

The Effects of Energy Restricted Diet on the Activities of Plasma Cu-Zn SOD, GSH-Px, CAT and TBARS Concentrations in Late Pregnant Ewes

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Abstract: The aim of the study was to determine the effects of low energy diets on the enzymatic antioxidant activities of ewes in late pregnancy. Thirty Chios ewes were used. Twenty pregnant ewes were divided into 2 groups of 10 ewes each (groups II and III), with 10 non-pregnant ewes being separated into a third group (group I). The ewes in groups I, II and III were fed rations containing 9.14 MJ ME/kg of dry matter (DM)-10.23% crude protein (CP), 10.20 MJ ME/kg of DM-15.04% CP, and 8.82 MJ ME/kg of DM-14.47% CP, respectively. Plasma copper-zinc superoxide dismutase (Cu-Zn SOD), glutathione peroxidase (GSH-Px), catalase (CAT) activities and thiobarbituric acid reactive substances (TBARS) concentrations were measured on days 105 and 148 of pregnancy. No significant differences for Cu-Zn SOD and TBARS were detected among the groups on days 105 and 148 of pregnancy. The catalase activities of group III were higher by $P < 0.05$ than those of both groups I and II on day 148 of pregnancy. The GSH-Px activities of group III were higher than those of group I on day 148 of pregnancy ($P < 0.05$). In a 2-day comparison of each group, only GSH-Px activities increased in group III ($P < 0.05$). It was concluded that the reason for the high plasma CAT and GSH-Px activities at the end of pregnancy is to protect the foetus from the deleterious effects of hydrogen peroxide.

Key Words: Pregnancy, ewes, energy restriction, antioxidant enzyme activity

Gebeliğin Son Dönemindeki Koyunlarda Enerji Kısıtlı Diyetle Beslemenin Plazma Cu-Zn SOD, GSH-Px, CAT Aktiviteleri ile TBARS Konsantrasyonları Üzerine Etkisi

Özet: Enerji kısıtlı diyetle beslemenin gebeliğin son dönemindeki koyunlarda antioksidan savunma sistemi üzerine olan etkilerinin ortaya çıkarılması amacı ile yapılan çalışmamızda 30 adet multiple gebelik oranı yüksek Sakız ırkı koyun kullanılmıştır. Gebe olan koyunlar her bir grupta 10 hayvan olacak şekilde iki gruba ayrılırken (grup II ve III), gebe olmayan 10 adet hayvandan da üçüncü bir grup oluşturulmuştur (grup I). Grup I, II ve III'te bulunan koyunlar sırasıyla 9,14 MJ ME/kg kuru madde (KM)-%10,23 Ham protein (HP), 10,20 MJ ME/kg KM-%15,04 HP ve 8,82 MJ ME/kg KM-%14,47 HP içeren rasyonla beslenmişlerdir. Tüm koyunlardan gebeliğin 105. ve 148. günlerinde kan örnekleri toplanmış ve plazma Cu-Zn süperoksid dismutaz (SOD), glutatyon peroksidaz (GSH-Px), katalaz (CAT) aktiviteleri ve tiobarbiturik asit reaktif maddeleri (TBARS) konsantrasyonları tespit edilmiştir. Gebeliğin 105. ve 148. günlerinde plazma Cu-Zn süperoksid dismutaz ve TBARS düzeyleri açısından gruplar arasında farklılık bulunamamıştır. Gebeliğin 148. gününde grup III'ün katalaz düzeyinin Grup I ve II'den $P < 0,05$ düzeyinde daha yüksek olduğu belirlenmiştir. Yine 148. günde Grup III'ün GSH-Px düzeyinin Grup I'den $P < 0,05$ düzeyinde daha yüksek olduğu saptanmıştır. Gruplar içinde günler arası yapılan kıyaslamada ise sadece Grup I'in GSH-Px aktivitesinin 148. gündeki artışının 105. günden $P < 0,05$ düzeyine daha yüksek olduğu belirlenmiştir. Sonuç olarak, gebeliğin sonunda plazma CAT ve GSH-Px konsantrasyonunun yüksek bulunmasının nedeni yavrunun hidrojen peroksidin zararlı etkilerinden korunmasıdır.

