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Effect of Sheep Dietary Urea Level on Serum Urea, Glucose and Amino Acids Concentrations

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Abstract:

The trial was carried out to evaluate the effects of dietary urea level on serum urea, glucose and amino acids concentrations. Eighteen clinically healthy ewes were previously adapted to urea feeding (initial body weight 33.81 kg and about 4 years age). Animals were randomly assigned to three groups; the control group was given basal diet which met energy and protein requirements for body weight maintenance and the other two groups were fed on basal diet with 1% and 1.5% urea on dry matter (DM) basis to replace part of the dietary crude protein. The duration of this study was lasted for 3.5 months. Animals were kept in individual pens. The estrous cycles were synchronized using two injections of prostaglandin ($PGF_{2\alpha}$). Blood samples were taken at the beginning of the experiment as well as at the first and second injection with $PGF_{2\alpha}$ and then just before slaughter. Three animals were slaughtered at follicular phase and the remaining three animals were at luteal phase in each group. The follicular and luteal phases were monitored 40 hrs and 10 days upon second injection with $PGF_{2\alpha}$. Serum glucose and urea concentrations were determined using spectrophotometer. Essential and non essential amino acids were determined using HPLC method in blood plasma collected at follicular and luteal phases. The results indicated that serum urea levels in treated groups were significantly higher than those in the control one ($P<0.05$), and vice versa with serum glucose concentrations. There were no significant differences in plasma essential and non essential amino acids during follicular and luteal phases among the three groups. It could be concluded that dietary 1% and 1.5% urea level changes serum concentrations of urea and glucose but did not affect plasma amino acids.

Key words: Dietary urea, Glucose, Amino acids, ewes.

Introduction

The process of reproduction is a coordinated function of many tissues, cell types and regulatory systems which is possible only when animals are provided with sufficient quantities of dietary nutrients. The livestock industry is faced with different situations as far as protein nutrition is concerned in different countries. In most of the third world countries, animals survive on poor quality roughages and crop residues

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which are deficient in many essential nutrients. The major constraint in such feeds is protein deficiency because the digestible crude protein content of these roughages is very low. In certain Asian and African countries, animals generally graze without much feed supplement (Leng, 1990). Thus, the availability of nutrients, particularly protein, remains inadequate most of the year except during or after rainy season, during this period the estrous cycle is normally exhibited (Kaur and Arora, 1995).

Ruminant animals are able to utilize efficiently non protein nitrogen (NPN) by converting it to microbial protein (Miller, 1979). Therefore the protein deficiency can be overcome by adding urea or other non-protein substances to the diet or by treating crop residues with urea or ammonia.

Urea treatment improves the nutritive value of crop residues and increases the nitrogen content, feed intake and rumen digestibility (Tingshuang *et al.*,2002; Abebe, 2010). Because urea is easy to handle and cheaper than any other source of proteins (Preston, 1986), so that it is economical to be used as protein supplement in ruminant ration (Mussa, 1996).

High producing cows are commonly fed diets containing high levels of crude protein to maximize milk production. A consequence of high dietary crude protein is elevated plasma urea nitrogen (PUN) concentrations which have been associated with decreased fertility in dairy cows (Butler et al., 1996). This effect is not limited to lactating dairy cows because dairy heifers fed a high protein diet had a 21% point decrease in conception rate (Elrod and Butler, 1993). Whereas elevated plasma urea nitrogen (PUN) concentrations have been associated with decreased fertility, elevated plasma glucose concentrations have been associated with increased fertility in cows (Butler,1998). However, mechanisms underlying the effects of urea nitrogen on fertility remain unclear.

The present study aimed at evaluating the effect of different urea levels in sheep ration on blood serum urea, glucose and amino acids.

Material and methods

The present study was carried out at the experimental farm of the department of animal production, Faculty of Agriculture, Assiut University throughout the period from January up to April 2009.

