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EFFECT OF ENERGY RESTRICTION ON SERUM CORTISOL AND ITS FAECAL METABOLITE (11,17-DIOXOANDROSTAN) IN PREGNANT EWES

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Abstract

The objective of the study was to investigate the effect of an energy restriction on serum cortisol and its faecal metabolite (11,17-dioxoandrostan) in *Chios* ewes in late gestation. Twenty pregnant and ten non-pregnant out of 38 randomly selected ewes were detected with ultrasound and were separated into three groups (n=10) on day 105 after mating: normal energy fed pregnant group (NE), restricted energy fed pregnant group (RE), and non-pregnant group (NP). Blood samples were taken on days 117, 130, and 144 of pregnancy and faecal samples were taken twice a week starting on day 106 of pregnancy from both pregnant and non-pregnant ewes. After extraction of serum and faecal samples, concentrations of cortisol and 11,17-dioxoandrostan (11,17-DOA) were measured. A significant decrease in RE was determined only on day 144 of pregnancy ($P \leq 0.05$). Between the two pregnant groups (NE and RE), a significant difference was found only on day 144; at this period, RE had lower serum concentrations. RE had significantly lower 11,17-DOA concentrations in late pregnancy than NE. In pregnant ewes, the levels decreased in a late gestational period. The present data shows that in ewes fed 14.47% crude protein and 8.82 MJ /kg of metabolic energy in late pregnancy, pregnancy toxaemia does not occur. We suggest that the determination of cortisol metabolites in faeces is more objective and safe than that of serum cortisol concentrations.

Key words: ewes, pregnancy, energy restriction, cortisol, 11,17-dioxoandrostan.

Female mammals invest large amounts of energy in foetal growth, lactation, and rearing of their young. In ewes, the most common insufficiency related to diet is energy deficiency. Insufficient energy leads to losses in both yield and body weight, lambing of weak lambs, and high mortality ratio due to pregnancy toxaemia (14). Daily basal nutritional requirement of the sheep for living is 8.4 MJ/kg of metabolic energy (ME) of dry matter (DM) and 9.4% crude protein (CP). Taking energy and diet requirements into account, the diet of pregnant ewes can be evaluated in two periods: the first 15 weeks and the last four weeks of pregnancy. Diet requirements in the last four weeks of pregnancy raises to 9.6 MJ/kg ME DM - 11% CP in single lambing ewes, and to 10 MJ/kg ME DM – 12.8% CP in twin lambing ewes (18).

Stress is revealed by the inability of an animal to cope with its environment, a phenomenon that is often reflected in a failure to achieve genetic potential for growth rate, milk yield, disease resistance, or fertility etc. (6). Stressful conditions cause an increased release of ACTH from the pituitary and glucocorticoids from the adrenal cortex (3, 16). The most important glucocorticoid secreted in sheep is cortisol.

After metabolisation by the liver, either the cortisol is excreted by the kidneys to be disposed with urine or it is sent to the gut along with bile to be excreted with faeces. The elimination of stress caused by blood sampling along with easy handling are the advantages of faecal sampling (17).

The object of the present study was to investigate the effect of an energy restriction on serum cortisol and its faecal metabolite (11,17-dioxoandrostan) in *Chios* ewes in late gestation.

Material and Methods

A group of 38 *Chios* ewes aged 4-6 years, with a high twin lambing rate from the farm of the University of Istanbul was used in the study. Oestrous synchronisation was performed according to Esen and Bozkurt (8). One *Chios* ram was added to each box of ten ewes for random mating. On the 105th d after mating, all the ewes were inspected with ultrasound and 24 ewes were detected as pregnant. Twenty pregnant ewes and ten non-pregnant were chosen randomly and used for this study. The animals were divided into three equal

groups: sufficient protein and normal energy fed ewes (NE), sufficient protein but restricted energy fed ewes (RE), and non-pregnant ewes (NP**).**

From the day of mating to day 105, the diet for all the ewes contained 10.23% of CP and 9.14 MJ/kg of ME with a serving size of 1 400 g/d/animal (Tables 1 and 2).

From day 106 of pregnancy until the birth, the animals were fed according to the diet chosen for their group (Table 1). Diet between the 106th d of pregnancy and parturition for the ewes of NE, RE, and NP groups contained 15.04% CP and 10.20 MJ/kg of ME, 14.47% CP and 8.82 MJ/kg of ME, and 10.23% CP, and 9.14 MJ/kg of ME, respectively (with 1 400 g/d/animal). After the 106th d pregnancy, the CP contents increased according to the demand of the foetus. All the ewes had water *ad libitum* and 700 g of (group specified) feed once at 09.00 am and once at 04.00 pm every day. The chemical analysis of the diet was performed according to AOAC (2) (Table 2).

