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Veterinary Sciences *and* Practices

ABOUT

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Veterinary Sciences and Practices publishes clinical and basic research articles, review articles, systematic reviews articles, and case reports.

The target audience of the journal includes specialists and professionals working and interested in all disciplines of veterinary medicine.

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Research Article *Araştırma Makalesi* DOI: 10.17094/vetsci.1471422

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.

Ethics Committee Approval: Ethics committee approval was obtained from Burdur Mehmet Akif Ersoy University Animal Experiments Local Ethics Committee (Date: 04/2022, Number: 887)

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The Effects of Different Fattening Methods and Sex on Fattening Performance in Native Turkish Geese

Yerli Türk Kazlarında Farklı Besi Yöntemleri ve Cinsiyetin Besi Performansı Üzerine Etkisi

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ABSTRACT

The animal material of the study consisted of native Turkish geese. Regardless of the sex of the chicks, the first 4 wk of age were fed together, and the 5 and 6 week of age were fed ad libitum in groups using the starter feed. From the sixth wk onwards, four fattening method were formed until the 16 week of age as feed, pasture + cracked barley, pasture + feed and pasture. In the feed, pasture + cracked barley, pasture + feed groups, general body weight increase averages are 39.81, 37.46 and 39.90 g, respectively; average feed daily consumption is 426.95, 207.99 and 200.76 g, respectively; feed conversion ratio were 10.72, 5.55 and 5.03, respectively. At the end of the study, body weights were determined as 4209, 4108, 4239 and 3971 g in the feed, pasture + cracked barley, pasture + feed groups and pasture groups, respectively (P<0.01). As a result, the average body weight of geese was found to be similar with some literature data, which is lower than some literature data. This may be due to the fact that the geese used in the study are native Turkish geese and no selection studies have been performed on them. Although the highest body weight increase was determined in the pasture + feed group, it was concluded that it would be more appropriate to make pasture feed since the geese fed economically in the pasture provide body weight increase similar to other groups. It was determined that fattening with only feed is not economical for goose breeding and does not provide much daily weight gain compared to other groups.

Keywords: Fattening methods, fattening performance, native Turkish goose, sex

ÖZ

Araştırmanın hayvan materyalini yerli Türk kazları oluşturmuştur. Kaz civcivlerinin cinsiyetine bakılmaksızın ilk 4 hafta birlikte, 5 ve 6. haftalar gruplar halinde başlangıç yemi kullanılarak ad libitum olarak beslenmiştir. Altıncı haftadan itibaren 16. haftaya kadar konsantre yem, mera + arpa kırması, mera + konsantre yem ve mera olmak üzere dört besi yöntemi oluşturulmuştur. Konsantre yem, mera+arpa kırması, mera+ konsantre yem gruplarında genel canlı ağırlık artış ortalamaları sırasıyla 39.81, 37.46 ve 39.90 g; ortalama günlük yem tüketimi sırasıyla 426.95, 207.99 ve 200.76 g; yemden yararlanma oranı ise sırasıyla 10.72, 5.55 ve 5.03 olarak bulunmuştur. Çalışma sonunda canlı ağırlıklar konsantre yem, mera+ arpa kırması, mera + konsantre yem ve mera gruplarında canlı ağırlıklar sırasıyla 4209, 4108, 4239 ve 3971 g olarak belirlenmiştir. Sonuç olarak kazların ortalama canlı ağırlıklarının bazı literatür verileriyle benzer ve düşük olduğu tespit edilmiştir. Bunun nedeni, çalışmada kullanılan yerli Türk kazları üzerinde herhangi bir seleksiyon çalışması yapılmamış olması olabilir. En yüksek canlı ağırlık artışı mera+yem grubunda belirlenmekle birlikte merada ekonomik olarak beslenen kazların diğer gruplara benzer şekilde canlı ağırlık artışı sağlaması nedeniyle mera besisi yapılmasının daha uygun olacağı sonucuna varılmıştır. Kaz yetiştiriciliğinde sadece konsantre yemle beslemenin ekonomik olmadığı ve diğer gruplara göre çok fazla günlük canlı ağırlık artışı sağlamadığı belirlenmiştir.

Anahtar Kelimeler: Besi performansı, besi yöntemi, yerli Türk kazı, cinsiyet.

This study was summarized from the first author's PhD thesis with same title.

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INTRODUCTION

The impact of scientific and technological developments leads to changes in the standard of living and nutritional habits of human beings. This change inevitably creates demands for an increase in animal protein needs and diversity. In order to meet the expressed demands, the products obtained from the animals that are the only source will be increased, and different animal species can be included in the production sector. The largest resource used to increase species diversity in animal production is poultry. An important part of this wide range extending from ostrich to quail is composed of water birds. In waterfowl, geese are in a different position with their breeds raised for different purposes and the variety they offer to production.[1-2]

In recent years, the demand for poultry meat with taste that differs from that of broiler chickens has increased among consumers. Among the various alternative poultry species, geese have interesting biological characteristics; such as a high growth rate, a good adaptation to free range and grazing, disease resistance and a high dietary meat quality. Besides, goose breeding is a part of the culture in certain region of Türkiye. Geese grown in summer and slaughtered in autumn are stored in special conditions. In winter, goose meat becomes indispensable for banquet tables[1-6]. Goose breeding is carried out in the Northeastern Anatolia region, especially in province of Kars, Ardahan and Muş in Türkiye. The total of three province constitutes approximately 53.2% of all Türkiye (TÜİK: https://biruni.tuik.gov.tr/).

Several studies have been conducted on the growth, slaughter and carcass traits of native Turkish geese breeds.[4,7,8.] However, there have been no studies comparing a native Turkish goose breed with different food methods. The farming traditionally made goose, geese are usually fed pasture-based, are also rarely barley in Türkiye. Based on this point, the effects of the use of food and cracked barley in addition to pasture and pasture were investigated on the growth characteristics of the study. In addition, with this study, it is aimed to develop different maintenance-fattening methods that will make goose breeding more economical and to increase the producer's income in this way.

MATERIALS AND METHODS

Animal Material

The animal material of the study consisted of native Turkish geese. In the study, four groups were formed as feed, pasture + cracked barley, pasture + feed and pasture. The experiment was carried out on 14 males and 14 females of the feed group, 13 males and 14 females of the pasture + cracked barley group, and 13 males and 15 males of the pasture + feed and pasture grups, a total of 111 birds. After being weighed one-d-old goslings were weighed, they were divided into 4 groups according to the sensitive sampling methods.[9] The room temperature was 32-35 °C in the 1 wk of age in fattening period, then gradually decreased by aproximatelly 2-3 °C every 3 days so that at 4 wk of age of goslings the temperature was 18-20 °C. Relative humidity was 50-65%.

Feed Material

Chick starter feed containing 2949 ME, kcal/kg energy and 21.32% crude protein was used in the feeding of birds. Starter feed was given to all geese during the first 6 wk. After 6 wk of age, birds were given 3034 ME, kcal/kg energy and 15.35% crude protein growth feeding to pasture + feed and feed groups (Table 1). The nutrient composition of barley used in the feeding of pasture + cracked barley group was calculated as 2784 ME, kcal/kg energy and 12.57% crude protein (Table 2).10 After the geese were divided into fattening method, they started to be left to pasture from the 6th wk. The first pasture analysis samples were taken to represent the pasture from 7 different points of the pasture on the day the geese were placed in the pasture and mixed and sent to the laboratory (0th, 30th and 65th days) (Table 3).

Method

The study was conducted at the private enterprise in Kars Türkiye. The birds were weighed after hatching and the wing number was fitted. Then they are grouped into 4 groups. Birds were fed at 0-4 wk together, and 5-6 wk divided into groups and fed with starter feed as *ad libitum*. These four group was fed from the 6 wk of age until the 16 wk of age as feed, pasture + cracked barley, pasture + feed and pasture. The geese have been provided with *ad libitum* water. The study started at the 6 wk because the hatched chicks feathers did not develop to protect them from the cold and therefore they might cause problems in living in nature conditions. While geese are in the pasture, the shelters were cleaned daily. For each group, sections of 3.5 m width and 4.5 m length of 15.75 m^2 were prepared and shelters were regularly ventilated. This area is arranged to be at least 0.5 m^2 per goose.

No additional lighting was applied for the lighting of the shelters, and daylight was used. The feed group geese are fed only as *ad libitum* and this group were never taken to

the pasture. Pasture + cracked barley group; It was taken to the pasture at 8.00 in the morning and taken to the shelters from the pasture at 17.00 in the evening, and the geese in this group were given cracked barley as *ad libitum* in their pasture return. Pasture + feed group; It was taken to pasture at 8.00 in the morning and taken to shelters from the pasture at 17.00 in the evening. This group was given feed as *ad libitum* on pasture return. The pasture group was left to the pasture at 8.00 in the morning and remained in the pasture until the evening and were taken inside at 17.00 in the evening. No additional feeding was made to the pasture group.

Table 1. Ingredient and analysis nutrients of the concentared fed

Ingredients, %	Starter feed	Finisher feed
Wheat	20.00	36.00
Corn	34.58	39.00
Vegetable oil	3.50	2.00
Soybean meal	22.00	8.63
Sunflower meal	8.51	6.00
Cottonseed meal	8.00	5.00
Antioxidant	0.09	0.09
Di-calcium phosphate	1.50	1.50
DL-methionine	0.25	0.20
Limestone	0.55	0.55
L-lysine hydrochloride	0.09	0.10
L-tryptophan	0.08	0.08
Sodium bicarbonate	0.20	0.20
Salt	0.30	0.30
Vitamin-mineral mix*	0.35	0.35
Dry matter	90.10	89.76
Crude protein	21.32	15.35
Crude fat	4.51	3.46
Crude fiber	8.40	8.58
Ash	5.72	4.61
Calcium	0.70	0.65
Available Phosphorous	0.37	0.35
Sodium	0.24	0.25
Methionine+cysteine	0.88	0.71
Lysine	1.06	0.67
Threonine	0.75	0.50
Tryptophan	0.35	0.25
Linoleic acid	2.77	1.92
ME, kcal/kg**	2949.30	3034.18

*: Premix provided the following per kg of basal diet: Vit A 1000000 IU; Vit D3 200000 IU; Vit E 1.0 g; Fe 3.0; Mn 2.4 g; Cu 0.45 g; Co 0.015 g; Zn 4.5 g; I 0.06 g; Se 0.015 g; Ca 21.99 g. **: It was found by calculation. ME: Metabolizable energy

Table 2. Nutritient value of cracked barley

Nutrients	%
Dry matter	92.50
Crude protein	12.57
Crude fat	2.24
Crude fiber	5.00
Ash	1.95
Sugar	5.05
Starch	48.00
N-free substance	70.74
Calcium	0.07
Phosphorous	0.13
Methionine+cystine	0.47
Lysine	0.43
Tryptophan	0.15
Arginine	0.17
Threonine	0.36
ME, Kcal/kg*	2784.00

*: It was found by calculation. ME: Metabolizable energy

Table 3. Nutrient value of Pasture

Parameters%	d 1	d 30	d 65	Analysis Methods
Dry matter	19.05	27.7	25.04	1974 RG14987
Crude protein	13.70	13.00	13.44	TS 4717 ISO 5983
Crude fat	3.05	2.67	2.80	1974 RG14987

Statistical Analysis

The statistical analyses of the study data were performed using the IBM SPSS v. 23 software package. The General Linear Model (GLM) detailed below in statistical notation was used for body weight and daily weight gain of geese. According to this model; Yijk = μ + ai + bj + a * bij + eijk equation is created. Model;

Yijk: Yield value of any geese examined,

μ: Means of population,

ai: Fattening method (i: 1-4; Feed, Pasture + Cracked barley, Pasture + Feed, Pasture),

bj: Sex (j: 1-2; Male, Female)

a * bij: Interaction between fattening method*sex

eijk: It is the error term.

Duncan test was used to compare the examined significant factors (SPSS 23.0).

RESULTS

Body weight means and standard errors by fattening method and sex are presented in Table 4. The mean hatching weight were determined as 92.71 g and averages 16 wk of age weight was determined as 4132 g. The mean body weights in final body weights of feed, pasture + cracked barley, pasture + feed and pasture groups were found 4209, 4108, 4239 and 3971 g, respectively. It was

observed that the mean weight obtained in pasture + feed and feed groups at 14 and 16 wk of age were higher than pasture + cracked barley and pasture groups. There was no significant difference between fattening method x sex interactions (P>0.05) (Table 4).

The daily weight gain means and standard errors by fattening methods and sex are shown in Table 5. The highest average daily weight gain from the beginning of the study to the end was found 73.88 g in 5-6 wk, and the lowest average daily weight gain was found 8.57 g in 14-16 wk of age. There were statistically significant differences between the sex between 4-5, 5-6, 6-7, 8-9 and 9-10 wk of age in terms of daily weight gain averages (P<0.05-P<0.01). The highest daily weight gains in feed and pasture + feed groups were found 5-6 wk of age, in pasture + cracked barley group was found 3-4 wk of age, in pasture group was found 4-5 wk of age (Table 5).

Table 4. Body weight means and standard errors by fattening method and sex (g)

Traits	n	Hatching	1 wk	2 wk	3 wk	4 wk	5 wk	6 wk
Overall	111	92.71±0.61	269.94±1.91	559.11±4.62	957.38±10.45	1442±19.82	1947±24.36	2464±24.80
Fattening method								
Feed	28	93.88±1.23	265.36±3.80	541.68±9.19	956.61±20.78	1444±39.41	1936±48.42	2473±49.46
Pasture+cracked barley	27	92.70±1.25	264.54±3.88	551.51±9.37	942.53±21.18	1469±40.16	1976±49.34	2467±50.41
Pasture + feed	28	91.89±1.23	278.37±3.82	589.97±9.21	982.42±20.83	1439±39.50	1933±48.54	2457±49.59
Pasture	28	92.37±1.23	271.51±3.81	553.27±9.20	947.96±20.84	1417±39.51	1942±48.55	2459±49.60
Sex								
Male	53	93.37±0.90	274.15±2.77	572.73±6.68	986.32±15.11	1496±28.66	2025±35.21	2580±35.97
Female	58	92.05±0.85	265.74±2.65	545.49±6.39	928.44±14.45	1388±27.40	1869±33.60	2348±34.39
Fattening method							0.920	0.996
Sex							0.002	<0.001
Fattening method X Sex							0.976	0.857

Traits	n	7 wk	8 wk	9 wk	10 wk	12 wk	14 wk	16 wk
Overall	111	2870±23.36	3168±24.52	3408±26.20	3627±27.20	3855±27.94	4012±27.86	4132±27.23
Fattening method								
Feed	28	2904±46.44	3209±48.74	3458±52.08	3693±54.07	3903±55.55	4085±55.38[a]	4209±53.92[a]
Pasture+cracked barley	27	2846±47.32	3149±49.67	3354±53.08	3547±55.10	3771±56.61	3954±56.43[ab]	4108±55.16[ab]
Pasture + feed	28	2861±46.56	3165±48.88	3432±52.22	3663±54.21	3912±55.69	4105±55.52[a]	4239±54.26[a]
Pasture	28	2870±46.55	3151±48.87	3388±52.21	3606±54.20	3836±55.69	3903±55.51[b]	3971±54.27[b]
Sex								
Male	53	3016±33.77	3332±35.45	3591±37.88	3832±39.32	4089±40.40	4240±40.27	4371±39.36
Female	58	2724±32.29	3004±33.88	3225±36.21	3423±37.59	3622±38.62	3775±38.50	3893±37.63
Fattening method		0.840	0.806	0.518	0.252	0.256	0.028	0.003
Sex		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Fattening method X Sex		0.256	0.101	0.233	0.109	0.198	0.394	0.533

a, b: Differences in in superscript letters within columns represent significant differences between groups (P<0.05).

In the 4-16 wk of age, daily weight gain (ADG, g), daily feed consumption (ADFD, g) and feed conversion ratio (FCR, %) of all geese in the study are presented in Table 6. The average ADG were determined as 39.81, 37.46 and 39.90 g, respectively; ADFD as 426.95, 207.99 and 200.76 g, respectively; FCR as 10.72, 5.55 and 5.03, respectively in feed, pasture + cracked barley, pasture + feed groups (Table 6).

