

Effects of Early and Late Lactation Period on Plasma Oxidant/Antioxidant Balance of Goats

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Summary

The purpose of the study was to investigate effects on oxidant and antioxidant balance of early and late lactation period in goats. Blood samples used in study were obtained from Halep goats in early and late lactation period. Oxidative stress index (OSI) and lipid hydroperoxide (LOOH) and total oxidant status (TOS) and total antioxidant status (TAS) were analyzed to determine oxidant and antioxidant balance. Plasma TOS, OSI and LOOH levels in early lactation period were significantly higher ($P<0.001$) than late lactation period of goats, while lower ($P<0.001$) level of TAS. It was concluded that lipid peroxidation decreased and antioxidant defence system mechanism in late lactation period in goats served as more positive than early lactation period.

Keywords: Goat, Lactation, Total antioxidant status, Total oxidant status, Oxidative stress

Erken ve Geç Laktasyon Periyodunun Keçilerde Plazma Oksidan/Antioksidan Dengesi Üzerindeki Etkileri

Özet

Bu çalışmada keçilerin oksidan ve antioksidan dengesi üzerinde erken ve geç laktasyon periyodunun etkilerinin araştırılması amaçlandı. Çalışmada kullanılan kan örnekleri erken ve geç laktasyon periyodunda bulunan Halep keçilerinden elde edildi. Oksidan ve antioksidan dengenin değerlendirilmesi için oksidatif stres indeksi (OSİ), lipid hidroperoksid (LOOH), total oksidan (TOS) ve total antioksidan düzeyleri (TAS) analiz edildi. Erken laktasyon periyodunda plazma TOS, OSİ ve LOOH düzeylerinin geç laktasyon periyodundaki keçilere göre daha yüksek ($P<0.001$) olduğu belirlenirken, TAS düzeyinin daha düşük olduğu belirlendi ($P<0.001$). Sonuç olarak, geç laktasyon periyodundaki keçilerde erken laktasyon periyoduna göre lipid peroksidasyonunun azaldığı ve antioksidan savunma sistemi mekanizmasının daha olumlu yönde işlediği kanısına varıldı.

Anahtar sözcükler: Keçi, Laktasyon, Total antioksidan seviye, Total oksidan seviye, Oksidatif stres


INTRODUCTION

Lactation period is a process that makes metabolic and physiological regulations necessary for maintaining the homeostasis during postpartum ¹. Lactation stages including early and late lactation period since the catabolic reactions increases at cellular level may be effected on some metabolic functions related to the level of the free radicals ^{2,3}. It has been reported that differences can be observed in the level of oxidative stress with respect to the stages of lactation period ^{2,4}. The changes occurring at the composition of the milk in lactation period are resulted from the various factors such as epithelial cell proliferations, secretion activity,

supplying of the nutrients and removing of the metabolic wastes by means of the blood ^{5,6}.

General knowledge about oxidative stress related-metabolic disorders increases, and the pathologic effects of these disorders generally are associated with the free radical molecules ⁷⁻⁹. While free radicals are continuously produced in metabolic processes, their levels increase in a remarkable rate as a result of the various pathological and non-pathological conditions ⁷. When the balance between oxidant and antioxidant status is disturbed, health problems

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may appear due to the oxidation of the biological substances⁸⁻¹⁰. Oxidative stress can be detected by some biological markers, but new methods have been developed that measure each antioxidant parameter to evaluate the TAS^{11,12}. The ratio of total peroxide to total antioxidant capacity is accepted as a measure to define the OSI¹³. It is reported that only measurement of TAS can be used to identify the dynamic balance between the plasma pro-oxidant and antioxidant status^{9,14}. When polyunsaturated fatty acids are oxidized with free radicals, lipid peroxyl radicals are formed via chain reactions which also produces LOOH¹⁵.

Changes may occur in oxidant and antioxidant status in the blood during transitional period from early lactation to late lactation period that could affect both the animal and the health of others which benefit from the milk and meat^{16,17}. In addition, it has not reached to sufficient number of studies relation to lactation period and comprehensive oxidative status on goats. Therefore, in this study, TAS, TOS, OSI and LOOH levels was examined to understand the balance between oxidants and antioxidants in the blood.