Animal and management:

A total number of eighteen clinically healthy ewes (previously adapted for urea feeding) of about 4 years of age and 33.81±2.17 kg av., body weight were randomly divided into 3 groups (six animals in each group). The control group was given basal diet which met energy and protein requirements for body weight maintenance according to NRC. (1985) and the other two groups were fed on basal diet supplemented with 1% and 1.5%

urea on dry matter (DM) basis to replace part of basal diet, CP. All ewes were placed in individual cement floor pens. Each ewe was fed one kilogram of the experimental diet daily for 90 days. Water was available at all times. Ingredients of the experimental rations fed to ewes are presented in table (1). Chemical analysis of corn, undecorticated cotton seed cake (UCSC), wheat straw and molasses used in formulating the experimental ration are presented in table (2).

Table (1) Ingredients of the experimental rations fed to ewes.

Ingredients	rations		
	Control	1%urea	1.5%urea
Corn %	36.0	45.9	40.4
Uncorticated cotton seed cake %	21.0	5.0	-
Wheat straw %	34.9	40.0	45.0
Molasses %	5.0	5.0	10.0
Urea%	-	1.0	1.5
Lime stone%	2.0	2.0	2.0
Sodium chloride %	1.0	1.0	1.0
Minerals %	0.1	0.1	0.1
Total	100	100	100
Crude protein %	9.49	9.40	9.49

Table (2) Chemical analysis of corn, undecorticated cotton seed cake (UCSC), wheat straw and molasses used in formulating the experimental ration.

Composition%	ingredients			
	Corn	UCSC	Wheat straw	molasses
Dry matter	89.31	93.70	95.56	74.10
organic matter	88.12	88.09	86.85	66.00
Crude protein	8.23	24.36	3.60	3.00
Crude fiber	2.15	13.26	24.02	0.00
Ether extract	4.08	1.94	0.56	0.00
Nitrogen free extract	73.66	48.53	58.67	63.0
Ash	1.19	5.61	8.71	8.10

Estrus cycle synchronizing:

For synchronizing of estrus cycle, each ewe received two intramuscular injections of 125µg of prostaglandin ($PGF_{2\alpha}$). The first injection was given after 60 days, then after ten days second injection was given.

Determination of follicular and luteal phases:

Animals were slaughtered after the second injection(three after 40 hrs and three after 10 days). After slaughtering of ewes, reproductive systems were removed and kept in sealed plastic bag for each ewe, in container contains normal saline at (37.5°C), then transferred to the laboratory within 20 minutes. In the laboratory, the ovaries

were taken and examined. The follicular and luteal phases were monitored through the presence of graffian follicles and corpora lutea in the ovaries respectively.

Blood sampling:

Blood samples were obtained by jugular vein puncture from all animals at four times: at the beginning of experiment before animals get adapted to urea, immediate after first and second prostaglandin ($PGF_{2\alpha}$) injections, then just before slaughtering. About 10 ml of blood sample was collected from each animal into dry clean centrifuge tubes. Except blood samples collected at just before slaughtering, dry clean centrifuge tube samples divided into two parts, the first was kept in heparin as an anticoagulant while the other was kept in dry clean centrifuge tube without heparin.

Serum Samples were allowed to clot at room temperature and separated by centrifugation at 400 rpm. for 15 minutes. While plasma samples were immediately centrifuged. Serum and plasma were decanted into dry clean Eppendrofe tubes and stored at -20 °C until subsequent analysis.

Determination of serum glucose and urea concentrations:

Blood serum was analyzed for urea and glucose using spectrophotometer with appropriate commercial kits. Serum urea nitrogen was measured according to Patton and Crouch (1977) method using assay kits supplied by Diamond, Egypt. Serum glucose was measured according to Trinder (1969) method using assay kits supplied by diamond, Egypt.

