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Research Article

Effect of Energy Deficiency during Late Pregnancy in Chios Ewes on Free Fatty Acids, β-Hydroxybutyrate and Urea Metabolites*

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Abstract: Pregnant Chios ewes with high twin-lambing rates fed a low energy ration during the final periods of pregnancy were studied. The aim of this study was to determine whether pregnancy toxemia occurred in these ewes by monitoring the changes in the concentrations of serum free fatty acids (FFAs), plasma β-hydroxybutyrate (β-HB) and urea. Thirty-eight Chios ewes were used. The animals were divided into 3 groups: pregnant normal energy (PNE), pregnant low energy (PLE), and non-pregnant normal energy (N-PNE). On day 105 of gestation, the animals were begun to be fed with the treatment rations. The feeding regime was continued until lambing. Blood samples were taken into tubes with and without anticoagulant from the jugular vein on days 120, 127, 134, 141, and 148 during gestation. The ewes in PLE had higher concentrations of FFAs on days 134 and 148 compared to those in PNE. β-HB concentrations in PLE animals were always higher than those in PNE. No difference was found between PNE animals and N-PNE animals. Plasma urea concentration in PNE decreased on day 127, and then continuously increased to a significant level on day 148. It is concluded that an energy intake of greater than 10 MJ/kg ME should be ensured for pregnant ewes in late pregnancy, although an energy level of 8.0 MJ/kg ME does not cause a serious energy deficiency and does not cause pregnancy toxemia.

Key Words: Free fatty acids, β-hydroxybutyrate, urea, ewes, gestation, energy deficiency

Sakız Irkı Koyunlarda İleri Gebelikte Enerji Noksanlığının Serbest Yağ Asitleri, β-Hidroksibütirat ve Üre Metabolitleri Üzerine Etkisi

Özet: Bu çalışmada deneysel olarak enerji eksikliği oluşturulan ikizlik oranı yüksek Sakız ırkı gebe koyunlarda, gebeliğin son döneminde düşük enerji ile beslenmenin koyunlarda gebelik toksemisi gibi metabolik bir hastalığın oluşup oluşmadığını serum serbest yağ asitleri, plazma β-hidroksibütirat ve plazma üre konsantrasyonlarının seviyelerindeki değişikliklere bakarak saptamak amaçlanmıştır. Bu çalışmada 38 adet Sakız ırkı koyun kullanılmıştır. Hayvanlar, normal enerjili gebe grup, yetersiz enerjili gebe grup ve normal enerjili gebe olmayan grup olacak şekilde üç gruba ayrılmışlardır. Gebeliğin 105. gününden itibaren hayvanlar bulundukları gruplara uygun bir şekilde beslenmeye başlanmışlardır. Bu durum hayvanların doğumuna kadar devam etmiştir. Kan numuneleri gebeliğin 120, 127, 134, 141 ve 148. günlerinde koyunların vena jugularisinden heparinli vakumlu tüplerle alınmıştır. Yetersiz enerjili gebe koyunların 134. ve 148. günlerindeki serum serbest yağ asitleri konsantrasyonları normal enerjili gebe gruptakilere göre yüksek çıkmıştır. Yetersiz enerjili gebe gruptaki koyunların plazma β-hidroksibütirat konsantrasyonları normal enerjili gebe gruptakilere göre daima yüksek çıkmıştır. Normal enerjili gebe koyunlarda plazma üre düzeyleri 127. günde anlamlı ölçüde azalmış, daha sonra artmaya devam etmiş ve yine 148. günde anlamlı ölçüde yüksek bulunmuştur. Sonuç olarak koyunlar gebeliğin son dönemlerinde 10 MJ/kg ME'den daha yüksek enerji içeren yemlerle beslenmelidir ve gebeliğin son dönemlerinde koyunların 8,0 MJ/kg ME ile beslenmesi gebelik toksemisine yol açmamıştır.

Anahtar Sözcükler: Serbest yağ asitleri, β-hidroksibütirat, üre, koyun, gebelik, yetersiz enerji

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Introduction

Pregnancy and lactation are known as the most critical periods in ovine nutrition. Energy requirements of pregnant ewes significantly increase during the last period of pregnancy. Sufficient growth and development of the fetus depend on feeding of the dam with a balanced ration in the last 6 weeks of gestation, during which 70%-80% of fetal growth occurs (1). It has been reported that nutrition in the last period of gestation affects lambing performance, milk yield, birth and growth weights of lambs, and survival rates. Deficiency in any of the main nutrients including water, lipids, carbohydrates, proteins, minerals and vitamins affects the growth and reproductive performance of sheep (2). Previous studies (2,3) revealed that nutrition deficiency resulted in low yield conditions. Nutrient deficiency, if it occurs during the gestation of ewes, causes underdevelopment of the fetus.

