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EFFECT OF UREA SUPPLEMENTATION ON SOME METABOLIC PROFILES IN BLOOD SERUM OF SHEEP BEFORE AND AFTER GRAZING

(With 4 Tables)

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تأثير اضافة اليوريا على بعض صور التمثيل الغذائى فى سيرم دم الأغنام قبل وبعد خروجها للرعى

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أجريت هذه الدراسه على عدد ١٢ من ذكور الحملان الاوسيمى الناميه عمرها سبعة أشهر ومتوسط أوزانها ٢٩ كجم لمعرفة أثر اضافة مستويات مختلفه من اليوريا على بعض صور التمثيل الغذائى لهذه الأغنام قبل وبعد خروجها إلى المرعى .

وقد تم وضع الحملان فى أربع مجموعات أختيرت عشوائيًا حيث شملت كل مجموعه ثلاثة حملان . المجموعه الضابطه تمت تغذية الحيوانات بها على عليقه متزنه بدون أضافة اليوريا أما الثلاث مجموعات الاخرى فقد أضيفت اليها اليوريا بنسب ١٪ ، ٥ ر ١٪ ، ٢٪ من الماده الجافه على التوالى . وقد استمرت هذه التجربه مدة ٦ أشهر . الثلاث أشهر الأولى ظلت الحملان داخل الأسطبل أما الثلاث أشهر التاليه فقد خرجت الحيوانات للرعى صباحًا لمدة ثلاث ساعات يوميًا . وقد روعى تقديم العليقه يوميًا بأنتظام صباحًا ومساءًا أثناء فترة تواجد الحيوانات بالاسطبل أما فى فترة خروج هذه الحيوانات للمرعى فقد روعى تقديم العليقه المركزه أثناء فترة الظهيره فقط.

وقد أوضحت الدراسه أن مستوى كل من البروتين الكلى ، الجلوبيولين ، الكوليسترول الكلى ، نتروجين اليوريا ، البليروبين الكلى وأنزيم جلوتاميك أو كسالوستيك يميل إلى الانخفاض فى سيرم دم المجموعات المعامله باليوريا مقارنة بالمجموعه الضابطه . فأضافة ١٪ يوريا للعليقه قلل من نسبة نتروجين اليوريا بنسبة ٢٩٪ بينما العليقه المحتويه على ٥ ر ١٪ يوريا قلت فيها نسبة البليروبين الكلى بمقدار ٩ ر ٣٠٪ وأنزيم جلوتاميك أو كسالواستيك بمقدار ٩٪ . وعند تغذية الأغنام على يوريا بمقدار ٢٪ فان مستوى كل من البروتين الكلى ، الجلوبيولين ، الكوليسترول الكلى وأنزيم جلوتاميك أو كسالواستيك انخفض بصورة غير معنويه وذلك بالمقارنه بالمجموعه الضابطه .

ومن ناحيه أخرى ارتفعت نسبة الجلوكوز والدهون الكليه فى سيرم دم الآغنام المعامله باليوريا عن المجموعه الضابطه . فالأغنام المضاف إلى عليقتها ١٪ يوريا أرتفعت فيها نسبة الدهون الكليه بمقدار ٣ ر ٢٣٪ بينما أرتفعت نسبة الجلوكوز بنسبة ٧ ر ٢٩٪ فى سيرم دم الأغنام المغذاه على عليقه بها ٥ ر ١٪ يوريا بينما الأغنام المضاف للعليقه ٢٪ يوريا أرتفعت فيها نسبة الجلوكوز والدهون الكليه بمقدار ٥ ر ٣٩٪ ، ٤٠ ر ٩٪ فى سيرم الدم على التوالى .

كذلك أظهرت النتائج فى فترة الرعى أنخفاض معنوى فى نسبة كل من البروتين الكلى ، الجلوبيولين ، البليروبين الكلى وأنزيم جلوتاميك أو كسالواستيك وزيادة معنويه فى نسبة كل من الالبيومين ، الدهون الكليه وأنزيم جلوتاميك بيروفيك ترانس امينيز وذلك بالمقارنه بالفتره داخل الاسطبل (عدم الرعى).