MATERIAL and METHODS

Two-three years old 25 Halep goats lived in early and late lactation period were used in this study. Goats were allowed to graze on natural pasture from 07:30 to 17:30 and kept in pens from 17:30 to 07:30 during the trial. During the study, they were fed with 75% tender whole crop barley and 25% mustard straw (23% dry matter, 9.2% organic matter, 14% crude protein, 0.5% crude lipid, 24% crude cellulose, 7.2% crude ash, and 54.3% nitrogen free extract). Fresh water was available *ad libitum*. Kids were not weaned and goats were not milked throughout lactation period. The management of the goat did not change during the experimental period.

The blood samples were taken from *V. jugularis* at the end of March, the first week of April and first week of August. The samples taken into the tube with EDTA were centrifuged for 10 minutes at 3000 rpm. Plasma samples were kept at -20°C until the analysis. TAS, TOS, OSI and LOOH levels were evaluated by the methods stated below.

Measurement of Total Antioxidant Status

TAS levels were measured by using commercially available diagnostic kits (Gaziantep, Turkey) via auto analyzer (Aerose[®], Abbott[®], Illinois, USA)¹⁸. In this method, hydroxyl radicals are produced, which are very strong biological radical. In the analysis, ferrous ion solution which is found in Reagent 1 is mixed with H₂O₂ which is found in Reagent 2. Then consecutively, by hydroxyl radicals strong radicals are produced like the brown coloured dianisidyl radical cation. With this method, antioxidant status of the sample is measured against the strong free radical reactions, which is initiated by producing hydroxyl radical. Results are expressed as mmol Trolox Eq/L.

Measurement of Total Oxidant Status

TOS level was measured by using commercial diagnostic kit (Gaziantep, Turkey) at auto analyzer (Aerose[®], Abbott[®], Illinois, USA)¹⁹. The oxidants found in the sample, convert ferrous ion-o-dianisidine complex into ferric ion. Oxidation reaction is increased by glycerol molecules during the reaction. Ferrous ion forms a colour complex with xylenol orange in acidic media. Density of the colour is related to total quantity of oxidant molecules which are found in the sample. Measurement was calibrated by hydrogen peroxide (H₂O₂) and the results are given as micro moles in a liter H₂O₂ equivalent (μmol H₂O₂ Eq/L)

Measurement of Lipid Hydroperoxide

Lipid hydroperoxide level is evaluated in "xylenol orange" containing media by using ferrous ion oxidation method²⁰.

Oxidative Stress Index

The rate of TOS and TAS were used as oxidative stress index (OSI).

Statistical Analysis

Results of all parameters were expressed as mean (X) ± standard deviation (SD) for each group. The results were evaluated statistically using paired samples t-test on SPSS packet programme (SPSS 16.0 for Windows). A p-value less than 0.001 was considered to be statistically significant.

RESULTS

Levels of TOS, OSI and LOOH in early and late lactation period of goats were shown in Fig. 1 and also levels of TAS in Fig. 2. In early lactation period according to late lactation period was determined that TAS levels were significantly decreased while TOS, OSI and LOOH levels were significantly increased.

In the blood samples taken from early and late lactation period, OSI and LOOH levels were 2.53±0.32 and 1.46±0.25 arbitrary unite, 10.92±1.41 and 8.31±0.63 μmol H₂O₂/L (P<0.001), respectively. TOS levels was determined to be 20.01±1.69 and 14.14±2.36 μmol H₂O₂/L while TAS levels was determined to be 0.83±0.04 and 0.99±0.07 mmol trolox Eq/L (P<0.001).