Table 5. Daily weight gain means and standard errors by fattening method and sex (g)

Traits	n	0-1 wk	1-2 wk	2-3 wk	3-4 wk	4-5 wk	5-6 wk	6-7 wk
Overall	111	25.32±0.28	41.31±0.67	56.90±1.44	69.20±1.63	72.22±1.72	73.88±1.67	58.04±1.70
Fattening method								
Feed	28	24.50±0.59	39.47±1.33	59.28±2.86	69.59±3.25	70.36±3.42	76.73±3.31	61.60±3.38
Pasture+cracked barley	28	24.55±0.57	41.00±1.35	55.86±2.92	75.09±3.31	72.63±3.48	70.14±3.37	54.12±3.44
Pasture + feed	27	26.64±0.56	44.52±1.33	56.07±2.87	65.11±3.26	70.78±3.43	74.90±3.32	57.62±3.38
Pasture	28	25.59±0.56	40.26±1.32	56.38±2.87	67.02±2.25	75.12±3.42	73.75±3.32	58.81±3.39
Sex								
Male	53	25.82±0.40	42.66±0.96	59.09±2.08	72.76±2.36	75.70±2.48	79.24±2.41	62.39±2.46
Female	58	24.81±0.39	39.96±0.92	54.71±1.99	65.64±2.26	68.75±2.37	68.52±2.30	53.69±2.35
Fattening method						0.752	0.561	0.484
Sex						0.046	0.002	0.012
Fattening method X Sex						0.683	0.240	0.078

Traits	n	7-8 wk	8-9 wk	9-10 wk	10-12 wk	12-14 wk	14-16 wk
Overall	111	42.59±1.35	34.23±1.11	31.32±0.99	16.30±0.62	11.17±0.38	8.57±0.28
Fattening method							
Feed	28	43.55±2.69	35.55±2.21^{ab}	33.57±1.97	15.02±1.22	12.98±0.76^{a}	8.90±0.55^{b}
Pasture+cracked barley	28	43.26±2.74	29.35±2.25^{b}	27.60±2.01	15.94±1.25	13.11±0.77^{a}	10.97±0.56^{a}
Pasture + feed	27	43.46±2.61	38.11±2.20^{a}	33.00±1.98	17.78±1.23	13.83±0.76^{a}	9.59±0.55^{b}
Pasture	28	40.07±2.60	33.93±2.21^{ab}	31.10±1.99	16.46±1.22	4.77±0.75^{b}	4.84±0.55^{c}
Sex							
Male	53	45.14±1.96	36.96±1.60	34.39±1.43	18.36±0.89	11.39±0.55	8.76±0.40
Female	58	40.03±1.87	31.52±1.53	28.25±1.37	14.24±0.85	10.96±0.53	8.39±0.38
Fattening method		0.762	0.048	0.148	0.452	0.001	0.001
Sex		0.062	0.016	0.003	0.001	0.578	0.499
Fattening method X Sex		0.406	0.209	0.170	0.477	0.009	0.001

a,b,c: Differences in in superscript letters within columns represent significant differences between groups (P<0.05).

Table 6. ADG, ADFD and FCR by fattening methods (4-16 wk)

Wk	Feed			Pasture + Cracked Barley			Pasture + Feed		
	ADG	ADFD	FCR	ADG	ADFD	FCR	ADG	ADFD	FCR
4-5	70.36	374.49	5.32	72.63	163.00	2.24	70.78	124.42	1.76
5-6	76.73	461.73	6.02	70.14	196.47	2.80	74.90	147.46	1.97
6-7	61.60	421.29	6.84	54.12	200.02	3.70	57.62	183.27	3.18
7-8	43.55	446.85	10.26	43.26	216.40	5.00	43.46	200.51	4.61
8-9	35.55	476.48	13.40	29.35	229.53	7.82	38.11	196.01	5.14
9-10	33.57	459.81	13.70	27.60	237.73	8.61	33.00	218.58	6.62
10-12	15.02	425.67	28.34	15.94	202.44	12.70	17.78	262.30	14.75
12-14	12.98	368.86	28.42	13.11	208.63	15.91	13.83	226.66	16.39
14-16	8.90	407.36	45.77	10.97	217.71	19.85	9.59	247.61	25.82
4-16	39.81	426.95	10.72	37.46	207.99	5.55	39.90	200.76	5.03

ADG: daily weight gain (g), ADFD: daily feed consumption (g), FCR: feed conversion ratio (%)

DISCUSSION

In the study, the most appropriate fattening method was tried to be determined by giving pasture, pasture + cracked barley, pasture + feed and feed in geese cultivated by almost every family, and the possibilities of saving fattening period and labor. Also in this study, the effects of barley on the growth characteristics of the region as a traditional method of goose breeding were investigated.

The differences between body weight averages at 14 and 16 wk of age were statistically significant (P<0.05-P<0.01). The average hatching weight of geese is 92.71 g, the average hatching weight of male geese is 93.37 g and the

average hatching weight of female geese is 92.05 g. Male geese have a heavier body weight than female geese since the first weeks. Saatcı et al.[3] in a study on the effects of sex, color and fattening period on body weights of native Turkish geese; the results of this study are similar. Similarly, the results obtained in the study were similar to the values reported by Knizetova et al.[11] in Bohemian, Italian White and their hybrids. In the 4th wk of the study, the mean body weight of geese was similar to that reported by Ünal et al.[12] determined in geese fed with rations containing different protein levels, and Aksu and Kaya[13] determined in geese fed with 4 different rations with the same level of energy.

In the 8 wk of the study, the highest body weight was determined in the feed group, and the averages of the other 3 feed groups were quite close to each other. The difference between the body weight of male geese and female geese is gradually increasing. Average body weights were lower than similar studies.[14-16.] Again, in the 10 wk of the study, the highest body weight was determined only in geese fed to the shaft. This is followed by the feed + pasture group. Body weights of 10th wk of age male and female geese was similar to that reported by Tilki et al.[17], and was lower than that reported by Aksu and Kaya[13], Eroglu and Erisir[18.] This value was found to be higher than that detected by Tilki et al.[8], Şahin et al.[19] and Rayan et al.[20.]

In the 14 and 16 wk of the study, the highest body weight was determined in the feed and feed + pasture group and it was found statistically significant in the differences between the groups. The difference between the body weights of male geese and female geese has been further expanded. On the 16 wk of the study, the mean body weights were higher than that reported by Tumova and Uhlirova[21] for Czechoslovakian geese and Akbaş et al.[22] for Lindovskaya geese, but lower than that determined Kucharska-Gaca et al.[23] and Lin et al.[24] for White Koluda geese. This result was similar to that reported by Tilki et al.4, Boz and Sarica[25] for Native Turkish geese. Guy et al.[26] reported that body weights were 4784 and 5602 g in 119 days, 5993 and 6074 g in 168 days for geese fed with pasture and concentrate feed. The body weights of the geese in the pasture and concentrate feed fattening group are close to each other and this result is similar to with our study.

It was determined that the ADG continued to increase until the 6 wk and started to decrease after this week. When the ADG between the groups are evaluated, the best ADG was determineted in the group that only consumes feed in 5-6 wk of age. Secondly, it was determined geese in the pasture + feed group in all weeks. Similar results, Lui et al.[16] found the highest body weight increase in grinded and grain-fed geese between 29-49 days. Tilki et al.4 reported that the increase in ADG after 8th wk showed a decreasing tendency, but in this study, it was observed that ADG decreased in all groups starting from the 6th week.

The highest average ADFD in the fattening method was found to in 8-9th wk geese. The geese in the pasture + cracked barley and pasture + feed groups consumed 51.30% and 52.98% less feed than geese consuming only feed in general. In the 5-6 wk of the study, ADFD was made in a similar study by Arroyo et al.[27] was found close to the value determined fed with pellet feed on the 44 day. Average ADFD values in pasture + cracked barley and pasture + feed groups were found to be lower than that reported by Arroyo et al.27 the values that the geese fed with pellet feed determined in 53-55 days. These results were higher than that reported by Uhlirova et al.[28] for Czech and hybrid Novohdradska geese, Abou-Kassem et al.[29] for Egyptian geese and Mancinelli et al.[30] for Romagnola geese. In the present study, ADFD values determined for pasture + cracked barley, pasture + feed group were found to be similar to the values determined by Lui et al.16, Wang et al.[31]. These results are similar to those reported for dry feed and wet feed geese by Liu et al.32, for sorghum dried distillers grains with solubles geese by Wang et al.[31], who found that feed conversion ratio between 3.87-4.81 and feed intake between 193.0-239.3 g.

The best feed conversion ratio was determined in the pasture + feed and pasture + cracked barley group, which was higher in the feed group. In the study, the rate of FCR for pasture + cracked barley and pasture + feed group was similar to the values reported by Mancinelli et al.[30], Elminowska et al.[33], and lower than those reported by Arroyo et al.[34], Chen et al.[35]. In adition, FCR in all feed groups were found to be lower than the value determined by Wang et al.[36] for Sichuan white geese and Ölmez et al.[37] for native Turkish Geese. Aslan and Oztürk[38] stated that by using rouhghage isntead of some of the concentrated feed in geese diets, savings in feed costs can be achieved and geese can be produced more economically.

It is seen that there are some differences between the body weight, ADG increase and FCR values determined in the study and other study results. Factors such as breed, origin, age, care, fattening type and fattening period can be counted as the reason for these differences.

In Türkiye, goose breeding is generally made only on pasture. Large businesses are almost non-existent. In this form of breeding, feed and labor expenses are minimized and geese that can use the pasture well are fed in this way

until the slaughter season.

As a result, the average body weight of geese was found to be similar with some literature data, which is lower than some literature data. This may be due to the fact that the geese used in the study are native Turkish geese and no selection studies have been performed on them. Although the highest body weight increase was determined in the pasture + feed group, it was concluded that it would be more appropriate to make pasture feed since the geese fed economically in the pasture provide body weight increase similar to other groups. It was determined that feeding with only feed is not economical for goose breeding and does not provide much ADG compared to other groups.

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Investigation of the Effects of Hesperidin on Bisphenol-A Induced Neurotoxicity in Rats

Ratlarda Bisfenol-A'nın Neden Olduğu Nörotoksisite Üzerine Hesperidinin Etkilerinin Araştırılması

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ABSTRACT

Bisphenol A (BPA) is an adhesive substance used in the production of food packaging, electronic devices, dental sealants and polycarbonate plastics. This substance, which can leak into products during industrial processes, can be taken into the body through contact or consumption. BPA causes oxidative damage in the body and toxicity to organs. This study was conducted on 52 male rats. The rats were randomly distributed into 4 separate groups, with 13 animals in each. Experiment groups were formed as follows: Control: 1 ml of olive oil was administered intragastrically for 14 days. Hesperidin (HESP): HESP was administered intragastrically at a dose of 50 mg/kg for 14 days. BPA: BPA dissolved in olive oil was administered intragastrically at a dose of 100 mg/kg for 14 days. BPA+HESP: BPA at a dose of 100 mg/kg and HESP at a dose of 50 mg/kg were administered intragastrically for 14 days. Brain tissue samples from the rats were collected on the 15th day of the experiment while the rats were under sevoflurane anesthesia. Histopathological and biochemical analyzes were performed on the brain tissues of the rats. As a result of the study, it was observed that HESP had a protective effect on BPA-induced neurotoxicity in rats and triggered the antioxidant mechanism responsible for defense in the cell. It was opined that the degenerative and necrotic tissue damage caused by BPA in the brain tissue decreased with the effect of Hesperidin.

Keywords: Bisphenol A, hesperidin, MDA, neurotoxicity, rat

ÖZ

Bisfenol A (BPA), gıda ambalajı, elektronik cihazlar, diş dolguları ve polikarbon plastiklerin üretiminde kullanılan yapışkan bir maddedir. Bu madde, endüstriyel işlemler sırasında ürünlere sızabilir ve temas veya tüketim yoluyla vücuda alınabilir. BPA, vücutta oksidatif hasara ve organlara toksisiteye neden olabilir. Bu çalışma, 52 erkek sıçan üzerinde gerçekleştirilmiştir. Sıçanlar, her birinde 13 hayvan bulunan 4 ayrı gruba rastgele dağıtılmıştır. Deney grupları şu şekilde oluşturuldu: Kontrol: 1 ml zeytinyağı, 14 gün boyunca intragastrik olarak uygulandı. BPA maruz kalan gruplara HESP dozunda 100 mg/kg intragastrik uygulama ile verildi. HESP: HESP, 50 mg/kg dozunda 14 gün boyunca intragastrik olarak uygulandı. BPA: BPA, zeytinyağında çözülmüş olarak 100 mg/kg dozunda 14 gün boyunca intragastrik olarak uygulandı. BPA+HESP: BPA, 100 mg/kg dozunda ve HESP, 50 mg/kg dozunda 14 gün boyunca intragastrik olarak uygulandı. Deneyin 15. gününde, sıçanlar sevofluran anestezisi altındayken sıçanlardan beyin dokusu örnekleri toplandı. Sıçanların beyin dokularında histopatolojik ve biyokimyasal analizler yapıldı. Çalışma sonucunda, HESP'nin BPA tarafından indüklenen nörotoksisite üzerinde koruyucu bir etkisi olduğu ve hücre savunmasından sorumlu antioksidan mekanizmayı tetiklediği gözlemlendi. BPA'nın beyin dokusunda neden olduğu dejeneratif ve nekrotik doku hasarının, HESP'nin etkisi ile azaldığı düşünülmüştür.

Anahtar Kelimeler: Bisfenol A, hesperidin, MDA, nörotoksisite, rat

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Atıf: Bolat M, Laçin BB, Çelebi F. Ratlarda Bisfenol-A'nın Neden Olduğu Nörotoksisite Üzerine Hesperidinin Etkilerinin Araştırılma. *Vet Sci Pract*. 2024;19(1):17-24.

INTRODUCTION

BPA is a widely used fabrication chemical that is a colorless, crystalline solid with a distinct phenolic odor. It is commonly used in the production of food packaging, electronic devices, building materials, dental filling materials, toys, plastic, and feeding bottles.[1-6] BPA has become a global environmental pollutant because of its widespread use in industrial production and contact with food products.[7,8] BPA is recognized as an endocrine disruptor due to its ability to disrupt the regular functioning of hormones within the body. By interfering with hormones, BPA has the potential to cause a range of health issues, including reproductive disorders, developmental problems, and metabolic disorders. This substance, taken into the body orally and through the respiratory tract, interacts with many receptors in the hormonal system and causes severe damage to the endocrine system. As a result of these damages, toxicities are observed in the brain, liver, and other organs.[9] It has been established that BPA can induce neuropsychological and behavioral disorders by crossing the blood-brain block.[10] Exposure to neurotoxic substances such as BPA leads to the oxidation of the protein and lipid structures of cells in the brain, resulting in the overproduction of reactive oxygen and nitrogen molecules (ROS and RNS, respectively).[11-13] The increase in oxygen radicals and RNS leads to the activation of apoptotic pathways, which result in cell death by damaging the cell's organelles, macromolecules, and membrane structure. Chronic exposure triggers an inflammatory process in the brain that contributes to neurodegenerative diseases. Against this damage, brain tissue has various antioxidant defense mechanisms created by different enzymes, such as superoxide dismutase (SOD). Glutathione in its reduced form (GSH) is one of the critical internal antioxidants found in brain tissue and possesses strong scavenging properties against hydroxyl radicals (OH.).[14] Alongside these protective mechanisms, researchers are working on alternative approaches to mitigate the current side effects.[15] An example of such an approach involves utilizing safe, low-side-effect, and readily available herbal antioxidants in the treatment process. In recent experimental studies, HESP, a compound in the flavonoid group found in citrus fruits, green tea, and some vegetables, has been investigated for its antioxidant effects to eliminate and treat the toxic effects of BPA.[16-20] It has been reported that this compound, frequently utilized in both industry and cosmetics, possesses numerous properties, including anticarcinogenic, antiallergic, neuroprotective, immunomodulatory, and anti-diabetic effects. The objective of this study was to investigate the protective effects of HESP in BPA-induced neurotoxicity in rats and to contribute to the literature in line with the data obtained.

MATERIALS AND METHODS

Chemicals

BPA (≥99%) (Cas No: 80-05-7) and HESP (Cas No: 520-26-3) were purchased from Sigma-Aldrich Co. (St Louis, MO, USA). Malondialdehyde (MDA) (Cat No: SL0475Ra), Superoxide Dismutase (SOD) (Cat No: SL0664Ra), Glutathione (GSH) (Cat No: SL0998Ra), and Nitric Oxide (NO) (Cat No: SL0531Ra) commercial ELISA kits were purchased from SunLong Biotech Co.LTD.

Animals

The study utilized experimental animals sourced from the Medical Experimental Research and Application Center at Atatürk University. We used 52 male Sprague Dawley rats with an average weight of 250-300 g (2.5-3 months old). The animals in the experimental groups had unrestricted access to meet their daily nutritional and water requirements. This study was approved by the Animal Ethics Committee of Animal Experiments of the Veterinary Faculty at Atatürk University (18.08.2022/182).

Experimental Protocol

All rats were weighed, and by random assignment, four different experimental groups were formed, each consisting of 13 rats. Active substance applications were made to the experimental groups at 9:00 for 14 days. BPA application was made 1 hour after the HESP application. Experiment groups were formed as follows: Control: 1 ml of olive oil was administered intragastrically for 14 days. HESP: HESP was administered intragastrically at a dose of 50 mg/kg[21,22] for 14 days. BPA: BPA dissolved in olive oil was administered intragastrically at a dose of 100 mg/kg[22,23] for 14 days. BPA+HESP: BPA at a dose of 100 mg/kg and HESP at a dose of 50 mg/kg were administered intragastrically for 14 days.

