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Investigation of Paraoxonase and Ceruloplasmin Concentrations in Hair Goats Grazing at Different Heights

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ABSTRACT

The aim of this study was to investigate the changes in serum paraoxonase and ceruloplasmin concentrations in hair goats grazed at different altitudes. A total of 80 hair goats from different regions of Adana were included in the study and divided into four groups. The first group grazed at an altitude of 10-99 meters above sea level, the second at 240-250 meters, the third at 750-800 meters and the fourth group was grazed at an altitude of 1200-1500 meters. Blood samples were collected from the jugular vein during the summer months and placed into red-capped tubes. Paraoxonase and ceruloplasmin levels were analyzed by spectrophotometric method. The results of paraoxonase in 240-250 meter (1113.91±387.54 U/mL) and 750-800 meter (974.27±295.20 U/mL) were found statistically higher than 10-99 meter group (651.72±253.84 U/mL; p<0.01). Ceruloplasmin levels were significantly different between the 10–99 meter (10.15±3.04 U/mL) and 750–800 meter (7.13±1.94 U/mL) groups, and also between the 240–250 meter (9.97±3.96 U/mL) and 750–800 meter (7.13±1.94 U/mL) groups (p<0.05). Studies have shown that antioxidant parameters of populations accustomed to living at high altitudes may be lower than those later moved to higher altitudes. In populations normally living close to sea level, relocation to high altitudes induces hypoxia, which activates the body's defense systems and leads to an increase in antioxidant enzymes. It is thought that the reason why the highest ceruloplasmin concentration was observed in hair goats grazed at 10-99 meters may be that the temperature is higher at those levels and heat stress increases the acute phase proteins and likewise heat stress causes to a decrease in paraoxonase enzyme activity.

Keywords: Altitude, antioxidant, hair goats

Farklı Yüksekliklerde Otlayan Kıl Keçilerinde Paraoksonaz ve Serüloplazmin Konsantrasyonlarının Araştırılması

Süreç

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Teşekkür
Sonuçların bir kısmı, 23-25 Mayıs 2024 tarihlerinde Van-Türkiye'de düzenlenen "3. Uluslararası Veteriner Biyokimya ve Klinik Biyokimya Kongresi"nde sözlü bildiri olarak sunulmuştur.

ÖZ

Bu çalışmanın amacı, farklı rakımlarda otlatılan kıl keçilerinde serum Paraoksonaz ve Serüloplazmin konsantrasyonlarındaki değişiklikleri ortaya koymaktır. Çalışmaya Adana'nın farklı bölgelerinde yaşayan 80 kıl keçisi dâhil edilmiş ve bu keçiler dört gruba ayrılmıştır. Birinci grup deniz seviyesinden 10-99 metre yükseklikte, ikinci grup 240-250 metre yükseklikte, üçüncü grup 750-800 metre yükseklikte ve dördüncü grup ise 1200-1500 metre yükseklikte otlatılmıştır. Yaz aylarında jugular venden alınan kan örnekleri, kırmızı kapaklı tüplere alınmıştır. Paraoksonaz ve Serüloplazmin analizleri spektrofotometrik yöntemle gerçekleştirilmiştir. Paraoksonaz sonuçları, 240-250 metre (1113,91±387,54 U/mL) ve 750-800 metre (974,27±295,20 U/mL) gruplarında, 10-99 metre grubuna (651,72±253,84 U/mL; p<0,01**) göre istatistiksel olarak anlamlı şekilde yüksek bulunmuştur. Serüloplazmin sonuçlarında ise 10-99 metre (10,15±3,04 U/mL) ile 750-800 metre (7,13±1,94 U/mL) grubu arasında, ayrıca 240-250 metre (9,97±3.96 U/mL) ile 750-800 metre (7,13±1,94 U/mL) grubu arasında istatistiksel olarak anlamlı fark saptanmıştır (p<0,05*). Yapılan çalışmalar, yüksek rakımda yaşamaya alışık popülasyonlarda antioksidan parametrelerin, sonradan yüksek rakıma taşınanlara göre daha düşük olabileceğini göstermektedir. Normalde deniz seviyesine yakın yerlerde yaşayan ve sonradan yüksek rakımlara çıkan bireylerde, hipoksiye bağlı olarak vücut savunma sistemleri aktive olmakta ve buna bağlı olarak antioksidan enzim düzeylerinde artış beklenmektedir. 10-99 metre rakımda otlatılan kıl keçilerinde en yüksek serüloplazmin konsantrasyonunun gözlemlenmesinin, bu seviyelerde sıcaklığın daha yüksek olmasına ve buna bağlı olarak ısı stresinin akut faz proteinlerini artırmasına bağlanabileceği; benzer şekilde, ısı stresinin paraoksonaz enzim aktivitesinde azalmaya neden olduğu düşünülmektedir.

