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Determination of Serum Biochemical Profile and Oxidant-Antioxidant Activities in Damascus Goats at Different Ages

Şam Keçilerinde Farklı Yaşlarda Serum Biyokimyasal Profilinin ve Oksidan-Antioksidan Aktivitelerinin Belirlenmesi

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ABSTRACT

This study aimed to research changes in biochemical profile and oxidant-antioxidant activities of Damascus goats of different ages. The 45 non-gravid Damascus goats of different ages were included in the research. They were divided into three groups of 15 goats according to age: goat kids (- ≤ 6-months age), young goats (2-3-year age) and old goats (5-8-year age). Biochemical parameters and oxidant-antioxidant activities were determined in serum samples using commercial kits and colorimetric methods. The lowest serum urea, blood urea nitrogen, aspartate aminotransferase, total protein, albumin, globulin and magnesium levels were observed in goat kids ($P < .05$). Concentrations of glucose, inorganic phosphorus, non-esterified fatty acids, albumin/globulin and alanine aminotransferase decreased with age in the goats ($P < .05$). Serum total oxidant capacity, total antioxidants capacity and oxidative stress index levels were increased in goat kids compared to old goats ($P < .05$). Furthermore the highest serum glutathione and glutathione preoxidase activities were observed in young goats ($P < .05$). An increase was determined in concentrations of serum β-carotene and bilirubin in old goats compared to goat kids ($P < .05$). In contrast, serum catalase activity, arylesterase, ceruloplasmin and uric acid values did not differ between the groups. The results obtained on the biochemical profiles and oxidant-antioxidant activities of Damascus goats will contribute to monitoring this breed's age-related health and nutritional status and establishing reference values.

Keywords: Antioxidants, age, blood parameters, damascus goat

ÖZ

Bu çalışmada, farklı yaşlardaki Şam keçilerinin biyokimyasal profillerindeki değişikliklerin ve oksidan-antioksidan aktivitelerin araştırılması amaçlandı. Araştırmaya 45 adet gebe olmayan farklı yaşlardaki Şam keçisi dahil edildi. Keçiler yaşlarına göre üç gruba ayrıldı: keçi yavruları (≤ 6-aylık), genç keçiler (2-3-yaş) ve yaşlı keçiler (5-8-yaş). Serum örneklerinde biyokimyasal parametreler ve oksidan-antioksidan aktiviteler ticari kitler ve kolorimetrik yöntemler kullanılarak belirlendi. En düşük serum üre, kan üre azotu, aspartat aminotransferaz, total protein, albumin, globulin ve magnezyum seviyeleri keçi yavrularında gözlemlendi ($P < ,05$). Glukoz, inorganik fosfor, non-esterifiye yağ asitleri, albumin/globulin ve alanin aminotransferaz konsantrasyonları keçilerde yaşla birlikte azaldı ($P < ,05$). Serum total oksidan kapasitesi, total antioksidan kapasitesi ve oksidatif stres indeksi düzeyleri keçi yavrularında yaşlı keçilere göre yüksekti ($P < ,05$). En yüksek serum glutatyon ve glutatyon peroksidaz aktiviteleri genç keçilerde gözlemlendi ($P < ,05$). Keçi yavrularına kıyasla yaşlı keçilerde serum β-karoten ve bilirubin konsantrasyonlarında artış tespit edildi ($P < ,05$). Buna karşın serum katalaz aktivitesi, arilesteraz, seruloplazmin ve ürik asit değerleri gruplar arasında farklılık göstermedi. Elde edilen sonuçlar, bu ırkın yaşla ilişkili sağlık ve beslenme durumunun izlenmesine ve referans değerlerin belirlenmesine katkı sağlayacaktır.

