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Effect of Urea on Hematological and Selected Biochemical Parameters of Growing Somali Lambs

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ABSTRACT

Nine sheep were assigned into three groups to evaluate the effect of urea on their hematology and biochemistry. Sheep of first group received water without urea and served as control group whereas sheep of second and third groups received urea in drinking water at 0.1 and 0.2% respectively for four weeks. The present findings revealed that 0.2% of urea caused significant increase in body weight gain compare to control. Significant decrease was also observed in mean values of neutrophil percentage of sheep received 0.2 % urea (48.8 ± 4.1) compare to that of sheep received 0.1% urea (67.7 ± 1.0) and control group (63.3 ± 8.2), respectively. Moreover, percentage of lymphocyte of sheep received 0.2 % urea was increased significantly (50.7 ± 4.1) compare to that of sheep received 0.1% urea (31.9 ± 1.00) and control group (36.2 ± 8.2). Serum biochemistry indicated that, sheep received 0.1% and 0.2% of urea dissolved in drinking water caused significant increase in the values of glucose (60 ± 1.1; 62 ± 2.1 mg/dl, respectively), ALT (17 ± 1.1; 18 ± 2.1 U/l), AST (105 ± 1.2; 107 ± 2.1 U/l), creatinine (1.5 ± 0.1; 1.6 ± 0.1 mg/dl) and phosphorus (4.1 ± 1.9; 5.2 ± 0.1 mg/dl) compared to control (50 ± 2.1; 14 ± 1.1; 99 ± 2.1; 1.1 ± 0.1 and 2.0 ± 0.2, respectively). The present study concluded that, urea in drinking water improved body weight gain and blood picture in Somali sheep however, liver and kidney function was disturbed.

***Keywords*:** Urea, sheep, liver function, kidney, minerals, hematology

True protein supplements are expensive ingredients in diets for sheep. Therefore, partial or total substitution of a true protein supplement with a non protein nitrogenous source (NPN) can significantly reduce feeding cost. Urea is the most commonly used NPN source in sheep diets due to availability and low cost. However, the addition of urea to animal diet is under limitations to avoid the risk

of hyper ammonia. The biochemical understanding of urea metabolism by ruminal microbes is essential for nutritional planning and efficient utilization of urea. Urea dissolves quickly in water and is rapidly hydrolyzed to ammonia because of rumen microbial urease activity in ruminants (Helmer and Bartley, 1971). The rumen microorganisms hydrolyze urea into ammonium and ammonia to synthesize their own protein. The conversion of urea into ammonium and ammonia is increased at a pH 7.0 or higher (Visek, 1968). Most of produced ammonium is utilized by rumen micro flora, whereas small amount of ammonia produced is escaped into circulation due to its lipophilic properties (Bartley *et al.,* 1976; Froslie, 1977). Detoxification of ammonia into urea occurs in the liver via urea cycle (Visek 1968). Elevated level of ammonia (hyper ammonia) overwhelm the detoxifying capacity of the liver (Chalmers *et al.,* 1971) as reflected in higher level of ammonia in blood and cerebrospinal fluids inducing ammonia toxicity (Davidovich *et al.,* 1977; Visek, 1968). Higher level of ammonia in blood inhibits the tricarboxylic acid cycle with subsequent lower energy production and induction of metabolic acidosis (Haliburton and Morgan, 1989). Liver enzymes as Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and muscle leakage enzymes, creatin kinase are useful biochemical markers to diagnose ammonia toxicity (Thrall *et al.,* 1996). Glucose and urea levels were elevated without any changes in total protein values in plasma of lambs fed diet mixed with urea (Eryavuz *et al.,* 2003). Significant elevation of glucose, phosphorus, creatinine, ALT, PCV, lymphocytes and total leucocyte counts was observed in fattening suakin lambs fed diet mixed with urea doses of 0.5 and 1% of the concentrate feed (Al-Shami *et al.,* 2012). Other studies (Mandour *et al.,* 2012) demonstrated that 1.5% urea supplementation did not affect growth performance, hematological and biochemical indices of New Zealand rabbits. Based on pervious data, it is clear that, results regarding the effect of urea on hematology and blood biochemistry of sheep are scarce and conflicting. Therefore, the objective of this study is to evaluate the influence two levels of urea dissolved in drinking water (0.1 and 0.2%) on hematology and blood biochemistry of growing Somali lambs.

MATERIALS AND METHODS

Location

The study was conducted in sheep farm of the collage of Veterinary Medicine and Animal Resources, King Faisal University, Al-Ahsa, Saudi Arabia.