Blood samples from both pregnant and non-pregnant ewes were taken from the jugular vein into 10 ml vacuumed serum tubes at 07:00 a.m. on the 117, 130, and 144 d after mating. The serum was separated by centrifugation at 3 000 rpm for 10 min. Faecal samples were collected starting from day 106 of pregnancy until delivery, twice a week at 07:00 a.m. before feeding. The extraction of cortisol metabolite from faeces was performed according to the method described by Palme and Möstl (19) and their concentration was measured by an enzyme immunoassay.

The statistical comparisons of serum cortisol and faecal cortisol metabolite between groups were evaluated with Students' *t*-test, furthermore between days of blood and faecal sampling within groups by variation analysis (Duncan test). A statistical significance was considered at $P \leq 0.05$. SPSS statistical software package (version 10.0) was used for statistical analysis. All results were displayed as mean ± SE.

Results

The mean serum cortisol levels of NE, RE, and NP and the statistical comparison among various days within groups are shown in Table 3. A statistical significance was found only on day 144 between NE and RE ($P \leq 0.01$). Likewise, a significant decrease was found in RE only on day 130 ($P \leq 0.05$) and in NP on day 104 ($P \leq 0.05$).

Table 1
Content and chemical composition of diet fed to pregnant and non-pregnant ewes

Ingredients	NE[1] (%)	RE[2] (%)	NP[3] (%)
Pasture grass	34.00	65.00	75.00
Cracked barley	1.60	3.80	5.00
Cracked corn	28.00	1.00	7.50
Sunflower meal	21.00	18.50	1.10
Wheat fine brain	3.00	4.30	8.00
Wheat brain	6.00	3.00	1.50
Cracked wheat	6.00	4.00	1.50
Zeolite [a]	0.10	0.10	0.10
Vitamin premix [b]	0.10	0.10	0.10
Salt	0.10	0.10	0.10
Marble powder	0.10	0.10	0.10

[a] 1 kg zeolite contains 48.58% SiO_2, 14.72% Al_2O_3, 11.11% CaO, 11.65% MgO, 9.19% F_2O_3, 2.50% LiO, 1.28% Na_2O, 0.44% K_2O, 0.38% TiO_2, 0.16% MnO, 0.06% Cr_2O_3, and 0.03% P_2O_5; [b] 1 kg vitamin premix contains 10 000 000 IU of vitamin A, 1 500 000 IU of vitamin D_3, 25 g of vitamin E, 20 g of niacin, 7 g of pantotenic acid, 2.5 g of vitamin B_2, 1.5 g of vitamin B_1, 1.5 g of vitamin B_6, and 15 mg of vitamin B_{12}.
[1]Normal energy fed pregnant group, [2]Restricted energy fed pregnant group, [3]Non-pregnant group.

Table 2
Chemical composition of diet fed to pregnant and non-pregnant ewes

Nutrient	NE[1]	RE[2]	NP[3]
Dry matter (%)	88.13	87.58	88.02
Crude protein (%)	15.04	14.47	10.23
Crude fat (%)	5.21	3.29	3.90
Crude ash (%)	5.41	6.91	6.54
Crude fibre (%)	16.83	26.24	26.90
Ca (%)	0.73	0.70	0.89
P (%)	0.51	0.52	0.49
Metabolic Energy (MJ/kg KM)	10.20	8.82	9.14

[1]Normal energy fed pregnant group, [2]Restricted energy fed pregnant group, [3]Non-pregnant group.

Table 3
Serum cortisol levels in pregnant and non-pregnant ewes (ng/mL, n=10)

Groups	Days 117	130	144
NE[1]	9.07 ± 0.70	11.24 ± 0.93	9.22 ± 0.98
RE[2]	9.74 ± 1.01 [a]	10.09 ± 1.82 [a]	4.55 ± 0.87 [b] **
NP[3]	9.18 ± 1.57	8.12 ± 1.03	11.46 ± 2.15

[1]Normal energy fed pregnant group, [2]Restricted energy fed pregnant group, [3]Normal energy fed non-pregnant group,
[a, b, c] Means within the same line with different letters differ (P≤0.05),
* Means within the same column with different letters differ, (NE *vs* RE and NP),
** P≤0.01.

Table 4
Faecal 11,17-dioxoandrostan concentrations on different days in each group (ng/mL, n=10)

Groups	Days 106 – 121	122 – 137	138 – 153
NE[1]	124.8 ± 13.2 [c]	205.3 ± 15.0 [b]	314.9 ± 41.5 [a]
RE[2]	77.1 ± 5.1 [b] **	109.2 ± 7.8 [a] ***	119.1 ± 8.7 [a] ***
NP[3]	40.1 ± 3.0 ***	44.7 ± 3.8 ***	40.2 ± 5.1 ***

[1]Normal energy fed pregnant group, [2]Restricted energy fed pregnant group, [3]Normal energy fed non-pregnant group,
[a, b, c] Means within the same line with different letters differ (P≤0.05),
* Means within the same column with different letters differ, (NE *vs* RE and NP),
** P≤0.01,
*** P≤0.001.