Anahtar Kelimeler: Antioksidanlar, kan parametreleri, Şam keçisi, yaş

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INTRODUCTION

Rapid population growth remains a major obstacle to improving food security in some countries, even as the world's population stops growing in the present century. Therefore many farm animal species and native breeds are in danger of extinction due to their low productivity. Damascus goats are used in crossbreeding studies to increase the milk yield of domestic goat breeds raised in hot climate conditions because of their high milk yield. It is a breed of Syrian origin and is grown in many countries of the world (Turkey, Syria, Lebanon, Egypt, Cyprus and Israel). Damascus goats make better use of pastures in high temperature conditions than sheep. They are cost effective as they consume bush, heather, thorns and straw, and adapt well to arid and semi-arid climatic conditions.[1]

Biochemical parameters are used in ruminants to assist in the clinical diagnosis of metabolic, several parasitic diseases and infectious. These parameters help realistically evaluate management practices, nutritional status and health conditions. Furthermore in healthy animals, biochemical and haematological parameters and antioxidant status are known to change due to age, race, gender, environmental conditions, nourishing, stress, and several reasons. Therefore, reference intervals are needed for the appropriate age range specific to each animal species in order to evaluate biochemical test results more accurately.[2,3]

Ageing is the process that covers the changes that occur over time from the molecular level to the functional organs. It is reported that the cause of ageing is the destruction that occurs due to oxidative stress in the ordinary life process. The term oxidative stress (OS) is an imbalance between oxidant and antioxidant molecules in favor of oxidants.[3]

Oxidative stress can cause tissue damage by damaging cell components.[3,4] It causes tissue damage by damaging cell components. It appears that oxidized DNA, protein, and carbohydrates increase with age, and the levels of oxidized metabolic products formed depend on the rate of free radical production, which varies by species.[3,4,5] Lipid peroxidation, an indicator of oxidative stress, is a series of reactions that produce free radicals in cell membranes, and is measured by malondialdehyde (MDA).[6] The antioxidant systems, which can prevent oxidative stress through providing genesis and/or scavenging of oxidants, consist of three components: (i) primary or enzymatic antioxidant enzymes [glutathione peroxidase (GPx) and catalase (CAT)]; (ii) low molecular mass non-enzymatic antioxidants [i.e. glutathione (GSH), β-carotene, bilirubin], (iii) and proteins (i.e, ceruloplasmin and uric acid) that can sequestrate free transition metals.[7]

Although Damascus goats are widely bred, a limited number of studies have been reported on the effects of age on the serum oxidant/antioxidant activities and biochemical values. To our knowledge, studies on this breed have mostly focused on trace elements in colostrum, hormonal profile, gene sequence and breeding. Therefore, this study aims to determine the blood biochemical profile and serum oxidant-antioxidant status of Damascus goats in different age groups.

MATERIALS AND METHODS

Animals and Experimental Design

This study used 45 healthy non-gravid Damascus goats of different ages. The goats were divided into three groups according to age: goat kids (- ≤ 6-months age, n= 15), young goats (2-3-year age, n= 15) and old goats (5-8-year age, n= 15). Animals were obtained from from Helalköy A.Ş in Isparta/Turkey. The sample collection was taken in June 2022 and the ambient temperature was around 21°C on average for June. The approval number of the study has been given before the study has been started from Burdur Mehmet Akif Ersoy University Animal Experiments Local Ethics Committee (Date: 04/2022, Number: 887).

All blood samples were taken in the morning of the same day, while the goats were in the stable before feeding. Blood samples were collected from the jugular vein (10 mL) into sterile vacuum tubes without anticoagulant (Venoject®; Sterile Terumo Europe, Leuven, Belgium). Afterwards, serums were obtained by centrifugation at 3000 rpm for 10 minutes. The obtained serum samples were frozen at -80°C and stored until the analysis.

Biochemical Analysis

Serum cholesterol (Chol), triglycerides (TG), non-esterified fatty acids (NEFAs), blood urea nitrogen (BUN), uric acid, bilirubin, magnesium (Mg), inorganic phosphorus (P), calcium (Ca), glucose (GLU), total proteins (TP), albumin (ALB), globulin (GLOB), urea, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma glutamyl transferase (GGT) were measured with an automatic analyzer using commercial test kits (Gesan Chem200).