Collection and Homogenization of Brain Tissues

On the 15th day of the experiment, the rats were euthanized under sevoflurane anesthesia by decapitation. Tissues were stored at -80°C. Tissue sections taken in equal amounts from brain tissues on the analysis day were transferred to capped tubes. It was completed with 1.5 ml of PBS (Phosphate buffered saline) solution with a pH of 7.4. Tubes were placed in the homogenizer device to perform the homogenization process. Afterward, the tissues were homogenized for 80 seconds at 5,000 rpm, followed by centrifugation at 7,000 rpm for 5 minutes. The

resulting supernatants were then carefully transferred into clean tubes.

Assessment of MDA levels, NO quantity, SOD and GSH enzyme activities in brain tissue:

The necessary analyzes for the determination of MDA levels, NO quantity, SOD and GSH activities in rats were performed according to the protocol using commercial rat ELISA kits. In line with the data obtained, evaluations were made between the groups.

Histopathological Examination

Upon the culmination of the evaluation, brain tissue specimens underwent fixation in a meticulous 10% formaldehyde solution for a duration of 48 hours. Subsequently, they were delicately enshrined within paraffin blocks through the standard course of tissue processing. Precise sections, each measuring a mere 4 µm in thickness, were meticulously extracted from every block. These sections were then meticulously prepared for histopathological scrutiny through a nuanced staining process employing hematoxylin-eosin (HE). The resulting slides were subjected to intense examination utilizing a luminous microscope of unparalleled quality (Olympus BX 51, JAPAN). The assessments were conducted with a discerning eye, discerning the histopathological nuances, and categorizing the sections based on their distinct characteristics: absent (-), mild (+), moderate (++), or severe (+++).

Statistical Analysis

After the completion of the studies, the statistical analysis of more than two independent groups was conducted using one-way ANOVA within the SPSS 20.00 (IBM SPSS Corp., Armonk, NY, USA) statistical data software. Subsequently, Tukey test was utilized for the acquisition and evaluation of quantitative values. The resulting values were presented as mean ± standard error of the mean (± SEM), and statistical significance was defined as $P < .05$. For histopathological analyses Kruskal Wallis test was used for comparison between groups, and Mann Withney U test was used for comparison of paired groups.

RESULTS

Biochemical Analyses

This study investigated the possible effects of Hesperidin on BPA-induced neurotoxicity in rats. The administration of BPA resulted in a notable increase in the MDA levels within the brain tissues when compared to the control group ($P < .05$). HESP demonstrated a significant reduction in the BPA-induced increase of MDA ($P < .05$). The MDA level in the HESP group was observed to be comparable to that of the control group. (Figure 1).

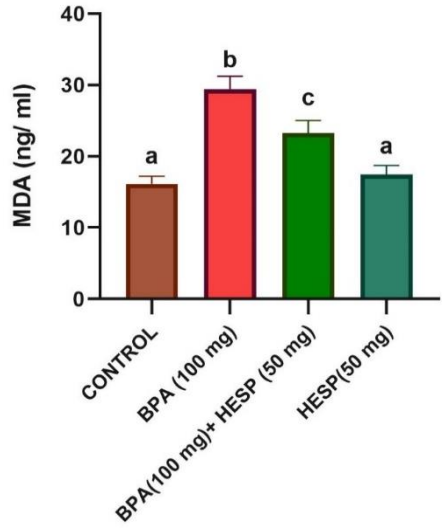

Figure 1. Representation of MDA levels in brain tissues taken from rats. Results were expressed as mean ± SD. Different letters indicate statistical differences between groups (P<0.05, n=8). SD: standard deviation

The introduction of BPA led to a pronounced decline in SOD activity within cerebral tissues, showcasing a marked difference from the control group ($P < .05$). Notably, HESP effectively thwarted the BPA-induced reduction in SOD activity, demonstrating a significant protective effect ($P < .05$). Furthermore, SOD activity levels in the HESP group were observed to closely mirror those of the control group, with no statistically significant difference noted ($P > .05$) (Figure 2).

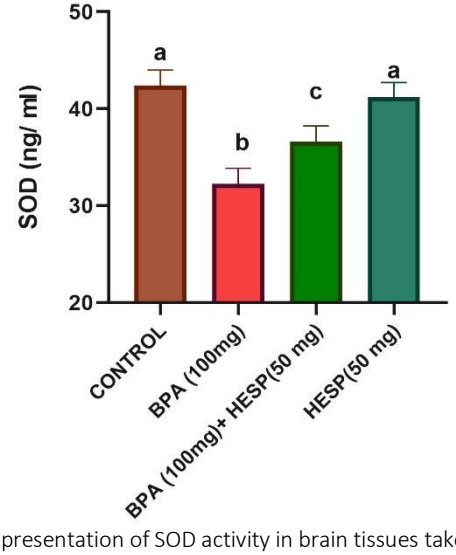

Figure 2. Representation of SOD activity in brain tissues taken from rats. Results were expressed as mean ± SD. Different letters indicate statistical differences between groups (P<0.05, n=8). SD: standard deviation

The administration of BPA markedly decreased the activity of glutathione (GSH) in brain tissues compared to the control group ($P < .05$). However, the detrimental effects of BPA on GSH activity were effectively alleviated by HESP ($P < .05$). Remarkably, the GSH activity in the HESP group did not exhibit a significant difference from those in the control group ($P > .05$) (Figure 3).

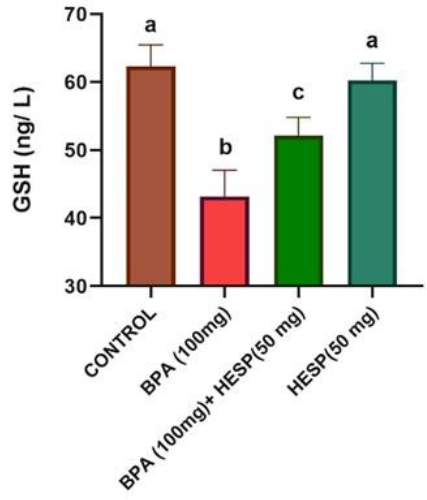

Figure 3. Representation of GSH activity in brain tissues taken from rats. Results were expressed as mean ± SD. Different letters indicate statistical differences between groups (P<0.05, n=8). SD: standard deviation

The administration of BPA led to a significant elevation in the NO levels within brain tissues compared to the control group ($P < .05$). HESP effectively mitigated the BPA-induced increase in NO ($P < .05$). Notably, the NO levels in the HESP group were comparable to those in the control group ($P > .05$) (Figure 4).

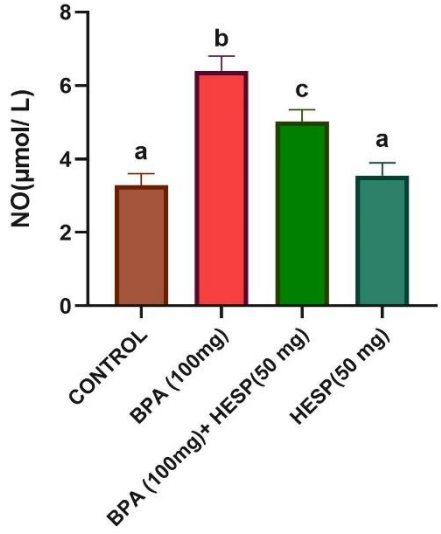

Figure 4. Representation of NO activity in brain tissues taken from rats. Results were expressed as mean ± SD. Different letters indicate statistical differences between groups (P<0.05, n=8). SD: standard deviation

Histopathological Findings

Control and HESP: When the brain tissues were examined histopathologically, normal histological appearance was detected.

BPA: Significant neuronal degeneration and necrosis, along with pronounced vascular hyperemia, were evident.

BPA+HESP: Moderate vascular hyperemia was noted, accompanied by moderate neuronal degeneration and mild necrosis (Figure. 5). A statistically significant difference was observed compared to the BPA group ($P < .05$). The histopathological findings are presented in Table 1.

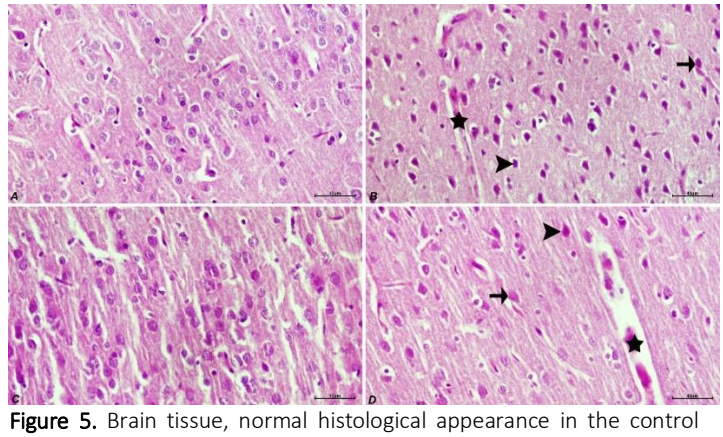

Figure 5. Brain tissue, normal histological appearance in the control group (A) and HESP group (C), degeneration (arrow) and necrosis (arrowhead) in BPA group (B) and BPA+HESP (D) group neurons, hyperemia in vessels (star), H&E, Bar: 40µm.

Table 1. Histopathological findings and scoring in brain tissue

Groups	Degeneration in neurons	Necrosis in neurons	Hyperemia in the veins
Control	-	-	-
BPA	+++	+++	+++
HESP	-	-	-
BPA+HESP	++	+	++

(-): absent, (+): mild, (++): moderate, (+++): severe.

DISCUSSION

BPA, widely used in the industrial field, is a substance that seriously harms health. Hence, individuals are consistently exposed to BPA in their everyday lives. Both in vivo and in vitro research have demonstrated the accumulation of BPA in various tissues, leading to the onset of diseases.[24-28] Research indicates that BPA induces oxidative stress, can

modify neurogenesis, and leads to neurological damage and cognitive disorders in organisms.[29,30] In this study, rats were selected to evaluate the existing complications in animals exposed to environmental toxic substances such as BPA. The objective was to evaluate HESP protective potential against BPA-induced neurotoxicity through biochemical and histopathological assessments.

HESP, a derivative of flavonoid, boasts various pharmacological advantages, notably encompassing significant anti-inflammatory and antioxidant properties. HESP has demonstrated noteworthy efficacy in alleviating inflammation, relieving pain, combating fungal and viral infections, and exhibiting potent antioxidant and anticancer activities.[31-33] Furthermore, recent studies indicate that HESP may be beneficial in neurodegenerative diseases, psychiatric disorders, demyelinating diseases, as well as ischemic-reperfusion injury and neuroinflammatory conditions.[34-36] Building upon these critical insights, we delved into investigating the potential protective effects of HESP against oxidative stress induced by BPA induced brain damage. This study signifies that HESP could play a crucial role in maintaining the health of brain tissue and mitigating the effects of oxidative stress.

The first indicator of cellular damage is the peroxidation of lipids in the cell membrane. MDA is one of lipid peroxidation's main products, reflecting the degree of membrane damage. A few studies have noted an elevation in MDA levels in specific rat tissues due to BPA exposure.[37,38] The study conducted by Abdou et al., investigates the neurotoxicity induced by BPA in male rats. The research reveals that administering specific doses of BPA to rats leads to a significant increase in the levels of MDA in brain tissues.[39] In the study conducted by Morsy et al., it was observed that the levels of MDA increased, indicating heightened oxidative stress in the hippocampus following exposure to BPA in rats.[40] These findings underscore the deleterious impact of BPA on hippocampal neurotoxicity and memory function. Our findings demonstrate that the application of HESP mitigated the increase in MDA levels induced by BPA-related neurotoxicity. Previous studies have found that HESP exhibits neuroprotective properties and supports the clearance of free radicals, leading to a reduction in lipid peroxidation products.[41-43] This is beneficial in terms of reducing oxidative stress.

An effective endogenous antioxidant defense mechanism neutralizes oxidative stress in the body.[44] Superoxide dismutase (SOD) is an essential endogenous antioxidant that reduces superoxide radicals in the cell and forms the first line of defense against oxidative damage. One of the antioxidants required for converting this harmful hydrogen peroxide into water and molecular oxygen is GSH. GSH, a crucial non-enzymatic antioxidant, is a tripeptide composed of cysteine, glycine amino acid, and glutamic acid. It is involved in the inhibition of lipid peroxidation. Some studies have shown that exposure to BPA reduces SOD and GSH activities in brain tissue. The research carried out by Ishtiaq and colleagues delves into the neurotoxic effects induced by BPA in male rats. The study uncovers that the administration of specific doses of BPA to rats results in a notable decrease in the activity of SOD and GSH in brain tissues.[45] Similarly, in a study conducted by T. Geetharathan on pregnant rats, a decrease in the activity of antioxidant enzymes SOD and GSH was observed in BPA-induced brain damage.[46] Consistent with previous research, our study compellingly demonstrates that the co-administration of hesperidin and BPA is significantly associated with an increase in SOD and GSH activities in the brain tissue of rats. On the other hand, studies have reported that HESP improves cognitive and motor functions by increasing the levels of antioxidant enzymes in the brain tissue and sera of rats.[41-43]

Nitric oxide (NO) reacts with the superoxide radical ($O2^{\cdot-}$) and turns into an oxidant factor called peroxynitrite. Peroxynitrite can react with the cell's DNA, lipid, and protein structures and inactivate the antioxidant forms GSH and GPx. Studies have shown that it increases NO activity due to BPA application in brain tissues. In the study conducted by Ayazgök and colleagues, it was observed that the exposure to BPA in SH-SY5Y neuroblastoma cells resulted in an increase in NO activity.[47] Furthermore, according to the study conducted by Xinyu Li and colleagues, it was observed that Hesperidin inhibits nitric oxide production in LPS-stimulated BV-2 microglial cells.[36] In alignment with these findings, research by Li C and the team demonstrated that Hesperidin suppresses nitric oxide production in the RAW246.7 macrophage cell line. These instances underscore the anti-inflammatory potential of Hesperidin in modulating nitric oxide levels in different immune cell types.[48] Consistent with prior research, our study convincingly demonstrates that the simultaneous application of HESP and BPA resulted in a significant reduction in NO levels in the brain tissue of rats.

It has been demonstrated in some experimental studies that BPA causes neuronal damage in brain tissue.[39,40] In this study, it was determined histopathologically that BPA caused severe degenerative and necrotic damage to

neurons in the brain tissue. It has been reported that HESP has protective activity against the toxic effects of BPA in experimental studies conducted in various tissues. It has been demonstrated by this study that Hesperidin, which is used against the neurotoxic effect of BPA, also protects the brain tissue against this toxic effect. As a result of the study, it was demonstrated histopathologically that HESP also has a protective effect in brain tissue against BPA toxicity.

In conclusion, this study was conducted to investigate the effects of HESP on BPA-induced neurotoxicity in brain tissues, some biochemical parameters and histopathological changes. With this research, it has been shown that Hesperidin successfully reverses BPA-induced changes in oxidative stress, changes in biochemical parameters, and inflammation. It has also been observed that Hesperidin prevents tissue damage by inhibiting lipid peroxidation and activating antioxidant enzymes.

Ethics Committee Approval: Animal Ethics Committee of Animal Experiments of the Veterinary Faculty at Atatürk University (18.08.2022/182)

Peer-review: Externally peer-reviewed.

Author Contributions: Concept -M.B., F.Ç.; Design-M.B., F.Ç.; Supervision-M.B.; Resources- B.B.L.; Data Collection and/or Processing-M.B.; Analysis and/or Interpretation-M.B.; Literature Search-M.B.; Writing Manuscript-M.B., F.Ç.; Critical Review-M.B., F.Ç.

Declaration of Interests: The authors declare that there is no conflict of interest.

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Hakem Değerlendirmesi: Dış bağımsız.

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Research Article *Araştırma Makalesi* DOI: 10.17094/vetsci.1471538

The Intensity of Lipid Peroxide Oxidation Processes and the System State of Antioxidant Protection of Broiler Chicken Due to the Action of the Synbiotic Preparation in Complex with the Disinfectant

Lipid Peroksit Oksidasyon Proseslerinin Yoğunluğu ve Dezenfektanla Kompleks İçinde Sinbiyotik Preparatın Etkisine Bağlı Broyler Tavuğun Antioksidan Korumasının Sistem Durumu

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ABSTRACT

Developing methods for increasing the immune reactivity and antioxidant potential of the bird's body during critical periods of growth is an urgent task today. The purpose of the research was to find out the influence of the synbiotic "Biomagn" in combination with the disinfectant "Diolide" on the intensity of the processes of peroxide oxidation of lipids and the activity of the system of antioxidant protection in the organism of chickens. The research was carried out on 2 groups of chickens, 100 in each, starting from 1 to 41 days of age: the control group was fed with standard compound feed (SCF); the chickens of the experimental group were fed with SCF, and the synbiotic preparation "Biomagn" based on 0.5 kg per ton of compound feed. The experimental group received a solution of the preparation "Diolide" with water. For conducting immunological research, blood was taken from chickens at different age periods: from 10-, 27-, 31-, and 41-day-old chickens. The use of the synbiotic preparation "Biomagn" in combination with the disinfectant "Diolide" in the chickens of the experimental group normalized the intensity of the processes of lipid peroxidation and oxidative modification of proteins in the poultry's organism - a decrease was established ($P < .05–.001$) in GPO content and TBK-active products and aldehyde derivatives oxidative modification of proteins in the blood compared to the control. The detected event was determined by increased activity of the enzyme link of the antioxidant protection system of the organism of chickens.

