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Serum Ceruloplasmin Levels in Ewes Fed Deficient-Energy During Late Pregnancy

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Abstract: The aim of the study was to investigate the changes in serum ceruloplasmin levels of ewes fed deficient-energy during late pregnancy. Thirty Chios ewes at 4-6 years of age were used in the study. On day 105 after random mating, the ewes were subjected to ultrasound examination to determine pregnancy. After that they were divided into three groups: Pregnant Normal Energy (PNE, n = 10), Pregnant Deficient Energy (PDE, n = 10) and Non-Pregnant Normal Energy (N-PNE, n = 10). From the mating day up to the day 105, all ewes were fed in according to the ration of N-PNE group. On day 106 of gestation, the animals were begun to be fed the treatment rations. Blood samples were taken from the jugular vein into the tubes before feeding in the morning on days 120, 127, 134, 141 and 148 during gestation. Serum ceruloplasmin levels were not statistically different between groups except day 148. On day 148, serum ceruloplasmin levels in the pregnant deficient group were significantly higher than in the other two groups ($p<0.05$). They were significantly higher in the pregnant normal energy group than in the non-pregnant normal energy group and were significantly lower in the pregnant normal energy group than in the pregnant deficient group ($p<0.05$). In the study, serum ceruloplasmin level became a sensitive indicator of feed deficiency in the last days of pregnancy. Thus, variations in this antioxidant in ewes with pregnancy toxemia may be of considerable clinical importance.

Key words: Ceruloplasmin, energy deficiency, ewe, late pregnancy, Turkey

INTRODUCTION

Energy deficiency is the most common nutritional deficiency for ewes. It can reduce conception rate, lambing rate and milk production. Energy deficiency leads to greater susceptibility to parasite infestation and is also the primary cause of pregnancy toxemia (ketosis) in late pregnancy (Ates *et al*., 2008).

Almost 70% of fetal growth occurs during late pregnancy. Rapid fetal development necessitates increased amounts of all nutrients, particularly protein and energy. Poor nutrition during late pregnancy can have a negative effect on the upcoming lamb crop. Ewes that were not adequately fed during the last 6 weeks before parturition are likely to have weaker lambs at birth resulting in a high mortality rate. Performance of lambs born under these conditions is also likely to be reduced. If the nutrition is not adequate, ewe will not milk to her genetic potential. Severe energy deficiency during pregnancy may lead to ketosis (Johnson, 1992).

Lipid peroxidation is a normal procedure that occurs continuously at low levels. Lipid peroxidation reactions are in part toxic to cells and their membranes. However, they are continuously controlled by countervailing biologic mechanisms. The severe oxidative stress produces reactive oxygen free radicals and induces uncontrolled lipid peroxidation. Since, cell membranes primarily consist of lipids, the uncontrolled lipid peroxidation can cause cell injury and death (Halliwell and Chirico, 1993).

Ceruloplasmin is a major protein that circulates in the blood plasma and functions as the copper transporter that is able to transport 90-95% of copper in serum (Ryden, 1984). It functions as a ferroxidase by catalyzing the oxidation of Fe^{2+} to Fe^{3+}. Ceruloplasmin is an acute phase protein and is synthesized by liver in response to the tissue damage and inflammation. It is an important intravascular antioxidant and protects the tunica intima against free radical injury (Sirajwala *et al*., 2007). It also inhibits the lipid peroxidation and the deoxyribose degradation stimulated by iron and copper salts. It can protect the lipids and the erythrocyte membranes from copper and iron-induced damage. The alternative proposals are that the ceruloplasmin can decompose lipid peroxides or scavenge organic oxygen radicals (Gutteridge, 1983).

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The ceruloplasmin synthesis and/or secretion may be affected by inflammation, hormones and copper. Factors as cancer, inflammation, exercise, trauma, copper deficiency and pregnancy increase its level up to 3 fold (Sirajwala *et al*., 2007).

The developing fetus is dependent on the mother for the supply of copper during pregnancy. The adequate supplies are necessary for the normal fetal development (McArdle, 1995). The maternal copper deficiency can cause infertility, abortion and stillbirth in ruminants. The copper deficiency during pregnancy can also lead to the parturition of offsprings with congenital disease of nervous system (Davis and Mertz, 1987). It had been suggested that the ceruloplasmin levels may increase during pregnancy (McArdle, 1995).

Normal pregnancy is associated with the increase in oxidative stress and lipid peroxidation (Wand *et al*., 1991). The diet deficiency during late pregnancy increases the lipid peroxidation, alters the vitamin status and leads to the anemia in progeny. The research on diets is an important area of fetal origins of diseases that require the significant attention (Fetoui *et al*., 2007). The aim of the study was to investigate the changes in serum ceruloplasmin levels of ewes fed deficient-energy during late pregnancy.