Analysis of Oxidants and Antioxidants

Serum MDA activity was measured by a method described by Yoshioka et al[6] based on thiobarbuturic acid (TBA) reactivity at 532 nm. The levels of total oxidant capacity (TOC) and total antioxidants capacity (TAC) were measured

via commercial kits (Rel Assay Diagnostic, Turkey) as previously described by Erel.[8,9] The TAC and TOC results were expressed as mmol Trolox equivalent/L and µmol H_2O_2 equivalent/L, respectively. The TOC/TAC ratio determined the oxidative stress index (OSI). (OSI (AU) = TOC (µmol H_2O_2 equivalent/L)/TAC (µmol Trolox equivalent/L). The activity of arylesterase (ARES) was analyzed with commercially available kits (Rel Assay Diagnostics Kit; RL0055 Mega Tip).

The measurement of GSH content was performed by a method reported by Beutler et al[10] The absorbance was measured at 412 nm and the results were given mmol/L. The GPx activity was determined at 340 nm according to the spectrophotometric method developed by Paglia and Valentine[11], using t-butylhydroperoxide as substrate. The activity was expressed as IU/L. The concentration of CAT was measured by the decomposition of H_2O_2 at 240 nm according to the method of Aebi[12], and is expressed as IU/L in serum. Serum β-carotene and ceruloplasmin activities were measured with spectrophotometrically (Shimadzu, Japan) by methods of Suzuki and Katoh[13] and Sunderman and Nomoto[14], respectively.

Statistical Analysis

The SPSS Software (version 21.0; SPSS, Inc., Chicago, USA) was used for the statistical data analyses. Whether thedata showed normal distribution or not was evaluated with the Shapiro-Wilk test. While comparisons were made with the ANOVA test to the groups with normal distribution, the Kruskal-Wallis test was applied to the groups that did not show normal distribution. Data were presented as mean ± standard deviation and $P < .05$ was considered as significant.

RESULTS

The results for some biochemical parameters of different ages in the study groups were given in Table 1. Serum urea, BUN, AST, TP, ALB, GLOB and Mg concentrations were statistically lower in kids than in young and old goats ($P <$ .05). The concentration of P were significantly higher in kids and young than in old goats ($P < .05$). Serum GLU, ALB/GLOB and ALT values were highest in goat kids ($P <$ .05). Significantly lower NEFA concentrations were observed in kids than in old goats ($P < .05$). When the kids, young and old goats were compared, no significant difference was found between serum Chol, TG, GGT and Ca levels.

There was no statistically difference in MDA level between all groups. The TOC and TAC concentrations were significantly higher in kids than young and old goats ($P <$ .05). It was determined that OSI level higher statistically in the goat kids ($P < .05$) (Table 2)

Table 1. Some serum biochemical parameters in different ages healthy Damascus goats.

	Groups		
Parameters	Goat kids (0-6 months)	Young goats (2-3 years)	Old goats (5-8 years)
BUN (mg/dL)	3.12 ± 0.86^{b}	12.21 ± 2.59^{a}	13.92 ± 2.41^{a}
Urea (mg/dL)	6.69 ± 1.84^{b}	26.13 ± 5.55^{a}	29.78 ± 5.18^{a}
Chol (mg/dL)	53.45 ± 18.38	58.76 ± 12.24	63.95 ± 12.26
TG (mg/dL)	15.67 ± 6.89	16.71 ± 4.88	13.64 ± 6.72
GLU (mg/dL)	56.46 ± 7.35^{a}	44.30 ± 9.74^{b}	38.75 ± 6.89^{b}
AST (U/L)	67.73 ± 13.67^{b}	87.25 ± 18.67^{a}	87.48 ±10.23^{a}
ALT (U/L)	21.88 ± 1.86^{a}	15.74 ± 2.52^{b}	15.18 ± 2.46^{b}
GGT (U/L)	44.53 ± 11.63	49.86 ± 12.19	51.08 ± 10.12
TP (g/dL)	4.35 ± 0.44^{b}	6.22 ± 0.34^{a}	6.62 ± 0.52^{a}
ALB (g/L)	2.31 ± 0.25^{b}	3.05 ± 0.15^{a}	2.90 ± 0.12^{a}
GLOB(g/L)	2.03 ± 0.36^{c}	3.23 ± 0.41^{b}	3.72 ± 0.52^{a}
ALB/GLOB (g/L)	1.13 ± 0.21^{a}	0.95 ± 0.09^{b}	0.79 ± 0.11^{c}
Ca (mg/dL)	7.66 ± 0.61	7.73 ± 0.41	7.40 ± 0.45
Mg (mg/dL)	1.37 ± 0.23^{b}	1.68 ± 0.43^{a}	1.86 ± 0.47^{a}
P (mg/dL)	4.41 ± 1.12^{a}	4.67 ± 0.78^{a}	3.47 ± 0.65^{b}
NEFAs (mmol/L)	0.67 ± 0.14^{a}	0.55 ± 0.15^{ab}	0.52 ± 0.15^{bc}