مما سبق يتضح أن تغذية الحملان على مصادر مختلفه من اليوريا يمكن أن يؤدى إلى زيادة كفاءة هذه الحيوانات مع تحسن ملحوظ فى صور التمثيل الغذائى التى تم أختبارها فى هذه الدراسه.

SUMMARY

This trial was carried out using twelve growing Ossimi rams previously adapted to urea feeding (initial body weight 29 kg and 7 months age), to evaluate the effects of different urea levels and feeding system (pre and post grazing) on some blood metabolites. Animals were divided into four groups of three animals each: control group was daily fed basal diet without urea and three treated groups were fed basal diet with 1%, 1.5% and 2% urea on dry matter (DM) basis. The duration of the study was 6 months. Animals were kept in a stall for three months (pre grazing period) then they were allowed to graze three hours daily in the morning for the other 3 months. During the pregrazing period, allowed daily feed was divided into two equal portions, while in the grazing period the concentrate portion of the diet was offered to rams in mid afternoon. The results indicated that blood serum total protein, globulin, total cholesterol, urea nitrogen, total bilirubin and glutamic oxaloacetic transaminase (GOT) levels tended to be lower in urea treated groups with different response compared with untreated group. Addition of 1% urea to the diet decreased blood urea nitrogen by 29% (P<0.01). Diet contained 1.5% urea decreased total bilirubin by 30.9% (P<0.05) and GOT by 9% (P<0.05). When sheep were fed 2% urea diet, serum total protein, globulin, total cholesterol and GOT levels insignificantly (P>0.05) decreased when compared with the control. On the other hand, serum glucose and total lipids were significantly higher in urea treated groups when compared with the control. Rams fed 1% urea diet had 23.3% (P<0.05) more serum total lipids. Serum glucose was higher by 29.7% (P<0.05) when 1.5% urea diet was fed than the other groups. Rams fed diet supplemented with 2% urea had 39.5% (P<0.01) and 9.4% (P<0.10) more serum glucose and total lipids, respectively. However, grazing rams exhibited significnatly higher levels of serum total protein, globulin, total bilirubin and GOT with lower levels of serum albumin, total lipids and glutamic pyrovic transaminase (GPT) than ungrazed group. The study

declared that feeding with different urea levels improved the nutritional status (daily gain and feed efficiency) of growing rams without adverse effect on the selected serum constituents.

INTRODUCTION

Protein is one of the most limiting nutrients for both humans and animals throughout most of the world. Among the large types of animals, only ruminants are able to utilize efficienently non protein nitrogen (NPN) by conversion to microbial protein *(MILLER, 1979)*. Feeding nitrogen to the ruminants diets improves the voluntary consumption of food *(Campling et al. 1962 and Umunna, 1982)*, stimulates microbial proliferation and activity resulting in increased dry matter intake *(UMUNNA, 1982)*. Daily gain and feed efficiency were also increased by urea addition to the basal diet *(MOUSA, 1993)*.

Effect of feeding nitrogen sources on ruminal metabolism and some blood serum biochemical values of farm animals are discussed by many workers with contravertion results. Such differences could be related to scheme of feeding, length of experiment, breed and age of tested animals,...ect. *ALTINTAS et al. (1984)* found that pH was significantly less and total volatile fatty acids (VFA) were significantly high in Merino labms supplemented with 3% urea in basal diet. In local lambs (30 kg) receiving 45% of digestible protein (DP) as urea N in the diet, *El-KAPANI et al. (1985)* indicated that a significant increase was found in blood ammonia nitrogen, serum urea N, serum glucose and transaminase activity. Feeding purified diets containing urea as the only source of dietary nitrogen caused a reduction of plasma amino acids of Holstein Friesian steer calves (*DANILSON et al. 1987*). *KAPOOR et al. (1987)* reported that buffalo calves fed diet containing 2% urea had higher molar proportion of propionic acid in rumen fluid, higher blood glucose, lower total bacterial count, total VFA and molar proportions of acetic and valeric acids and transaminases enzymes (glutamic oxaloacetic and glutamic pyrovic transaminases; SGOT and SGPT, respectively). *ABDUL-RAZZAQ and BICKERSTAFFE (1983)* found that grazing sheep produced a propionic fermentation associated with high blood glucose, and blood urea and increased efficiency of utilization of feed for growth.