MATERIALS AND METHODS

Thirty Chios ewes at 4-6 years of age were used in the study. Chios ewes were housed in three separate boxes. 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 (Oztabak *et al*., 2005). They 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. After that they were divided into three groups: pregnant normal energy (PNE, n = 10), pregnant deficient energy (PDE, n = 10) and non-pregnant normal energy (N-PNE, n = 10).

From the mating day up to the day 105, all ewes were fed in according to the ration of N-PNE group as indicated in Table 1. On day 106 of gestation, the animals were begun to be fed the treatment rations (Table 1). The feeding regime continued until lambing. All ewes were fed 700 g of the corresponding rations in the morning (08:00) and the evening (16:00) (totally 1400 g day^{-1}). Water was provided *ad libitum*.

The feeds given to each group were analysed chemically (Table 2) (AOAC, 1990). Blood samples were taken from the jugular vein into the tubes 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 serum. The serum samples were stored at -20°C until analyzed. Serum ceruloplasmin concentrations were analyzed according to the method described by Colombo and Richterich (1964).

Table 1: Composition of the rations (%)

Ingredients	N-PNE	PNE	PDE
Grass hay	75.00	34.00	65.00
Cracked barley	5.00	1.60	3.80
Cracked maize	7.50	28.00	1.00
Sunflower meal	1.10	21.00	18.50
Rasmol[a]	8.00	3.00	4.30
Wheat bran	1.50	6.00	3.00
Cracked wheat	1.50	6.00	4.00
Mineral premix[b]	0.10	0.10	0.10
Vitamin premix[c]	0.10	0.10	0.10
Salt	0.10	0.10	0.10
Marble powder	0.10	0.10	0.10

[a]Fine bran, flour and embryo of wheat. [b]1 kg mineral premix: 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, PDE = Pregnant Deficient Energy, N-PNE = Non-Pregnant Normal Energy

Table 2: Chemical analysis of rations

Ingredients	N-PNE	PNE	PDE
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^{-1} DM)	9.14	10.20	8.82

N-PNE = Non-Pregnant Normal Energy, PNE = Pregnant Normal Energy, PDE = Pregnant Deficient Energy

Serum ceruloplasmin concentrations were statistically compared between groups using one-way ANOVA (Analysis of variance, Duncan's test). SPSS (1999) was used for statistical analysis. All results were expressed as mean±standard error. A significance level of $p<0.05$ was employed in the analysis of data from the treatment groups.

RESULTS AND DISCUSSION

The mean serum ceruloplasmin levels and standard errors of groups are indicated in Fig. 1. Serum ceruloplasmin levels were not statistically different between groups except day 148. On day 148, serum ceruloplasmin levels in the pregnant deficient group were significantly higher than in the other two groups ($p<0.05$). They were significantly higher in the pregnant normal energy group than in the non-pregnant normal energy group and were significantly lower in the pregnant normal energy group than in the pregnant deficient group ($p<0.05$).

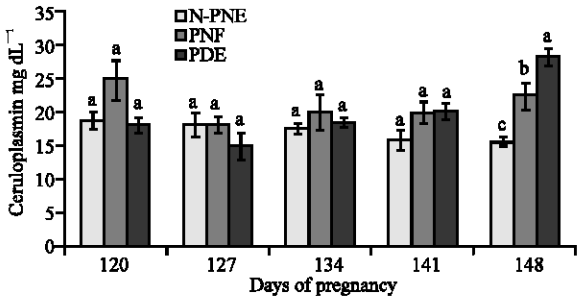

Fig. 1: Statistical comparisons of serum ceruloplasmin levels in ewes fed deficient energy or normal energy during late pregnancy (n = 10, mean±standard error). a-c: Different superscripts indicate significant differences ($p<0.05$). N-PNE = Non-Pregnant Normal Eenergy, PNE = Pregnant Normal Energy, PDE = Pregnant Deficient Energy

Serum ceruloplasmin concentrations increase in the stress conditions (Cousins, 1985). Starcher and Hill (1965) suggested that any stress-related change in the serum ceruloplasmin involves adrenal steroids. Both ACTH (adrenocorticotropic hormone) and hydrocortisone increased serum ceruloplasmin concentrations in chickens (Starcher and Hill, 1965). Curtis and Butler (1980) also found that both ACTH and β-methasone increased serum ceruloplasmin levels in chickens. A single injection of ACTH rose serum ceruloplasmin concentrations in rabbits (Alias, 1971). Epinephrine or norepinephrine injected into chicks had similar effects (Freeman *et al*., 1973). Meyer *et al*. (1958) reported that both epinephrine and estradiol increased serum ceruloplasmin levels in rats. Estradiol-17β appears to stimulate the ceruloplasmin synthesis (Haram *et al*., 1983).