BUN: Blood urea nitrogen, Chol: cholesterol, TG: Triglyceride, GLU: Glucose, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, GGT: Gamma glutamyl transferase, TP: Total protein, ALB: Albumin, GLOB: Globulin, Ca: Calcium, Mg: Magnesium, P: inorganic phosphour, NEFAs: Non-esterified fatty acids. Data presented as mean ± standard deviation. a,b,c: Values within a row with different superscripts differ significantly at $P < .05$.

Table 3 shows, statistically differences in GSH, GPx, β-caroten and bilirubin levels were observed between different age groups. Significantly higher activities of GSH in serum were determined in young compared to kids and old goats ($P <$ .05). The concentration of GPx enzyme was observed to be higher in kids and young than in old goats ($P <$ .05).

The CAT levels did not differ when comparing young and old goats, but tended to decrease with age. In contrast, β-carotene and bilirubin levels were observed to be significantly higher in old goats ($P < .05$). There was no statistically significant difference in ceruloplasmin, arylesterase and uric acid values between all groups.

Table 2. Serum MDA, TOC, TAC and OSI levels in different ages healthy Damascus goats.

	Groups		
Parameters	Goat kids (0-6 months)	Young goats (2-3 years)	Old goats (5-8 years)
MDA (µmol/L)	27.02 ± 10.33	26.28 ± 7.07	24.44 ± 2.85
TOC (µmol H_2O_2 Equiv/L)	8.51 ± 2.12[a]	4.17 ± 0.96[b]	4.67 ± 2.45[b]
TAC (µmol Trolox Equiv/L)	3.03 ± 0.08[a]	2.86 ± 0.06[b]	2.80 ± 0.05[b]
OSI	2.80 ± 0.52[a]	1.42 ± 0.34[b]	1.66 ± 0.93[b]

MDA: Malondialdehyde, TOC: Total oxidant capacity, TAC: Total antioxidant capacity, OSI: Oxidative stress index Data presented as mean ± standard deviation. [a,b]: Values within a row with different superscripts differ significantly at $P < .05$.

Table 3. Activities of serum enzymatic and non-enzymatic antioxidants in healthy Damascus goats of different ages.

	Groups		
Parameters	Goat kids (0-6 months)	Young goats (2-3 years)	Old goats (5-8 years)
Glutathione (mmol/L)	0.92 ± 0.01[b]	1.03 ± 0.06[a]	0.94 ± 0.03[b]
Glutathione Peroxidase (IU/L)	22.43 ± 6.01[a]	28.10 ± 6.71[a]	15.16 ± 3.19[b]
Catalase (IU/L)	12.03 ± 9.20	13.85 ± 9.91	8.38 ± 6.51
Arylesterase (IU/L)	22.01 ± 3.69	17.87 ± 5.02	22.67 ± 8.42
β-caroten (µg/dL)	2.57 ± 1.26[b]	3.77 ± 1.36[b]	8.68 ± 4.56[a]
Ceruloplasmin (mg/dL)	38.07 ± 6.10	34.19 ± 3.69	34.95 ± 11.38
Uric acid (mg/dL)	3.56 ± 0.89	3.74 ± 0.59	3.67 ± 0.46
Bilirubin (mg/dL)	0.86 ± 0.08[b]	0.90 ± 0.11[b]	1.05 ± 0.24[a]

Data presented as mean ± standard deviation. [a,b]: Values within a row with different superscripts differ significantly at $P < .05$.

DISCUSSION

Blood parameters are used not only in the diagnosis of diseases, but also in the evaluating of the health status of living organisms, as they provide precise information about metabolic events in the body. In the present study, we investigated some biochemical parameters, lipid peroxidation levels, total oxidant-antioxidant capacity, and serum enzymatic and non-enzymatic antioxidant activities in healthy Damascus goats according to age.