Mean 11,17-DOA levels of NE, RE, and NP and the statistical comparison among various days within groups are shown in Table 4. Significant differences were found in NE and RE between day 106 and 121 (P≤0.01), between days 122 and 137 (P≤0.001), and between days 138 and 153 (P≤0.001). In NP, a significant decrease was found between days 106 and 121 and between days 138 and 153, in comparison to the first period of faeces sampling (P≤0.05).

Discussion

Plasma glucocorticoid levels are commonly used as indicators of animal reproduction activities and stress response (3, 16, 17). Cortisol is the main anti-stress steroid hormone and the most important glucocorticoid that is secreted in ewes (8). The energy requirement of animals increases in late pregnancy significantly. Pregnancy toxaemia occurs in the last six weeks of pregnancy in ewes carrying one large, or more commonly two or three lambs (1), if the ewe cannot receive at least half of the required energy from nutrition during this period (8). It is reported that pregnancy toxaemia causes enough stress to raise the cortisol level in the circulation (8). Ford *et al.,* (10) and Henze *et al,* (12) reported that cortisol levels increase in ewes with pregnancy toxaemia. Casamassima *et al.* (4) determined that sheep fed normal diet had plasma cortisol concentrations of 9-11 ng/mL. Forbes *et al.* (9) found that Suffolk raced sheep fed *ad libitum* 8.8 MJ/kg ME and 13.5% CP had mean cortisol concentrations of 10.7 ng/mL. Christison and Johnson (5) reported that in heifers exposed to high temperatures for a long time, the cortisol levels decreased, which was caused by an adaptation mechanism. Ronchi *et al.* (21) showed that a decrease in cortisol concentration in feed restricted heifers exposed to high temperatures depends on heat stress, not on feed restriction. Ronchi *et al.* (21) reported also that feed restriction has no effect on cortisol excretion, while Rhind *et al.* (20) suggested that race and diet have significant effects on plasma cortisol levels. Fırat and Özpınar (8) showed that cortisol levels decreased significantly in developing pregnancy. On the contrary, Luna-Munoz *et al.* (15) and Fowden *et al.* (11) reported that in ewes, plasma cortisol concentrations increased near parturition.

In this study, a significant decrease (P≤0.05) in serum cortisol level was found in RE on day 144 of pregnancy. Between two pregnant groups (NE and RE), a significant difference was only found on day 144 of pregnancy, whereas RE had lower concentrations. A significant difference between NE and NP could not be found. Because of decreasing cortisol concentrations in ewes in late pregnancy, the obtained results are at variance with the data of Luna-Munoz *et al.*(15) and Fowden *et al.* (11), but are in accord with the data of Fırat and Özpınar (8). In this study, the last period of pregnancy of ewes was only in summer time when the temperature rises. As Christison and Johnson (5) suggested, heat stress decreases cortisol levels. In pregnant and sometimes in non-pregnant ewes low serum cortisol levels may occur due to heat stress.

The variation of the diet in this study caused a significant difference only on day 144 of pregnancy. In this period, serum cortisol levels of RE were found lower than those of the NE. We suggest that the reason may be an insufficiency of cholesterol and fat in diet and growth of the foetus. As known, fatty acids are metabolised to acetyl CoAs, from which cholesterol is

synthesised, and cortisol is synthesised from cholesterol (22). We suggest that the reason why serum cortisol concentrations in RE did not increase is that pregnancy toxaemia did not occur. The elevating energy requirement of the foetus in the last period of pregnancy and the insufficient intake of energy, cause pregnancy toxaemia and this leads to an increase in blood cortisol concentration (8, 10, 12).

In this study, RE showed significantly lower faecal cortisol metabolite 11,17-DOA concentrations in the last period of pregnancy than NE. The levels increased during the developing pregnancy. The levels of the NP were always found significantly lower than in the pregnant ewes. It was thought that faecal 11,17-DOA is a much better indicator than serum cortisol to monitor the cortisol level of the animal. Furthermore, some researchers had found that the differences in plasma cortisol were reflected by faecal 11,17-DOA levels. This study shows similarity to the work of Jensen *et al.* (13) who showed that in the last half of pregnancy, plasma cortisol level elevates 2-3 times, and to those of Fowden *et al.* (11) and Henze *et al.* (12) who demonstrated high concentrations of 11,17-DOA in pregnant ewes with developing pregnancy. It is possible that the reason why faecal cortisol levels in RE were lower than NE is that the diet was poor of cholesterol and fat and therefore cortisol synthesis was lower. In the study of Bamberg *et al.* (3) it was found that the increase in faecal corticosteroid metabolites did not reflect the little elevation of cortisol in blood of rats injected with ACTH. Similarly, in this study all 3 groups showed no correlation between serum cortisol and faecal 11,17-DOA concentrations, because disposed faecal steroid amounts reflect the production, not the amount in blood as reported by Möstl *et al.* (16).

The present data shows that ewes fed 14.47% CP and 8.82 MJ/kg ME in late pregnancy does not cause pregnancy toxaemia. We suggest that determination of faecal cortisol metabolite (11,17-DOA) is more objective and safer than the determination of serum cortisol concentrations.

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