DISCUSSION

Free radicals including reactive oxygen and nitrogen species (ROS and RNS) are produced continuously during aerobic metabolism and their levels may increase dramatically during increased production requirements or as a result of some pathological events^{21,22}. In the free radical level may be changes during the postpartum and lactation stages including early and late lactation periods

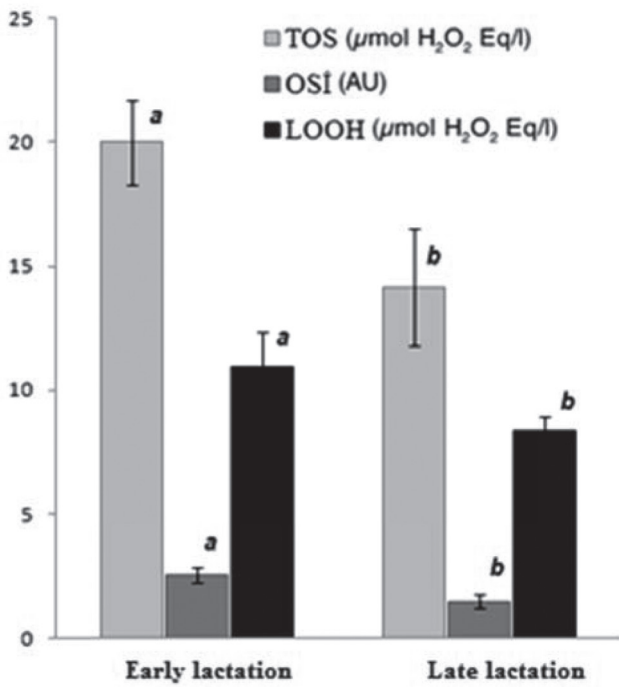


Fig 1. TOS, OSI and LOOH levels in early and late lactation period of goats, **a,b:** Values with different letter indicates significant differences ($P<0.001$)

Şekil 1. Keçilerde erken ve geç laktasyon periyodunda TOS, OSI ve LOOH düzeyleri, **a,b:** Farklı harf ile gösterilen değerler istatistiksel olarak farklıdır ($P<0.001$)

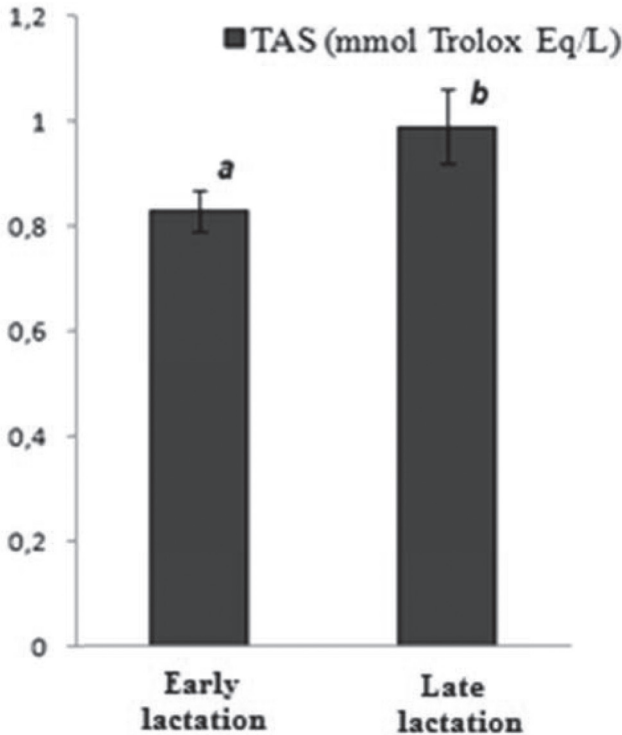


Fig 2. TAS levels in early and late lactation period of goats. **a,b:** Values with different letter indicates significant differences ($P<0.001$)

Şekil 2. Keçilerde erken ve geç laktasyon periyodunda TAS düzeyleri, **a,b:** Farklı harf ile gösterilen değerler istatistiksel olarak farklıdır ($P<0.001$)