In general, serum levels of BUN and urea are used clinically to describe of urinary function, nutritional and hydration status. However, their concentrations also increase during high fever, anorexia, inflammaton and increased protein catabolism.[15] It has been reported that the urea concentration in adult goats is higher than in goat kids.[2] In this study, serum urea and BUN levels were higher in adult goats than in goat kids, and it was thought that this might be due to renal dysfunction due to systemic inflammation or an increase in protein catabolism. Furthermore, in the current study, an important increase in ALB, GLOB and TP concentrations were observed with age in goats, while the ALB/GLOB ratio decreased. These findings are most probably due to the gradual build-up of immunoglobulins.

Cholesterol and TG concentrations in goats are changeable depending on age, sex, dietary, transition period, lactation and pregnancy.[16,17] Karaşahin et al[18] showed no difference in age-related cholesterol levels in male Hair goats. In another study, it was reported that TG concentrations in the sheep serum decreased with age.[19] In this study, cholesterol and TG levels were not statistically different between groups, but cholesterol levels tended to increase with age. Differences between studies may be due to nutritional status and physiological differences between breeds.

The energy status of goats may be evaluated by GLU levels in blood. GLU levels in the blood are considered as indicators of pancreatic hormonal function and dietary intake.[16] In previous studies, it was shown that GLU levels are higher in young goats and mares than in older.[20] In our study, the highest serum GLU concentrations were observed in goat kids. This may be due to different dietary sources and high colostrum intake. When the uptake of high-energy substrates is low in ruminants for pregnancy and milk production, NEFAs levels increase due to

increased lipolysis. It has been reported that growth hormone levels in the plasma of buffaloes decrease with lipolysis and lower NEFAs levels are observed as aging.[21] In this study, serum NEFAs concentrations were higher in kids than in old goats.

Enzymatic activities such as ALT, AST, and GGT in the liver are used to determine hepatic cell function and integrity. AST enzyme activity is increased in liver injury, passive congestion, muscle wasting, and proventriculus tension.[22] In this study, the increase in AST activity with age may be due to glucocorticoid activity, liver cell damage or disease-induced stress. These findings were in line with results of Piccione et al[23] and Njidda et al[24]. In contrast, ALT levels of goats decreased significantly with age, while GGT levels did not change. It has been shown that ALT activity in adult is significantly lower than in young animals.[25] Another study, it was reported that ALT activities in ruminants may be affected by the breeding season.[26] Similarly, the low ALT activities in the this study may be due to the fact that blood samples were collected outside the breeding season.

In the present study, it was determined that age significantly affects serum macroelements except Ca. Devrim et al[27] showed no significant differences were reported in Ca values between the monthly age groups in intensively fed, goats after 4 months of age for 12 months. In this study, the highest serum P values were detected in goat kids. The higher amount of P in young animals compared to adults may be due to growth hormone that increases renal phosphate reabsorption or the composition of the meals.[28] Previous studies have shown similar results for ruminant serum P values.[25] Mg values in our study determined in old goats were significantly higher compared with goat kids. Enhanced use of Mg for bone mineralization and the reduced availability of this element in digested food may cause decreased Mg in early life in kid goats.

In ruminants, oxidative stress has been linked to various pathological conditions, including retained placenta, udder edema, and mastitis. These conditions, in turn, may negatively impact reproductive performance. Additionally, it can also affect the milk yield, immune system, parasitic infections and metabolic functions in the energy production process.[29] The oxidative damage indicated by the individual animal's oxidative stress index, which reflects the risk of developing a disease, can be more reliably monitored through the use of an oxidative stress parameters. Concentrations of MDA, TOC and OSI are commonly used parameters in oxidative stress. Yatoo et al[30] reported that MDA concentration in the young goats was higher than older goats. In this study, serum MDA concentration was not different between groups. Additionally, in kids, serum TOC, TAC and OSI levels were higher compared to elderly goats, and in Damascus goats, oxidative damage did not increase with age. The low oxidative stress in young and old goats suggests that the animals were probably well fed, and in good health and welfare conditions. Furthermore, the oxidation of NEFAs in ruminants were reported to lead to an increase in free radical production and ultimately results in the development of oxidative stress.[29] Our study shown that oxidative status might be related to the metabolic changes in goat kids, and supported by significant positive correlation between MDA, TOC, OSI and NEFAs concentrations.