Keywords: Antioxidant protection, chickens, chlorine dioxide, lipid peroxide oxidation, probiotics.

ÖZ

Büyüme sürecinin kritik dönemlerinde kanatlılarda vücudun bağışıklık tepkisini ve antioksidan potansiyelini artırmaya yönelik yöntemler geliştirmek günümüzde acil bir görevdir. Araştırmanın amacı, "Biomagn" sinbiyotiğinin "Diolide" dezenfektanı ile birlikte tavukların organizmasındaki lipid peroksit oksidasyon süreçlerinin yoğunluğu ve antioksidan koruma sisteminin aktivitesi üzerindeki etkisini belirlemektir. Araştırma, 1 günlük yaştan 41 günlük yaşa kadar yetiştirilen ve her birinde 100 tavuk bulunan 2 grup üzerinde gerçekleştirilmiştir: kontrol grubu standart karma yem (SKY) ile beslenmiştir; deney grubundaki tavuklar ise SKY ve 0,5 kg/ton oranında "Biomagn" sinbiyotik preparatı ile beslenmiştir. Deney grubuna "Diolide" preparatının su ile çözeltisi verilmiştir. İmmünolojik araştırmalar için tavuklardan farklı yaş dönemlerinde (10-, 27-, 31- ve 41 günlük tavuklardan) kan alınmıştır. Sinbiyotik preparat "Biomagn" ile dezenfektan "Diolide"nin birlikte kullanımı, deney grubundaki tavukların organizmasındaki lipid peroksidasyon süreçlerinin yoğunluğunu ve proteinlerin oksidatif modifikasyonunu normalleştirmiştir. Kanlarındaki GPO içeriği ve TBK-aktif ürünler ile aldehit türevleri oksidatif modifikasyonu kontrol grubuna göre azalmıştır ($P <$,05–,001). Tespit edilen etki, tavukların organizmasının antioksidan koruma sisteminin enzim bağlantısının aktivitesindeki artışla belirlenmiştir.

Anahtar Kelimeler: Antioksidan koruma, klor dioksit, lipid peroksit oksidasyonu, probiyotikler, tavuk

INTRODUCTION

Modern intensive production technologies in poultry farming are characterized by the presence of many factors that do not comply with the evolutionary poultry physiology, especially in broiler chickens. In most cases, this causes a stressful situation, which leads to significant violations of the biochemical homeostasis in the poultry's organism, which is explained by the action of catabolic hormones, the release of which increases under conditions of stress. At the same time, free radical processes and peroxidic oxidation of lipids increase, which contributes to a decrease in their productivity and the occurrence of immunodeficiency.[1,2]

The prerequisite for the development of oxidative stress is the accumulation of active oxygen species and free radicals, which influences the development of pathologies of various genesis.[3]

Processes of peroxide oxidation, which are required for the normal functioning of biochemical, biophysical, and physiological systems, occur in all cells of living organisms. The formation of products of lipid peroxidation (LPO) and oxidative protein modification (OPM) are normal functional processes in the organism, with which vital functions are connected. The intensity of changes in free radical processes in the poultry's organism depends on the concentration of oxygen in the tissues, therefore LPO is a physiological process since mitochondria membranes maintain a stationary level of LPO that has a certain functional value and reflects the degree of influence of molecular oxygen on mitochondrial lipids under normal physiological conditions.[4,5]

An increase in the content of LPO products in membranes weakens their barrier function and increases permeability to organic substances, and ions, and as a result, sulfhydryl groups are destroyed, which causes enzyme inactivation; thus, LPO processes are considered as one of the mechanisms of underlying cellular pathology at the basis of many negative effects, such as cytotoxic, genotoxic, mutational and oncogenic effects.[6] The physicochemical stability of eukaryotic cell membranes is ensured by the balanced processes of POL and ORM and the rotation of protein and lipid components and is associated with the protective and adaptive reactions of the body. The pathogenesis of many diseases is accompanied by the activation of peroxide oxidation processes.[7]

Given this, complex preparations have been developed in recent years to ensure the pro-oxidant-antioxidant balance of the organism and prevent its disturbance. As the most physiologically adaptogenic substances that are part of these preparations, compounds of an antioxidant nature have become more and more widely used. Various preparations can have antioxidant activity according to the mechanism of action, affecting both the central mechanisms of regulation and exhibiting a local effect.[8] The products being developed should effectively regulate the level of peroxide processes and indirectly affect the oxidative metabolism of the organism as a whole, and in immunocompetent cells, in particular. Given this, in the field of veterinary medicine, the research was directed at the search for biologically active substances with immunocorrective and antioxidant properties.[9]

The results of the search in the literature show that over the last few years, the number of data on the beneficial effects of probiotics has increased, especially those that are important for mediating reactions to oxidative stress. It became known that probiotics can modulate the redox status of the recipient due to their ability to chelate metal ions, and antioxidant systems, thus regulating signaling pathways and enzymes that produce reactive forms of oxygen and intestinal microbiota.[10,11]

There is scientific interest in finding potential probiotic strains that may exhibit powerful antioxidant properties along with health benefits. *In vitro* and *in vivo* research have ascertained that probiotics exhibit antioxidant potential.[12]

Despite the existing data on the influence of probiotics on the antioxidant and immune defense systems of the organism, the feasibility and safety of their use require additional research and scientifically based analysis.

Manufacturers offer a wide selection of probiotics with different compositions, quality, action, and use. However, sometimes, but not always, probiotics meet the claimed properties. However, practice shows that the use of probiotics is promising in the prevention and treatment of poultry diseases, especially in combination with disinfectants that are used to disinfect premises and the water supply system. Disinfection in the presence of poultry is carried out carefully and cautiously because the poultry is in close contact with fences, inventory, and equipment.[13,14,15]

Along with this, it is important to clean and disinfect the water supply system of drinking water, because microorganisms can be fed in the sediment with vitamins and other additives to drinking water and as a result, a "biofilm" is formed. One of the efficacious active substances of the disinfectant, which is effective for water disinfection, is chlorine dioxide.[16,17,18]

The research conducted by many scientists has established that excessive concentrations of chlorine dioxide in drinking water did not show any toxicity in the subchronic oral toxicity test. At the same time, it demonstrated favorable disinfecting activity and a tendency towards a higher safety profile.[19,20]

Therefore, the creation and use of complex preparations with immunomodulatory and antioxidant properties based on probiotics will provide an opportunity to increase the immunobiological reactivity antioxidant potential and resistance of the poultry organism to technological stresses. At the same time, the use of safe disinfectants in the water supply system is an urgent problem in modern conditions of industrial poultry farming.

The purpose of the research was to find out the influence of the synbiotic preparation "Biomagn" in combination with the solution of the disinfectant "Diolide" on the intensity of the processes of peroxide oxidation of lipids and the activity of the antioxidant defense system of the organism of broiler chickens during their growing period.

MATERIALS AND METHODS

The study was conducted at the poultry farm, located in Lviv oblast on broiler chickens from ROSS-308 cross. Chicken aged 1 to 41 days after hatching were used in the experiments. Poultry was held in the coops with free access to feedstuff and water supply, under the technological conditions, recommended for broiler breeding (temperature and insolation levels) by local standard - ONTP-2005. Two groups of broiler chickens were formed for the experiment (control and experimental), 100 chickens per group. The control group of the poutry was fed with standard compound feed-stuff (SCF) recommended for the ROSS-308 cross of broilers. The experimental group was similarly fed with SCF and supplied with "Biomagn" synbiotic preparation at a dose of 0.5 kg per ton of feedstuff. The preparation was used by the following scheme: for the first time on 1st day after hatching for seven days, and the next treatment was performed on the 22nd day, for seven days also.

Biomagn synbiotics include *Bacillus subtilis, Bacillus licheniformis, Bacillus coagulans*, fermentation products of *Lactococcus lactis, Bacillus subtilis, Bacillus licheniformis*, as well as betaine, acidity regulator, thistle meal, cellulose, emulsifier, magnesium chloride, chitosan, xylanase, and protease. The chickens of the experimental group were given a solution of the drug "Diolide" with water throughout the experiment (41 days) (active substances sodium chlorite and sodium chloride) at a dose of 1 mg/l per chlorine dioxide in accordance with its technical reglementation. This preparation was developed by employees of the State Research Institute for Laboratory Diagnostics and Veterinary-Sanitary Examination (Kyiv).

Biochemical researches of blood were performed by samples from chickens after decapitation at various ages: 10-, 27-, 31-, and 41 days after hatching. The blood samples were examined for the following: content of reduced glutathione (RG; Butler E., 1963); content of lipid hydroperoxides (LHP; V.V. Myronchuk, 1998); concentration of TBK-active products according to the method of E.N. Korobeynikov (1989); activity of superoxide dismutase (EC SOD; 1.15.1.1) according to the method of E.E. Dubinina with co-authors. (1983); activity of glutathione peroxidase (GP; EC 1.11.1.9; Moin V.M., 1986); and content of ketone and aldehyde derivatives of oxidative modification of proteins (OPM_{370}, OPM_{430}) according to the method described by Levine et al. (1990). Biochemical researches were carried out according to the specified methods, which are described in the handbook.[2]

Concentration of Lipid Hydroperoxides

The measurement of LHP (lipid hydroperoxides) level was performed in accordance with the methods of trichloroacetic acid-induced protein precipitation and ethanol-induced lipid extraction. Ammonium thiocyanate interacts with lipid ethanol extracts and initiates the colour reaction. Extinction recording of the colored product was performed spectrophotometrically (λ 480 nm). LHP level (EU/ml) was calculated as the difference between the control and experimental samples.

Concentration of Thiobarbituric Acid Reactive Substances

Evaluating the concentration of TBARS (thiobarbituric acid reactive substances) is based on the principle of malondialdehyde and thiobarbituric acid interaction under the conditions of acidity and a high temperature. The result of malondialdehyde and thiobarbituric acid interaction is the colour reaction. The coloured product extinction recording was performed spectrophotometrically (λ 535 nm, and λ 580 nm) and TBARS level was calculated as μmol/ml.

Activity of GP (EC 1.11.1.9)

The measurement of GP (glutathione peroxidase) enzymatic activity is performed in the presence of GSH before and after adding tertiary butyl hydroperoxide. Evaluating the GP activity is based on the principle of GSH oxidation rate. SH-groups of GSH molecule are oxidized in the presence of 2-nitrobenzoic acid. Dinitrophenyl anion is formed as a result of GSH oxidation. Extinction recording of the coloured product was performed spectrophotometrically (λ 412 nm)

and GP activity was calculated in mmol GSH/min×mg of protein.

Concentration of GSH

The evaluation of the GSH (reduced glutathione) level is based on the principle of thionitrophenyl anion formation (coloured product) after binding of 2-nitrobenzoic acid to SH-group of GSH molecule. The value of GSH concentration depends on the intensity of the colour reaction. The coloured product extinction recording was performed spectrophotometrically (λ 412nm) and GSH content was calculated as μmol/ml.

Activity of SOD (EC; 1.15.1.1).

The measurement of SOD (superoxide dismutase) enzymatic activity was performed in the presence of NADH and phenazine methosulfate. The evaluation of the SOD activity is based on the principle of nitroblue tetrazolium reduction. The intensity of inhibition of the nitroblue tetrazolium reduction process indicates the intensity of enzyme activity. The absorbance recording was performed spectrophotometrically (λ540nm) and SOD activity was calculated as Units/mg of protein*min.

The content of aldehyde and ketone derivatives of oxidative modification of proteins.

The level of intensity of oxidative destruction of proteins was evaluated by the reaction of carbonyl derivatives of the amino acid reaction with dinitrophenylhydrazine. The content of carbonyls was calculated by measuring the optical absorption at 370 nm and 430 nm, taking into account the absorption coefficient of 22000 M-1 cm-1. Carbonyl groups were determined spectrophotometrically by the difference in absorbance at 370 nm (aldehyde derivatives, OMP370) and 430 nm (ketone derivatives, OMP430). During the determination, after adding 0.9 ml of THO and 1 ml of 2,4 dinitrophenylhydrazine to 0.1 ml of serum, incubation was carried out at room temperature and the mixture was centrifuged for 45 min at 3000 rpm. Next, the mixture was washed with ethanol-acetate mixture 3 times. After adding urea and heating for 5 minutes in a boiling water bath, measurements were made at the indicated wavelengths. The concentration of aldehyde and ketone derivatives of oxidative modification of proteins was expressed in nmol/mg of protein.

The experiment plan was designed taking into account Council Directive 2010/63/EU (Council Directive 2010/63/EU, 2010) on the protection of animals used for scientific purposes and the European convention for the protection of vertebrate animals used for experimental and other scientific purposes (Strasbourg, 1986),[22-24] and was approved by the Bioethics Commission dated November 7, 2022.

Digital data were processed by the biometric method of variational non-parametric analysis using Microsoft Excel program of the Microsoft Office Professional XP table editor package and Origin 6.1 program. The differences between values were considered statistically significant: $P < .05$; .01 and .001.

RESULTS

Free-radical processes caused by active forms of oxygen are the basis of lipid peroxidation (LPO). Lipid peroxidation is a physiological process. LPO occurs most intensively in biological systems, where the rate of metabolism is particularly high. In particular, a large number of free radicals are formed in mitochondria, which contain electron transport systems. However, the formation of ROS also occurs in microsomes, in the nuclear and plasma membranes, as well as in the cytoplasm.[25]

The process of formation of free radicals, hydroperoxides, peroxides, and diene conjugates ensures the renewal of cell membrane lipids, thereby maintaining structural homeostasis, and also forms a protective mechanism in the body at the physiological and biochemical levels under the influence of stress factors. According to modern ideas, the activation of the process of lipid peroxidation in biological membranes and fluids serves as a trigger (primary mediator) for starting a stress reaction. The intensity of LPO in body tissues is assessed by their content of diene conjugates, lipid hydroperoxides, and TBC-active products, respectively, the initial, intermediate, and final products of LPO, as well as by the degree of chemiluminescence, which correlates with the content of TBC-active products in them.[3,4,25]

The research demonstrated (Table 1) that the content of intermediate and final products of LPO in the broiler chicken blood plasma of the control group was increased during the growing of poultry. The most intensive growth of LPO processes was recorded in chickens during the period of active growth. At the same time, in the experimental group of 27-, 34-, and 41-day-old chicken, the content of GPO and TBC-active products in the blood plasma had the level of, respectively, 7.1, 19.5 and 28.0% ($P < .001$) that was less than in the control group of chicken (12.6 ($P < .01$); 20.5 and 28.2% ($P < .001$). This indicates the inhibitory influence of developed synbiotic preparation in complex applications together with disinfectant on the content of intermediate and final products of LPO.

Table 1. Lipid peroxide oxidation products levels in the blood plasma of broiler chickens (M±m; n=5)

Indicator	Groups	Periods of research			
		10th day	27th day	34th day	41th day
TBK-active products, μmol/ml	C	1.61±0.029	1.74±0.046	1.85±0.052	1.95±0.035
	E	1.61±0.034	1.52±0.041**	1.47±0.0408***	1.40±0.054***
LHP, unit E/ml	C	0.41±0.016	0.42±0.014	0.47±0.012	0.50±0.010
	E	0.40±0.012	0.39±0.013	0.38±0.013***	0.36±0.010***

*: statistically probable differences between the investigated indicators in chickens of the experimental group, compared to the control group: *p<0.05; **P < .01; *** P < .001. TBK-active products: thiobarbituric acid reactive substances; LHP: lipid hydroperoxides.

Similar changes were detected, but to a lower extent, in the content of products of protein oxidation in the chicken blood serum. The data, presented in Table 2, demonstrate that synbiotic preparation use in combination with disinfectant produces a reduction in oxidation modification of proteins in chicken blood serum. OPM aldehyde and ketone derivatives' content was lower in the blood of poultry from experimental group; however, the differences with control group were detected only in OPM aldehyde derivatives content in 41-day-old chicken.

Table 2. Aldehyde (OPM_{370}) and ketone (OPM_{430}) derivatives of oxidative modification of protein levels in chicken blood serum (M±m; n=5)

Indicator	Group	Periods of research			
		10th day	27 th day	34 th day	41st day
OPM_{370} nmol/mg protein	C	4.93±0.38	5.31±0.56	4.91±0.14	5.12±0.33
	E	4.91±0.45	4.58±0.46	4.09±0.35	3.87±0.34*
OPM_{430} nmol/mg protein	C	12.82±1.16	13.10±0.51	10.40±0.96	9.84±0.94
	E	12.18±0.97	11.39±1.05	9.46±0.48	8.76±0.87

*: statistically probable differences between the investigated indicators in chickens of the experimental group, compared to the control group: * P < .05; ** P < .01; *** P < .001. OPM: oxidative modification of proteins.