since the catabolic reactions increases at cellular level with metabolic and physiological regulations necessary for maintaining the homeostasis¹⁻³. While molecular oxygen is need to continue of normal cellular functions in mammals, excess level of ROS can cause cell and tissue damages and lead to a case referred to as oxidative stres^{3,7,22}. It is the antioxidant capability including several antioxidative matters that will ensure to protect cells from the disruptive effects of oxidant stress, and the influences of diet on overall antioxidant capacity types, it is a serious point to be able to quantitatively measure TAS or antioxidant power inside of biological specimens^{21,22}. Evaluation of the oxidative and antioxidative status can also be performed by the measurement of TAS, TOS, OSI and LOOH levels^{18,20}. In this study, it was used assay of TAS levels to determine combined action of all antioxidants present in the sample was based upon to measured against the strong free radical reactions, which is initiated by producing hydroxyl radical. The level of TAS was lower during early lactation, in agreement with other reports related with antioxidants, and is presumably due, in part, to the utilization of anti-oxidants in colostrums production period²³. Changes in free radical and antioxidant concentration appear to represent homeorhetic processes that normally occur in early lactation. Given the lack of reference values for oxidative stress indicators and TAS levels for lactation periods in goats, and the fact that few studies have been carried out in this topic the causes of oxidative stres are difficult to determine.

It was also claimed that animals in the early lactation period were shown more effort to meet its energy needs and so, there is a decrease in total protein and lipid content of the adipose tissue^{24,25}. Transition phase from early lactation period to late lactation period is regulated by a homeostatic mechanism comprising metabolic interactions and body fuel distribution, and this mechanism is primarily based on glucose²⁶. Turk et al.²⁸ reported that in lactating cow serum glucose concentrations were in low levels in early lactation period and these levels increased towards to the middle of the lactation period. In early lactation period, non-esterified fat acids (NEFA), which form as the result of lipid mobilization, are used to meet energy need^{27,28}. It has been stated that free radical level will also be higher because of NEFA oxidation that increases in hepatocyte mitochondrium²⁹. A decrease in the antioxidant capacity may be possible depending on the function of secretion and synthesis in the liver of ruminant in which hepatomegaly is seen due to elevated intracellular lipid rate resulting from increased NEFA synthesis^{30,31}. The relationship between blood NEFA and triglycerides in the peripartum period by performed studies has been well documented^{32,33} and reported that increase in the oxidative metabolism has implied peroxidation of fatty acids leading to formation of lipid peroxides³⁴. One way to determine

if injury originated from ROS is occurring within tissues is to measure end products of free radical oxidation pathways. Lipid peroxidation consists when ROS react with polyunsaturated fatty acids (PUFA) ^{22,26}. The evaluation of LOOH levels in plasma would be a sign of early stages of this lipid peroxidation injury. Castillo et al.⁹ reported that there is no statistically change in mean serum TAS levels between late lactation period and the first weeks of the early lactation periods, and they reported that TAS levels could show remarkable fluctuations during the first weeks of early lactation period. It has been stated that glutathion peroxidase (GSH-Px) activity of plasma connected with TAS as an indirect indicator of oxidative stress has showed higher values 4 weeks after calving compared with those measured 2 weeks before calving ³⁵. It was found an increase in malondialdehyde (MDA) levels related to TOS known as an oxidative stress indicator one week before and one week after calving compared to earlier and later time points, however with wide individual variations ^{34,36}. Measurement of LOOH levels in this study indicated significant differences between goats early and late lactation periods ($P < 0.001$). This finding are consistent with study reported in late lactation animals where MDA levels, another biomarker of lipid peroxidation ³⁴. Results for the present study suggest that antioxidant potential reduced and oxidative stress increased in early lactation according to goats at late lactation period. The depletion of critical antioxidant defense components in tissues may predispose to metabolic changes originated from oxidative stress as reported in fish ³⁷. Our results are coherent with similar studies related to lactation and oxidative parameters ^{34,35}. Concentrations of the oxidant and antioxidant parameters are measured separately by using different methods and it can be seen that with the methods used in this study oxidant and antioxidant balance can be evaluated in a shorter time period, with a lower cost and with safer results ^{18,38}. In our study it was identified that TAS levels are higher in late lactation period goats compared to early lactation period goats, and also TOS, OSI and LOOH levels are significantly lower. This situation may be related with decrease in lipid mobilization occurring in adipose tissue due to the transition from negative energy balance (NEB) to positive energy balance (PEB) in late lactation period.

