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RESEARCH ARTICLE

Determination of Changes in Some Biochemical Parameters and Oxidant-Antioxidant Balance After Food Intake in Sheep[#]

İbrahim DURMUŞ[1], Mustafa EVCİMEN[2], Mehmet Naci SALİM[3], İsmail KÜÇÜKKURT[4], Sinan İNCE[5], Abdullah ERYAVUZ[6*]

[1]*Afyon Kocatepe University, Suhut Vocational School, Department of Veterinary Health and Laboratuary, Afyonkarahisar/TURKEY.*
[2]*Afyon Kocatepe University, Faculty of Veterinaey Medicine, Department of Physiology, Afyonkarahisar/ TURKEY.*
[3]*Afyon Kocatepe University, Faculty of Veterinaey Medicine, Farm of Reseach Afyonkarahisar/ TURKEY.*
[4]*Afyon Kocatepe University, Faculty of Veterinaey Medicine, Department of Biochemistry, Afyonkarahisar/TURKEY.*
[5]*Afyon Kocatepe University, Faculty of Veterinaey Medicine, Department of Pharmacology and Toxicology, Afyonkarahisar/TURKEY.*

#Bu çalışma Afyon Kocatepe Üniversitesi Bilimsel Araştırma Projeleri Koordinasyon Birimi tarafından desteklenmiştir (14.HIZ.DES.64).

***Corresponding author e-mail:** eryavuz@aku.edu.tr

ABSTRACT

Daily variations in the concentration of urea, glutamine, glucose, cortisone, malondialdehyde (MDA) and antioxidant potential (AOP) were evaluated in the blood of sheep fed once a day. The study was carried out on 10 Anatolian Merinos sheep, average 1.5 years old and healthy. Animals were fed once per day with 61.3 % forage and 38.7 % concentrate mixture throughout the experimental period for 15 days. The blood samples were taken from the animals at first hour before and at 1, 3, 5, 7, 9, 12, 18 and 24th hours after feeding. Urea concentration decreased at first hour after feeding compared to before feeding and started augmentation after that and reached to levels before feeding at 12th hour after feeding. Glucose concentration increased at first hour after feeding compared to before feeding and started decline after that and reached to levels before feeding at 5th hour after feeding. Glutamine and cortisone concentrations decreased from at first hour to at 7th hour after feeding compared to before feeding and reached to levels before feeding at 7th hour after feeding. MDA levels decreased at first and 5th hours after feeding compared to before feeding and started increasing after 7th hour and reached significantly higher levels than before feeding in samples taken from at 12th hour to at 24th hour after feeding. While AOP increased at 3th and 9th hours after feeding, it decreased at 5, 7 and 12th hours after feeding. We concluded that the blood metabolic profiles and oxidant and antioxidant balance in sheep vary depending on time after feeding. In order to maximize the diagnostic value of these the blood metabolic profiles, the most suitable time for blood collection seems to be just before the feeding in sheep fed once a day.

Key Words: Antioxidant, Cortisone, Glutamine, Metabolic profile, Oxidant, Sheep.

Koyunlarda Yemlemeden Sonra Kan Oksidan-antioksidan Denge ile Bazı Biyokimyasal Parametrelerdeki Değişimlerin Belirlenmesi

ÖZ

Bu proje çalışması, koyunlarda beslemeden sonra geçen zamana bağlı olarak kan metabolik profili ile oksidan-antioksidan dengede meydana gelen değişiklikleri tespit etmek amacıyla yapıldı. Araştırma; ortalama 1,5 yaşlı, sağlıklı 10 Anadolu Merinosu koyunda gerçekleştirildi. Hayvanlar 15 gün süren deneme boyunca % 61.3 kaba yem ve % 38.7 karma yemden oluşan rasyonla günde bir kez beslendiler. Hayvanlardan besleme öncesi ve beslemeden sonraki 1, 3, 5, 7, 9, 12, 18 ve 24. saatlerde kan örnekleri alındı. Kan örneklerinde, üre, glikoz, glutamin ve kortizol düzeyleri ile malondialdehid (MDA) ve antioksidan potansiyel (AOP) belirlendi. Üre düzeyleri besleme öncesine göre besleme sonrası ilk saatte düştü, bu saatten sonra artmaya başladı ve besleme önceki düzeylerine beslemeden sonraki 12. saatte ulaştı. Glikoz düzeyleri besleme öncesine göre beslemeden sonraki ilk saatte önemli oranda arttı, bu saatten sonra düşerek beslemeden sonraki 5. saatte besleme öncesi düzeylerine ulaştı. Glutamin ve kortizol düzeyleri besleme öncesine göre beslemeden sonraki ilk saatten 7.saate kadar önemli düzeyde düşüş gösterdi ve beslemeden sonraki 7.saatte besleme öncesi düzeylere ulaştığı bulundu. MDA düzeyleri beslemeden sonraki ilk ve 5. saat örneklerinde önemli düzeyde düşüş gösterdi ve 7. saatten sonra artmaya başlayarak 12 saatten 24. saate kadar alınan örneklerde önemli düzeyde arttığı tespit edildi. AOP beslemeden sonraki 3. ve 9. saatlerde önemli oranda artarken, 5, 7 ve 12. saatlerde önemli oranda azaldığı bulundu.