Animals

Animal care and handling procedures were follow the committee of scientific research ethics, King Faisal University, Al-Ahsa, Saudi Arabia. Animals were

housed with constant fluorescent lighting and ventilation. The animal house was cleaned daily to maintain animal health and well-being. Water was available ad-libitum throughout the experimental period. Sheep were maintained as a group of three in each room to prevent stress of isolation. Sheep were acclimatized for a week before the beginning of the experiment.

Experimental Design

A total number of nine healthy sheep with an average body weight of 20 ± 5 kg were obtained from the local market, Al-Ahsa, Saudi Arabia. Sheep were divided into 3 groups. The standard diet was available for all groups. Sheep of the first group received normal water and served as a control. Sheep of the second and third groups received water mixed with urea at a dose of 0.1% (100mg/l) and 0.2% (200mg/l), respectively.

Sampling and the Analytical Methods

At the end of the experiment, blood samples were collected from the jugular vein of all groups for estimation of total erythrocytic count (TEC), total leucocytic count (TLC), packed cell volume (PCV) and differential leucocytic count using standard hematological techniques (Feldman *et al.,* 2000). Haemoglobin percentage (Hb%) was assessed according to Drubkin (1947). The Mean corpuscular volume (MCV) was calculated ([hematocrite value × 10]/erythrocyte count) and expressed as (u^3). Mean corpuscular hemoglobin (MCH) was calculated ([hemoglobin% × 10]/ erythrocyte counts) and expressed as pictogram (pg) whereas Mean corpuscular hemoglobin concentration (MCHC) was calculated ([hemoglobin × 100]/ hematocrite value) and expressed as a percent (%). Similarly, blood samples were collected without anticoagulant for serum separation. Serum was separated by centrifugation for 10 min at 1200×g and was immediately frozen at –20ºC until the time of analysis. The sera were used for spectrophotometric determination of the activities of Aspartate Transaminase (AST) and Alanine Transaminase (ALT) as directed by Reitman & Frankle (1957). In addition, serum glucose, total protein, albumin and globulin values were determined spectrophotometrically as implied by the methods of (Trinder, 1969), Doumas *et al.,* (1981), Reinhold (1953) and Coles (1974), respectively. Serum blood urea nitrogen, and creatinine were determined according to the method described by Tabacco *et al.,* (1979) and Henry (1984), respectively. Furthermore, the obtained sera were used for spectrophotometric analysis of serum triacylglycerol (TAG), total cholesterol by using of enzymatic method of spin react kits according to the methods of Sidney & Bernard (1973) and Zak *et al.,* (1954), respectively. Calcium, phosphorus and magnesium were determined by using commercial kits on chemistry analyzer according to the manufacturer instructions.

Statistical Analysis

The obtained data on hematological and biochemical parameters were compared between groups by using one way analysis of variance (ANOVA). All data were presented as mean ± standard error of mean (SEM). All tests were performed using computer package of the statistical analysis system (SAS 1987).

RESULTS AND DISCUSSION

The data summarized in Table 1 indicated that, sheep received 0.2% urea drinking water showed significant increase in body weight (29.0 ± 0.5 kg) compare to sheep received 0.1% urea in drinking water (25.0 ± 0.5) and control group (25.0 ± 0.5). Similar results (Mandour *et al.,* 2012) were obtained in rabbits fed 1.5% urea compare to other group fed 0.5% and 0% urea for 4 weeks. Previous findings (Yono *et al.,* 1986; Isikwenu, 2010) support the significant increase in body weight in urea supplemented group compare to control group.

Table 1: Effect of oral administration of urea (0.5 and 1%) for six weeks on body weight gain (Kg).

Groups	Body weight (Kg)		Gain in body weight (Kg)
	Initial	Sixth weeks	
Control	25.0 ± 0.5^{b}	27.0 ± 0.5^{b}	2
Urea (0.1%)	25.0 ± 0.5^{b}	27.0 ± 0.5^{b}	2
Urea (0.2%)	29.0 ± 0.5^{a}	32.0 ± 0.3^{a}	3

Values are mean ± SD of 3 sheep; Means within the same column with different letters are significantly differed ($P \leq 0.05$).