In studies were focused on energy balance and milk yield and feeding system categories to avoid from this uncertainty ^{30,39}. Both early lactation and after parturition in dairy animals is a period of severe NEB characterized by variables such as reduced blood glucose and insulin concentrations and elevated blood growth factors concentrations ^{2,4,40}. In addition, NEB has been related to altered hormonal levels in the ability of the hypothalamus-hypophyseal axis that do not support a functional reproductive system during early lactation ⁴⁰. The energy requirements of a lactating animals are met through a combination of dietary intake and mobilization of body reserves ^{40,41}. In a study performed by Soryal et al.¹⁶ in goats,

it was found that lipid and content of additive substances reach the highest level in late lactation period ¹⁶. It has reported that oxidative stress increases due to NEB in early lactation period and the reason of this increase may effected from decreasing elements of antioxidant defence system ³⁰. The time taken to re-establish PEB after parturition is affected by the extent of fatty tissue reserves and the efficiency with which these reserves can be mobilized ⁴². It has been reported that differences in the level of oxidative stress can be observed with respect to the stages of lactation period as a result of NEB that take places after the parturition ^{2,4}. According to a study performed for the energy balance category, the results indicated that an increased energy reserve mobilization affected the level of oxidative stress but only in cows fed to achieve restricted milk production. Therefore, it has been defined that oxidative stress levels were related to lower biological antioxidant potential in animals fed to achieve restricted milk production, a finding that might suggest that only animals with very high body reserve mobilization experience oxidative stress ³⁰. It was reported that animals in good body condition at calving and in high NEB in early lactation had higher oxidative stress ². In the study evaluated the role of diet during pregnancy and lactation in rats in the induction of metabolic abnormalities, especially an oxidant/antioxidant imbalance was found that level of total antioxidant capacity and levels of antioxidant component increased following days in lactation period. In addition, it strongly has suggested that maternal fat and energy intake condition during lactation can play an important role in the development of metabolic disorders observed in their offspring, and that maternal oxidative stress can be singled out as the factor involved ⁴³.

In conclusion, elevated TOS, OSI and LOOH levels in early lactation period and increased TAS levels in late lactation period suggest that lipid peroxidation and oxidative stress decrease in late lactation period.

REFERENCES

- Bell AW:** Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. *J Anim Sci*, 73 (9): 2804-2819, 1995.
- Bernabucci U, Ronchi B, Lacetera N, Nardone A:** Influence of body condition score on relationships between metabolic status and oxidative stress in periparturient dairy cows. *J Dairy Sci*, 88 (6): 2017-2026, 2005.
- Nordberg J, Arnér ES:** Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. *Free Radical Biol Med*, 31 (11): 1287-1312, 2001.
- Celi P, Di Trana A, Claps S:** Effects of plane of nutrition on oxidative stress in goats during the peripartum period. *Vet J*, 184 (1): 95-99, 2010.
- Akers RM:** Major advances associated with hormone and growth factor regulation of mammary growth and lactation in dairy cows. *J Dairy Sci*, 89 (4): 1222-1234, 2006.
- Safayi S, Theil PK, Hou L, Engbaek M, Nørgaard JV, Sejrsen K, Nielsen MO:** Continuous lactation effects on mammary remodeling during late gestation and lactation in dairy goats. *J Dairy Sci*, 93 (1): 203-217, 2010.
- Róth E:** Oxygen free radicals and their clinical implications. *Acta Chir*

Hung, 36 (1-4): 302-305, 1997.