Anahtar Kelimeler: Antioksidan, Kıl Keçileri, Rakım

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Introduction

Goats are important farm animals in Turkey because of their easy care and feeding conditions. In Turkey, 80-90% of their nutrition is provided from natural pasture area, which is economic for farmers (Koyuncu and Taskin, 2016). Although Turkey is a country rich in natural pasture areas, it does not operate at full potential in terms of sheep and goat breeding (Kaymakci et al., 2004). Goats do not use only pasture areas, they prefer trees and shrubs for feeding too and this kind of feeding has a positive effect on biodiversity, which makes goats preferable for breeding (Yilmaz et al., 2012). The breed that forms the densest goat population in Turkey is the hair goat. Hair goats are combined breeds and their resistance to hard conditions is one of the most known characteristic for hair goats (Gunlu and Alasahan, 2010). Hair goats are mostly raised in Mediterrenean region and South east region of Turkey. Their population has increased in the last years and they can be raised mountainous villages and forested areas, which make them preferable (Gungor et al., 2021).

It is known that various conditions like nutrition, housing, age, sex, management, genetics, breed, temperature, humidity etc can change the biochemical profiles of farm animals. Even grazing can change the profile of goats (Mohammed et al., 2016). A study investigated the effect of heat and cold on heat adaptive and cold adaptive goat breeds. They found significant changes in hematological and biochemical profiles. So it is important to know the breeds and their adaptation on climate changes. High altitude in summer mostly results with decreased temperature and decreased oxygen consumption leads to higher hemoglobin and erythrocyte levels (Banerjee et al., 2015). Oxidative stress and hypoxia are related to each other and the cause of hypoxia in physiological conditions is seen in high altitudes. Increased oxidative stress depletes the antioxidant capacity of the body (Askew, 2002).

Ceruloplasmin is known for its anti- and pro-oxidant effect. Especially in chronic diseases it was found that ceruloplasmin levels in sick people increases (Demirpence et al., 2014). In a study ceruloplasmin and paraoxonase (PON) activity was found negatively related and in coronary artery diseases serum ceruloplasmin levels were increased (Gocmen et al., 2008). Both ceruloplasmin and PON have antioxidant effects but while ceruloplasmin is a positive acute phase protein (Fleck, 1989), PON is a negative acute phase protein (James and Deakin, 2004).

The aim of the study was to investigate the effect of altitude on serum PON and ceruloplasmin levels. For this purpose, 80 hair goats in different altitudes of Adana province were chosen and in summer during grazing in 0-99 m, 240-250 m, 750-800 m and 1200-1500 m heights blood samples were collected from hair goats for analysis.

Materials and Methods

The Ethical Statement of this study was approved by Cukurova University Local Ethics Committee of Ceyhan Faculty of Veterinary (Decision date and number: 12.12.2018; 1/11).

Animals and groups

Hair goats from the Adana province in Turkey, living in different regions of Adana, were included in the study. A total of 80 goats were enrolled and four groups were categorized according to the altitude. Table 1 presents the grouping of the goats, including the number of animals in each group, their sexes, and age ranges.

In summer, while grazing the goats in the pasture, the blood samples were collected from the jugular vein (V. jugularis) of each goat into red top tubes and serum samples were taken immediately by centrifugation at 3000 rpm for 10 minutes. Samples were stored at -80 ^{o}C until analysis.

Table 1. Groups and number of goats in every group.

Group Number	Group Name	Ages	Sex	n
1	10-99 m	2-5 years old	Female	23
2	240-250 m	2-5 years old	Female	21
3	750-800 m	2-5 years old	Female	18
4	1200-1500 m	2-5 years old	Female	18

Paraoxonase Analysis

PON activity was measured according to the method of Aviram and Rosenblat (2008). The working principle is based on the optic density at 412 nm of the yellow colored p-nitrophenol after the enzymatic hyrdrolisation of PON.

Ceruloplasmin Analysis

This analysis catalyzes the oxidation of p-phenylendiamin, which gives a purple color. The method of Ceron and Martinez-Subiela (2004) was used. In calculation Curzon and Vallet (1960)'s method was used.

Statistical Analysis

SPSS 21.00 version was used and after normality distribution with shapiro-wilk test one way ANOVA test was done and groups were compared according to the Levene's Test (Homogeneity of variances). In pairwise group comparison Bonferroni test was used and $p<0.05$ was accepted as statistically significant (Field, 2013). The positive or negative correlation between PON and ceruloplasmin was done with RStudio statistics 4.3.2 software.