Williams *et al*. (1977) showed that total maternal copper increased to almost 10% over controls by mid-pregnancy and thereafter rapidly to 50% higher by the end of pregnancy. King and Wright (1985) reported that effect of pregnancy is to increase the retention of dietary copper by pregnant female. Daszynaska *et al*. (1968) stated that serum ceruloplasmin and copper levels increased in pregnant cows. McArdle (1995) also noted that during pregnancy, serum levels of both copper and ceruloplasmin markedly rose. Lahey *et al*. (1953) noted a correlation between the pregnancy and the elevated serum ceruloplasmin levels. Chmielnicka and Sowa (2000) reported that pregnant rats had considerably higher serum ceruloplasmin activity than that of non-pregnant females. Lee *et al*. (1993) determined high activity of ceruloplasmin during second half of pregnancy in rats. Thomas *et al*. (1989) found an increase in maternal serum ceruloplasmin level towards end of pregnancy.

Vannucchi *et al*. (2002) reported that serum ceruloplasmin concentrations rose between first and second week of pregnancy in bitches and the progressive increase was verified from the third to the fifth week of pregnancy, followed by decreasing concentrations until the 8 weeks, with an important increase previous to parturition. They also stated that ceruloplasmin values were significantly greater for pregnants than for non-pregnant bitches during sixth and nineth (last) weeks (except for seventh and eighth weeks) and during the preparatory week previous to parturition, serum ceruloplasmin concentration increased once again, indicating the readapting to parturition. Same researchers (Vannucchi *et al*., 2002) also noted that serum estradiol-17β concentrations besides ceruloplasmin increased towards the end of the pregnancy and however, relationship between serum estradiol-17β and ceruloplasmin concentrations was not determined during the first half of pregnancy, suggesting that the hemodilution during pregnancy.

Various researchers (McArdle, 1995; Gurdogan *et al*., 2006) have shown that estrogen and progesterone increase ceruloplasmin production and secretion and it is feasible that the increase in ceruloplasmin occurs as a result of this stimulation. Similarly, Karp *et al*. (1986) reported that the rise of serum ceruloplasmin concentration in pregnancy has been attributed to estrogen, as concentrations of estrogen increase during pregnancy and the treatment of animals with estrogen has the same effect on ceruloplasmin. Mas and Sarkar (1992) also stated that the increase of maternal ceruloplasmin during pregnancy reflects a requirement for copper transport to fetus. In the current study, serum ceruloplasmin concentrations were not significantly different between non-pregnant and pregnant ewes in late pregnancy. This finding was similar to the findings of Vanucchi *et al*. (2002) who reported that ceruloplasmin values were not significantly different between non-pregnant and pregnant bitches on the seventh and eighth weeks of pregnancy. In the present study, pregnant ewes had the higher ceruloplasmin concentrations than non-pregnant ewes in the last week (day 148) ($p<0.05$). This result is in agreement with previous reports (Thomas *et al*., 1989; Vannucchi *et al*., 2002). The reason of this was probably the increase of estradiol-17β levels towards the end of the pregnancy period (Vannucchi *et al*., 2002), as estradiol-17β stimulates ceruloplasmin synthesis (Haram *et al*., 1983).

The body produces oxygen free radicals during normal respiration (Gunter and Gunter, 1994). When reactive oxygen free radicals interact with the polyunsaturated fatty acids in membranes or lipoproteins,

the process of lipid peroxidation begins. In the result of lipid peroxidation chain, fatty acids are converted to the primary product of lipid peroxides and to the secondary metabolites such as malondialdehyde. Under physiological conditions, antioxidant defense systems have evolved to counterbalance toxic actions of lipid peroxides by limiting the amount of them (Little and Gladen, 1999). The susceptibility of cells to the oxidative stress is a result of the overall balance between the degree of oxidative stress and the antioxidant defense capability. It is possible that during pregnancy, the increase in antioxidant activity occurs in response to normal oxidative stress due to pregnancy (Nakai *et al*., 2000).