Antioxidants prevent the formation of free radicals or harmful effects on metabolism and ensure that metabolic events continue in a healthily.[7] TAC provides biological information that describes the dynamic equilibrium between pro-oxidants and antioxidants in the serum of animals.[9] In our study, we determined that serum TAC levels were also rising against increased oxidative stress in goat kids. GSH is an important tripeptide molecule that plays a role in the cellular aging process. It is function by protecting the protein-SH groups of enzymes, haemoglobin or the cell membrane from oxidation.[7] In this study, the highest serum GSH levels were observed in young goats. These results were similar to previously reported values in mares and dogs.[25,31,32] Besides, cysteines in ALB which one of the important and effective antioxidants in plasma, form disulfide with molecules such as glutathione.[33] In our study, a positive correlation was determined between the highest ALB levels and GSH levels in young goats. Enzymatic antioxidants such as GPx and CAT protect biological macromolecules from oxidative damage. GPx enzyme catalyze conversion of reduced form of glutathione to its oxidize form and removal of H_2O_2, and a parallel decrease in reactive oxygen metabolite levels is also expected. Other antioxidant molecules such as CAT enzyme is a heme protein located in peroxisomes and converts H_2O_2, generated in the cytocol or peroxisomes.[7] There are different reports of CAT and GPx antioxidant activity according to age. Şimsek et al[34] reported that CAT activity in Angora goats did not change with age. Another study showed that plasma GPx activity increased with age, but CAT activity decreased in Saanen goats.[35] In our study, serum GPx and CAT activities were higher in kids and youngs than in older goats. This increases may be the result of increased oxidative stress in Damascus goat kids triggering the compensatory response of the antioxidant defense system.

β-carotene, a low molecular mass non-enzymatic antioxidant, has peroxyl radical scavenging ability and singlet oxygen quenching properties.[36] Tekeli et al[35] found that plasma β-carotene levels were higher in mother Saanen goats than in youngs. Although it is known to have toxic effects at high concentrations, bilirubin is considered to be a member of the antioxidant family.[37] It has been reported that the mean bilirubin concentration in newborn calves is high and after decreases until the 14th day.[38] In our study, we found a significant increase in serum β-carotene and bilirubin activities of goats with age. This increase is thought to be an indicator of adaptation to the aging process.

Ceruloplasmin is a protein that has antioxidant effects. By binding copper ions, it has neutralize free radicals and reduce cellular damage. This effect occurs by reducing reactive oxygen species and protecting against oxidative damage to the cell membrane and other cellular components. Additionally, it helps to remove free radicals produced during the oxidation of iron.[39] Kartal et al[40] reported that serum ceruloplasmin levels were higher in Hair goats kid than in olds. Conversly, in this study, ceruloplasmin levels did not change between groups.

Uric acid acts as an antioxidant by inactivating superoxide, peroxynitrite anion, singlet oxygen, hydroxyl and chelate transition metals.[41] Few studies in the literature have examined age-related uric acid change in goats. In our study, uric acid levels were not different in Damascus goats between age groups.

Paraoxonase activity (PON1) could hydrolyze aromatic esters, such as phenylacetate, the term ‘‘Arylesterase’’ was introduced for the enzyme hydrolyzing both substrates.[42] To the best of our knowledge, arylesterase activity as known antioxidants, has not been evaluated in different age Damascus goats. Taha et al[43] reported that serum arylesterase activities in 1-year-old male and female camels were significantly lower than in 2-year-old camels. Conversly, in our study, there was no significant difference in serum arylesterase activity between Damascus goats.

In conclusion, this study is the first published reference values related with serum biochemical profile and oxidant-antioxidant activities in Damascus goats of different ages and may be helpful for comprehending the metabolic profile of this breed. The oxidant-antioxidant parameters evaluated were detected in ruminants plasma in many studies, but we could not find any study examining the oxidant-antioxidant activities depending on age in the serum of Damascus goats. The results obtained can assist in observing Damascus goats nutritional and health status, and we think our findings may be useful for future studies in this regard.

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