Determination of plasma amino acids concentrations:

Blood plasma essential and non essential amino acids were measured in heparinized samples which were collected at just before the slaughter from ewes during follicular and luteal phases. Amino acids were determined in research laboratories of faculty of Agriculture Cairo University using automatic amino acid analyzer (AAA 400 Ingos Ltd). Acid hydrolysis was carried out according to the method of Block *et al.*(1958). The dried ground sample was hydrolyzed with 6 N HCl (10ml) in a sealed tube at 110°C in an oven for 24 hour. The excess of HCl was then freed from 1ml hydrolyzed under vacuum with occasionally addition of distilled water, then evaporated to dryness. The HCl free residue was dissolved in exactly one ml of diluting buffer (0.2M, pH2.2).

Statistical analysis:

Data were analyzed using General Linear Model (GLM) procedure of SAS (SAS institute, 1998) according to the following model. Duncans multiple range test was used to compare among means of the control and treated groups. The model of analysis was as follows: $Yij = \mu + Ti + Eij$ Where:μ= the mean. Ti= the effect of treatment. Eij = the random error

Results and discussion

Effect of dietary urea level on serum urea nitrogen (SUN):

Mean values of serum urea nitrogen of the three experimental groups are presented in table (3) and illustrated in figure (1). Results showed that SUN levels increased with time of the groups fed on urea. Also SUN concentrations were significantly ($P < 0.05$) greater of the ewes fed urea at compared with the control group. Furthermore, dietary urea 1.5% elevated SUN significantly ($P < 0.05$) compared to dietary urea 1%, at second injection and at slaughtering.

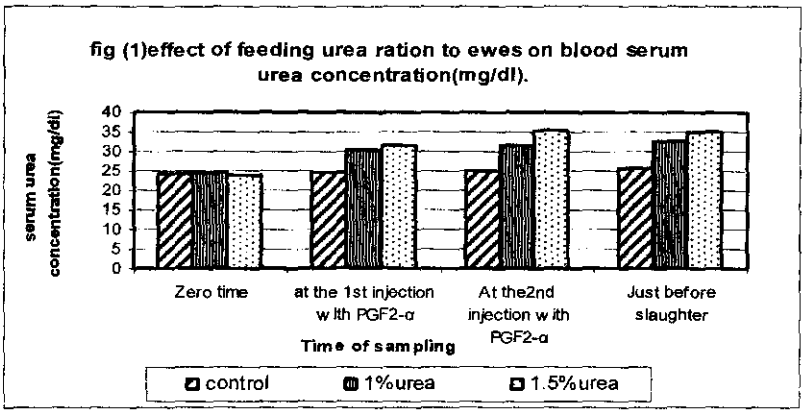
The mean values of serum urea for all treatments was within the normal range of (17-42.86 mg/dl of sheep) as indicated by Kaneko (1980) under normal conditions. Our results agreed with the results of Bishonga *et al.*(1996); Abd-El-Aziz(2001); Taghizadeh *et al.* (2007); Mohamed (2008) and Elkholly *et al.*(2009). Who reported that blood urea level was significantly higher in sheep fed on diets containing urea than those fed on diets without urea.

On the other hand, El-Khdrawy (1991) found that there were no significant differences in blood urea concentration of cattle fed urea ration and those fed ration free of urea. Moreover, Abd-El Rehim (1997) reported that SUN level in Ossimi sheep was not significantly affected by ammonia contained ration. Concentrations of serum urea are related in part to the quantity of ammonia absorbed from the rumen and converted to urea in the liver, which reflect both protein degradation to ammonia in the rumen and availability of fermentable carbohydrate to provide energy for utilization of ammonia for microbial protein synthesis. Concentrations of SUN also reflect the amount of urea produced by the liver to synthesis glucose from amino acids (Wilson et al., 1998). Increased SUN concentration with increasing non protein nitrogen (urea) or dietary rumen degradable protein probably can be explained by increased absorption of ruminal ammonia, resulting in higher ammonia being detoxified in the liver to form urea (Bulter, 1998; Koening et al., 2004).

Table (3) Effect of dietary urea level on serum urea nitrogen concentration (mg/dl) in ewes.