At the same time, the results of this study indicated the inhibitory influence of tested compounds on the intensity of protein oxidative modification accumulation in chicken blood serum.

It is known that OPM also causes the formation of ROOH in the body, followed by ROH (o- and m-tyrosine), R(OH)2, carbonyl, and other oxidized derivatives; autooxidative glycosylation of proteins also occurs. It is believed that the negative effect of oxidatively modified proteins in cells is due to the fact that oxidized proteins are a source of free radicals that deplete the reserves of cellular antioxidants [32]

The decrease of LPO and OPM products' content in the blood of the experimental group of poultry was probably associated with the complex action of the developed product on the enzyme link of the antioxidant defense system (ADS). In particular, when studying indicators characterizing the glutathione link of ADS, attention is drawn to higher glutathione peroxidase activity in chicken blood in the experimental group in all periods of testing compared to the control group of poultry (Table 3). At the same time, at 34 and 41-day-old chickens, the activity of this enzyme was, respectively, 17 (P <.05) and 21.6% (P < .05) higher than in the control. At the same time, in the experimental group chicken blood, compared to the control group, a tendency of increase of reduced glutathione content was detected in all dates of testing.

Glutathione peroxidase (GPx) is an antioxidant enzyme that catalyzes in the body the reduction of hydrogen peroxide and organic hydroperoxides with reduced glutathione (GSH) to water or to hydroxy-derived organic compounds.[7, 8, 12, 25]

The activity of the glutathione system of antioxidant protection in the cell limits reduced glutathione (GSH), which is oxidized (GSSG) in the process of H_2O_2 reduction. At physiological concentrations of peroxides and a high level of glutathione reduction in cells, glutathione peroxidase is found mainly in reduced forms.[3,4,12,25]

Table 3. Reduced glutathione, glutathione peroxidase, and superoxide dismutase activity levels in broiler chickens' blood (M±m; n=5)

Indicator	Group	Period of research			
		10th day	27th day	34th day	41st day
GP GSH/min per mg protein	C	21.10±0.37	21.42±0.53	21.57±0.54	21.56±0.43
	E	21.79±0.36	24.78±1.55	25.88±1.44*	26.21±1.35*
RG, µmol/ml	C	0.26±0.018	0.27±0.008	0.28±0.009	0.28±0.005
	E	0.28±0.01	0.31±0.016	0.31±0.012	0.32±0.019
SOD, unit act./mg of protein*min	C	20.75±1.97	20.54±0.57	19.56±1.04	19.92±0.78
	E	21.86±0.69	24.04±1.27*	23.33±1.26	23.89±1.19*

*: statistically probable differences between the investigated indicators in chickens of the experimental group, compared to the control group: *p<0.05; **p<0.01; ***p<0.001. GP GSH: enzymatic activity of glutathione peroxidase in the presence of glutathione, RG: reduced glutathione, SOD: superoxide dismutase.

From the data presented in Table 3, we can see that the use of the synbiotic preparation in combination with a disinfectant in the chickens of the experimental group caused an increase in superoxide dismutase activity, an enzyme of the primary link of the antioxidant defense system.

Superoxide dismutase (SOD) is a key enzyme of the antioxidant system. It neutralizes superoxide radicals, turning them into less toxic hydrogen peroxide. Three forms of superoxide dismutase are known: Su/Zn-SOD; Mn-SOD and Fe-SOD. Metals perform a catalytic function, being successively reduced and oxidized in the active center of the enzyme.[3,4,6,8,25]

Research results showed that on days 27- and 41, the activity of this enzyme in the blood of broiler chickens of the research group was, respectively, 20% ($P < .05$) and 19.9% ($p<0.05$) higher than in the control group.

Among other research results obtained in this study, the complex use of the "Biomagn" synbiotic preparation and the "Diolide" disinfectant in broiler chickens are worthy of attention after demonstrating a stimulating influence on chicken growth intensity. During the growing period of poultry, the weight of broiler chickens treated with our products surpassed the growth of the counterparts of control group. At the same time, the average daily growth parameter of chickens treated with the developed product was 6.7% higher than in the control group of poultry. It was noticed that in chickens of the experimental group, the intensity of growth increased until the end of the experiment.

Therefore, based on the results of our research, it can be stated that the use of the "Biomagn" synbiotic preparation and the "Diolide" disinfectant regulated the intensity of oxidative processes in the organism of chicken. The products ensured a pro-oxidant-antioxidant balance, and increased the immune potential of chicken organisms, as it was noted in our previous works,[26,27]and had a positive effect on chicken growth intensity.

DISCUSSION

The results of our research were confirmed by the literature data about the adaptive and metabolic processes that occur in the organisms of broiler chickens during their growing period. In particular importance is the clarification of the role of the tested synbiotic product and disinfectant used in broiler chickens in the regulation of metabolic homeostasis, in particular, the pro-oxidant-antioxidant balance of the body during the period of decrease of the body's immune potential, both under normal and stress conditions.[28-31]

As noted earlier, the processes of LPO and OPM are largely associated with protective and adaptive reactions of the organism. Enhancement of peroxide oxidation processes plays a significant role in the pathogenesis of many diseases. The analysis of literature data showed that for an integral evaluation of the functional activity of the poultry's organism, it is necessary to investigate the intensity of LPO processes and the activity of enzymes of the antioxidant protective system. Therefore, the functioning of the

antioxidant system provides an appropriate level of protection, and products of free radical peroxide oxidation can act as indicators of tissue damage since their content can be used to analyze the intensity of free radical processes in various organism systems.[32,33] The research of LPO is widely used in the investigation of oxidative stress.[34,35.]

Our studies are also consistent with data[36-38], which found that certain strains of *Bacillus* exhibit antioxidant activity, which is assumed to promote the synthesis of antioxidant enzymes that protect the host from oxidative stress.

At the same time, Yu et al.[39] report that the probiotic *Bacillus coagulans* promotes the activation of antioxidant defense system enzymes (SOD, CAT, GP) and reduces MDA levels in the blood serum of broilers. However, the probiotic *Lactobacillus plantarum* in this case does not promote the activation of antioxidant defense system enzymes and causes a less pronounced decrease in MDA levels in the broiler bloom serum.[39]

Studies by other authors also indicate a positive impact of probiotics (*Bacillus subtilis*, *Bacillus licheniformis*) on the antioxidant defense system, which is associated with the activation of SOD, CAT, GP enzyme in the bloom serum [40,41], liver, ileum [41,43], and a decrease in the concentration of MDA in the bloom serum [40] and ileum [41,42] of broilers, both under normal conditions and under conditions of *Clostridium perfingens* – induced subclinical necrotizing enteritis.[40,41] Instead, Ji et al.[42] report only a decrease in MDA levels in the mucosa of the ileum of broilers under the influence of the probiotic *Bacillus subtilis M6*, while the MDA content in the mucosa of the jejunum and the activity of antioxidant defense system enzyme in the mucosa of the ileum and jejunum of broilers remained unchanged.[42] The authors suggest that the enhancement of antioxidant status under the influence of probiotics may be associated with the stimulation of the expression of Nrf-2, HO-1, SOD, and GP genes and an increase in the concentration of non-enzymatic antioxidants under the influence of the studied probiotics,[40] as well as with the chelating properties of probiotics relative to metal ions.[41]

In summary, these results suggest that *Bacillus* may play a useful role in oxidative defense due to its inherent antioxidant activity.

The results of our studies are consistent with the fact that probiotics (*Bacillus coagulans*, *Lactobacillus plantarum*, *Bacillus subtilis*, *Bacillus licheniformis*) have a positive effect on broiler growth performance and improve such animal parameters as total body weight, and average daily feed intake (ADFI), average daily gain (ADG) and feed to gain ratio (F/G).[29,30,37-42] The authors suggest that the improvement in broiler growth performance in this case is associated with increase in the number of *Ruminococcaceae* bacteria and a decrease in the number of *Desulfovibrio* bacteria in the broiler intestinal flora, which in turn may help reduce inflammation, anxiety, and depression.[39] Improvement of broiler growth performance under the influence of probiotics may be associated with increased immunity, regulation of metabolic functions, and composition of the intestinal flora, as well as increase in beneficial metabolites (extracellular digestive enzymes, lysozyme, antifungals proteins, antibiotics) produced by *Bacillus subtilis* and *Bacillus licheniformis.*[40]

In conclusion, the present findings demonstrate that synbiotic preparation "Biomagn" and "Diolide" disinfectant use in broiler chickens caused an inhibitory influence on LPO and OPM processes intensity, as evidenced by a decrease in the LPO intermediate and final products' content in blood plasma and aldehyde derivatives of OPM, which was accompanied by a positive effect on the growth of chickens. Under the influence of the studied drugs, a higher activity of the key enzymes of antioxidant protection - superoxide dismutase and glutathione peroxidase - was recorded in the blood of chickens against the background of the revealed tendency to increase the content of reduced glutathione, which helped ensure the pro-oxidant-antioxidant balance of the body.

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A Preliminary Study on the Investigation of Learning Ability of Arabian Horses Through

Arap Atlarının Öğrenme Yeteneklerinin Araştırılması Üzerine Bir Ön Çalışma

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ABSTRACT

Knowing the behavioural responses of horses while planning the training processes is crucial in constituting the appropriate training program. The purpose of the study was to examine some behavioural and physiological responses of Arabian horses participating in flat races against the reactivity to human and fear tests. Within the scope of this study, 15 female Arabian horses aged between 2 and 8 years were subjected to reactivity to passive human test, reactivity to active human test, and handling tests from reactivity to human tests and the novel surface test, the static novel object test, and the startling novel object tests from fear tests. Significant relationships ($P < .05$) were detected between the agonistic behaviours of horses and maximum heart rate values in the passive human test, active human test, static novel object test, and startling novel object test. Active human test, it was determined that as the agonistic behaviours of horses increased, the distance to approach humans increased ($P < .05$). The novel surface test observed that as the horses' agonistic behaviours increased, the number of attempts to cross the surface successfully also increased ($P < .01$). In the handling test, increased agonistic behaviours caused the test time to prolong ($P < .01$). As a result, it was determined that various processes in training were prolonged in horses with high agonistic behaviours during the tests. It has also been determined that the trainability of horses may differ within the same breed and sex. For this reason, it is recommended to determine behavioural responses and to plan horse training individually. By understanding the trainability of horses, it is possible to achieve maximum efficiency for their benefit.

Keywords: Behavioural tests, equid ethogram, horse, learning theory, welfare.

ÖZ

Atların eğitim süreçleri planlanırken davranışsal tepkilerinin bilinmesi, uygun eğitim programının oluşturulması açısından önemlidir. Bu amaçla davranışsal testlerden yararlanılmaktadır. Araştırmaya 2-8 yaş arası, on beş dişi Arap atı katılmıştır. Davranışsal Testler kapsamında İnsana Karşı Tepki ve Korku Testleri uygulanmıştır. Pasif İnsana Karşı Reaksiyon Testi, Aktif İnsana Karşı Reaksiyon Testi, Sabit Alışılmamış Nesne Testi ve Ürkütücü Alışılmamış Nesne Testi'nde atların agonistik davranışları ile maksimum kalp atım hızı değerleri arasında anlamlı ilişkiler ($P < ,05$) tespit edilmiştir. Aktif İnsana Karşı Reaksiyon Testi'nde atların agonistik davranışları arttıkça insanlara yaklaşma mesafesinin de arttığı tespit edilmiştir ($P < ,05$). Alışılmamış Yüzey Testi'nde, atların agonistik davranışları arttıkça, yüzeyi başarılı bir şekilde geçme girişimlerinin sayısının da arttığı gözlemlenmiştir ($P < ,01$). Etki-Tepki Testinde, agonistik davranışların artmasının test süresinin uzamasına neden olduğu tespit edilmiştir ($P < ,01$). Sonuç olarak, testler sırasında agonistik davranışları yüksek olan atlarda eğitimdeki çeşitli süreçlerin uzadığı tespit edilmiştir. Atların eğitilebilirliğinin aynı ırk ve cinsiyet içerisinde farklılık gösterebileceği belirlenmiştir. Sonuç olarak, atların davranışsal tepkilerinin belirlenmesi ve at eğitiminin bireysel olarak planlanması önerilmektedir. Atların eğitilebilirliğinin anlaşılması yoluyla, onların yararına maksimum verim elde etmek mümkün olabilecektir.

Anahtar Kelimeler: At, at etogramı, davranişsal testler, öğrenme teorisi, refah

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INTRODUCTION

Recently there have been different views in the scientific evaluation of animal welfare. Welfare is arguably the result of the animal's attempt to adapt to its environment.[1] Some physiological stress signs and behavioural responses happen during the adaptation of animals to different environmental conditions.[2, 3] An animal's ability to effectively cope with environmental conditions is viewed as a measure of its robust biological functioning. Conversely, its inability to adapt adequately is seen as an indication of welfare issues.[4] Horse training requires understanding the adaptations horses undergo during the process to ensure their welfare and reduce accident risks. Behavioural tests help tailor training programs accordingly, enhancing horse well-being and safety.

Understanding horses' habituation and sensitization abilities is crucial for tailoring effective training methods. Positive horse training seeks to enhance learning by diminishing horses' fear of humans, which can be assessed by the quality of horse-human interaction [5,6] Behavioural tests offer a means to evaluate horses' habituation and sensitization abilities.

In this study, Reactivity to human tests and fear tests are applied. The purpose of the study was to examine some behavioural and physiological responses of Arabian horses participating in flat races against the Reactivity to human and fear tests.

MATERIALS AND METHODS

Horses and Management

Fifteen female Arabian horses, aged 2-8 years, from a private horse farm were part of the study. In their daily routine, horses receive forage in the stable and then freely access the pasture from their boxes without human guidance. They undergo regular veterinary checks and hoof care but have limited interaction with humans beyond these practices.

Water and hay were ad libitum in both the stable and the paddock. Each box had an area of about 3 x 3 m (Table 2. The Test Arena and Behavioural Tests) and paddock had an area of about 40 x 60 m. Horses were given forage in their boxes twice a day, according to the routine in the stud.

The tests were always administered between 9:00 am and 3:00 pm on horses. The horses were in the paddock during the hours outside the test (07:30-18:30). During the research, they were released in the paddock after being tested.

Experimental Design

Behavioural tests, such as reactivity to human tests and fear tests, are used to evaluate horses' responses. Reactivity to human tests includes passive human test, active human test, and handling test, assessing horses' reactions to humans. Fear tests involve the novel surface test, static novel object test, and startling novel object test, evaluating horses' habituation-sensitization abilities.

In the study, reactivity to human tests was conducted in standardized 3 x 3 meter boxes where all horses were tested individually. Fear tests were carried out in the stable, with a 3-meter wide service road serving as the testing area for all horses (Table 2. The Test Arena and Behavioural Tests). The same groom and trainer/researcher participated in all tests, with the trainer/researcher consistently wearing a red vest. The term "trainer/researcher" denoted an unfamiliar person, while "groom" referred to a familiar person. Distances were measured using a Leica Geosystems DISTO Laser meter.

Heart rates and behavioural responses were assessed during the tests. The Polar Equine M400 Heart Rate Monitor was used to measure heart rate, while behavioural responses were recorded via camera and scored using the "Equine Agonistic Ethogram," comprising 12 agonistic behaviours presented in Table 1.[7] A higher ethogram score indicates increased agonistic behaviours during the habituation-sensitization process.

Statistical Analysis

One-way ANOVA was applied for approach distances and average heart rates in the Reactivity to human and Fear tests, and the Duncan test was used to compare groups with differences. Correlation analysis was performed to determine the relationships between the values obtained from the Reactivity to human and Fear tests. SPSS version 24 (IBM SPSS Corp., Armonk, NY, USA) package program was used to conduct the analyses.

