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SOME INDICES OF METABOLIC PROFILE AND OXIDATIVE STRESS IN SOHAGI EWES DURING TRANSITION PERIOD

(With 2 Tables and One Figure)

By

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(Received at 8/12/2011)

**بعض مؤشرات التمثيل الغذائي وضغوط الأكسدة في النعاج السوهاجي خلال
الفترة الانتقالية**

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اجريت هذه الدراسة لتقدير بعض مؤشرات التمثيل الغذائي وضغوط الاكسدة في النعاج السوهاجي خلال الفترة الانتقالية (قبل وبعد الولادة)، حيث تم استخدام عدد ٤٠ نعجة سوهاجي عمر ٢.٥ – ٤.٥ سنة قسمت الى مجموعتين، المجموعة الاولى ٢٥ نعجة عشر والمجموعة الثانية ١٥ نعجة فارغة جافة (غير عشر). تم تقسيم المجموعة الاولى حسب عدد الاجنة الى عشر فردي (١٥) وعشر توأمي (١٠). تم جمع عينات دم قبل وبعد الولادة باسبوعين لتحليل البروتين الكلى والجلكوز واليوريا والكرياتينين وانزيمات الكبد الناقلة لمجاميع الامين واكسيد النيتريك في سيرم الدم. اظهرت نتائج الدراسة عدم وجود تغيرات معنوية في مكونات سيرم دم النعاج الولادة الفردية قبل وبعد الولادة باستثناء مستوى اكسيد النيتريك. من ناحية اخرى كان هناك انخفاض معنوي (٥%) لمستوى البروتين الكلى والجلكوز في سيرم دم نعاج الولادة التوأمية، بينما كانت انزيمات الكبد واليوريا عالية المعنوية (٥%) في هذه الحيوانات بالمقارنة بالنعاج الفارغة الجافة (غير عشر) ، كما أظهرت هذه النعاج تحسن معنوي في مؤشرات التمثيل الغذائي بعد الولادة. كانت هناك فقط زيادة معنوية في انتاج اكسيد النيتريك في كلا النعاج- ذات الولادات الفردية والتوأمية- قبل وبعد الولادة بالمقارنة بالنعاج الفارغة الجافة (غير عشر). بينما اوضحت هذه الدراسة حساسية النعاج السوهاجي ذو الولادات التوأمية لميزان الطاقة السلبي وضغوط الاكسدة قبل الولادة ، اظهرت النعاج السوهاجي ذات الولادات الفردية عدم تأثر لاضطرابات الطاقة. هذا بالاضافة الى ان الدراسة اظهرت التأقلم الابيض في النعاج ذات الولادات التوأمية كنتيجة لتحسين مستوى الجلوكوز في الدم بعد الولادة بوقت قصير.

SUMMARY

The present study was carried out to assess some metabolic indices and nitric oxide in sexually mature, cycling; apparently healthy Sohagi ewes during transition period. At the beginning of the study, 40 ewes were randomly separated into two main groups: pregnant (n = 25) and non pregnant (n = 15) groups. Pregnant group was further subdivided retrospectively into single and twin bearing ewes' subgroups. Blood samples were collected from both main groups. Samples from pregnant ewes were collected at two weeks before and after parturition. Samples were assayed for serum total protein, glucose, urea nitrogen, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and nitric oxide. The results of this study showed no marked change in serum metabolites in single-bearing transition Sohagi ewes except for nitric oxide level. On the other hand, significantly lower ($p < 0.05$) levels of serum total protein and glucose concentrations were recorded in twin-bearing ewes while liver enzymes and serum urea nitrogen showed significant higher ($p < 0.05$) compared to control group. Marked improvement in serum metabolites had been recorded after parturition. Only level of nitric oxide production showed significant increase in both single and twin-bearing ewes before parturition compared to control one. While this study indicates the susceptibility of full-term twin-bearing pregnant Sohagi ewes to negative energy balance and oxidative stress before parturition, it showed tolerance of single-bearing pregnant ewes to energy disturbance. Additionally, twin-bearing pregnant Sohagi ewes showed high degree of metabolic adaptation as indicated by remarkable improvement in the blood glucose level shortly after parturition.