Pregnancy toxemia is a metabolic disease that commonly affects pregnant ewes and does during late gestation. Affected animals are generally pregnant with multiple fetuses and in their last month of gestation. It is characterized by hypoglycemia, increased concentrations of ketone bodies in the blood and elevated plasma concentrations of free fatty acids (FFAs) (4). In energy deficiency in ruminants, blood concentrations of FFAs and β-hydroxybutyrate (β-HB) increase as a result of usage of lipids (5,6). Blood concentration of β-HB can be used as an index for the energy condition of animals, if the metabolic energy (ME) level of the ration is lower than 10 MJ/kg in the last 3 months of gestation (7). The status of energy metabolism can be assessed by the blood levels of ketonic substances. Determining blood concentrations of glucose and β-HB is essential for the diagnosis of pregnancy toxemia in ewes (8). Monitoring blood glucose, ketone, protein, and urea levels of pregnant ewes for a certain time period may provide useful information for assessment of the status of anabolism or catabolism (9).

In the present study, pregnant Chios ewes with high twin-lambing rates fed a low energy ration during the final periods of pregnancy were used. The aim of this study was to determine whether pregnancy toxemia occurred in these ewes by monitoring changes in the concentrations of serum FFA, plasma β-HB, and urea.

Materials and Methods

Thirty-eight Chios ewes at 4 to 6 years of age were used. They were housed in 3 separate boxes ($3 \times 4 \times 5$ m). A 15-day flushing was applied to the ewes before estrus synchronization to increase the pregnancy rate. The ewes were synchronized for estrus to increase the twin rate and simultaneous pregnancy. For synchronization, sponges containing 60 mg of medoxyprogesterone acetate were applied to the ewes intravaginally using special applicators. After 15 days, the sponges were removed and 600 IU of PMSG per ewe was administered intramuscularly. Forty-eight hours after the injection, the ewes were placed in boxes with 1 Chios ram per 10 ewes for mating. On day 105 after random mating, the ewes were subjected to ultrasound examination to determine pregnancy. The ultrasound examination revealed that 24 of the 38 ewes were pregnant. The animals were divided into 3 groups: pregnant normal energy (PNE), pregnant low energy (PLE), and non-pregnant normal energy (N-PNE). On day 105 of gestation, the animals were begun to be fed with the treatment rations (Table 1). The feeding regime

Table 1. Composition of the diets (%).

Ingredients	PNE	PLE	N-PNE
Grass hay	34.00	65.00	75.00
Cracked barley	1.60	3.80	5.00
Cracked maize	28.00	1.00	7.50
Sunflower meal	21.00	18.50	1.10
Razmol[a]	3.00	4.30	8.00
Wheat bran	6.00	3.00	1.50
Cracked wheat	6.00	4.00	1.50
Zeolite[b]	0.1	0.1	0.1
Vitamin premix[c]	0.1	0.1	0.1
Salt	0.1	0.1	0.1
Marble powder	0.1	0.1	0.1

[a] Fine bran, flour and embryo of wheat.

[b] 1 kg zeolite: SiO_2 48.58 g, Al_2O_3 14.72 g, CaO 11.11 g, MgO 11.65 g, Fl_2O_3 9.19 g, LiO 2.5 g, Na_2O 1.28 g, K_2O 0.44 g, TiO_2 0.38 g, MnO 0.16 g, Cr_2O_3 0.06 g, P_2O_5 0.03 g.

[c] 1 kg vitamin premix: vitamin A 10,000,000 IU, vitamin D_3 1,500,000 IU, vitamin E 25 g, niacin 20 g, d-pantothenic acid 7 g, vitamin B_2 2.5 g, vitamin B_1 1.5 g, vitamin B_6 1.5 g, vitamin B_{12} 15 mg.

PNE = Pregnant normal energy, PLE = Pregnant low energy, N-PNE = Non-pregnant normal energy continued until lambing. All ewes were fed 700 g of the corresponding rations in the morning (08:00) and evening (16:00). Water was provided ad libitum. Two ewes died in the PNE group during the final periods of pregnancy. It was determined that 2 ewes had acute pneumonia in the PLE group and 4 ewes had metritis in the N-PNE group. In total 8 ewes were removed from the 3 groups in this study.

Feeding between days 0 and 105 of gestation: All ewes (n = 38) in all groups were fed a ration with 11% crude protein (CP) and 8.8 MJ/kg ME (1400 g/day) (Table 1, ration for N-PNE).