Results and Discussions

The results for PON levels between groups were statistically significant ($p<0.01$) in 10-99 m (651.72±253.84 U/mL) and 240-250 m (1113.91±387.54 U/mL) and 10-99 m and 750-800 m (974.27±295.20 U/mL). In table 2 the results of PON are given.

Ceruloplasmin measurements gave statistically significance ($p<0.05$) between 10-99 m (10.15±3.04 U/mL) and 750-800 m groups; and 240-250 m (9.97±3.96 U/mL) and 750-800 m (7.13±1.94 U/mL) groups. In table 2 the ceruloplasmin results and group comparisons are given.

Table 2. The statistical differences and Me±SD values of PON and ceruloplasmin levels.

		N	Me±SD	Std. Error	95% Confidence Interval for Mean		P value
					Lower Bound	Upper Bound	
PON	**10-99 m**	23	651.72±253.84[a]	52.93	541.95	761.49	0.001
	240-250 m	21	1113.91±387.54[b]	84.57	937.51	1290.32	
	750-800 m	18	974.27±295.20[b]	69.58	827.46	1121.07	
	1200-1500 m	18	841.86±473.36[ab]	111.57	606.46	1077.25	
Cerulo-plasmin	**10-99 m**	23	10.15±3.04[a]	0.63	8.83	11.47	0.014
	240-250 m	21	9.97±3.96[a]	0.86	8.17	11.77	
	750-800 m	18	7.13±1.94[b]	0.46	6.16	8.09	
	1200-1500 m	18	8.88±3.22[ab]	0.76	7.28	10.48	

*Different letters in each column for PON and ceruloplasmin levels show the statistical difference.

Considering the negative acute phase protein effects of PON and the positive acute phase protein effects of ceruloplasmin, the highest ceruloplasmin and lowest PON values were observed in the same group. The correlation between PON and ceruloplasmin can be seen in figure 1. According to the correlation plots, a negative correlation was found between levels of PON and ceruloplasmin. The negative correlation was stronger for altitude and ceruloplasmin levels. Because of the negative correlation of PON and ceruloplasmin, altitude and PON shown a weak positive correlation.

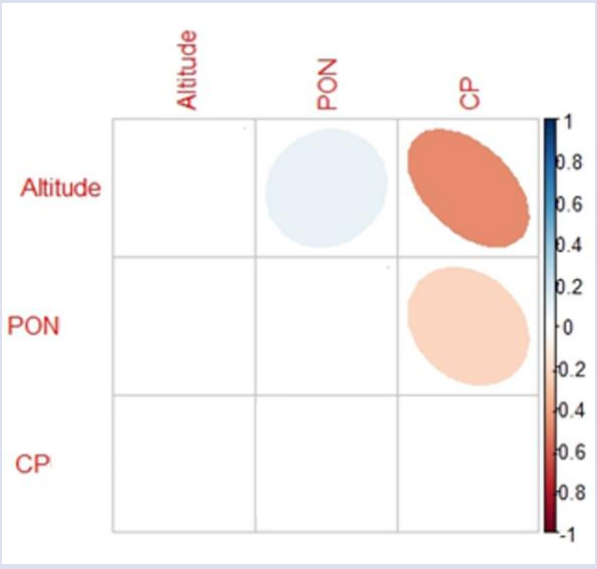

Figure 1. Pearson correlation matrix of altitude in PON and ceruloplasmin parameters of hair goats.

The study of Yildirim et al. (2008) investigated the effect of altitude on some acute phase proteins in 800 and 2600 meter and found that there was a significant increase in some positive acute phase proteins such as ceruloplasmin and alpha 1 antitripsin. Our study did not investigate higher altitudes, which is a limitation for this study. However, heat stress might explain high ceruloplasmin levels at the sea level. At 750-800 m ceruloplasmin concentration was lower than at 1200-1500 m but there was not a significant difference. So if the altitude could be higher maybe a significantly increased ceruloplasmin level could be seen because of elevated oxidative stress.

The physiologically healthy biochemical results of hair goats in Cukurova region, which is located in Adana province, were analysed according to altitude differences and it was found that the alkaline phosphatase (ALP), aspartate aminotransferase (AST), cholesterol, triglyceride and glucose levels showed significant differences between 87 and 756 meters altitudes. Due to the study they found that the normal values of healthy hair goats can differ compared with other goat species (Er and Ok, 2020). This emphasizes that the species of goats are important to know because the results of biochemical analysis can change.

In a study done with humans, altitude was classified as low and moderate. According to the results the highest PON levels were found in 200-400 m heights and the lowest was seen in 0-200 m height (Cabrera de Leon et al., 2011). If compared with the current study with goats the results are similar, but in our study there was a statistically significant difference and in the study of Cabrera de Leon et al. (2011) there was not a significant difference between groups.