- 8. Davies KJ:** Oxidative stress, antioxidant defenses, and damage removal, repair, and replacement systems. *IUBMB Life*, 50 (4-5): 279-289, 2000.
- 9. Castillo C, Hernández J, Valverde I, Pereira V, Sotillo J, Alonso ML, Benedito JL:** Plasma malonaldehyde (MDA) and total antioxidant status (TAS) during lactation in dairy cows. *Res Vet Sci*, 80 (2): 133-139, 2006.
- 10. Castillo C, Benedito JL, Lopez-Alonso M, Miranda M, Hernandez J:** Importance of oxidative stress in cattle: Its relationship with the physiological condition (pregnancy and parturition) and nutrition. *Arch Med Vet*, 33, 5-20, 2001.
- 11. Niki E, Noguchi N:** Evaluation of antioxidant capacity. What capacity is being measured by which method?. *IUBMB Life*, 50 (4-5): 323-329, 2000.
- 12. Yamaner F:** Oxidative predictors and lipoproteins in male soccer players. *Turk J Med Sci*, 40 (3): 427-434, 2010.
- 13. Demirbag R, Gur M, Yilmaz R, Kunt AS, Erel O, Andac MH:** Influence of oxidative stress on the development of collateral circulation in total coronary occlusions. *Int J Cardiol*, 116 (1): 14-19, 2007.
- 14. Ghiselli A, Serafini M, Natella F, Scaccini C:** Total antioxidant capacity as a tool to assess redox status: critical view and experimental data. *Free Radical Biol Med*, 29 (11): 1106-1114, 2000.
- 15. Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J:** Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell B*, 39 (1): 44-84, 2007.
- 16. Soryal K, Beyene FA, Zeng S, Bah B, Tesfai K:** Effect of goat breed and milk composition on yield, sensory quality, fatty acid concentration of soft cheese during lactation. *Small Rumin Res*, 58 (3): 275-281, 2005.
- 17. Suriyasathaporn W, Vinitketkumnuen U, Chewonarin T, Boonyayatra S, Kreausukon K, Schukken YH:** Higher somatic cell counts resulted in higher malondialdehyde concentrations in raw cows' milk. *Int Dairy J*, 16 (9): 1088-1091, 2006.
- 18. Erel O:** A novel automated method to measure total antioxidant response against potent free radical reactions. *Clin Biochem* 37 (2): 112-119, 2004.
- 19. Erel O:** A new automated colorimetric method for measuring total oxidant status. *Clin Biochem*, 38 (12): 1103-1111, 2005.
- 20. Nourooz-Zadeh J:** Ferrous ion oxidation in presence of xylenol orange for detection of lipid hydroperoxides in plasma. *Method Enzymol*, 300, 58-62, 1999.
- 21. Matés JM, Sánchez-Jiménez F:** Antioxidant enzymes and their implications in pathophysiological processes. *Front Biosci*, 4, 339-345, 1999.
- 22. O'Boyle N, Corl CM, Gandy JC, Sordillo LM:** Relationship of body condition score and oxidant stress to tumor necrosis factor expression in dairy cattle. *Vet Immunol Immunopathol*, 113 (3-4): 297-304, 2006.
- 23. Goff JP, Stabel JR:** Decreased plasma retinol, alpha-tocopherol, and zinc concentration during the periparturient period: Effect of milk fever. *J Dairy Sci*, 73 (11): 3195-3199, 1990.
- 24. Eknæs M, Kolstad K, Volden H, Hove K:** Changes in body reserves and milk quality throughout lactation in dairy goats. *Small Rumin Res*, 63 (1-2): 1-11, 2006.
- 25. Cañas RC, Quiroz RA, León-Velarde C, Posadas A, Osorio J:** Quantifying energy dissipation by grazing animals in harsh environments. *J Theor Biol*, 225 (3): 351-359, 2003.
- 26. Herdt TH:** Ruminant adaptation to negative energy balance. Influences on the etiology of ketosis and fatty liver. *Vet Clin North Am: Food Anim Pract*, 16 (2): 215-230, 2000.
- 27. Cebra CK, Garry FB, Getzy DM, Fettman MJ:** Hepatic lipidosis in anorectic, lactating Holstein cattle: A retrospective study of serum biochemical abnormalities. *J Vet Intern Med*, 11 (4): 231-237, 1997.