Feeding between day 106 of gestation and birth: The ewes in PNE were fed a ration containing 13% CP and 10 MJ/kg ME, while those in PLE were fed a ration containing 13% CP and 8.0 MJ/kg ME. The ration for the ewes in N-PNE contained 11% CP and 8.8 MJ/kg ME. Blood samples (10 ml) were taken from the jugular vein into tubes with and without anticoagulant before feeding in the morning on days 120, 127, 134, 141, and 148 during gestation. The samples were centrifuged at 3000 rpm for 10 min for separating plasma and serum. The plasma and serum samples were stored at –20 °C until analyzed. Serum FFA, plasma β-HB and urea concentrations were analyzed by commercial test kits (FA 115; RB 1007, Randox, UK; Berthalot BB241 enzymatic-colorimetric method, Spain, respectively) and SEAC ch 100 photometer.

Statistical Analysis

Parameters including FFAs, β-HB and urea were statistically compared between PNE and PLE and PNE and N-PNE ewes using an independent-sample t test. In addition, concentrations of FFA, β-HB and urea were compared between sampling days using one-way ANOVA (Duncan's test). SPSS was used for all statistical analysis. (SPSS for Windows. Standard version 10.00 SPSS Inc. Headquarters, Chicago, USA, 1999). All results were expressed as mean ± SD. A significance level of $P < 0.05$ was employed in the analysis of data from treatment groups.

Results

Mean serum FFA concentrations of pregnant ewes fed PNE and PLE rations and those of N-PNE ewes are presented in Table 2. The PLE ewes had higher concentrations of FFAs ($P < 0.05$) on days 134 and 148 compared to those in PNE. On the other sampling days, the FFA concentration of PLE ewes was generally higher than those in PNE ($P < 0.05$). PNE ewes always had a higher serum FFA concentration than N-PNE ewes. The FFA concentration of PNE ewes appreciably ($P < 0.05$) decreased on day 134 but then increased again. In PLE ewes, on the other hand, the level of this parameter increased as the pregnancy progressed. No noteworthy difference was seen in N-PNE ewes, as expected.

Mean plasma β-HB concentrations of PNE, PLE, and N-PNE ewes are presented in Table 2. Plasma β-HB concentrations in PLE animals were always higher than those in PNE animals ($P < 0.05$). Similarly, no difference was found between PNE animals and N-PNE animals. A tendency to increase in plasma β-HB concentrations of PNE was seen as the pregnancy progressed ($P < 0.05$). The level in PLE ewes continuously decreased until day 134 and then progressively increased. No difference was seen in plasma β-HB concentrations of N-PNE ewes between the sampling days.

Mean plasma urea levels of PNE, PLE, and N-PNE ewes are presented in Table 2. PLE ewes had a higher concentration of plasma urea than PNE ewes only on day 127. The difference between plasma urea levels of PNE and N-PNE ewes was significant only on day 148 ($P < 0.001$). Plasma urea concentration of PNE ewes decreased on day 127 ($P < 0.05$), and then continuously increased to a significant level on day 148 ($P < 0.05$). In PLE ewes, however, plasma urea concentration increased on day 127 ($P < 0.05$), decreased on day 134, and then increased. N-PNE ewes, which were fed a normal energy ration, had an increase in plasma urea concentration on day 127 and then a decrease on day 148 ($P < 0.05$).

Discussion

This study aimed to determine whether pregnancy toxemia occurred in pregnant ewes fed a low energy ration during the final periods of pregnancy by monitoring the changes in the concentrations of FFA, β-HB, and urea.

Feeding with a low energy diet during late pregnancy mobilizes FFAs from adipose tissue (10). During the final periods of pregnancy PLE ewes had higher serum FFA concentrations than PNE ewes, and PNE ewes had higher FFA concentrations than N-PNE ewes (Table 2). Symonds et al. (11) determined higher blood fatty acid levels in

Table 2 Mean (± s.d.) FFA, β-HB and urea concentrations in late pregnancy (mmol/l).