In a study conducted by Kurt et al. (2021) total oxidant capacity (TOC) of hair goats in Adana province was measured and the groups were categorized as 0-99 m, 100-500 m and higher than 500 meter. According to the results there was a significant difference between the groups and the highest total oxidant capacity (TOC) levels were measured in the >500 m group. Normally the closer to sea level the higher the heat stress but in this case the differences in oxygen pressure in high altitude could effect the reactive oxygen species and this could be the reason of high TOC levels (Bakonyi and Radak, 2004).

Kartal et al. (2021) investigated the effect of age on PON and ceruloplamin levels in hair goats from Adana. The results showed that there was not a significant difference between the groups in PON analysis but still the highest PON levels were found in 1.5-2 and 2.5-6 year old group. In ceruloplasmin levels, there was significant difference between 0-6 month and the last three groups.

After birth ceruloplasmin levels were higher and with age it slowly decreased. So working with 2-5 year old goats eliminate the effect of age on ceruloplasmin levels.

PON levels decrease at higher altitudes, which may result in lower HDL cholesterol and increased triglyceride levels. This could be a reason to be predisposed to atherosclerosis in people living at high altitudes. The study of Hirschler et al. (2018) investigated the PON levels in children living at high altitude (3700 meter) and low altitude (25 meter) and found that even the body mass index was significantly higher in lowlanders, HDL and PON activity were lower. In goats the highest altitude was 1500 meter so the differences could be because of a lower high altitude. But Hirschler et al. (2018) worked in October and November while the current study was studied in summer. Lower PON activity at the sea level could be the result of heat stress.

Two different heights (1432 and 3750 m) were selected and HDL, LDL, TC, TG and PON was measured. PON was higher in 1432 m height when the other parameters were higher in 3750 m height (Hirschler et al., 2019). So hypoxia results with high TG levels because of the hepatic lipid oxidation (Muratsubaki et al., 2003).

Living at high altitude is generally associated with a reduction in antioxidant profile as it is known. But a study conducted by Sinha et al. (2009a; 2009b) searched the adaptation in lowlanders when road to high altitude (4500 meter) and native highlanders. They found that there was a significant elevation in antioxidant enzymes in lowlanders after the trip to high altitudes. But in native highlanders antioxidant enzyme activity was lower than the lowlanders at the same altitude (4500 meter). Oxidative stress markers were found higher in lowlanders at high altitude compared with native highlanders. So an increase in oxidative stress will activate the defense system of the body and this could be the reason for the increase in antioxidant levels in lowlanders at high altitude (Halliwell et al., 1995).

A study investigated the effect of altitude on athletes. According to the study athletes blood samples were collected 24 h after exercise at the sea level and then athletes were ascended to high altitude (2400 m) for 4 weeks and 24 h after the last exercise second blood sample were collected. Results showed that MDA and GSH levels increased in high altitudes but SOD enzyme activity did not differ in this study (Belviranli et al., 2017). So elevated oxidative stress and antioxidant defense system can balance each other up to a certain exposure but the enzymatic antioxidant parameter (SOD) was not changed with altitude. This could be due to excessive hydrogen peroxide accumulation (Sinha et al., 2009c).

Conclusion

In conclusion, it can be stated that levels of serum PON and ceruloplasmin are related negatively and altitude has an effect on these parameters. But 1500 meter is mostly seen as moderate altitude so working in higher altitudes is necessary to see the differences better. But in this study the working area was limited to Adana province which is a region at the sea level, so the highest altitude there was 1500 meters height. The effect of altitude on biochemical parameters such as blood gases or antioxidant enzymes are mostly studied with humans and athletes. There are relatively few studies about farm animals. Goats are skilled climbers and this allows them to reach areas that many people/farm animals cannot reach. So this situation makes them important to study with goats at different altitudes to see how they are affected. There was no significant change in low (0-99 m) and high (1200-1500 m) altitudes. This may be attributed to the heat stress at sea level and adaptation to high altitudes. The significant difference in moderate levels in PON were higher than that at the sea level. This might again be explained by the heat stress because at 240-250 and 750-800 m heights humidity and heat is reduced and this leads to a decrease in oxidative stress too. On the other hand the significant difference in ceruloplasmin levels could be seen only in 750-800 m because of the same reason like in PON. To draw clearer conclusions, it could be better to know the copper and other positive acute phase protein values. Reference values of parameters determined in goats may vary depending on environment and breed. There are recommendations on creating a separate reference table of parameters for each region and breed in goats. Nonetheless, further research is needed for goats with different parameters and higher altitudes.

Conflict of Interest

The authors declared that there is no conflict of interest.

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