Key Words: *Transition period, Sohagi ewes, Energy balance, Nitric oxide.*

INTRODUCTION

There has been much interest over the past two decades to study the impact of transition (peripartum) period on animal health, reproduction and lactational performance in food animals. While several studies have been conducted in particular, on dairy cows (Shaver 1993; Grant and Albright 1995; James and Drackley 1999; Bernal-Santos

et al., 2003; Pushpakumara *et al.*, 2003; Zachut *et al.*, 2010; Silvestre *et al.*, 2011; do Amaral *et al.*, 2011), little was carried out on transition ewes. Current research concepts regarding metabolic alterations associated with transition ewes may be extrapolated from dairy cattle, however unexpected metabolic changes may occur. Several factors undoubtedly influence the metabolic process during transition period. Nutrition and energy demand, in particular are essential factors in the adaptation process of metabolism in pregnant ewes. Decreasing dry matter intake (DMI) and decreasing ruminal capacity in the last trimester stage can lead to negative energy balance and subsequent pregnancy toxemia (Radostits *et al.*, 2007; Schlumbohm and Harmeyer, 2008). High energy demand associated with rapid fetal growth during the last weeks of gestation contributes also negatively on energy balance of pregnant ewes. The influence of other factors such as body condition score is not fully understood because contradicted results were recorded in different studies (Kahn and Line, 2005). Nowadays scientists think about genetic predisposition in the metabolism of fat and carbohydrates in some breeds (Graber *et al.*, 2010).

The present study was designed to determine some indices of metabolic and oxidative stress in Sohagi ewes breed during transition period. The effects of pregnancy and parturition on some biochemical and oxidative parameters including total protein, blood glucose, blood urea nitrogen, creatinine, serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and nitric oxide were studied on sexually mature Sohagi ewes during transition period.

MATERIALS and METHODS

Animals and breed characteristics: Sohagi sheep flock had been formed by the College of Agriculture, University of Sohag in 2001. The breed was registered by Domestic Animal Genetic Resources Information System -International Livestock Research Institute, Addis Ababa, Ethiopia as an additional breed of sheep (DAGRIS- ILRI, 2007; Mahfouz *et al.*, 2008). Animals are shallow body, medium in size with long neck and legs. Head is medium with straight profile and ewes are mostly polled while rams are horned. Body is covered with coarse wool ranging from cream to white in color. Head is generally dark brown or creamy with dark rings around eyes. Ears are vestigial and tail varies in shape from wide base terminated into a sort segment to a lesser wide

base ending into cylindrical part extending well below the hocks. (Hamdon, 2009).

Experimental design: The study was conducted on 40 sexually mature, cycling; apparently healthy Sohagi ewes with an average body weight 36.10 ± 0.66 and age ranged from 2.5 to 4.5 years. Ewes were randomly divided into two main groups according to their pregnancy: Pregnant (n = 25) and non pregnant (n = 15). Pregnant group was further subdivided retrospectively into single and twin bearing subgroups. Blood samples were collected from both main groups. Samples from pregnant group were taken at two weeks before and after parturition. Samples were assayed for total protein, blood glucose, blood urea nitrogen, creatinine, serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and nitric oxide. The experiment acts in accordance with the guidelines and recommendations for the Institutional Animal Care and Use Committee of Sohagi University.

Study area: This experiment was carried out at the animal production experimental farm, Faculty of Agriculture, Sohag University, Sohag, Upper Egypt. The flock was housed in semi open pens, fed concentrates and roughage. Hay and green fodder (*Trifolium Alexandrium* and *Sorghim*) were used as roughages, free access to water and block common salt. Parturitions took place in February, June and October. Lambs were kept with their dams in individual boxes for three days after birth.

Clinical parameters

A clinical chart was designed to record various clinical indices of health. The appetite, dry matter intake, behavior and mental status (Demeanor) of transient ewes were monitored by inspection. Body temperature and pulse rate were digitally recorded using pulse oximeter for veterinary use (Model 340V, CA-USA).

Blood sampling

Blood samples were collected from pregnant and non pregnant groups. Pregnant ewes were sampled on days -14 and +14, from parturition. Samples were collected in clean, dry centrifuge tubes without anticoagulant. The samples were left for 30 min. at room temperature until clotting and then centrifuged at 3000 rpm for 20 minutes (Coles and Rich, 1992). The obtained clear non hemolyzed sera were separated and transferred into clean epindorff vials and then stored

at -20 °C for determination of blood glucose, total protein, blood urea nitrogen, creatinine, serum AST, ALT and nitric oxide.

Biochemical assays

All reagents and kits used in this study were previously tested in our laboratory. Spectrophotometric method by using Phillips Pye Unicam spectrophotometer (U.V. visible Mod. 800) was adopted to determine serum concentrations of the following constituents: glucose (Trinder), total protein (Biuret), urea (Urease–glutamate dehydrogenase), creatinine (Jaff'e reaction), AST and ALT (International Federation of Clinical Chemistry-modified method). All kits and reagents were obtained from Spectrum Reagents (Egyptian Company for Biotechnology, Cairo, Egypt).