Sonuç olarak, koyunlarda kan metabolik profil ile oksidan-antioksidan dengenin beslemeden sonra geçen zamana bağlı olarak değişmektedir.

Anahtar Kelimeler: Antioksidan, Kortizol, Glutamin, Metabolik profil, Oksidan, Koyun.

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INTRODUCTION

The costs of production related to nutrition in ruminant industry are among the problems of animal husbandry. Therefore, animal nutritionists suggest that an alternative to improve the profitability and the environmental sustainability of ruminant animal production is through the improvement of feed efficiency (Montanholi et al., 2010). This is also important to reduce feed costs, which make up a significant portion of the cost of animal production. A better understanding of the factors regulating feed efficiency and their potential as predictors of feed efficiency in ruminant animals is needed. Gonano et al. (2014) suggested that the characterization of blood metabolite concentrations over the circadian period and across physiological stages is important for understanding the biological basis of feed efficiency, and may culminate in indirect methods for assessing feed efficiency. For the feed to be economical for herbivorous animals, it needs to undergo microbial fermentation with a number of microorganisms in the rumen, which also counts for ruminants (Eryavuz, 2000; Kutlu and Serbester, 2014). Thus, because rumen fermentation increases during and after feeding, changes in rumen fermentation and the blood metabolic profile in the period after feeding can be seen in a 24-h period in animals fed once a day. Knowledge of the variation in the blood metabolic profile during a 24-h period is important for a better understanding of circadian cycle effects on metabolism and helps in assessment of production indices (Lee et al, 1978).

Since forage comprises up to 60 % of the diet for non-feedlot ruminants, fiber digestion in the rumen is an important factor in ruminant nutrition. Its digestion and subsequent fermentation by ruminal microbes provides much of the energy for the animal (Weimer, 1998). However, this leads to a long fermentation process of the diet in the rumen. As long as the fermentation continues in the rumen, the blood metabolite transition continues. This is why the ingredients of the feed consumed by animals and the regularity of the rumen fermentation constitute an important effect on the changes in blood metabolite levels (Kutlu and Serbester,2014). Hence, just like the levels (Herdt et al.,2000,Antunoviç et al.2004) of blood metabolite change depending on the breed, physiological status, age, and season, the levels also change depending on the feed consumed by the animals and the digestibility levels(Eryavuz et al. 2003). It can also enable an evaluation of the best time to collect blood in order to be able to correctly interpret metabolic mechanisms or status (Caldeira et al,1999).It also seems possible that the changes in the oxidant–antioxidant balance in the blood may occur during the rumen fermentation taken a long time. Thus, a constant metabolite transfer happens from the rumen into the blood. There is no study investigated the changes in the oxidant–antioxidant balance in the blood depending on the time after feeding in ruminants to date. Therefore, the purpose of this study was to evaluate daily variations in the concentration of urea, glutamine, glucose, cortisone, malondialdehyde and antioxidant potential AOP in sheep fed once a day. A better understanding of mechanistic processes altering the blood metabolite levels will help us to interpret metabolic mechanisms or status in animals fed a single daily meal.

MATERIALS and METHODS

Materials

A total of 10 male merino sheep, 1 years of age, having the same breeding and feeding conditions, weighing about 58 kg, were obtained from Afyon Kocatepe University Research and Application farm of Afyonkarahisar, Turkey. The animals were fed a 38.7 % concentrate and 61.3 % roughage rations for 15 days and feeding in the morning only one. The dry matter intake of sheep was 1.57 kg/day. The chemical analysis of the diet (NRC, 2007) is displayed in Table 1. The experimental protocols were approved by the Animal Care and Use Ethical Committee at Afyon Kocatepe University (91-09). On day fifteen, blood samples were collected from the jugular vein into tubes containing heparin as anticoagulant prior to feeding one hour and after 1, 3, 5, 7, 9, 12, 18, and 24 hours. All the animals were carefully monitored in a period.