Time of blood sampling	N	treatments		
		control	1%urea	1.5%urea
Zero time	6	24.38±0.21	24.62±0.21	23.89±0.21
at the first injection with $PGF_{2\alpha}$	6	24.45 ± 0.33^{b}	30.44 ± 0.47^{a}	31.44 ± 0.50^{a}
at the second injection with $PGF_{2\alpha}$	6	24.84 ± 0.22^{c}	31.45 ± 0.28^{b}	35.52 ± 0.31^{a}
Just before slaughter	6	25.64 ± 0.29^{c}	32.80 ± 0.36^{b}	35.13 ± 0.40^{a}

a, b, c: Values with the different superscripts letter in the same row differ significantly ($P<0.05$).



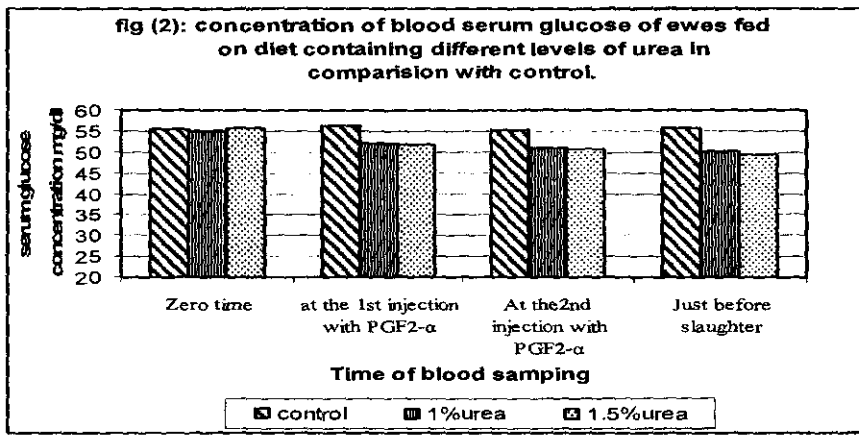
Effect of dietary urea level on serum glucose concentration:

Mean values of serum glucose concentrations are presented in table (4) and illustrated in figure (2). The results indicated that serum glucose concentration was significantly lower ($P<0.05$) of sheep fed urea ration than those fed on control one, during the three different periods of blood sampling. No significant changes in serum glucose concentration were found between ewes fed either 1% or 1.5%urea. All values serum glucose levels of all treatments lie within the normal range which were 52-56 mg/dl in sheep 2.5- 4 years (Eshratkhan *et al.*, 2008; Mustafa *et al.*,2009). Our results agreed with Leonard *et al.* (1977) data who reported that serum glucose concentrations were 3.7 and 4.1 mmol/L of sheep fed urea diet and those fed free urea one respectively. As well as, Knaus *et al.* (2002) observed that blood serum glucose level significantly decreased in steers fed diets containing urea in compared to those fed on untreated one. On the contrary, the present results disagreed with Daghash and Mousa (1994) and Elkholy *et al.* (2009) who reported that blood glucose levels were higher in sheep fed diets supplemented with urea than those fed on untreated one. Feeding ruminants on urea supplemented diets result in increased serum urea level which inhibits gluconeogenesis, the later is extremely important to neonatal and adult ruminants because it provides 75% and 90% of the total glucose needs to those animals (Young, 1977; Nafkove and Beitz, 2007).

Table (4) Effect of dietary urea level on serum glucose concentration (mg/dl) in ewes

Time of blood sampling	N	Treatments		
		control	1%urea	1.5%urea
zero time	6	55.48±0.35	54.93±0.34	55.76±0.36
at the first injection with $PGF_{2\alpha}$	6	56.21±0.60[a]	51.99±0.55[b]	51.71±0.54[b]
at the second injection with $PGF_{2\alpha}$	6	55.30±0.58[a]	50.88±0.55[b]	50.60±0.54[b]
Just before slaughter	6	55.64±0.29[a]	50.07±0.26[b]	49.51±0.25[b]

a, b: Values with the different superscripts in the same row differ significantly (P<0.05)



Effect of dietary urea level on serum amino acids:

Data of the plasma amino acids are summarized in tables (5, 6, 7 and 8). Results indicated that there were no significant differences in concentrations of plasma essential and non essential amino acids among groups at follicular phase (Control & 1% urea) or the luteal phase (Control & 1.5%). These results agreed with the findings of Bergen et al.(1973) who found that plasma amino acid concentrations were not significantly differ of sheep fed corn basal ration and those fed urea ration. Bishonga *et al.*(1996) also reported that there were no differences between levels of plasma constituents of sheep fed basal diet with 1.5 urea and those fed basal diet without urea.