Anahtar Sözcükler: Gebelik, koyun, enerji kısıtlaması, antioksidan enzim aktivitesi

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Introduction

Since 80% of foetus growth occurs in the last 2 months of pregnancy, ewes exhibit a dramatic increase in metabolism during this period (1). The energy level in the diet of pregnant ewes should be increased by 20%, as indicated by the National Research Council (NRC) (2). In years when high feed costs make the use of concentrate feed impractical, pasturing becomes a common alternative for sheep raisers. Under these conditions, the most common nutritional problem in pregnant ewes is energy deficiency (3). The energy expenditure of animals on low energy diets is likely to be less than those on high energy diets. Insufficient energy intake is thought to give rise to a fall in the mitochondrial proton leak, which is positively related to the reactive oxygen species (ROS) (4-6). On the other hand, long-term energy deficiency causes mobilisation in the body's fat deposits and an increase in ketone bodies, especially acetoacetate and β -hydroxybutyrate, relative to energy metabolism (7,8). Acetoacetate and β -hydroxybutyrate may be utilised in aerobic metabolism and their ratio is regulated dependent on the NADH:NAD ratio in mitochondria. There is a close correlation between energy expenditure and the available oxygen. The decrease in cellular oxygen consumption causes a dramatic loss in the amount of oxygen molecules produced in the mitochondria and a decrease in the amount of ROS (9,10). Ruminant embryos do not need high level oxidative metabolism and a large amount of oxygen in the early phases of pregnancy. Later, however, especially in the final months, they do have high levels of both oxidative and glycogenic metabolism (11,12). When the pregnant animal is subjected to some oxidative stress, antioxidant activities increase in the placenta. This increase indicates that the effects of free oxygen radicals are limited in the embryonic tissue and this is known as the placental block (13).

This study examines the effects of energy shortages on enzymatic antioxidant activities of pregnant ewes, especially in the last 6 weeks of pregnancy, when the growth of the foetus and the need for energy measures lead to peak values.

Materials and Methods

Animals and oestrus synchronisation: Thirty-eight Chios ewes were synchronised for oestrus. Following a 15-day adaptation period, sponges containing 60 mg of

medroxyprogesterone were inserted into the vagina using a special applicator. The sponges were removed after 5 days and 600 IU of pregnant mare's serum gonadotropin (Folligon, Intervet, Netherlands) was administered to each animal intramuscularly. Forty-eight hours later, a ram was added to each pen of 10 ewes for random mating (14).

Feeding and group formation: All ewes were fed a ration of 8.8 MJ ME/kg of dry matter (DM) and 11% crude protein (CP) (1400 g/day per head) in the first 3.5 months of gestation. All ewes were provided drinking water ad libitum. On day 105 after mating, the ewes were examined by ultrasound for pregnancy. The animals were divided into 3 groups, a normal energy (group II) group, a reduced energy (group III) group, and a non-pregnant (group I) group. Ten randomly selected ewes were assigned to each group and then placed in individual pens and held under the same environmental conditions. As of day 105 of gestation, the experimental feeding regime was initiated and continued until lambing (Table 1). All animals were fed 700 g twice a day (8:00 am and 04:00 pm) (1400 g/day per head). Drinking water was provided ad libitum. The feeds given to each group were analysed for CP and ME (Table 2) (15). The ewes in

Table 1. The content of diets fed to pregnant and non-pregnant ewes.

	Group III ³ (%)	Group I ¹ (%)	Group II ² (%)
Pasture grass	75.0	34.0	65.0
Cracked barley	5.0	1.6	3.8
Cracked corn	7.5	28.0	1.0
Sunflower meal	1.1	21.0	18.5
Wheat fine brain	8.0	3.0	4.3
Wheat brain	1.5	6.0	4.0
Cracked wheat	1.5	6.0	4.0
Vitamin-mineral premix ⁴	0.2	0.2	0.2
Salt	0.1	0.1	0.1

¹ Non-pregnant group

² Normal energy group

³ Energy restricted group

⁴ Vitamin-mineral premix provides (per kg of concentrate): 48.58% SiO₂, 14.72% Al₂O₃, 11.11% CaO, 11.65% MgO, 9.19% F₂O₃, 2.50% LO, 1.28% Na₂O, 0.44% K₂O, 0.38% TiO₂, 0.16% MnO, 0.06% CrO₃, 0.03% P₂O₅, 10,000,000 IU vitamin A, 1,500,000 IU vitamin D₃, 25,000 mg vitamin E, 20,000 mg niacin, 7000 mg pantothenic acid, 2500 mg vitamin B₂, 1500 mg vitamin B₁, 1500 mg vitamin B₆ and 15 mg vitamin B₁₂.

