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 oxidative 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

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

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 (OSI), lipid hidroperoksid (LOOH), total oksidan (TOS) ve total antioksidan düzeyleri (TAS) analiz edildi. Erken laktasyon periyodunda plazma TOS, OSI ve LOOH düzeylerinin geç laktasyon periyodundaki keçileri 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 azalışı ve antioksidan savunma sistemi mekanizmalarının daha olumlu yönde işlediğini kanıtladı.

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

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**INTRODUCTION**

Lactation period is a process that makes metabolic and physiological regulations necessary for maintaining the homeostasis during postpartum. 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. It has been reported that differences can be observed in the level of oxidative stress with respect to the stages of lactation period. 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.

General knowledge about oxidative stress related metabolic disorders increases, and the pathologic effects of these disorders generally are associated with the free radical molecules. 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. 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 substances. Oxidative stress can be detected by some biological markers, but new methods have been developed that measure each antioxidant parameter to evaluate the TAS. The ratio of total peroxide to total antioxidant capacity is accepted as a measure to define the OSI. It is reported that only measurement of TAS can be used to identify the dynamic balance between the plasma pro-oxidant and antioxidant status. When polysaturated fatty acids are oxidized with free radicals, lipid peroxyl radicals are formed via chain reactions which also produces LOOH.

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. In addition, it has not reached to sufficient number of studies relation to lactation period and comprehensive oxidative state 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 grass 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 (Aeroset®, Abbott®, Illinois, USA). 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 (Aeroset®, Abbott®, Illinois, USA). 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 xylene 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 “xylene orange” containing media by using ferrous ion oxidation method.

**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 requirments or as a result of some pathological events. In the free radical level may be changes during the postpartum and lactation stages including early and late lactation periods.
since the catabolic reactions increases at cellular level with metabolic and physiological regulations necessary for maintaining the homeostasis. 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 stress. It is the antioxidant capability including several antioxidative matters that will ensure to protect cells from the disruptive effects of oxidative stress. Given the multiplicity of antioxidant matters and pathways, their centrality in the prevention 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. Evaluation of the oxidative and antioxidative status can also be performed by the measurement of TAS, TOS, OSI and LOOH levels. In this study, it was used assay of TAS levels to determine combined action of all antioxidants present in the sample was based upon 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 antioxidants in colostrum production period. Changes in free radical and antioxidant concentration appear to represent homeostatic 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 stress 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. 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 glucose. Turk et al. reported that in lactating cow serum glucose concentrations were in low levels in early lactation period and these levels increased towards 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. It has been stated that free radical level will also be higher because of NEFA oxidation that increases in hepatocyte mitochondria. 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. The relationship between blood NEFA and triglycerides in the peripartum period by performed studies has been well documented and reported that increase in the oxidative metabolism has implied peroxidation of fatty acids leading to formation of lipid peroxides. One way to determine

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**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)
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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). The evaluation of LOOH levels in plasma would be an sign of early stages of this lipid peroxidation injury. Castillo et al. 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 glutathione 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. 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. 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. Our results are coherent with similar studies related to lactation and oxidative parameters. 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. 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. 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. In addition, NEB has been related to altered hormonal levels in the ability of the hypothalamus-hypophyseal axis that do not support a functional reproductve system during early lactation. The energy requirements of a lactating animals are met through a combination of dietary intake and mobilization of body reserves. In a study performed by Soryal et al. in goats, it was found that lipid and content of additive substances reach the highest level in late lactation period. It has reported that oxidative stress increases due to NEB in early lactation period and the reason of this increase may effect from decreasing elements of antioxidant defence system. 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. 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 place after the parturition. 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. It was reported that animals in good body condition at calving and in high NEB in early lactation had higher oxidative stress. 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.

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.

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