Table 1. Ethogram of Agonistic Horse Behaviour during the Behavioural Tests

Horse Behaviour* (McDonnell and Haviland, 1995)[7]	Description
Alert	Rigid stance with the neck elevated and the head oriented toward the object or animal of focus. The ears are held stiffly upright and forward and the nostrils may be slightly dilated.
Approach	Forward movement in a straight or curving path. The approach can be at any gait or speed. The head may be elevated and ears forward or the head may be lowered and ears pinned back.
Avoidance/retreat	Movement that maintains or increases an individual's distance from an approaching animal. The head is usually held low and ears turned hack. The retreat can be at any gait but typically occurs at the trot.
Balk	Abrupt halt or reversal of direction with movement of the head and neck in a rapid sweeping dorsolateral motion away from an apparent threat while the hind legs remain stationary. The forelegs may simuhaneously lift off the ground.
Bite Threat	The ears are pinned and lips retracted. Similar to a bite (Opening and rapid closing of the jaws with the teeth grasping the flesh of another animal) except that no contact is made. The neck is stretched and ears pinned back as the head swings toward the target stallion.
Head Bump	A rapid lateral toss of the head. Usually the eyes remain closed and the ears forward.
Head bowing	Head bowing is a repeated, exaggerated, rhythmic flexing of the neck such that the muzzle is brought toward the point of the breast.
Kick	One or both hind legs lift off the ground and rapidly extend backwards, with apparent intent to make contact. The forelegs support the weight of the body and the neck is often lowered.
Kick retreat	Similar to a kick, but without sufficient extension or force to make. The hind leg(s) lifts slightly off the ground and under the body in tense "readiness", usually with no subsequent backward extension of the hind leg(s). This action is often indistinguishable from the preparation for an actual kick.
Nip	Similar to a bite, but with the mouth less widely opened and the teeth closing on only a small piece of flesh.
Push	Pressing of the head, neck, shoulder, chest, body or rump against another in an apparent attempt to displace or pin
Stomp	One foreleg is raised and lowered, sharply and firmly striking the ground, usually repeatedly.

*The study examined agonistic behaviours through horse-human interactions.

RESULTS

All horses (100%) in scope of the study were defined as good-tempered by their breeders. Also, has been reported that 26.67% of horses are skittish and 73.33% of them are calm.

The findings of the reactivity to human tests are given in Table 3. In the passive human test, it was determined that 46.60% of the horses contacted the familiar person and 53.30% of them contacted the unfamiliar person. While the average distance of horses approaching a familiar person was 70.67±22.33, the average distance they approached an unfamiliar person was 60.00±22.34. The ethogram score of horses against both familiar and unfamiliar person ranged from 0 to 2, with the highest rate being 0 (66.60%).

In the active human test, it was observed that 6.70% of the horses contacted an unfamiliar person. The average distance of horses approached an unfamiliar person was determined as 160.00±21.93. The ethogram score was between 0 and 5 during the test and the highest rate was observed in the 3 scores (33.33%).

In the handling test, the ethogram score during approach to the horse varied between 0 and 4 and the highest rate was 0 (60.00%), it changed between 0 and 5 during contact and when the lead rope was attached, and the highest rate was 0 (60.00%), it has been determined that it varies between 0 and 5 while being led and the highest rate was 1 (40.00%). During the behavioural tests, the average heart rate values of the horses were determined as 53.67±2.76 and 41.66±1.06 in the passive human test, against a familiar and an unfamiliar person, respectively, as 47.80±2.38 in the Active human test, and as 52.40±2.06 in the handling test.

The findings of the fear tests are given in Table 4. In the novel surface test, 53.30% of the horses were successful on the first trial, 80.00% on the second, and 86.60% on the third trial. It was determined that the ethogram score ranged from 0 to 8 during the horse's first pass over the novel/frightening surface, with the highest score being 0 (40.00%).

In the static novel object test, it was determined that 100% of the horses sniffed the object, 86.60% of them touched the object, 53.30% of the horses bit the object, and 6.60% of the horses did not show interest in the object. 93.30% of the horses passed by the object. Horses that "passing by the object" was defined as follows, the horse's passing without any resistance, without negative reinforcement with the help of a halter, without frightening behaviours, at most 25cm ahead of the object and in walk. When the behaviours of the horses while passing by the object were examined, it was determined that the ethogram score ranged between 0-5 and the highest value was 0 (73.30%).

Table 2. Test Arena and Behavioural Tests

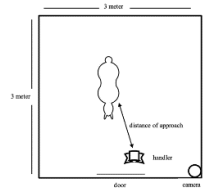
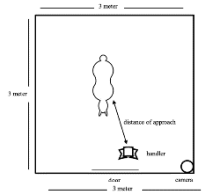
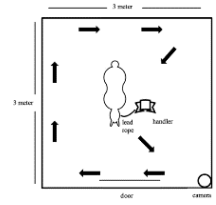
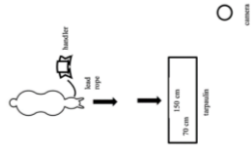
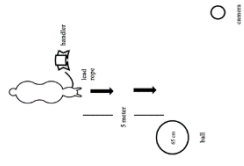
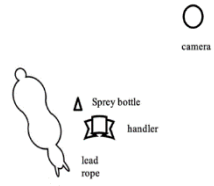
Reactivity to passive human test	In the reactivity to passive human test, the horse's approach time and distance to the passive person were assessed. The trainer/researcher entered the horse's box with passive body language, stood still by the door for 3 minutes without providing any stimulus to the horse.	
Reactivity to active human test	In the reactivity to active human test, the horse's approach time and distance to the moving person were assessed. The distance was measured between the horse's forefoot and the trainer/researcher's foot. During this test, the trainer/researcher provided auditory and tactile stimuli to the horse in the box for 3 minutes.	
Handling test	During the Handling Test, horses were led by the trainer in the box for three rounds. Behavioural responses of the horse during various management tasks, such as the trainer's approach, touching, attaching the lead rope, and leading, were evaluated.	
Novel surface test	In the novel surface test; horses were passed over a tarpaulin (70 x 150 cm, red) with led by the trainer. Passing through the novel surface with horses has been tried 3 times. Horses that crossed the surface with all their feet were defined as successful.	
Static novel object test	In the Static novel object test, a ball with a diameter of 65cm was placed at a point 5m from the horse and approach time of the horse to the object, distance of approach, and behavioural responses in this process were evaluated. At the same time, the reactions of the horses to the object (sniffed, touched, bit, lack of interest) were also examined during the test.	
Startling novel object test	In the Startling novel object test, water was sprayed towards the horse's shoulder using a spray bottle. The test involved two stages: initially spraying water on the ground and then on the horse's body. It aimed to assess horses' behavioural responses to objects with a gradual increase in stimulus intensity. Additionally, the horses' reactions to the object, such as sniffing, touching, biting, or showing lack of interest, were examined during the test.	

Table 3. Reactivity to human tests (N=15)

Tests	Reactivity to human Tests						
	Passive human test		Active human test	Handling Test			
	Familiar person	Unfamiliar person	Unfamiliar Person	Approaching	Touching	Attaching the lead rope	Leading
Contact (%)	46.60	53.30	6.70				
Approach Distance (cm) ($\bar{x} \pm S\bar{x}$)	70.67±22.33[b]	60.00±22.34[b]	160.00±21.93[a]** *				
Etogram Score (%)							
0	66.60	66.60	20.00	60.00	60.00	60.00	26.66
1	20.00	20.00	13.33	26.66	20.00	13.33	40.00
2	13.30	13.30	20.00	0.00	13.33	20.00	20.00
3	0.00	0.00	33.33	6.67	0.00	0.00	6.67
4	0.00	0.00	6.70	6.67	0.00	0.00	0.00
5	0.00	0.00	6.70	0.00	6.67	6.67	6.67
Heart Rate (pcs/minute)							
Maximum	38-100	36-50	42-103		51-88		
Average ($\bar{x} \pm S\bar{x}$)	53.67±2.76[a]	41.66±1.06[b]	47.80±2.38[a]		52.40±2.06[a]		**

**: $P < .01$. a, b: Differences between means with different letters in the same row are significant.

Table 4. Fear tests (N=15)

Tests	Fear tests			
	Novel Surface Test		Static novel object test	Startling novel object test
Success in the 1st trial (%)	53.50	Sniffed (%)	100.00	86.60
Success in the 2nd trial (%)	80.00	Touched (%)	86.60	33.30
Success in the 3rd trial (%)	86.60	Bit (%)	53.30	6.60
		Lack of interest (%)	6.60	6.60
Etogram Score (%)				
0	40.00		73.33	33.33
1	26.66		6.67	6.67
2	6.67		0.00	40.00
3	0.00		0.00	20.00
4	6.67		6.67	0.00
5	0.00		13.33	0.00
6	13.33		0.00	0.00
7	0.00		0.00	0.00
8	6.67		0.00	0.00
Heart Rate (pcs/minute)				
Maximum	62-156		48-103	44-98
Average ($\bar{x} \pm S\bar{x}$)	63.46±3.31[a]		53.46±2.41[b]	50.53±2.20[b] **

**: $P < .01$. a, b: Differences between means with different letters in the same row are significant.

In the startling novel object test, it was determined that 86.60% of the horses sniffed the object, 33.30% of them touched the object. 60% of them bit the object, and 20.00% of them did not show interest in the object. When the behaviours of the horses towards the frightening object (sprey bottle) were examined, it was determined that the ethogram score ranged between 0-3 and the highest value was 2 (40.00%). During the behavioural tests, the average heart rate values of the horses were determined as 63.46±3.31, 53.46±2.41 and 50.53±2.20 for the novel surface test, static novel object test and startling novel object test, respectively. Correlations between the data

obtained from the reactivity to human and fear tests are given in Table 5, Table 6, Table 7 and Table 8.

As a result of the correlation analysis conducted to determine the relationships between the values obtained from the reactivity to human and fear tests; Significant positive relationships ($P < .05$) were detected between agonistic ethograms of horses and maximum heart rate values in the reactivity to passive human test (against a familiar person), reactivity to active human test, static novel object test and startling novel object test. In the reactivity to active human test, it was determined that as the ethogram value of horses increased, the approach distance also increased ($P < .05$). In the novel surface test, it was observed that as the ethogram values of the horses increased, the number of attempts to successfully pass over the surface also increased ($P < .01$). In the handling test, the increase in the ethogram value caused the test time to extend.

Table 5. Correlation values of variables in reactivity to passive-familiar human test, passive-unfamiliar human test, active-unfamiliar human test

Reactivity to Passive-Familiar Human Test				
	Approach distance	Etogram	Maximum heart rate	Average heart rate
Approach distance	-			
Etogram	0.339	-		
Maximum heart rate	0.315	0.682**	-	
Average heart rate	0.250	0.371	0.737**	-
Reactivity to Passive-Unfamiliar Human Test				
Approach distance	-			
Etogram	-0.133	-		
Maximum heart rate	0.025	0.047	-	
Average heart rate	0.012	-0.180	0.908**	-
Reactivity to Active-Unfamiliar Human Test				
Approach distance	-			
Etogram	0.519*	-		
Maximum heart rate	0.187	0.652**	-	
Average heart rate	0.326	0.619*	0.920**	-

*: $P < .05$, **: $P < .01$

Table 6. Correlation values of variables in reactivity to Handling Test

	Etogram at approaching	Etogram at touching	Etogram at leading	Maximum Heart rate	Average Heart rate	Test times
Etogram at approaching	-					
Etogram at touching	0.179	-				
Etogram at leading	0.058	0.851**	-			
Maximum heart rate	0.126	0.408	0.404	-		
Average heart rate	0.078	0.184	0.452	0.731**	-	
Test times	0.311	0.836**	0.755**	0.199	0.121	-

**: $P < .01$

Table 7. Correlation values of variables in reactivity to novel surface test

	Number of tries	Etogram	Maximum Heart rate	Average Heart rate	Test times
Number of tries	-				
Etogram	0.741**	-			
Maximum heart rate	0.513	0.498	-		
Average heart rate	0.424	0.427	0.965**	-	
Test times	0.349	0.205	0.143	0.039	-

**: $P < .01$

Table 8. Correlation values of variables in reactivity to static novel object test, startling novel object test

Static novel object test					
	Cruosity score	Etogram	Maximum Heart rate	Average Heart rate	Test times
Cruosity score	-				
Etogram	-0.402	-			
Maximum heart rate	-0.375	0.589*	-		
Average heart rate	-0.340	0.630*	0.975**	-	
Test times	0.398	0.161	-0.359	-0.384	-
Startling novel object test					
	Cruosity score	Etogram	Maximum Heart rate	Average Heart rate	Test times
Cruosity score	-				
Etogram	-0.377	-			
Maximum heart rate	-0.282	0.442	-		
Average heart rate	-0.025	0.672**	0.839**	-	

*: $P < .05$, **: $P < .01$

DISCUSSION

Passive and active human tests were found to produce consistent results in the studies of Hausberger and Muller[8], Jezierski et al.[9] and Lansade and Bouissou.[10] Similarly, handling tests produced repeatable results in several studies. [11,12] Consistency of fear reaction in three different situations: novel surface, novel static and startling object tests, was also confirmed.[10,12,13] Therefore, the above-listed tests were employed in our study to be evaluated for their practical usefulness.

In this study, it can be seen that the percentage of contact with unfamiliar person (53.30%) of horses is higher than the familiar person (46.60%) in the passive human test. When the approach distances of the horses were examined, it was seen that the distance between horses with familiar person (caretaker) (70.67±22.33cm) is similar to unfamiliar person (trainer/researcher) (60.00±22.34cm). The results of the research suggest that people's body language and behaviour are important in the approach distance of horses to people, rather than whether the person is familiar or not. Lundberg et al.[14] revealed in their research that horses' heart rates decreased upon reunion with both the owner and stranger. Therefore, they stated that whether the person is known or not by the horse does not make a difference in mitigating the effect of the stressful event. Ijichi et al.[15] examined the stress responses of horses during new handling procedures while being guided by their owners and a stranger, they found no difference in horses' performance (crossing time), behaviour or in physiological responses (heart rate, eye temperature) in terms of caregiver familiarity. Similar to our research results, Ijichi et al.[15] concluded that an unknown handler may be as effective as the owner in influencing horses' responses when exposed to potentially stressful situations. Also, Hartmann et al.[16] and Liehrmann et al.[17] deduced that they did not find any effect of the familiarity of the handler in novel object and novel surface tests.

Within the scope of the research, it was observed that although familiar and unfamiliar people used the same body language, the approach distance was similar. Similar to this finding, Lansade and Bouissou[10], in a study they conducted on the reactivity of horses to humans, reported that this indicates the horse's personality rather than the familiar or unfamiliar of the person and the active or passive body language. Górecka-Bruzda et al.[18], also stated in their research that there is a correlation between the timidity of horses and their reactions to humans. In addition to this in the study, the fact that the horse's percentage of contact with the unfamiliar person of horses is higher than the familiar person's contact, although not statistically significant, suggests that the horse has become habituated to repetitive practices. Larssen and Roth[19] investigated the effects of positive reinforcement on the contact-seeking behaviour of horses, they revealed that having the same person perform the tests and therefore increasing physical contact may be an indication that the experimenter is not seen as an unfamiliar in the second test. Similar to our research results, they also stated that it was possible for the horses to habituated to the testing area and to focus more easily on the experimenter in the case of repeated testing. According to the results, it was seen that the approach distance to the unfamiliar person in the reactivity to active human test (160.00±21.93 cm) was significantly ($P < .01$) higher than the results of the reactivity to passive human test (70.67±22.33 and

60.00±22.34cm). At the same time, when the ethogram score in both tests was compared, it was determined that the active human test was higher. This result reveals that horses are more sensitive to human active body language than passive body language and it could cause more stress and horses show more agonistic behaviours. Similarly, Birke et al.[20], examined the reactions of horses to human approach, and it was revealed that approaching horses quickly increased both the tendency to horse move quickly and the distance travelled significantly.

In the reactivity to passive human test, the maximum heart rate of horses was 38-100 pcs/min when they saw a familiar person, while it was 36-50 pcs/min when they saw an unfamiliar person. In the reactivity to active human test, the heart rate of the horses was 42-103 pcs/min. It has been determined that in the reactivity to passive human test, the average heart rate value for the reaction to an unfamiliar person (41.66±1.06) was significantly ($P < .01$) lower than the other tests (53.67±2.76 and 47.80±2.38). In the research, in the reactivity to passive human test, the processes of approaching a familiar person and then an unfamiliar were applied. Therefore, the research results reveal that horses become habituated to human passive body language with repeated applications. It is thought that these results are due to the habituation of the situation of humans in the test (waiting with passive body language), regardless of whether the horse recognizes the person or not. In addition, in the reactivity to human tests determined a positive significant ($P < .05$) relationship between the agonistic behaviour of horses and their heart rate. This suggests that horses with more agonistic behaviour during the tests had higher physiological stress levels.

In the handling test, a positive significant ($P < .01$) relationship was found between the agonistic behaviours of the horses during handling the horse to the test duration. Therefore, it was seen that the increase in the ethogram level of horses caused the test period to extend. This reveals that an increase in the agonistic behaviours of horses prolongs the duration of the handling process. At the same time, it is seen that the heart rate of horses is the highest in the handling test among human reaction tests. It is thought that a longer duration of training and increased stress may increase the risk of accidents and injuries. For this reason, it is thought to be important to evaluate horses' responses to behavioural tests. A study by Christensen et al.[21], on horse training, draws attention to the importance of knowing the habituation and sensitization abilities of horses in reducing the response related to frightening behaviours.