Table 2. Chemical analysis of diets fed to pregnant and non-pregnant ewes.

	Group I ¹ (%)	Group II ² (%)	Group III ³ (%)
Dry matter (%)	88.02	88.13	87.58
Crude protein (%)	10.23	15.04	14.47
Crude fat (%)	3.90	5.21	3.29
Crude ash (%)	6.54	5.41	6.91
Crude fibre (%)	26.90	16.83	26.24
Ca (%)	0.89	0.73	0.70
P (%)	0.49	0.51	0.52
Metabolisable energy (MJ/kg DM)	9.14	10.20	8.82

¹ Non-pregnant group (n = 10)

² Normal energy group (n = 10)

³ Energy restricted group (n = 10)

groups I, II and III were fed rations containing 9.14 MJ ME/kg of DM-10.23% CP, 10.20 MJ ME/kg of DM-15.04% CP, and 8.82 MJ ME/kg of DM-14.47% CP, respectively.

Blood Sampling: Blood samples were taken from the jugular veins of the ewes on days 105 and 148 of gestation. The samples were collected into vacume-tubes containing heparin. The plasma of the blood samples was separated by centrifugation at 3000 rpm for 10 min. The plasma samples were stored at -20 °C until analysis.

Methods

Assay of TBARS: Lipid peroxidation and products (TBARS) were determined in plasma according to the modified method described by Buege and Aust (16), using $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ as the molar extinction coefficient. The results were expressed as mmol/l.

Assay of GSH-Px activity: GSH-Px activity was determined in plasma according to the method described by Pleban et al. (17). GSH-Px oxidises GSH to GSSG in the presence of H_2O_2 . GSSG is immediately converted to GSH by glutathione reductase and NADPH. The oxidation of NADPH is monitored by the measurement of the decrease in absorbance at 340 nm.

Assay of Cu-Zn SOD activity: Cu-Zn SOD activity was determined in plasma according to the method described by Sun et al. (18), by inhibition of nitroblue tetrazolium (NBT) reduction with xanthine-xanthine oxidase used as a superoxide generator. One unit of SOD is defined as the

amount of protein that inhibits the rate of NBT reduction by 50%.

Assay of CAT activity: CAT activity was determined in plasma using the modified method described by Yasmineh et al. (19). CAT activity was expressed as kU/l.

Statistical Analysis: The data were analysed using SPSS (version 11.5). The same plasma antioxidant enzymes of each group were compared using one-way ANOVA. The paired-samples t test was used to examine the differences between days 105 and 148 of pregnancy in each group. The results were considered significant if $P < 0.05$. All data were expressed as the mean \pm S.D.

Results

No significant difference in Cu-Zn SOD and TBARS measurements were found between the 3 groups. Only the CAT activities of group III on day 148 of pregnancy were significantly higher than those of groups I and II. The GSH-Px mean value of group III was significantly higher than that of group I on day 148 of pregnancy, no significant differences being found in day 105 values of either parameter. In terms of within-group comparisons, only the GSH-Px in group III showed a considerable increase in the average measurements from day 105 to day 148. Table 3 shows plasma Cu-Zn SOD, GSH-Px, CAT activities and TBARS concentrations, respectively, of the 3 groups, measured on both days 105 and 148 of pregnancy.