Table 1. Ingredient compositions of the diet (%).

The ingredients of diet	%
wheat straw	30,98
alfalfa hay	8,45
Barley grain, ground	54,08
Sunflower meal	5,63
Limestone	0,84
Chemical composition of the diet	
Metabolical energy (Mcal/kg)	2,36
Crude protein (%)	10,7
Metabolical protein (%)	7,5
Rumen degrade protein (%)	6,9
ByPass Protein (%)	3,9

Methods

The blood plasma was separated by centrifugation at 3000 rpm for 10 min at 4 °C. Whole blood malondialdehyde (MDA) levels were measured by the double heating method of Draper and Hardley (1990). The method is based on spectrophotometric measurement of the purple color generated by the reaction of thiobarbituric acid with MDA. The plasma total antioxidant activity (AOA) was determined using the method described by

Koracevic et al. (2001). Plasma glucose (201-07-1421, specific sheep kit, Sun RED, USA), glutamine (201-07-2039, specific sheep kit, Sun RED, USA), urea (201-07-1028, specific sheep kit, Sun RED, USA) and cortisol (201-07-0067, specific sheep kit, Sun RED, USA) were measured using an enzyme linked immunoassay (ELISA).

Statistical analysis

Statistical analysis were performed with the SPSS 16 computer program (SPSS Inc. Chicago, IL, USA). The results were expressed as mean ± SEM. Significant differences between groups were analyzed by one way ANOVA. Significant differences among the means were determined by using Duncan's multiple-range test at $p < 0.05$.

RESULTS

Daily variations in some biochemical parameters and oxidant-antioxidant balance of sheep fed once a day are given in Figure 1 for urea, Figure 2 for glutamine, Figure 3 for glucose, Figure 4 for cortisol, Figure 5 for MDA and Figure 6 for AOP.

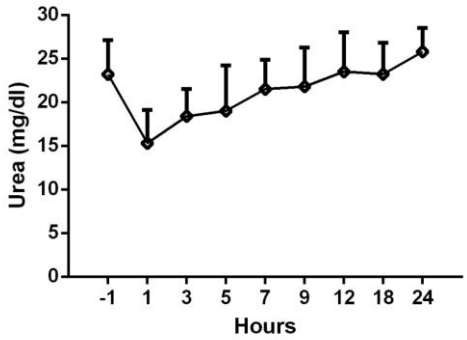

Figure 1: Daily variations in the plasma concentration of urea of sheep fed once a day (n=10).

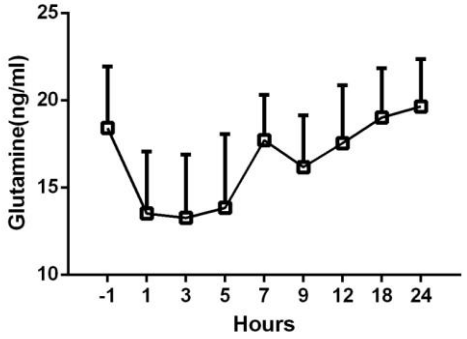

Figure 2: Daily variations in the plasma concentration of glutamine of sheep fed once a day (n=10).

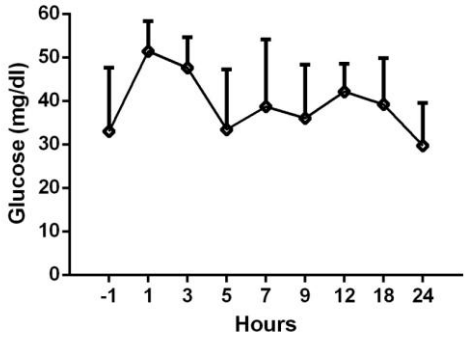

Figure 3: Daily variations in the plasma concentration of glucose of sheep fed once a day (n=10).

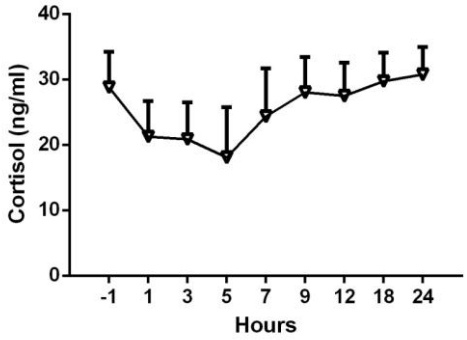

Figure 4: Daily variations in the plasma concentration of cortisol of sheep fed once a day (n=10).

