reasonable since BTG was neither affected by level of CP nor by RDN:UDN ratio. The BCH was significantly (P<0.05) affected by the mentioned interaction, where, as expected higher (P<0.05) values were observed in blood samples withdrawn from lambs fed diets containing medium level of CP and formulated with high RDN:UDN ratio. BCH concentration related to dietary lipid content and tissue catabolism (Miner et. al., 1990), How the CP level as interacted with RDN:UDN ratio affected BCH, is unknown, However, the response was limited to medium level only, where, higher level was not characterized with higher BCH concentration.

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