Nitric oxide determination

Nitric oxide (NO) in biological systems was measured indirectly by reaction kinetics according to Green *et al.* (1982).

Statistical analysis

Statistical Package for the Social Sciences program for windows version 10.0.1 (SPSS, 1999) was used to analyze the obtained data. All data were presented as mean \pm standard error (SE). Differences between groups were determined by LSD Post hoc test. Significance level was set at $P \leq 0.05$.

RESULTS

Clinical course

No signs of clinical toxemia of pregnancies such as star gazing, recumbency and coma were recorded in pregnant ewes during the period of study. Sohagi ewes in last trimester stage of pregnancy were alert and able to stand without assistant but reluctant to move with slight reduction in feed intake. Body temperature (°C) and pulse rate (beats/min) were 39.3 ± 0.20 , 93 ± 6 , and 85 ± 5 , respectively. Recently parturient ewes showed slight decrease in DMI, mild pyrexia (39.5 ± 0.50) and tachycardia (103 ± 5) in first 24 hours postpartum.

Finding of serum biochemical analysis

Levels of indices under study in terms of serum glucose, total protein, urea, creatinine, liver enzymes and nitric oxide concentrations evaluated in blood serum in transition Sohagi ewes were presented in Tables 1 and 2.

Serum total protein and glucose concentrations

Serum total protein concentrations 14 days before and after parturition were 5.77 ± 0.17 g/dl and 6.25 ± 0.18 g/dl, respectively in single-bearing ewes while they were significantly decrease ($p < 0.05$) in twin-bearing ewes (4.42 ± 0.23 and 4.95 ± 0.15 g/dl, respectively) compared to control one ((Figure 1A). Non significant improvement was recorded in serum total protein concentration before and after parturition in twin bearing animals. On the other hand, serum glucose level was significantly lower ($p < 0.05$) in twin-bearing group (53.76 ± 2.50 mg/dl) before parturition compared to control one (62.70 ± 1.44 mg/dl). Marked improvement (significantly increase, $p < 0.05$) was shown in serum glucose level (66.62 ± 1.39 mg/dl) two weeks after parturition in twin bearing animals. Single bearing ewes showed no significant change in their serum glucose level (Figure 1B).

Serum urea and creatinine concentrations

Mean serum urea concentration in twin-bearing ewes before parturition were significantly higher (30.93 ± 2.88 mg/dl) compared to control one (24.65 ± 1.05 mg/dl), however significant improvement was recorded after parturition in the same group (Figure 1B). Serum creatinine concentrations showed no significant difference among different groups (Figure 1A).

Liver Enzymes

AST and ALT enzyme activities were significantly higher (124.18 ± 4.76 and 50.41 ± 2.25 U/l) in twin-bearing (72.26 ± 1.40 and 35.10 ± 1.40 U/l) compared to control one, however they showed marked reduction in their activities after parturition. No significant changes in the activity of liver enzymes among single bearing animals (Figure 1B).

Nitric oxide production

Nitrite levels as an indication of nitric oxide production had been showed to be significantly elevated in both single and twin-bearing groups before parturition compared to control group. Slight but not significantly, improvement in NO concentrations was recorded postpartum in both groups (Figure 1A).

Table 1: Mean (\pm S.E.) of some metabolic indices concentration in transition Sohagi ewes regardless type of bearing

Parameter	Non pregnant (n = 15)	Pregnant (n = 25)	
		Pre	Post
TP (g/dl)	6.28 \pm 0.22	5.24 \pm 0.18 ^{ab}	5.74 \pm 0.18
Glucose (mg/dl)	62.70 \pm 1.44	58.50 \pm 1.41 ^{ab}	63.40 \pm 0.96
Urea (mg/dl)	24.65 \pm 1.05	27.54 \pm 1.46 ^{ab}	25.77 \pm 1.05
Creatinine (mg/dl)	0.96 \pm 0.30	1.01 \pm 0.03	0.99 \pm 0.04
ALT (U/l)	35.10 \pm 1.40	43.33 \pm 1.93 ^{ab}	38.39 \pm 1.23
AST (U/l)	72.26 \pm 1.40	94.88 \pm 5.69 ^{ab}	80.39 \pm 2.91
NO (μ M/l)	2.18 \pm 0.10	4.21 \pm 0.24 ^{ab}	3.64 \pm 0.19

Table 2: Mean (\pm S.E.) of some metabolic indices concentration in single and twin -bearing transition Sohag ewes