The objective of this study was to determine the changes in serum metabolites associated with feeding different levels of Non protein Nitrogen (Urea) to growing rams.

MATERIAL and METHODS

Animals and feeding:

This experiment was carried out in the Experimental Farm of the Department of Animal production, Assiut University on September 1991. Twelve Ossimi rams, adapted for urea feeding were used. The initial average age was seven months and average body weight was 29 kg. This study was conducted to evaluate the effect of different urea containing rations at different grazing systems on performance and some selected metabolites of rams. Animals were divided into four groups and randomly distributed and adapted for urea containing diet as stated by *MOUSA (1993)*. The duration of the study lasted for 6 months divided into two periods, pre grazing period that was extended for 3 months and post grazing period for 3 months.

Treatments included a contral group fed a basal diet without urea and three treated groups which were fed basal diet with 1%, 1.5% and 2% urea on dry matter (DM) basis. Non traditional feed mixture consisted of corn, wheat and rice bran, cottondseed meal, limestone and mineralized salt with no fixed proportion according to the availability of the ingredients. Diet composition, chemical analysis and feeding values are shown in table 1. Feed was divided into two equal partions and offered to each ram twice daily. Water was available all day. For grazing period, animals were left to graze for about 3 hours in the morning then the concentrate portion of the diet was offered to them in mid afternoon.

Blood samples and procedures:

Blood samples were taken from the jugular vein of all animals between 9 and 10 a.m. each month of the experimental period (during urea and grazing system) before feeding. Blood samples were collected using a clean dry plastic syringe and then transferred to centrifuge tubes. Serum was separated by centrifugation at 3000 rpm for 15 min. decanted into plastic tubes and stored at-20 $^{\circ}$C until further analysis. Serum total protein was determined using assay kit supplied by Bio Adwic, Adwia, (Egypt). Serum albumin level was estimated using assay kit supplied by bioMerieux (France). Globulin level was obtained mathematically by difference. Serum glucose, cholesterol, total lipids, total bilirubin, creatinine and urea nitrogen levels were estimated using assay kits supplied by Diamond Diagnostic (Egypt). Transaminases enzymes activity (Glutamic oxaloacetic and glutamic pyruvic transaminases; GOT & GPT, respectively) were determined using assay kits supplied by Weiner lab. Rosario (Argentine).

Statistical analysis:

Results were subjected to analysis of variance using general linear model (GLM) procedures of *SAS (1987)*, for personal computers, for complete randomized design (CRD) as explained by *COCHRAN and COX (1957)*. Urea levels effect, feeding system and interaction between them were included in the statistical model. When interaction was not found mean separation was conducted using the SNK procedure as explained by *SOKAL and ROHLF (1981)*.

RESULTS

The results of this trial are presented in tables 3 and 4. Serum total protein and its fractions levels were insignificantly ($p>0.05$) affected by urea levels (Table 3) in the diet, but differ significantly according to changing in feeding system (Table 4). Serum total protein and globulin concentrations numerically decreased as the levels of urea in the diet increased and tended to be lower by 14% and 39% for the rams receiving 2% urea in the basal diet compared with untreated group in the present study. Concentrations of serum total protein and globulin levels were lower in post grazing than pregrazing period. Serum total protein and globulin levels were significantly lower ($p< 0.01$) by 24% and 92% in grazed rams, respectively compared to ungrazed one (Table 4). Serum albumin levels were similar among treatments. On the other hand, grazing rams had 16.8% ($p< 0.05$) more serum albumin level compared with ungrazing group.

The main effect of urea levels on serum glucose was significant ($p<0.01$) while the main effect of grazing system was not significant (Table 3), yet the interaction between urea treatments and feeding system was significant ($p<0.05$). Across treatments, glucose concentrations in blood serum tended to increase with increasing urea levels in the diets resulting in 29.7% and 39.5% ($p<0.01$) increases for rams fed 1.5% and 2% urea diet in the present study, respectively than control group (65.76 and 70.70 vs 50.69, mg/dl). Numerical change was obseved in serum glucose level with changing the grazing system (10.30% lower in grazed animals than ungrazed ones).