Little and Gladen (1999) reported that maternal lipid peroxide levels revealed a moderate increase compared with those in non-pregnant controls. The rising levels may be related to the increase in serum lipids, since serum lipids spontaneously autooxidize to form lipid peroxides (Nakai *et al*., 2000). Lipid peroxidation is also stimulated in the placenta during pregnancy (Poranen *et al*., 1998). It is known that oxygenation of both maternal and fetal tissue frequently oscillate during parturition (Stipek *et al*., 1995). It has been also confirmed that ischemia-reperfusion leads to the production of free radicals (Zini *et al*., 1992). Furthermore, previous researchers (Falconer and Powles, 1982) have investigated maternal response to the pain and stress of parturition in terms of the release of certain hormones and have determined increases in epinephrine and norepinephrine levels throughout parturition. Epinephrine and norepinephrine cause reduction in uterine blood flow (Rosenfeld and West, 1977). These findings demonstrate the slight increase in lipid peroxide levels with the marked increase in antioxidant levels immediately after delivery (Nakai *et al*., 2000).

Dietary deficiency has been linked to risks for development of certain diet-related diseases such as diabetes and obesity, glucose intolerance and endocrine dysfunction. Dietary deficiency also reduces immune competence, decreases resistance to infections and impairs immune responses (Fetoui *et al*., 2007). These effects can be mainly ascribed to the participation of reactive oxygen species generated by dietary deficiency, leading to oxidative stress (Robinson *et al*., 1997). High demand for nutrients and energy is necessary for the hematopoietic and other tissues that present high rate of renewal and cellular proliferation (Fetoui *et al*., 2007). Pieri *et al*. (1991) suggested that the undernutrition induces the modifications in lipid composition of cell membrane and Domenicali *et al*. (2001) noted that dietary deficiency alters the status of antioxidant defense systems. According to Wohaieb and Godin (1987), rats experienced changes in free radical-scavenging mechanisms including antioxidant enzymes after 72 h fast. Moreover, dietary deficiency led to the depletion of antioxidant stores in organs and increased the generation of free oxygen radicals, particulary in liver (Robinson *et al*., 1997).

Transition metals such as iron (Fe^{2+}, Fe^{3+}) react with the superoxide hydrogen peroxide and the lipid peroxides to produce the oxidizing oxygen radicals that generate oxidative damage and initiate lipid peroxidation (Walsh, 1994). Once lipid peroxidation is initiated, it becomes self propagating and continues until it is interrupted by an antioxidant. Major antioxidant action of plasma is to bind the transition metal ions such as iron and copper, in forms that will not induce free radical reactions. This binding is achieved by antioxidants such as ceruloplasmin (Halliwell and Gutteridge, 1990). Normal pregnant females have an increase in the oxidative stress and lipid peroxidation that occur, while normal pregnancy advances (Wand *et al*., 1991). Ceruloplasmin has an important antioxidant activity which is paradoxically due to its ferroxidase activity. Fe^{2+} causes the production of toxic hydroxyl radicals. Since ceruloplasmin oxidizes Fe^{2+} to Fe^{3+} and increased formation of Fe^{3+}-transferrin, it limits production of hydroxyl radicals and thereby can protect against oxidative cell injury. Ferroxidase activity of ceruloplasmin proportionally increases with levels of ceruloplasmin during normal pregnancy (Agroyannis *et al*., 1993). An imbalance seems to occur between the oxidative stress and the anti-oxidative ability of plasma during normal pregnancy (Wand *et al*., 1991).

In the present study, serum ceruloplasmin levels were significantly higher in the pregnant deficient energy group than in the pregnant normal energy group on day 148 ($p<0.05$). Whereas, in the other days of late pregnancy except day 148, there were not significant differences between the pregnant deficient energy group and the pregnant normal energy group. On the last week before parturition, stress level increases in the pregnant animal (Falconer and Powles, 1982). This is related to the rise in the levels of some hormones such as estradiol-17β, cortisol, ACTH, epinephrine and norepinephrine.

In this period, the rising levels of lipid peroxides and these hormones cause to increase of synthesis and secretion of ceruloplasmin which is an antioxidant (Starcher and Hill, 1965; Falconer and Powles, 1982; Vannucchi *et al*., 2002). Feed deficiency is already a separate stress factor (Robinson *et al*., 1997; Fetoui *et al*., 2007). Thus, it may be suggested that the reason of significantly rising of serum ceruloplasmin concentration in the pregnant deficient energy group compared to the other pregnant group on day 148 was a combination of all the discussed factors.

CONCLUSION

Normal pregnancy is associated with an increase in oxidative stress and lipid peroxidation. The beneficial action of ceruloplasmin is due to its antioxidant capacity. In the study, serum ceruloplasmin level became a sensitive indicator of feed deficiency in the last days of pregnancy. Thus, variations in this antioxidant in ewes with pregnancy toxemia may be of considerable clinical importance.

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