Parameter	Non pregnant (n = 15)	Pregnant (n = 25)			
		Single (n = 15)		Twin (n = 10)	
		Pre	Post	Pre	Post
TP (g/dl)	6.28 \pm 0.22	5.77 \pm 0.17	6.25 \pm 0.18	4.49 \pm 0.21 ^{ab}	4.95 \pm 0.15
Glucose (mg/dl)	62.70 \pm 1.44	61.79 \pm 0.93	61.34 \pm 0.98	53.76 \pm 2.50 ^{ab}	66.62 \pm 1.39
Urea (mg/dl)	24.65 \pm 1.05	25.19 \pm 1.15	24.74 \pm 1.02	30.93 \pm 2.88 ^{ab}	27.86 \pm 2.34
Creatinine (mg/dl)	0.96 \pm 0.30	1.01 \pm 0.06	0.99 \pm 0.06	1.02 \pm 0.03	0.93 \pm 0.23
ALT (U/l)	35.10 \pm 1.40	38.43 \pm 1.95	37.17 \pm 1.57	50.41 \pm 2.25 ^{ab}	40.30 \pm 1.92
AST (U/l)	72.26 \pm 1.40	74.59 \pm 1.26	72.87 \pm 1.16	124.18 \pm 4.76 ^{ab}	92.09 \pm 5.30
NO (μ M/l)	2.18 \pm 0.10	3.80 \pm 0.26 ^a	3.47 \pm 0.25	4.79 \pm 0.38 ^{ab}	3.91 \pm 0.27

a = LSD compared to non pregnant groups

b = LSD before compared to after parturition

Figure 1A.

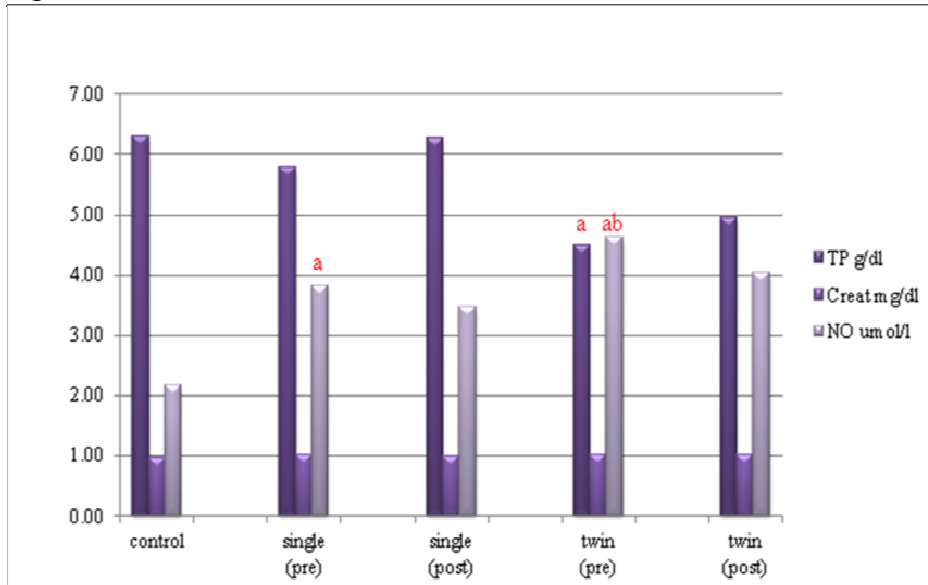
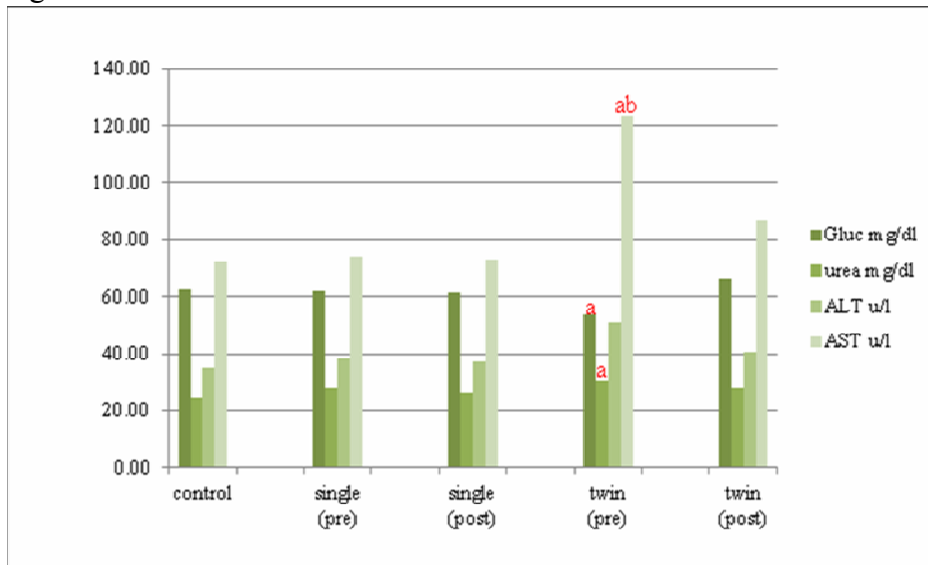


Figure 1B.