On the contrary, these results disagreed with what obtained by Young *at al.*(1973) who found that plasma amino acid levels were higher in steers fed on ration with 1.2 urea than those fed basal diet without urea. The differences in plasma amino acid concentrations within each group may refer to the differences of amino acid absorption from small intestine of the ruminant, for ex-

ample, absorption of lysine and histidine is higher than the absorption of leucine and phenylalanine. Young *et al.*(1973) indicated that the most responsive amino acids were valin, isoleucine, leucine and lysine. However in some instances the phase of the trial during which the samples were collected influenced the results. The results of the presents study indicated that there is a non significant fluctuation of the concentrations of essential and non essential amino-acids in ewes fed urea compared to control. Horn and Beeson (1969) have suggested that lower levels of plasma amino acids may indicate a more balanced amino acid pattern presented to the tissue and a subsequent increase in tissue protein synthesis, rather than an actual decrease in production. However, considerably less protein reaches the abomasum in animals fed urea than those fed soy protein (Potter et al., 1969; Tucker and Fontenot, 1970). This may suggest a decline in the quantity of amino acids absorbed rather than more efficient tissue utilization. Factors that stimulate microbial protein synthesis in vivo need to be determined.

Table (5) Effect of dietary 1% urea on plasma essential amino-acid concentrations (g/dl) during follicular phase in ewes

Amino Acids	**Symbol**	**Control mean ± SE**	**1% urea mean ± SE**
Threonine	Thr	0.434±0.007	0.441±0.043
Valin	Val	0.493±0.007	0.440±0.036
Methionine	Met	0.017±0.001	0.013±0.001
Isoleucine	Ile	0.149±0.005	0.164±0.009
Leucine	Leu	0.731±0.016	0.723±0.040
Tyrosine	Tyr	0.236±0.001	0.237±0.017
Phenyalanine	Phe	0.259±0.009	0.249±0.018
Histidine	His	0.244±0.011	0.222±0.024
Tryptophan	trp	0.354±0.083	0.307±0.135
Arginine	Arg	0.249±0.002	0.250±0.021

Table (6) Effect of dietary 1% urea on plasma non essential amino-acid concentrations (g/dl) during follicular phase in ewes.

Amino Acids Names	**Symbol**	**Control mean ± SE**	**1% urea mean ± SE**
Aspartic	Asp	0.744±0.010	0.740±0.041
Serine	Ser	0.498±0.005	0.530±0.038
Glutamic	Glu	0.87±0.006	0.888±0.060
Proline	Pro	0.011±0.001	0.011±0.002
Glycine	Gly	0.477±0.006	0.500±0.037
Alanine	Ala	0.566±0.015	0.567±0.033
Cysteine	Cys	0.099±0.001	0.076±0.024
Lysine	Lys	0.664±0.010	0.653±0.051

Table (7) Effect of dietary 1.5% urea on plasma essential amino-acid concentrations (g/dl) during luteal phase in ewes

Amino Acids Names	Symbol	Control mean ± SE	1.5% urea mean ± SE
Threonine	Thr	0.443±.0.048	0.465±0.055
Valin	Val	0.477±0.050	0.475±0.062
Methionine	Met	0.017±0.003	0.014±0.001
Isoleucine	Ile	0.150±0.008	0.167±0.019
Leucine	Leu	0.789±0.072	0.806±0.086
Tyrosine	Tyr	0.244±0.021	0.260±0.034
Phenylalanine	Phe	0.281±0.029	0.284±0.031
Histidine	His	0.263±0.027	0.265±0.031
Tryptophan	Trp	0.339±0.073	0.371±0.039
Arginine	Arg	0.252±0.016	0.276±0.033