- 28. Turk R, Juretić D, Geres D, Svetina A, Turk N, Flegar-Mestrić Z:** Influence of oxidative stress and metabolic adaptation on PON1 activity and MDA level in transition dairy cows. *Anim Reprod Sci*, 108 (1-2): 98-106, 2008.
- 29. Mudron P, Rehage J, Qualmann K, Sallmann HP, Scholz H:** A study of lipid peroxidation and vitamin E in dairy cows with hepatic insufficiency. *J Vet Med A*, 46 (4): 219-224, 1999.
- 30. Pedernera M, Celi P, García SC, Salvin HE, Barchia I, Fulkerson WJ:** Effect of diet, energy balance and milk production on oxidative stress in early-lactating dairy cows grazing pasture. *Vet J*, 186 (3): 352-357, 2010.
- 31. Turk R, Juretić D, Geres D, Turk N, Rekić B, Simeon-Rudolf V, Robić M, Svetina A:** Serum paraoxonase activity in dairy cows during pregnancy. *Res Vet Sci*, 79 (1): 15-18, 2005.
- 32. Bell AW:** Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. *J Anim Sci*, 73 (9): 2804-2819, 1995.
- 33. Vandehaar MJ, Yousif G, Sharma BK, Herdt TH, Emery RS, Allen MS, Liesman JS:** Effect of energy and protein density of prepartum diets on fat and protein metabolism of dairy cattle in the peripartum period. *J Dairy Sci*, 82 (6): 1282-1295, 1999.
- 34. Castillo C, Hernandez J, Bravo A, Lopez-Alonso M, Pereira V, Benedito JL:** Oxidative status during late pregnancy and early lactation in dairy cows. *Vet J*, 169 (2): 286-292, 2005.
- 35. Wullepit N, Raes K, Beerda B, Veerkamp RF, Fremaut D, De Smet S:** Influence of management and genetic merit for milk yield on the oxidative status of plasma in heifers. *Livestock Sci*, 123 (2-3): 276-282, 2009.
- 36. Yilmaz M, Karapehlivan M, Kaya İ:** Effects of zinc sulphate on transcaucasian barb, (*Capoeta capoeta* [Guldenstaedt, 1773]) plasma nitric oxide, malondialdehyde and total sialic acid levels. *Kafkas Univ Vet Fak Derg*, 18 (1): 61-64, 2012.
- 37. Kaya İ, Karapehlivan M, Yilmaz M, Ersan Y, Koç E:** Investigation of effects on plasma nitric oxide, malondialdehyde and total sialic acid levels of glyphosate in Kars Creek transcaucasian barb (*Capoeta capoeta* [Guldenstaedt, 1773]) in Turkey. *Fresenius Environ Bull*, 21 (1A): 123-126, 2012.
- 38. Horoz M, Bolukbas C, Bolukbas FF, Sabuncu T, Aslan, M, Sarifakiogullari S, Gunaydin N, Erel O:** Measurement of the total antioxidant response using a novel automated method in subjects with nonalcoholic steatohepatitis. *BMC Gastroenterol*, 5, 35-40, 2005.
- 39. Celi P:** The role of oxidative stress in small ruminants' health and production. *Rev Bras Zootecn*, 39, 348-363, 2010.
- 40. Butler ST, Marr AL, Pelton SH, Radcliff RP, Lucy MC, Butler WR:** Insulin restores GH responsiveness during lactation-induced negative energy balance in dairy cattle: Effects on expression of IGF-I and GH receptor 1A. *J Endocrinol*, 176 (2): 205-217, 2003.
- 41. Serbester U, Çınar M, Hayırlı A:** Sütçü ineklerde negatif enerji dengesi ve metabolik indikatörleri. *Kafkas Univ Vet Fak Derg*, 18 (4): 705-711, 2012.
- 42. Grimard B, Humblot P, Ponter AA, Mialot JP, Sauvant D, Thibier M:** Influence of postpartum energy restriction on energy status, plasma LH and oestradiol secretion and follicular development in suckled beef cows. *J Reprod Fertil*, 104 (1): 173-179, 1995.
- 43. Bouanane S, Benkalfat NB, Baba Ahmed FZ, Merzouk H, Mokhtari NS, Merzouk SA, Gresti J, Tessier C, Narce M:** Time course of changes in serum oxidant/antioxidant status in overfed obese rats and their offspring. *Clin Sci*, 116 (8): 669-680, 2009.