Discussion

Energy deficit feeding has diverse effects on placental life quality and growth in ewes (1). In this study, the ewes' diet contained 9.14 MJ ME/kg of DM and 10.23% CP for the 10 weeks of pregnancy. However, for the last 6 weeks, group II with no energy restriction received 10.20 MJ ME/kg of DM and 15.04% CP and group III under energy restriction received 8.82 MJ ME/kg of DM and 14.47% CP. The NRC (2) has stated that the energy requirement of pregnant ewes is 8.8 MJ ME/kg of DM and 10% CP for the first 3 months of gestation. In the terminal 4 weeks, the energy requirement increases to 9.6 MJ ME/kg of DM and 11% CP animals pregnant with 1 foetus, and to 10 MJ ME/kg of DM and 12% CP for animals pregnant with two foetuses. The intense increase in foetal development during the last weeks of pregnancy

Table 3. The plasma Cu-Zn SOD, CAT, GSH-Px activities and TBARS concentrations measured on days 105 and 148 of pregnancy in the 3 groups of subject ewes.

	Days	Group I ¹	Group II ²	Group III ³
Cu-ZnSOD (U/ml)	105	11.27 ± 2.07	11.51 ± 1.81	10.24 ± 2.76
	148	12.22 ± 4.68	12.31 ± 2.01	11.84 ± 1.92
CAT (kU/l)	105	2.06 ± 1.08	0.90 ± 0.45	2.17 ± 1.10
	148	2.06 ± 0.85 ^b	1.57 ± 1.35 ^b	3.75 ± 2.79 ^a
GSH-Px (U/l)	105	15.91 ± 7.23	15.54 ± 6.62	10.86 ± 4.26
	148	9.31 ± 5.35 ^b	20.55 ± 7.55 ^{ab}	25.90 ± 9.42 ^{a*}
TBARS (mmol/l)	105	9.64 ± 0.61	9.13 ± 0.62	9.39 ± 0.31
	148	9.64 ± 0.61	9.13 ± 0.62	9.39 ± 0.31

¹ Non-pregnant group (n = 10)

² Normal energy group (n = 10)

³ Energy restricted group (n = 10)

^{a,b,c} Means within the same line with different letters differ (P < 0.05)

* P < 0.05 (day 105 vs. day 148)

also gives rise to an increased energy requirement in both foetus and ewe (1). Feeding pregnant animals a diet inadequate in energy contents through the 6 terminal weeks of pregnancy causes mobilisation of fat deposits, which are physiological markers of a negative energy balance (8). Symonds et al. (20) reported that pregnant ewes with satisfactory energy intakes for the 4 terminal weeks have free fatty acid activities in their blood at levels considerably higher than the normal energy intake. In ruminants, mobilisation of fat deposits causes elevated levels of ketone bodies. These compounds are quantitatively important as energy sources, since tissues such as the heart, brain and kidney cortex utilise β -hydroxybutyrate and acetoacetate in preference to glucose. (21). While increased acetoacetate concentrations give rise especially to the production of reactive oxygen radicals, they also activate NADPH-cytochrome C reductase, which inhibits superoxide dismutase production (22). On the other hand, NADPH-cytochrome C reductase inhibits microsomal NADPH-dependent lipid peroxidation by over 90% (16). Our study has led us to conclude that these inhibitory effects are likely to be the causative factor, since there seems to have been no statistically significant difference between the low energy uptake group (group III) and the other 2 groups (groups I and II).

Mover and Ar (23) reported that at the end of pregnancy in rats a 30% increase was found in GSH-Px and catalase activities, suggesting that the reason for the increase was foetus growth. Gutman et al. (24) reported very high uterus peroxidase activity in pregnancy, and claimed that the reason for this activity was to protect the embryo from the deleterious effects of hydrogen peroxide stemming from hydroxyl radicals which are released as a result of the Fenton reaction during pregnancy. The increase in CAT and GSH-Px activities in group III on day 148 of pregnancy may be explained by production of hydrogen peroxide that has been enhanced by the mobilisation of fatty acids from the body deposits during pregnancy. This idea was strengthened by the fact that in group III, the GSH-Px level on day 148 of pregnancy was higher than that on day 105 of pregnancy (P < 0.05). In conclusion, the foetus grows very rapidly during the last 6 weeks of pregnancy, causing an increase in fatty acid consumption from the mother's fat reserves. Plasma CAT and GSH-Px were increased in pregnant ewes. This applied especially to ewes on the low energy diet. One possible reason for this is the placental protection of the rapidly growing foetus from the deleterious effects of hydrogen peroxide, produced and accumulated throughout pregnancy.

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