No significant differences in serum total cholesterol level were observed across treatments or grazing system (Table 3) but that level tended to be lower by 23.4% ($p>0.01$) when 1.5% urea diet was fed than the control (36.65 and 45.24, mg/dl) and 11.9% lower in grazed rams than ungrazed ones (37.66 and 42.14, mg/dl).

Significant ($p<0.05$) differences of serum total lipids were obtained with grazing systems and interaction between utrea levels and grazing feed system ($p<0.01$) was also found. In table 3, serum total lipids were higher by 23.3% and 9.4% in rames fed 1% and 2% urea diets, respectively as compared to control rams (1096 and 973 vs 889, mg/dl) and tended to be higher ($p<0.05$) by 17.9% in grazed rams than ungrazed ones (1041 and 883 mg/dl).

Concentrations of urea nitrogen in blood serum (BUN) did not differ significantly ($p>0.05$) with grazing system (table 4). Urea N concentration was the lowest ($p<0.01$) when 1% urea diet was fed and the highest when both control and 2% urea were fed (table 3).

Urea supplementation resulted in lowered serum total bilirubin (table 3) which decreased to be lowest value by 30.9% in 1.5% urea group than control (0.55 and 0.72, mg/dl). However, grazed group having 46% ($p<0.01$) lower concentration of serum total bilirubin than ungrazed rams (0.52 and 0.76, mg.dl).

Transaminases enzymes in blood serum (glutamic oxaloacetic; GOT and glutamic pyruvic transaminases; GPT) indifferently responsed to urea supplemented in the basal diet and grazing system for Ossimi rams (Table 3 and 4). The highest GOT level was found with control diet and low urea level, while the lowest value was obtained when 1.5% urea diet was fed ($p<0.05$). However, GPT level was similar among urea treatments. The effect of feeding system on GOT was in opposit trend than that of GPT (Table 4). Grazing rams were significantly ($p<0.01$) lower by 9% and higher by 26.7% in GOT and GPT levels, respectively, than ungrazing ones.

DISUCSSION

According to table (2) rams fed 2% urea were better than the others. This level increased daily gain and decreased in the feed consumed by bout 45% and 44%, respecyively, compared with the control and both 1% and 1.5% urea levels. Moreover, feed intake did not differed.

Improved feed efficiency, due to addition of urea in the diets, (Table 2) could be explained by altering ruminal total volatile fatty acids (VFA) proportions toward more ruminal propionate *(KAPOOR et al,. 1987)*. Propionic acid is used more efficiently than acetate by the host ruminant. Possibly it could reduce heat increament *(BLAXTER and WAINMAN, 1964)*, spare amino acids used normally for gluconeogenesis *(LENG et al, 1967; REILLY and FORD, 1971)* and stimulate body protein *synthesis (POTTER et al, 1968 and ESKELAND et al., 1974)*.

In spite of the high level of amino acids in blood due to NPN sources fed *(MILLER, 1979)*, the decrease of serum total protein in both of urea fed and grazed rams (Table 3) may be explained by altering amino acids in the liver towards more gluconeogenesis and stimulating body protein and total lipids as well as serum albumin synthesis than serum total protein. In the present study, 2% urea fed rams had higher serum glucose and increase in daily gain efficiency than other groups (Table 2 and 3). This result can be explained by increased serum albumin due to fed different nitrogen resources (Table 3 and 4), taking in consideration that serum albumin is synthesized in the liver from amino acids *(HARPER, 1975)*.