In the novel surface test, as horses made more attempts to pass over the surface across trials (1st Trial- 2nd Trial- 3rd Trial), the rate of successful passage also increased. This indicates habituation to the novel surface. Additionally, it was noted that the habituation process varies individually among horses. The results show that the trainability of horses may differ even if they are of the same breed and sex. The ethogram score of all horses passing the novel surface in the first trial was between 0-1. It was determined that the scores of the horses that did not pass the surface on the third attempt were above 5. In the data obtained from the novel surface test, a positive and significant relationship was determined between the number of surface crossings and the agonistic behaviours. This shows that the increase in agonistic behaviour in horses may reduce their success in crossing novel surfaces in behavioural tests and prolong the test durations.

Also, it was determined that the maximum heart rate of the horses that did not pass the novel surface on the first attempt was higher (85 pcs/min) than the other horses. The maximum heart rate of the horses which could not pass the surface at all was the highest (over 100 pcs/min). This suggests that frightening surfaces might cause more stress in horses with lower habituation ability than horses with higher habituation ability. Forkman et al.[22] drew attention to the importance of the fear test in examining the emotional states of animals and stated the importance of standardization of these tests in the development of applied ethology. Pierard et al. (2017)[23] pointed out that the existence of personality in animals is widely accepted in their studies, but it is necessary to develop valid and reliable methods to measure and interpret it.

According to the static and startling novel object tests, the rates of the horses' responses to the object were sniffing (86.60-100.00%), touching (33.30-86.60%), and biting (6.60-53.30%), respectively. In this test, almost all horses (93.40%) showed interest in the object. Although it was not statistically significant in the static and startling novel object tests, a negative relationship is observed between curiosity behaviour and agonistic behaviours and heart rates. This situation reveals the importance of benefiting from the curiosity behaviours of horses in training. Thus, there are many studies on the positive effect of the clicker method, which is based on curiosity behaviour and supports the training of horses by following the target stick, on the habituation processes of horses [24,25,26,27,28] and was reported to be a popular technique used in pet training today.[29] In the startling novel object test, the rate of interest toward the object was lower (80.00%). It was also

observed that the ethogram score was higher in the static novel object test compared to the startling novel object test. In this case, it can be said that horses show higher curiosity behaviour towards objects that cause less startle response. This situation also coincides with the way the target stick is used in the clicker method.

Researchers have measured fearfullness in horses in many studies.[30-32] Vidament et al.[33] stated that they developed experimental methods in their laboratories to determine the personalities of horses and that these simplified tests measured the reactions of horses in fear situations. When the heart rate values in the fear tests were examined in this study, the average heart rate values of the novel surface test (63.46±3.31) were found to be significantly ($P < .01$) higher than the other tests (53.46±2.41 and 50.53±2.20). This situation is also compatible with the higher ethogram values. These results show that startling stimuli in behavioural tests increase the occurrence of agonistic behaviours in horses and also increase heart rate levels. Additionally, there is a positive significant ($P < .05$) relationship between ethogram and heart rate during the tests. This reveals that the physiological and behavioural stress levels of horses during their habituation process could be determined with the help of behavioural tests. In Olczak et al.'s[34] study examining the link between horses' learning ability and fear across various tests, heart rate was measured before and after the fear test. The findings showed a significant increase in heart rate during the fear test compared to the pre-test level, aligning with our study's results. The research also revealed that fear can impact performance on certain types of learning tests.

In conclusion, the results of behavioural tests indicate that the trainability of horses may vary, even among those of the same breed and sex. It has been observed that increased agonistic behaviours during tests prolong training processes. Such behaviours and prolonged training periods can heighten the risks of accidents and injuries. Therefore, it's crucial to assess horses' behavioural responses through tests and plan their training individually. Utilizing learning theory in horse training is vital for both horse welfare and human safety. Additionally, standardized and reliable behavioural tests in applied ethology research can optimize efficiency by understanding horses' training tendencies, potentially reducing losses in the sector.

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Molecular Detection of Ovine Listeric Abortion in Nineveh Governorate, Iraq

Irak'ın Nineveh Vilayetinde Koyun Listerik Abortunun Moleküler Tespiti

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ABSTRACT

Listeriosis is an important abortifacient sheep disease, and is considered one of the most risky bacterial zoonotic disease worldwide**.** The study was carried out in 50 sheep flocks were located in the Nineveh governorate, Iraq during November and December 2022. A total of 300 specimens of blood, abomasal content and brain (100 each) were obtained from local breed ovine aborted fetuses (in the last stage of gestation) to be tested for molecular detection of *Listeria monocytogenes L. monocytogenes* DNA was detected in a total of 61(20.3%) specimens, distributed as: 35(57.4%), 15(24.6% and 11(18.0%) strains from fetal brain, abomasal content and blood specimens respectively using direct genus-specific conventional polymerase chain reaction (prfA gene) C- PCR. Two *L. monocytogenes* strains (HMB1 listeriolysin, HMB 2 listeriolysin) deposited in GenBank under accession numbers LC769365.1, and LC769366.1. Al *L. monocytogenes* strains were positive for three genes (*InlJ, InlA, and* hlyA) except act A gene was detected in 46 (75.4%) strains. In conclusion, *L. monocytogenes* is one of the important causative agent of abortion in sheep flocks in Nineveh governorate, Iraq, and greater fetal brain specimens were positive for listerial infection compared with other specimen.

Keywords: Listerosis, abortion, ovine, listeriolysin, Iraq

ÖZ

Listeriyoz, dünya çapında en riskli bakteriyel zoonotik hastalıklardan biri olarak kabul edilen önemli bir abortif koyun hastalığıdır. Çalışma, Kasım ve Aralık 2022 tarihlerinde Irak'ın Nineveh vilayetinde bulunan 50 koyun sürüsünde gerçekleştirildi. Listeria monocytogenes'in moleküler tespiti için yerel ırk koyun abort fetüslerden (gebeliğin son aşamasında) toplam 300 adet kan, abomazum içeriği ve beyin örneği (her birinden 100 adet) alındı. L. monocytogenes DNA'sı, doğrudan cinse özgü konvansiyonel polimeraz zincir reaksiyonu (prfA geni) C-PCR kullanılarak alınan toplam 61 (%20,3) örnekte tespit edildi. Örneklerin dağılımı: fetal beyin örneklerinden 35 (%57,4) suş, abomazum içeriği örneklerinden 15 (%24,6) suş ve kan örneklerinden 11 (%18,0) suş şeklindeydi. İki L. monocytogenes suşu (HMB1 listeriyolizin, HMB 2 listeriyolizin) GenBank'a LC769365.1 ve LC769366.1 erişim numaraları altında kaydedildi. Tüm L. monocytogenes suşları, akt A geni hariç olmak üzere üç gen (InlJ, InlA ve hlyA) için pozitif bulundu. Akt A geni 46 (%75,4) suşta tespit edildi. Sonuç olarak, L. monocytogenes, Irak'ın Nineveh vilayetindeki koyun sürülerinde yavru atmanın önemli nedenlerinden biridir ve diğer örneklerle karşılaştırıldığında daha fazla fetal beyin örneği listeriyal enfeksiyon açısından pozitif bulunmuştur.

Anahtar Kelimeler: Listeriyoz, abort, koyun, listeriyolizin, Irak

INTRODUCTION

Globally, ovine abortion is a common clinical issue and is mostly caused by *L. monocytogenes*.[1] It is a gram-positive, facultative intracellular microorganism that invades and colonizes mammalian cells. It is responsible for three main aspects in ruminants such as abortion in the last trimester of gestation, either sporadically or as outbreak, septicemia and encephalitis.[2] Silage, hay, bedding, and water were considered as major sources and possible reservoirs of *L. monocytogenes* in the farming.[2,3] A third trimester listeric infection may cause fetal death and placental retention with minor maternal sequelae, while near term infection potentially causes serious complications for pregnant dams including dystocia, severe metritis, and septicemia.[2,4]

The pathogenesis of *L. monocytogenes* is boosted by a numerator of essential virulence factors, counting endotoxin (encoded by inlA and inlB), hemolysin (hlyA), phosphatidylinositol-specific phospholipase C (PI-PLC, plcA), phosphatidylcholine-specific phospholipase C (PC-PLC, plcB) and actin polymerizing protein (actA)[5].

Isolation of the bacteria provides a definite diagnosis of the infection, but it takes a elongated time to cultivate on the agar media, L. monocytogenes can be recovered from an agar plate that has been refrigerated or preserved at room temperature for up to 3 weeks, hence it is preferred to use virulence genes to identify it.[6] Molecular techniques have been considered superior to traditional diagnostic methods, especially in recent years where it was relied upon in the diagnosis and classification of bacteria and the identification of virulence factors.[7,8] In Iraq, ovine Listeric abortion is inadequately investigated. Prior studies have been restricted to the bacterial isolation and molecular detection of microorganisms from foodstuffs and aborted cows.[9,10]

The purpose of present study to molecular diagnosis of ovine listeric abotion in Nineveh governorate, Iraq.

MATERIALS AND METHODS

Sample Collection

During November - December 2022, one hundred aborted fetuses from 50 flocks in the Iraqi Nineveh governarate were screened for the presence of *L. monocytogenes*. A 300 specimens were collected from the blood, abomasal content, and brain of the ovine aborted fetus in the last stage of gestation. Each obtained materials were collected separately in a sterile plastic bag and quickly transferred to the laboratory in cooled condition.

Conventional Polymerase Chain Reaction

A 25 mg of foetal tissue specimens were homogenized in phosphate buffered saline (pH 7.4) using mortar and pestle. DNA extraction was executed from the tissue homogenates using the commercial DNeasy Blood &Tissue Kit (Presto™ Mini gDNA Bacteria Kit, Geneaid Biotech Ltd /Taiwan) according to the manufacturer's instructions. DNA extraction were stored at -85 °C until required for PCR analyses. DNA from *L. monocytogenes* ATCC-7644 was used as a positive control, and a DNase-free distilled water was used as a negative control.

For the detection of the genus *Listeria, prs* gene amplification assays were carried out by PCR, using the luniversa l primers UNI-F and UNI-R (5'-'TTAGTGGCGGACGGGTGA and -3' GGTATCTAATCCTGTTTGCTC that amplify a 700-bp fragment of the 16s rRNA gene,[8] and for *Listeria monocytogenes*, the primers *prfA*-R-*prfA*-F (5'- GATACAGAAACATCGGTTGGC and 3' GTGTAATCTTGATGCCATCAG -) that generate a 274-bp fragment of the *prf A* gene.[11] The thermocyclar program were mentioned in table 1. The thermocyclar program for the *prfA* primers (primers *L. monocytogenes)* were presented in table 2.[12]

Table 1: PCR thermocycler program for *Listeria* spp.

The steps	Temp °C	Period	No of cycles
Initial denaturation	95	5 Min.	1
Denaturation	95	45 Sec.	
Annealing	63	45 Sec.	30
Extensions	72	45 Sec.	
Final extensions	72	7 Min.	1

Temp: temperatures, Min: minutes, Sec: seconds

Table 2: The thermocyclar program for the *prfA* primers

The steps	Temp. °C	Period	No. of cycles
Initial denaturation	95	5 Min.	
Denaturation	94	45 Sec.	
Annealing	56	30 Sec.	30
Extensions	72	1 Sec.	
Final extensions	72	5 Min.	1

Temp: temperatures, Min: minutes, Sec: seconds.

All strains of bacteria were examined for virulence-associated genes (InlJ, InlA, *hlyA*, and *actA).* The primers and it is sequences were listed in Table 3.

Table 3: Target genes and primer sequences used.

Target genes	Sequences (5'-3')	Product size (bp)	Reference
InlJ-R	TGTAACCCCCGCTTACACAGTT	238	Liu, et al. 13
InlJ-F	AGCGGCTTGGCAGTCTAATA		
InlA-R	ACGAGTAACGGGACAAATGC	800	Liu, et al. 13
InlA-F	CCCGACAGTGGTGCTAGATT		
hly -R	GCCTGCAAGTCCTAAGACGCCAATC	707	Hudson ,et al. 14
hly-F	CTTGCAACTGCTCTTTAGTAACAGC		
actA-F	CGCCGCGGAAATTAAAAAAAG	890	Suárez ,et al. 15
actA-R	ACGAAGGAACCGGGCTGCTAG		

The PCR technique program for the *InlJ, InlA, and* hlyA and actA genes were studied as follows: initial denaturation at 94°C for five minutes, followed by thirty cycles of denaturation at 94°C for 30 seconds, annealing at 50 ,52 and 58°C for thirty seconds for *InlJ, InlA, and* hlyA and actA genes respectively, and extension at 72°C for 2.5 minutes for *InlJ, InlA* and for 1 minute for other genes, then extension at 72°C for seven minutes for all genes. Final extension at 10°C for ten for four genes minutes.

PCR technique amplification products were analyzed electrophoretically on a 1% horizontal agarose gel.[16]

Statistical Analysis

The results were analyzed using Chi-square tests (STATA v.14.0) at confidence level 95%.

RESULTS

L monocytogenes was molecular detected in a total of 61 (20.3%) aborted fetal organs, distributed as: 35 (57.4%), 15 (24.6%) and 11 (18.0%) strains from fetal brain, abomasal content and blood respectively (Table 4), (Figure 1).

Table 4: Percentages of aborted fetal specimens positive for *L. monocytogenes* in Nineveh governorate, Iraq

Type of samples	Number of examined	Number of positive	%
Brain	100	35	35*
Abomasal content	100	15	15
Blood	100	11	13
Total	300	61	20.3
Type of samples	100	35	35*

* Significantly high in comparison to other specimens at P<0.05

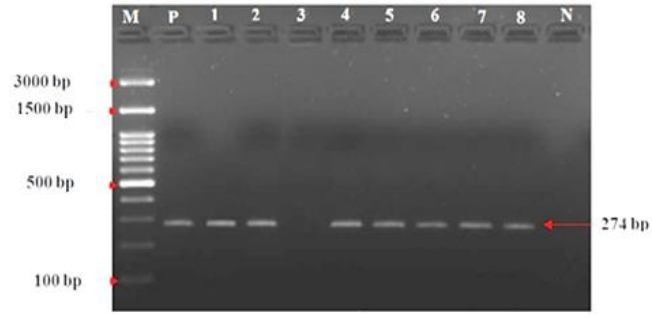

Figure 1: Electrophoresis and ethidium staining showing as a result of PCR procedures for *Listeria monocytogenes*, M: Represent marker. (1-8): Form positive result for *L.* monocytogenes with band size 274 bp, P: Positive control (L. monocytogenes ATCC: 7644), N: Negative control.

All *L. monocytogenes* strains were positive for three genes (*InlJ, InlA*, and hlyA) except for actA gene, detected only in 46 (75.4%) strains (Figs 2-4). Two abortigenic *L. monocytogenes* strains (HMB1 listeriolysin, HMB 2 listeriolysin) deposited in GenBank under accession numbers LC769365.1, and LC769366.1, and accession numbers of abortigenic two *L. monocytogenes* strains were positive for virulence-associated gene were deposited in the GenBank online database (Table 5).

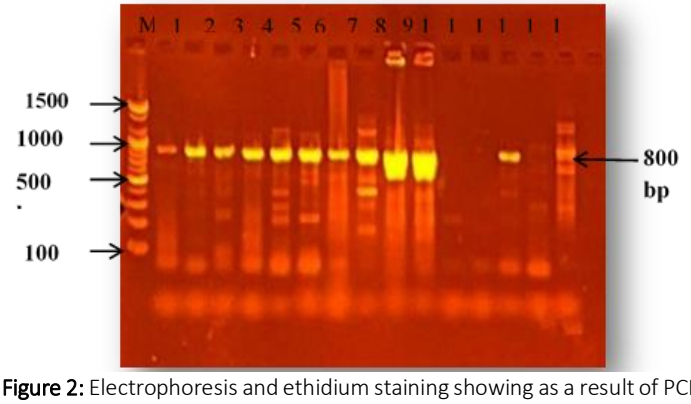

Figure 2: Electrophoresis and ethidium staining showing as a result of PCR procedures for Listeria monocytogenes Inter A., M: Represent marker (1-15) form positive result for Int A. with band size 800 bp.

Table 5: Strains and accession numbers of the L. monocytogenes isolated from aborted sheep fetuses in the Nineveh governorate, Iraq.

Strains	Accession numbers	Sources
HMBJ1 internalin J gene	LC769367.1	Aborted fetus blood-sheep
HMBJ2 internalin J gene	LC769368.1	Aborted fetus blood-sheep
HMBinlA1 internalin A gene	LC769373.1	Aborted fetus blood-sheep
HMBinlA2 internalin A gene	LC769374.1	Aborted fetus blood-sheep
HMBLLO1 listeriolysin O gene	LC769369.1	Aborted fetus blood-sheep
HMBLLO 2 listeriolysin O gene	LC769370.1	Aborted fetus blood-sheep
HMBactA1 actin-assembly inducing protein precursor gene,	LC769371.1	Aborted fetus blood-sheep
HMBactA2 actin-assembly inducing protein precursor gene,	LC769372.1	Aborted fetus blood-sheep

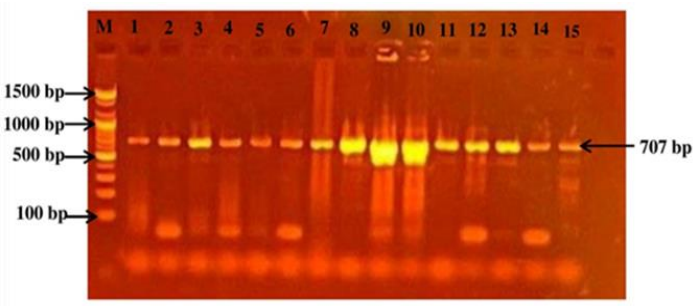

Figure 3: Electrophoresis and ethidium staining showing as a result of PCR procedures for Listeria monocytogenes Listerio Lysin O., M: Represent marker (1-15) form positive result for Listerio Lysin O. with band size 707 bp.