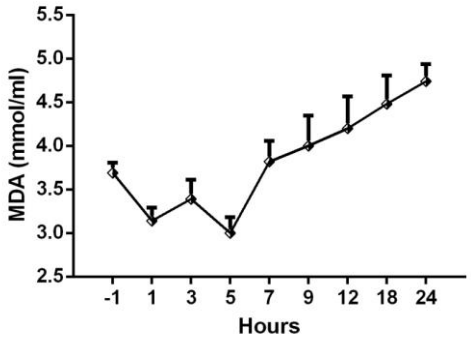

Figure 5: Daily variations in the blood malondialdehyde levels of sheep fed once a day (n=10).

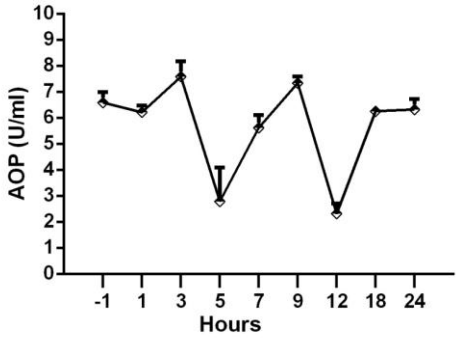

Figure 6: Daily variations in the plasma antioxidant potential levels of sheep fed once a day (n=10).

DISCUSSION

The metabolite levels in the blood of ruminant animals are used as an indicator of basic metabolic processes (i.e. indicators of whole body, liver and muscle metabolism) and show differences depending on the ingredients of the diet (Eryavuz et al., 2003), number of meals (Froestchel et al., 1990), feeding time and physiological status (Antunovic et al., 2011). Blood parameters may also facilitate the indirect selection for feed efficient ruminants (Gonano et al., 2014). Hence, the detection of changes in biochemical and hematologic parameters is commonly performed to state the nutritional and health status of animals. In this study, the animals were fed at 8:30 in the morning with diet met all nutritional requirements specified by NRC (2007) for 15 days. On the 15th day of the study, blood samples were taken from the sheep 1 h before and 1, 3, 5, 7, 9, 12, 18, and 24 h after feeding to determine the AOP and the levels of urea, glutamine, glucose, cortisol, and MDA. The determination of the metabolite levels in the blood is a potentially useful application in practice to state the efficacy of the diet consumed by ruminant animals (Gonano et al., 2014). Therefore, attempts in this study were made to detect the changes in six hematological parameters related to metabolism throughout the day in sheep fed once a day. This information constituted relevant data about the levels of glucose, urea, and glutamine; parameters related to the energy and protein metabolism in the liver; changes in the MDA levels, AOP, and oxidant–antioxidant balance in the blood (Eryavuz et al., 2015); and effects of stress on the cortisone levels in the blood (Montanholi et al., 2013). Blood urea concentration is the result of the balance between urea uptake (from the gastrointestinal tract and from liver metabolism) and excretion (urine, feces, milk) and thus mostly responsive to feeding and digestive function. The blood urea levels in the present study increased significantly at 1 h after feeding and dropped until the seventh hour after feeding. As of this hour, the levels increased and reached, even exceeded, the levels before feeding at the 12th hour (Figure 1). This result supported the report of Nikkah et al. (2008) fund that blood urea rose at 2 hours after feeding in cows and indicated that the amino acid breakdown, which is used to create energy in the body, dropped right after the feeding but increased later on. Remod et al. (1993) observed a 100% increase in the urea flow into the rumen before feeding and 5 h after feeding in sheep fed two times a day. The increase in blood urea concentration as of the seventh hour in this study is accordance with the observation of Remod et al. (1993). The decrease in blood urea concentration as of the seventh hour also showed that the energy spent for urea synthesis in the liver decreased as well (Eryavuz et al.,2008). The plasma urea concentrations obtained in this study (15.3–25.8 mg/dL) were close to the levels found in the study performed by Altıntaş and Fıdancı (1993) (17.1–42.8 mg/dL) on sheep. This study showed that the blood glutamine levels, similar to the urea levels, significantly increased 1 h after feeding and dropped until the seventh hour after feeding. The levels increased as of this hour until they reached, even exceeded, the levels before feeding (Figure 2). Urea and glutamine syntheses are two ways to detoxify the ammonia levels in the mammalian liver, which occur in coordination, in form of an upper and lower pair, to eliminate the ammonia that escapes from ureagenesis. This is why glutamine and urea are harmless carriers of ammonia in the bloodstream (Newsholme et al., 2003; Wright et al., 2010). The findings of this study pointed out that the volatile fatty acid levels, which were transferred from the rumen into the blood after feeding as a result of digestion of carbohydrates in the rumen and fermentation, were very high until the seventh hour after feeding. The findings also showed that the amino acid breakdown began to increase as of the said hour to increase the glucose level in the blood. Moreover, a study conducted with newborn pigs showed (Van der Schoor et al., 2001) that amino acid oxidation decreased as soon as the oxidation of glucose in the intestines increased. This study demonstrated that the glucose levels significantly increased 1 h after feeding and decreased as of the fifth hour but then again increased as of the 12th hour until they reached the levels before feeding at the 24th hour (Figure 3). Huntington (1997) reported that the plasma glucose level of cattle, fed with a high content of concentrate feed, was formed to 44% via the absorption of organic acids from the rumen (especially propionic acid) and their conversion into glucose in the liver, to 33% through post-ruminal glucose absorption and to 23% through conversion of amino acids and other carbon sources into glucose. After feeding, the propionic acid levels increased as a result of the digestion of easily dissoluble carbohydrates in the feed and declined depending on the time elapsed after feding (Remond et al.,1993). The increase in the plasma glucose level right after feeding may be caused by the increased conversion of propionic acid, absorbed from the rumen, into glucose in the liver. The increase in the blood glucose level after feeding also leads to an increase in the insulin level; it weakens glucagon secretion, which in turn decreases gluconeogenesis. Thus, intravenous administration of glucagon to sheep decreases feed intake (Deetz and Wangsness, 1981). The increase in the plasma glucose levels as of the 12th hour in this study might have resulted from the glucose derived from amino acids in the liver. The plasma glucose levels obtained in this study (29.7–51.4 mg/dL) are consistent with the normal levels observed in the study by Altıntaş and Fidancı (1993) (30–80 mg/dL).