Parameter	Days	(n)	PNE	(n)	PLE	(n)	N-PNE
FFAs	120	10	$0.67 \pm 0.20^{a,1}$	9	0.93 ± 0.29^{b}	10	$0.39 \pm 0.11^{a,2}$
	127	10	$0.80 \pm 0.23^{a,1}$	9	$1.15 \pm 0.22^{a,b}$	10	$0.33 \pm 0.05^{a,2}$
	134	9	$0.49 \pm 0.08^{b,1}$	10	$1.16 \pm 0.24^{a,b,2}$	9	0.36 ± 0.09^{a}
	141	9	$0.76 \pm 0.23^{a,1}$	8	$1.07 \pm 0.16^{a,b}$	8	$0.39 \pm 0.10^{a,2}$
	148	9	$0.79 \pm 0.06^{a,1}$	10	$1.29 \pm 0.19^{a,2}$	9	0.31 ± 0.04^{a}
β-HB	120	9	0.63 ± 0.13^{b}	10	1.10 ± 0.20^{a}	10	0.86 ± 0.17^{a}
	127	9	0.85 ± 0.20^{a}	10	0.98 ± 0.23^{a}	10	0.90 ± 0.08^{a}
	134	8	0.89 ± 0.11^{a}	9	0.96 ± 0.21^{a}	10	0.79 ± 0.15^{a}
	141	10	0.87 ± 0.13^{a}	10	1.06 ± 0.22^{a}	9	0.82 ± 0.08^{a}
	148	9	0.95 ± 0.20^{a}	8	1.10 ± 0.28^{a}	8	0.79 ± 0.07^{a}
Urea	120	10	5.94 ± 0.87^{b}	10	5.54 ± 0.81^{c}	10	5.75 ± 0.50^{a}
	127	9	$4.67 \pm 0.48^{c,5}$	10	$6.93 \pm 1.15^{a,b,6}$	9	6.06 ± 0.79^{a}
	134	10	6.42 ± 0.93^{b}	9	5.66 ± 0.81^{c}	10	6.09 ± 0.23^{a}
	141	8	6.63 ± 0.90^{b}	9	$6.46 \pm 0.93^{b,c}$	10	6.01 ± 0.81^{a}
	148	9	$7.50 \pm 1.07^{a,3}$	9	7.69 ± 1.15^{a}	9	$5.71 \pm 0.28^{a,4}$

[a b c] Different superscripts indicate significant differences in the same column ($P < 0.05$).

[1-2] Statistical difference is significant between 2 groups within lines ($P < 0.01$).

[3-4] Statistical difference is significant between 2 groups within lines ($P < 0.001$).

[5-6] Statistical difference is significant between 2 groups within lines ($P < 0.05$).

PNE = Pregnant normal energy, PLE = Pregnant low energy, N-PNE = Non-pregnant normal energy

FFAs = Free fatty acids, β-HB = β-hydroxybutyrate,

pregnant sheep fed an energy deficient ration in the last 4 weeks of gestation compared to those fed a sufficient energy ration. Likewise, Gonda et al. (8) observed that FFA levels in rams increased as the energy level of their FFA ratios was decreased. Results of the FFA analysis of samples in the present study were close to those reported previously (8,10-13). Higher levels of FFAs found in pregnant ewes might be explained by increased levels of cortisol due to stress caused by pregnancy (14). Another reason for this situation may be increased sensitivity of ewes to epinephrine hormone, which causes increases in serum FFA concentrations, in the last weeks of gestation (15).

The level of β-HB in circulation is an indicator of nutritional status. A β-HB level greater than 0.85 mmol/l in ewes is a sign of energy deficiency (7). In the present study, β-HB levels were not appreciably different between the treatment groups of ewes. However, the β-HB levels slightly increased as the pregnancy progressed (Table 2). Bell et al. (16) reported that β-HB levels in pregnant ewes were 0.4 mmol/l before day 120, 0.5 mmol/l on day 120, and 0.7 mmol/l on day 135 of the gestation period. Scott and Woodman (17) found that β-HB levels increased to 7.2 mmol/l in pregnant ewes with energy deficiency. This observation was consistent with the results of some previous reports (10,16,17). The finding in the present study that plasma β-HB levels greater than 0.85 mmol/l in PNE ewes indicates that the energy level was inadequate even in these ewes and higher levels of energy are required in the last stages of pregnancy. The β-HB level determined in PLE ewes was not as high as that in ewes with pregnancy toxemia, as reported by other researchers (18,19). Therefore, it can be assumed that pregnancy toxemia did not occur in ewes in this group.

Hyperuremia occurs generally in ewes suffering from poor nutrition in the last period of pregnancy (9). In the current study, the difference between plasma urea levels of PNE and N-PNE ewes was significant only on day 148. Likewise, except for on day 127, plasma urea levels of PLE and PNE ewes were not different (Table 2). El-Sherif and Assad (20) reported that plasma urea levels started increasing during week 10 of pregnancy in Barki race ewes and reached a peak level at birth while they were unchanged in non-pregnant ewes. Similarly, in the present study, plasma urea concentrations increased on day 148 in pregnant ewes while they were unchanged in N-PNE ewes. The reason for this increase could be the effect of increased cortisol levels on the catabolism of proteins in the body (21). This observation was similar to the results reported by other researchers (13,20,22,23). These results may indicate that the energy deficiency described in the present study was not severe enough to cause depletion of body proteins (20) or the absence of pregnancy toxemia did not cause a clear increase in the levels of plasma urea in PLE ewes.

In the light of the findings of the current study, it is concluded that an energy intake greater than 10 MJ/kg ME should be ensured for ewes in the last period of pregnancy, although an energy level of 8.0 MJ/kg ME does not cause a serious energy deficiency or pregnancy toxemia.

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