The increase in serum glucose level in urea-fed rams compared with untreated ones (Table 3) could be due to the increase in the ratio of propionate to acetate in the rumen as mentioned by *KAPOOR* et al . *(1987)*. *COLLIER (1985)* indicated that the VFA are absorbed into the portal circulation and carried out to the liver, where all propionates are extracted from the blood for glucose production. Propionate and valerate may provide up to 70 of exogenous glucose precursors in ruminants *(BALDWIN and SMITH, 1979)*. Much propionate produced in the rumen is absorbed and transformed to glucose in the liver. High levels of glucose is then released into the peripheral circulation. Propionate is a potent glucogenic compound and account for about two-thirds of the total glucose produced in animals fed large amount of concentrates *(BERGMAN, 1983)*. On the other hand, the decrease in blood glucose observed in the trial with grazing rams could be explained by enhanced cellular uptake of glucose or decreased the rate of gluconeogenesis due to decrease propionate proportion in grazing rams. Grazing rams consumed high fiber containing diet which changed the fermentation pattern of the rumen towards the acetate production *(CHURCH, 1979)*. Meanwhile, fall in serum glucose level in grazing rams is probably due to increased secretion of insulin hormone induced by feeding *(BERTONI* et al. *1992)*. Insulin depressed the amount of glucose in the circulation by inhibiting gluconeogenesis and glucogenolysis and stimulating the uptake and utilization of glucose by such tissues as liver, muscle and fat *(KIPNIS, 1973)*.

Urea levels as weel as grazing system increased serum total lipids (Table 4). This might be resulted from increased triglycerides (the main source of total lipis synthesis) due to increased fatty acids synthesis from rumen microorganism. These fatty acids are then taken by adipocyte and converted to triglycerides by estrification and increased serum glucose availability in NPN fed, which is essential for triglycerides synthesis by forming glycerol 3-phosphate (*LEAT, 1983)* which is a specific precursor for glycerol (*BERGMAN, 1983)*.

Blood urea level of controls was higher than that of 1% urea diet (Table 3). This may be because the addition of urea improved digistibility and feed efficiency in growing rams *(MOUSA, 1993)* which in turn increased the energy supply to the blood and hence decreased the breakdown of amino acids. This could be proved by the fact that increasing urea levels in the diet to 1.5% and 2% insignificantly ($p>0.05$) raised the concentration of blood urea in serum but significantly ($p<0.01$) increased glucose concentration in blood serum due to increased propionate proportion in the rumen. *MADSEN, (1983)* reported that when amino acids are utilized in the liver for gluconeogenesis, the amino groups are converted into urea. Thun serum urea can be used as an indicator for gluconengenesis from labile protein.

Decreased serum bilirubin in different levels of urea-fed rams (Table 3) might result from improved nutritional status (daily gain and feed efficiency) of those rams *(SHETAEWI and ROSS, 1990)*. Avreciprocal relationship between serum bilirubin and nutritional status was suggested in earlier studies. Serum bilirubin in ewe lambs and mature ewes was greater at parturition than before breeding as reproted by *HALLFORD and GALYEAN (1982)*. in humans, *BLOOMER* <u>et</u> <u>al</u>. *(1971)* reproted that fasting was associated with hyperbilirubinemia, which was attributed mainly to decreased hepatic removal of endogenously produced plasma bilirubin. The same results were observed with grazing animals (Table 4).

The differences in serum levels of transaminases enzymes (GOT and GPT) are dependent on the biosynthesis of amino acids by rumen bacteria (*ALLISON, 1969)* and amino groups of alanine and glutamine taken up by the liver*(MADSEN, 1983)*. These amino acids account for about 17% and 11% of the total hepatic uptake of amino acids *(WOLFF* <u>et</u> <u>al</u>, *1972)*. In addition, alanine is converted into pyrovate by GPT while glutamine is converted into glutamate by GOT enzymes. These amino acids (pyrovate and glutamate) are involved in urea formation *(RODWELL, 1975)*. *BERGMAN (1983)* reported that alanine and glutamine are released continuously by muscle and are removed by liver for glucose and urea synthesis. The higher level of blood urea concentration of controls is clearly explained by the results of these enzymes (Table 3).

CONCLUSION

It can be concluded, from these results, that addition of certain levels of NPN to sheep diets decreased feeding costs and increased efficiency of utilization of feed for growth which increased the amount of protein for human and other non ruminants without any harmful affect upon metabolic profiles in these animals.