Mean value of TP, creatinine and nitric oxide (Fig. 1A), glucose, urea and liver enzymes (Fig. 1B) in non pregnant, single and twin bearing Sohagi ewes

a = LSD compared to non pregnant group

b = LSD before compared to after parturition subgroups

DISCUSSION

Nutritional and other stress factors associated with pregnancy and parturition play a pivotal role in energy homeostasis. The nutritional requirements of ewes increase during late pregnancy due to the rapid fetal growth. Additionally, lactation in response to dramatic hormonal change during first few weeks of parturition puts further stress and increases energy demand. Improper management of ewes during transition period will result in negative energy balance due to lipomobilization in response to shortage in energy supply (Radostits *et al.*, 2007).

Serum glucose level

Our data primary showed marked change in serum glucose level in the last two week of pregnancy irrespective to bearing type (Table 1). Further classification of pregnant ewes according to their bearing type revealed that twin bearing ewes were more susceptible to negative energy balance than single bearing ones, however marked improvement in serum glucose levels was recorded two weeks after parturition. The lower serum glucose levels in twin-bearing ewes in late pregnancy could be attributed to lipomobilization syndrome in response to high energy demand associated with pregnancy in more than one fetuses (Radostits *et al.*, 2007). This finding was in an agreement with several authors (Bickhardt and KÖnig, 1985; Kleeman *et al.*, 1988; Firat and Özpınar, 1996; Hamadeh *et al.*, 1996; West, 1996). To contrary, some authors reported different findings of blood glucose level. Al-Dewachi (1999) reported high blood glucose levels in pregnant ewes while Vernon *et al.* (1981) did not record significant changes in serum glucose levels during pregnancy and lactation.

Serum total protein

A significant decrease in serum total protein level was also recorded in twin bearing ewes two weeks before and after parturition compared to non pregnant ewes while it showed no significant change in single bearing animals. Decrease in serum total protein may be attributed to increase demand for amino acids in response to fetal growth and colostrums production during late pregnancy and early parturition, respectively (Davson and Segal, 1980; Jainudee and Hafez, 1994). To contrary, Brozostowski *et al.* (1996); El-Sherif and Assad (2001) reported gradually increase of total protein to the reference value during lactation (Kaneko, 1989) but they reported similar findings in late

pregnancy. Similarly, Gonzales-Montana *et al.* (1994) reported twin pregnancies produce significantly lower protein levels when compared to a single pregnancy.

Serum urea and creatinine

In this study, while serum urea level was higher in twin bearing animals two week before parturition, mild improvement has been noticed after parturition. Similarly, El-Sherif and Assad (2001) reported serum urea to increase in pregnant ewes from 10th week of pregnancy, reaching a maximum level at parturition. The evaluation in serum urea nitrogen may be due to reduced glomerular filtration and urea clearance in ewes during late pregnancy and lactation (Rodriguez *et al.*, 1996). Contrary, Firat and Özpınar (1996) recorded insignificant differences in serum urea levels between multiple and single pregnancy in sheep. No significant changes were recorded in creatinine level in different groups in the present study.

Liver enzyme

ALT and AST activities showed no rise in single bearing animals while they showed significant increase in twin bearing ewes in the last two week of pregnancy. This temporary rise of liver enzyme in twin bearing ewes could be due to lipomobilization syndrome (Radostits *et al.*, 2007).

Nitric oxide

Nitrite concentrations in single and twin bearing ewes were increased. The increased nitrite concentrations reflect the increased nitric oxide synthesis, which may in part mediate the cardiovascular adaptations to stress associated with pregnancy and parturition (Yang *et al.*, 1996).

CONCLUSION

This study showed that changes occur in the blood metabolite concentrations during transition period in Sohag ewes. The magnitude of these changes was greater in twin-bearing sheep which could be attributed to increased nutrient demands in response to fetal growth. In conclusion, the present study has indicated that energy imbalance is likely to occur in twin bearing Sohagi ewes during transition period while both single and twin bearing Sohag ewes were susceptible to oxidative stress.

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