The results of hematological examination are summarized in Table 2. The obtained results indicated that there are significant decrease in the mean values of neutrophil percentage in sheep received 0.2 % urea (48.8 ± 4.1) compare to that of sheep received 0.1% urea (67.7 ± 1.0) and control group (63.3 ± 8.2), respectively. However, the percentage of lymphocyte in sheep received 0.2 % urea was increased significantly (50.7 ± 4.1) compare to that of sheep received 0.1% urea (31.9 ± 1.00) and control group (36.2 ± 8.2), respectively. Meanwhile, the mean values of TEC, TLC, Hemoglobin, PCV, MCV, HCV and MCHC showed non significant variation in sheep received 0.1% (11.3 ± 2 10^{12}/L; 11.5 ± 0.7 10^{9}/L; 11.2 ± 2.0 g/dl; 34.3 ± 5.9 %; 30.5 ± 0.4 fl; 9.9 ± 0.0 pg; 32.6 ± 0.2 g/dl) and that received 0.2% urea (12.7 ± 1.0; 9.9 ± 2.3; 13.8 ± 0.8; 32.0 ± 1.6; 25.0 ± 0.7; 11.1 ± 1.6; 31.6 ± 4.8) compare to the control group (12.1 ± 1.2; 12.5 ± 0.4; 13.0 ± 0.9; 33.6 ± 5.9; 27.0 ± 2.0; 10.7 ± 0.3; 33.4 ± 4.2), respectively. The significant increase in lymphocyte percentage and decrease in neutrophil percentage in lambs received either 0.1 or 0.2% urea come in accordance with previous work (Al-Shami *et al.,* 2012) reported the same effect on Suakin lambs supplemented by dietary 0.5 and

Table 2: Effect of oral administration of urea (0.1 and 0.2%) for six weeks on hematological parameters

Variables	Control	Urea (0.1%)	Urea (0.2%)
TEC (10^{12}/L)	12.1 ± 1.2	11.3 ± 2.0	12.7 ± 1.0
TLC (10^{9}/L)	12.5 ± 0.4	11.5 ± 0.7	9.9 ± 2.3
Neutrophils (%)	63.3 ± 8.2[a]	67.7 ± 1.0[a]	48.8 ± 4.1[b]
Lymphocytes (%)	36.2 ± 8.2[b]	31.9 ± 1.00[b]	50.7 ± 4.1[a]
Esinophils (%)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Basophils (%)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Monocytes (%)	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0
Hb (g/dl)	13.0 ± 0.9	11.2 ± 2.0	13.8 ± 0.8
PCV (%)	33.6 ± 5.9	34.3 ± 5.9	32.0 ± 1.6
MCV (fl)	27.0 ± 2.0	30.5 ± 0.4	25.0 ± 0.7
MCH (pg)	10.7 ± 0.3	9.9 ± 0.0	11.1 ± 1.6
MCHC (g/dl)	33.4 ± 4.2	32.6 ± 0.2	31.6 ± 4.8

Values are mean ± SD of 3 lamb; Means within the same row with different letters are significantly differed ($P \leq 0.05$).

1% urea. However, earlier report in rabbits (Mandour *et al.,* 2012) demonstrated that, 0.5 and 1% dietary urea did not change neither lymphocyte nor neutrophil percentages. The current study demonstrated that, the values of other hematological parameters in lambs received urea were comparable to control. All of these values were close to the range that reported earlier in Suakin lambs (Al-Shami *et al.,* 2012). PCV is a blood toxicity reduction index and its abnormal level point to the presence of a toxic factor which has a drastic effect on blood formation (Oyawoye and Ogunkunle, 1998). Therefore, the non-significant difference among treatment for PCV suggests good detoxification of urea. Because of hematological parameter especially PCV and hemoglobin were positively correlated with the nutritional status of the animal (Adejumo, 2004), the unchanged hemoglobin values of all lambs in the current study indicated that the provided diets contained good quality proteins that met lambs nutritional requirements. In addition the unchanged TEC in all treatments indicated that all lambs remained healthy throughout the experimental and being an indication of non-allergic condition, free parasitism and any foreign body in circulation (Hillyer, 1994).

The biochemical findings are presented in Table 3. The analysis of serum revealed that, sheep received 0.1% and 0.2% of urea dissolved in drinking water caused significant increase in the values of glucose (60 ± 1.1; 62 ± 2.1 mg/dl, respectively), ALT (17 ± 1.1; 18 ± 2.1 U/l), AST (105 ± 1.2; 107 ± 2.1 U/l), creatinine (1.5 ± 0.1; 1.6 ± 0.1 mg/dl) and phosphorus (4.1 ± 1.9; 5.2 ± 0.1 mg/dl) compared to the control (50 ± 2.1; 14 ± 1.1; 99 ± 2.1; 1.1 ± 0.1 and 2.0 ± 0.2, respectively).