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Table (1): Chemical composition and feed values of the basal diet*.

Item	0.0% Urea	1.0% Urea	1.5%Urea	2.0% Urea
Chemical compositions				
Dry matter	89.0	90.0	91.0	89.5
Crude protein	10.3	12.7	13.6	15.0
Crude fat	3.5	3.6	3.5	3.4
Crude fiber	18.8	18.6	18.8	18.9
NFE	58.1	55.9	54.8	53.6
Ash	9.3	9.2	9.3	9.1
Organic matter	79.9	80.8	81.7	80.4
Feeding values				
TDN	60.5	58.0	60.8	61.5
DCP	6.1	8.6	9.3	10.8
UN	0.0	22.0	30.9	37.3

* Mousa, (1993).

Table (2): Effect of feeding different urea levels to Ossimi rams on feed intake, average daily gain and feed efficiency.*

Treatments	Feed intake (g/head/day)	Daily gain (g/head/day)	Feed efficiency (gain/feed)
Control	798	90.7	0.114
1.0% Urea	804	108.7	0.135
1.5% Urea	795	100.7	0.127
2.0% Urea	805	132.0	0.164
SE	2.40	8.80	0.010

* Mousa, (1993).
SE : Standard error of least-squares means

Tabel (3): Some serum metabolites concentrations in Ossimi rams fed different urea levels.

Item*	Treatments				
	control	1% Urea	1.5% Urea	2% Urea	SE
T.protein, g/dl	6.24	5.64	5.81	5.45	0.12
Albumin, g/dl	3.14	2.99	3.01	3.22	0.08
Globulin, g/dl	3.10	2.65	2.79	2.23	0.12
Glucose, mg/dl	50.69^{b}	50.76^{b}	65.76^{ab}	70.70^{a}	2.41
T.Cholesterol, mg/dl	45.24^{a}	39.07	36.65^{b}	38.63	1.59
T.lipids, mg/dl	889.00	1096.00	890.00	973.00	32.80
Urea nitrogen, mg/dl	32.16^{a}	24.89^{b}	30.53^{ab}	31.74^{a}	1.09
Creatinine, mg/dl	0.42	0.43	0.54	0.39	0.03
T.bilirubin, mg/dl	0.72	0.68	0.55	0.61	0.03
GOT, (U/L)	33.75^{a}	33.65^{a}	30.87^{b}	32.62^{ab}	0.32
GPT, (U/L)	19.17	18.37	18.85	19.87	0.08

*Values are least-squares means of 3 rams/treatment.
SE = Standard error of least- squares means.
a,b,c,d Means of the same subclass in the same row not having a common superscript differ a,d (P<0.01) and c,d (P<0.05).
GOT = Glutamic oxaloacetic transaminase.
GPT = Glutamic pyruvic transaminase.

Tabel (4): Some serum metabolites concentrations in Ossimi rams before and after grazing.

Item *	Treatment		
	Pregrazing	Postgrazing	SE
T.protein, g/dl	6.40^{a}	5.18^{b}	0.12
Albumin, g/dl	2.85^{d}	3.33^{c}	0.08
Globulin, g/dl	3.54^{a}	1.84^{b}	0.12
Glucose, mg/dl	62.39	56.56	2.41
T.Cholesterol, mg/dl	42.14	37.66	1.59
T.lipids, mg/dl	883.00^{d}	1041.00^{c}	32.80
Urea nitrogen, mg/dl	28.92	30.74	1.09
Creatinine, mg/dl	0.47	0.42	0.03
T.bilirubin, mg/dl	0.76^{a}	0.52^{b}	0.03
GOT, (U/L)	34.17^{a}	31.26^{b}	0.32
GPT, (U/L)	16.86^{b}	21.37^{a}	0.08

*Values are least-squares means of 12 rams/treatment.
SE = Standard error of least- squares means.
a,b,c,d Means of the same subcalss in the same row not having a common superscript differ a,b (P<0.01) and c,d (P<0.05).
GOT = Glutamic oxaloacetic transaminase.
GPT = Glutamic pyruvic transaminase.