Glucocorticoids play a key role in energy metabolism, influencing the animals' performance (Montanholi et al., 2010). The animals under stress have a sympathetic response causing a fight and flight response that increases the energy expenditures. A previous study referred to the existence of a potential relationship between cortisol metabolites excreted in the feces and feed efficiency (Montanholi et al., 2013) and also between feed intake and blood cortisol levels. This study showed that the plasma cortisol levels (Knott et al., 2010) significantly decreased after the feeding until the seventh hour and then started to increase until the samples attained the levels before feeding (Figure 4). It is also stated that the plasma cortisol levels may determine the effects of stress while extracting the samples from the animals (Möstl and Palme, 2002). The plasma cortisol levels obtained in this study were between the cortisol levels (18.7–38.0 ng/mL) of cattle (Colditz et al., 2007; Curley et al., 2008) and the levels (21.5–47.1 ng/mL) reported in sheep (Knott et al.,2010). MDA, which is formed as a result of free radical oxidation of polyunsaturated fatty acids in cell membranes, is also used as an indicator of oxidative damage caused by free radicals (Bulbul et al., 2008). In this study, the blood MDA levels significantly decreased right after feeding and reached the lowest level in the sample at the fifth hour after feeding. As of the seventh hour, the MDA level started to increase again, and in the sample of the 24th hour, it reached its most important and highest level (Figure 5). This finding showed that the production of free radicals in ruminant animals, which were fed once a day, decreased until the seventh hour and then started to increase again. If the level of cortisol, which is one of the hormones that display the effects of stress, is the lowest during the fifth hour, it can be said that the decrease in MDA levels influences the decrease in cortisol levels. Antioxidant defense mechanisms prevent damages caused by free radicals in tissues (Dündar and Aslan, 2000). This study showed that AOP significantly decreased at the 5th, 7th, and 12th hours after feeding and significantly increased in the 3rd and 9th hour samples (Figure 6). Because of rumen fermentation, the blood levels of metabolites and hormones changed after feeding. The observed change in the plasma antioxidant defense might have resulted from the changes in the levels of vitamins and minerals with antioxidant effect, absorbed during rumen fermentation in the lower digestive tract, and from differences in the metabolite and hormone levels (Nikkhah 2011; Gonano et al., 2014).In conclusion, the results in the present study demonstrated a change in metabolite and oxidative stress levels in the blood of sheep depending on the time after feeding and indicate that the altered postprandial rhythms of peripheral blood metabolites can closely link to timing after feeding. Daily variations in those metabolites may be of importance when interpreting these blood indicators of metabolic state. In order to maximize the diagnostic value of blood metabolites, the most suitable time for blood collection seems to be just before feeding in ruminants fed once a day. Conflict of interest. The authors declare they have no conflict of interest.

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