Table (8) Effect of dietary 1.5% urea on plasma non essential amino-acid concentrations (g/dl) during luteal phase in ewes

Amino Acids Names	Symbol	Control mean ± SE	1.5% urea mean ± SE
Aspartic	Asp	0.770±0.059	0.811±0.092
Serine	Ser	0.517±0.039	0.567±0.088
Glutamic	Glu	0.886±0.059	0.944±0.097
Proline	Pro	0.011±0.001	0.010±0.002
Glycine	Gly	0.493±0.038	0.516±0.070
Alanine	Ala	0.606±0.057	0.611±0.069
Cysteine	Cys	0.104±0.010	0.109±0.009
Lysine	Lys	0.693±0.055	0.715±0.071

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تأثير مستوى اليوريا الغذائية في عليقة الأغنام على مستويات اليوريا والجلوكوز والأحماض الأمينية في الدم

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الملخص العربي:

أجريت هذه الدراسة على عدد 18 من النعاج السليمة صحياً، عمرها حوالي 4 سنوات ومتوسط أوزانها33.81 كجم. وقد تم أقلمتها على علائق اليوريا لفترة15 يوماً، وذلك لمعرفة أثر إضافة مستويات مختلفة من اليوريا على تركيز اليوريا، الجلوكوز والأحماض الأمينية الضرورية وغير الضرورية في سيرم الدم. تم تقسيم النعاج عشوائياً إلى ثلاثة مجموعات، حيث اشتملت كل مجموعة على 6 نعاج غذيت مجموعة الكنترول على عليقة خالية من اليوريا وتغطي إحتياجات الطاقة والبروتين اللازمين لحفظ حياة الحيوان، بينما تم أحلال اليوريا بنسبة 1%، 1.5% من المادة الجافة محل بروتين العليقة للمجموعتين الثانية والثالثة على التوالي. استمرت التجربة 3.5 أشهر، تم وضع الحيوانات في حظائر انفرادية. لتوحيد دورة الشبق عند النعاج قيد التجربة، تم حقنها بهرمون البروستاجلاندين ($PGF_{2\alpha}$). أخذت عينات الدم في بداية التجربة، ومع كل من الحقنة الأولى والثانية بالبروستاجلاندين ($PGF_{2\alpha}$) ثم قبل الذبح مباشرةً. حدد الطور الحويصلي بذبح النعاج بعد مرور 40 ساعة على الحقنة الأولى، بينما تم تحديد الطور الصفراوي بعد 10 أيام من الحقنة الثانية. تم تقدير اليوريا والجلوكوز في سيرم الدم باستخدام المطياف الضوئي، أما الأحماض الأمينية فقد قُدرت في بلازما دم النعاج أثناء الطور الحويصلي والطور الصفراوي وذلك باستخدام طريقة HPLC. وقد أوضحت الدراسة أن مستوى اليوريا كان مرتفعاً معنوياً ($P < 0.05$) في سيرم الدم للمجموعتين التي غذيت على علائق اليوريا، بينما تركيز الجلوكوز كان منخفضا معنوياً ($P < 0.05$) في المجموعتين التجريبيتين مقارنة بمجموعة الكنترول. إما بالنسبة لمستويات الأحماض الأمينية الضرورية وغير الضرورية فإنها لم تتأثر معنويا في جميع المعاملات. خلصت الدراسة إلى أن زيادة تركيز اليوريا في سيرم الدم له تأثير معنوي على كل من مستوى اليوريا والجلوكوز في الدم، بينما لم يؤثر على الأحماض الأمينية الضرورية وغير الضرورية.

الكلمات التصنيفية: اليوريا الغذائية، الجلوكوز، الأحماض الإمينية، النعاج.