Table 3: Effect of oral administration of urea (0.5 and 1%) for six weeks on selected biochemical parameters.

Variables	Control	Urea (0.1%)	Urea (0.2%)
Glucose (mg/dl)	50.0 ± 2.1[b]	60.0 ± 1.1[a]	62.0 ± 2.1[a]
Total Protein (g/l)	6.5 ± 0.2	6.4 ± 0.1	6.4 ± 0.2
Albumin (g/l)	3.6 ± 0.2	3.7 ± 0.1	3.8 ± 0.1
Globulin (g/l)	2.9 ± 0.2	2.7 ± 0.1	2.6 ± 0.1
A/G ratio	1.2 ± 0.1	1.3 ± 0.1	1.5 ± 0.2
Total cholesterol (mg/dl)	50 ± 2.2	52 ± 3.5	47 ± 2.1
TAG (mg/dl)	80 ± 4.1	78 ± 3.4	77 ± 4.1
ALT (U/l)	14 ± 1.1[b]	17 ± 1.1[a]	18 ± 2.1[a]
AST (U/l)	99 ± 2.1[b]	105 ± 1.2[a]	107 ± 2.1[a]
BUN (mg/dl)	23.1 ± 1.6	23.8 ± 2.1	22.3 ± 2.2
Creatinine (mg/dl)	1.1 ± 0.1[b]	1.5 ± 0.2[a]	1.6 ± 0.2[a]
Calcium (mg/dl)	10.4 ± 0.2	10.5 ± 0.8	10.3 ± 0.7
Phosphorus (mg/dl)	2.0 ± 0.2[b]	4.1 ± 1.9[a]	5.2 ± 0.1[a]
Magnesium (mg/dl)	1.5 ± 0.9	1.7 ± 0.2	1.6 ± 0.1

Values are mean ± SEM of 3 lamb; Means within the same row with different letters are significantly differed ($P \leq 0.05$).

Meanwhile, the mean values of Total protein, Albumin, globulin, A/G ratio, Total cholesterol, TAG, BUN, Calcium, Magnesium showed non-significant variation in sheep received 0.1% (6.4 ± 0.1; 3.7 ± 0.1; 2.7 ± 0.1; 1.3 ± 0.1; 52.0 ± 3.5; 78 ± 3.4; 23.8 ± 2.1; 10.5 ± 0.8 and 1.7 ± 0.2) and that received 0.2% urea (6.4 ± 0.2; 3.8 ± 0.1; 2.6 ± 0.1; 1.5 ± 0.2; 47.0 ± 2.1; 77 ± 4.1; 22.3 ± 2.2; 10.3 ± 0.7 and 1.6 ± 0.1) compared to the control group (6.5 ± 0.2; 3.6 ± 0.2; 2.9 ± 0.2; 1.2 ± 0.1; 50.0 ± 2.2; 80 ± 4.1; 23.1 ± 1.6; 10.4 ± 0.2 and 1.5 ± 0.9). The significant increase of glucose, ALT, AST, creatinine and phosphorus in sheep received 0.1% and 0.2% of urea dissolved in drinking water compared with control as observed in the current study come in accordance with previous results (Tiwari *et al.,* 2001) in buffalo calves, lambs (Eryavuz *et al.,* 2003; Al-Shami *et al.,* 2012), rabbits (Mandour *et al.,* 2012) and goats (Jain *et al.,* 2005). The increased level of glucose might be attributed to increased levels of volatile fatty acids in the rumen (Guyton and Hall, 1988; Jain *et al.,* 2005), or due to increased hepatic gluconeogenesis via amino acids catabolism (Wilson 1988). The rapid hydrolysis of urea to ammonia caused hyper ammonia in the rumen and subsequently elevated the blood urea nitrogen (Eryavuz *et al.,* 2003; Jain *et al.,* 2005) as observed in the current study. The significant elevation of ALT and AST in urea treated animals indicated hepatocellular damage and caution should be taken during urea administration to Somali sheep. The hepatocellular stress was confirmed by significant elevation of creatinine in urea treated animals

as a result of kidney affection. During stress, catabolism takes place in muscle and the stored phosphocreatinine converted into pyrophosphate which is utilized by the tissues as a source of energy, and creatine is excreted in the form of creatinine in the urine (Jain *et al.,* 2005). The present study concluded that, urea in drinking water improved body weight gain and blood picture in Somali sheep however, liver and kidney function was disturbed. In addition, The hepatorenal dysfunction observed in urea treated animals encourages further investigations of this topic at cellular and molecular levels.

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