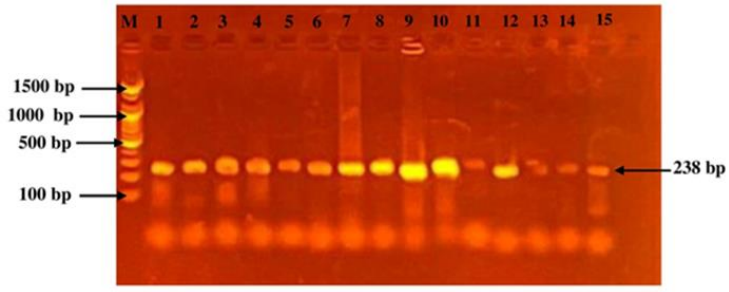

Figure 4: Electrophoresis and ethidium staining showing as a result of PCR procedures for Listeria monocytogenes Int J, M: Represent marker (1-15) form positive result for Int J. with band size 238 bp

DISCUSSION

Abortion in ruminants remains a difficult issue worldwide. It can result in unprofitable loss for farmers and an problematic of public health.[1] Abortion can be multicausal agents, and an extensive diagnostic research is essential to reach the accurate diagnosis[2].

In this study, we utilized molecular tool, for detection of abortigenic *L. monocytogenes*. Different procedures have been used to identify listeriosis, involving bacteriological, molecular and serology analyses.[17,18] Molecular approaches for identifying *L. monocytogenes* are becoming more frequently utilized because they are exceedingly reliable, and have high differentiation power within and between organisms that exhibit similar characteristics compared to cultural methods,[19] while the ordinary known microbiological methods routinely used for isolating *L. monocytogenes* in different samples usually need binary enrichment steps (enriched with Listeria selective supplements) which are later inoculated on the surface of the selective Listeria agar.[7] Additionally, *L. monocytogenes* are auxotrophic for seven amino acids including leucine, isoleucine, valine, methionine, arginine, cysteine, and glutamine and the bacteria also require four additional vitamins including riboflavin, thiamine, biotin, and thioctic acid[6]. After examination of 300 specimens were collected from ovine aborted fetuses, we identified *L. monocytogenes* DNA in 61 (20.3%), distributed as: 35 (57.4%), 15 (24.6%) and 11 (18.0%) strains from fetal brain, abomasal content and blood specimens respectively. The identification of clinical and ecological isolates of *L. monocytogenes* is significant since the same type has been shown to circulate within farms or geographical zones. *L. monocytogenes* genotypes related with human outbreaks were identified in dairy cows; thus, characterization of listerial isolates has implications for public health.[20]

Abortigenic *L. monocytogenes* strains in Iraq have not been studied with molecular characterization. The presence of *L. monocytogenes* has been identified serologically in 19.7% of camels in Kirkuk city, Iraq.[21] and in 11.5% in sheep flocks in Nineveh governorate, Iraq.[22]

In general, Listeria findings are many times smaller than our results. Researchers in Brazil, testing using molecular methods, found that 4 or 6.25% of the 64 materials analyzed were positive for *L. monocytogenes*.[23] Similarly Shoukat et al.[24] was detected of *L. monocytogenes* in 2.83% of aborted ewes in Kashmir Region, India, and 8.3% in Denmark.[25] Likewise, *L. monocytogenes* was isolated from aborted ewes in in Sharkia Governorate, Egypt[26], and thirty-one *Listeria* isolates out of 240 samples were recovered from diseased sheep with a prevalence rate of 12.9% in Egypt.[27]

These present findings of 20.3% as *L. monocytogenes* is slightly lower than what was reported by Wagner et al.[28],

who reported listerosis in 25% of aborted ewes in Austria. There may be many reasons for this variation in the results, including differences in diet type, specimen counts, and geographic location[1,3].

All *L. monocytogenes* strains were positive for three genes (*InlJ, InlA, and* hlyA) except actA gene was detected in 46 (75.4%) strains. The presence of two virulence genes in most *L. monocytogenes* isolates indicates that these isolates are virulent and can cause disease.[5]

A previous study also reported that the prevalence of hlyA gene among isolates was as high as 98.4%, while the prevalence of other virulence genes iapA, plcA, and plcB were 85.7%, 73%, and 68.2%, respectively.[29] In contrast to this study, Laximan et al.,[30] targeted virulence cluster genes (hlyA, iap, plcA, actA, and prfA) to identify *L.monocytogenes* in milk samples.

In conculsion, this study indicated that *L. monocytogenes* could be a noteworthy pathogen associated with ovine abortion cases in Nineveh governarate, and most of the brain specimens were positive for listeric infection compared to the other specimens.

Ethics Committee Approval: The institutional care of animals and utilization authority of the Veterinary Medicine College, University of Mosul, acknowledged this research (authorization number UM.2022.032).

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Measuring Stress in Animals By Noninvasive Methods

Hayvanlarda Stresin Noninvasiv Metotlarla Ölçülmesi

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ABSTRACT

Stress is a biological response in the form of physiological, biochemical, hematological, and behavioral changes to internal or external stimuli that threaten the homeostasis of living beings. Effects that activate the defense system in living things are defined as stressors, and it is possible to talk about many different stress factors. Factors that cause stress can be divided into environmental, physical, social or emotional. Stress reactions, which begin with the effect of the stressor, vary according to the duration and severity of exposure to stress. In animal welfare, stress has many negative effects on organism. These negative effects may cause many problems and, shape future by adding problems such as stress and chain links in animals. Just as well-being is for humans, well-being is very important for animals. To determination of glucocorticoids or metabolites in the bloodstream of an organism under stress, noninvasive methods that provide reliable stress measurement without interfering with the organism have recently become increasingly popular. In this review article study, nineteen articles from various parts of the world were examined. In this review, measures of stress by non-invasive methods by looking at GlucoCorticoid Metabolites (GCM) and the latest developments in this field are discussed. In this review article study, nineteen articles from various parts of the world were examined. As a result of the articles reviewed, non-invasive methods for measuring stress may aid and improve our understanding of stress biology and animal welfare. Applying this method to many animal species and biological materials will provide accurate results and support animal welfare.

Keywords: Animal welfare, cortisol, glucocorticoid metabolites, non-invasive, stress.

ÖZ

Stres, canlıların homeostasisini tehdit eden iç veya dış uyarılara karşı, fizyolojik, biyokimyasal, hematolojik ve davranışsal değişiklikler şeklinde verilen biyolojik bir cevaptır. Canlılarda savunma sistemini harekete geçiren etkiler stresör olarak tanımlanmakta olup bir çok farklı stres faktörlerinden bahsetmek mümkündür. Strese neden olan faktörler ise çevresel, fiziksel, sosyal veya duygusal olarak ayrılabilir. Stresörün etkisiyle başlayan stres reaksiyonları, strese maruz kalma süresine ve şiddetine göre değişir. Hayvan refahında stresin organizma üzerinde birçok olumsuz etkisi bulunmaktadır. Bu olumsuz etkiler birçok soruna neden olabilmekte ve hayvanlarda stres, zincir halkaları gibi sorunları da ekleyerek geleceği şekillendirebilmektedir. İnsanlar için olduğu gibi hayvanlar için de refah çok önemlidir. Stres altındaki bir canlının kan dolaşımındaki glukokortikoidlerinin veya metabolitlerinin belirlenmesi için canlıya müdahale etmeden, güvenilir bir şekilde stres ölçümü sunan noninvazif yöntemler son zamanlarda giderek daha popüler bir hale gelmiştir. Bu derlemede stresin noninvasiv metotlarla GlikoCorticoid Metabolitleri (GCM) bakılarak yapılan ölçümleri ve bu alandaki son gelişmeler ele alınmıştır. Yapılan bu derleme makalesi çalışmasında dünyanın çeşitli yerlerinden on dokuz makale ele alınarak incelenmiştir. İncelenen makaleler sonucunda stresi ölçmeye yönelik invazif olmayan yöntemler, stres biyolojisi ve hayvan refahı konusundaki anlayışımıza yardımcı olabilir ve bunu geliştirebilir. Bu yöntemin birçok hayvan türü ve biyolojik materyalde uygulanması hem doğru sonuçlar verecek hem de hayvan refahını destekleyecektir.

Anahtar Kelimeler: Glikokortikoid metabolitleri, hayvan refahı, kortizol, non-invaziv, stres.

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INTRODUCTION

Stress is a biological response in the form of anatomical, physiological, and behavioral changes to internal or external stimuli that threaten the homeostasis of living things.[1] In animal husbandry, stress is the sum of the organism's responses to unsuitable environmental conditions, which can lead to many undesirable consequences, from discomfort to death.[2] Stress; it can be determined by health, yield, behavior, and physiological parameters.[3]

In animal welfare, stress has many negative effects on organism. These negative effects may cause many problems and, shape future by adding problems such as stress and chain links in animals. Just as well-being is for humans, well-being is very important for animals.

The definition of animal welfare has been defined as "If an animal is healthy, comfortable, well fed, safe, can exhibit behavior patterns specific to its species, if it is not afraid, painless, and not under stress, that animal is well-off."[4]

Welfare refers to the long-term wellness of an animal, which is the result of its acquired experiences of the living conditions that it copes with. It is very important to optimize physical and climatic environmental conditions to keep health, welfare and production of the livestock.[5,6]

There has been an increased interest in animal welfare recently, but assessing animal welfare is both a difficult and complex subject. The definition of well-being is more than the absence of stress, but stress also plays an important role in good well-being. Stressors are stimuli that cause an imbalance of homeostasis. An animal's defense responses to stimuli, on the other hand, are defined as stress responses. The brain, on the other hand, has a central role in linking stressors to stress responses.[7]

In order to overcome stressful situations, glucocorticoids secreted from the adrenal glands are at the forefront of the war. They are usually measured in plasma samples as parameters of adrenal activity, but the collection of blood samples is uncomfortable and further stresses the animal. Therefore, non-invasive methods for the determination of glucocorticoids or their metabolites are better for animal welfare and stress.[7]

Stress Factors

The effects that activate the defense system in living things are defined as stressors. In this sense, it is possible to talk about quite different stress factors. Factors causing stress can be environmental, physical, social, or emotional.[8]

Extremely hot and cold conditions, precipitation, toxins in feed, poor care conditions, uncomfortable bedding, use of inappropriate litter, errors during transportation, some infections and chemicals, hunger, thirst, fear, noise, injury, diseases, social interactions between species or animals, frequency of settlement, social status, psychological stress, trauma, new or foreign environment, social isolation, punishment, problems in human-animal relations, alarm vocalizations, social disturbances and handling, social isolation, etc. are stressors.[1,8,9]

Effect of Stress

Stress in animals changes physiological (respiration, pulse, blood pressure, body temperature, heart rate), biochemical (hormone, enzyme, electrolyte levels, fatty acids, cortisol), hematological (heterophil, lymphocyte, hematocrit values, and rates), and behavioral (aggression, restlessness, nervousness, feed, and water consumption) parameters.[1]

Stress Response

Stress reactions begin with the influence of the stressor. This period is defined as the alarm phase. It continues with the adaptation phase against stress. If the adaptation phase is successfully passed, the creature returns to normal life. If the stressor cannot be dealt with, the organism enters the exhaustion phase.[8]

Changes in biological activities during stress are defined as the biological cost of stress. The organism has limited energy resources. If the biological cost is not at a level that will affect the resources necessary for the continuation of physiological functions, the animal is not at risk. However, if the stress is severe and long-lasting enough to consume the necessary resources for physiological functions, it results in a pathophysiological condition.[8]

Once the central nervous system perceives a threat, it devolops a biological responce or defence that consists of a combination of the four general biological defnece responses: the behavioural responce, the autonomic nervous system responses, the neuroendecorine system response or the immune response (Figure 1).[10]

Stress factors reveal neural and hormonal activities. Exposed stimuli affect the hypothalamus and turn into a neural hormonal factor. Corticotropin releasing hormone (CRF) secreted from the hypothalamus stimulates the anterior pituitary and releases adrenocorticotropic hormone (ACTH).

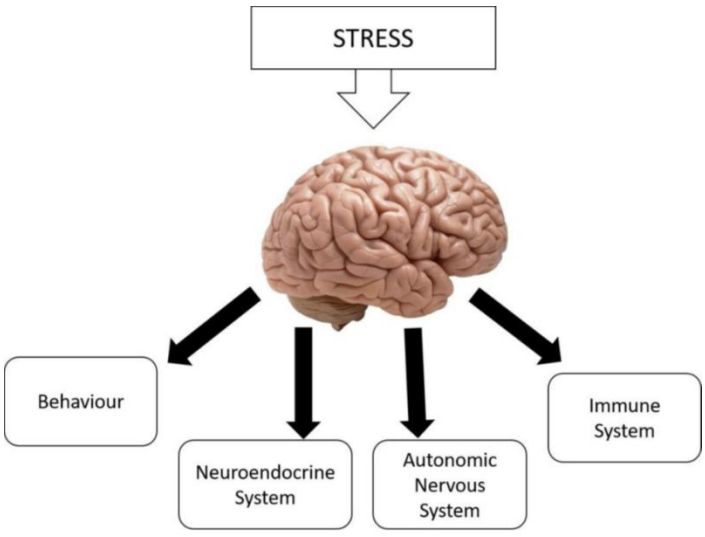

Figure 1. The general types of biological responses available to the animal for coping with stress.[10]

ACTH comes to the adrenal glands through the bloodstream and further increases the secretion of glucocorticoids. As a result of interaction with the stressor for a certain period of time, the first response in the organism is usually to fight. The body prefers to fight first rather than adapting to the stressor. This chain of events is called the fight-or-flight mechanism.[11]

While the sudden release of adrenaline or noradrenaline from the adrenal medulla continues this chain of events, an increase in energy production is inevitable. In addition, more energy production is required for the series of events in the nervous system. For this reason, neurogenic amines activate the hepatic adenylcyclase enzyme, which is necessary and effective in energy reactions, enabling the conversion of glycogen to glucose in the liver.[11]

In the alarm circuit, which starts with the effect of the stressor, hypochloremia occurs and blood density increases. Adrenaline secreted from the adrenal medulla and noradrenaline released from sympathetic nerve endings increase pulse, blood pressure and respiratory rate.[12,13]

In cases where the effect of the stressor is very long and continuous, it reduces the animal's relationship with the environment and even suspends its reproductive functions in order to maintain homeostasis and meet the increased metabolic needs. This passive and vulnerable response is called “protection or withdrawal” or “adaptation”.[14] At this stage, while the release of corticoids from the adrenal cortex becomes active; activated corticoids increase the metabolic effects of catecholamines and prolong the duration of action.[15-17] After the release of ACTH from the anterior pituitary lobe, the production of corticosterone increases, while the thymus, spleen, and peripheral lymph nodes shrink. At the same time, the pituitary lobe enlarges and the weight of the adrenal glands increases. While the number of lymphocytes in the circulation decreases, the number of heterophiles increases.[11] Glucocorticoids that occur in the adrenal cortex tissue and pass into the adrenal medulla regulate the conversion of noradrenaline to adrenaline by activating the phenyletolamine N methyl transferase enzyme.[12] Continuous stimulation of the adrenal cortex causes corticosteroids to remain in the circulation in high concentration and continuously.

Cardiovascular and gastrointestinal diseases, hypercholesterolemia, metabolic disorders, and changes in immunological functions may occur in animals. As a result of these, inflammatory events are suppressed, defense reactions related to lymphocytes are slowed down and antibody production is prevented. The magnitude of the effect on the immune system is greatly influenced by genetic factors and nutrition.[10] If the effect of the stressor continues for a long time and in this case, the defense mechanisms are insufficient, the "exhaustion" in the body of the animal. Then pathological changes may occur as a result of adrenal insufficiency and death comes at the end.[18,19]

Stress causes some rapid and temporary changes in the body then permanent and irreversible changes happen as a result of the continuation of stress. In addition, the growth, yield and reproduction of the sick animal comes to a standstill and the animal struggles to protect its health. Allostasis is the body's process of preventing problems that may occur against stimuli and maintaining homeostasis. In order to be successful in this process, both general and specific physiological systems and behavioral coping mechanisms are activated to a large extent.[20]

Why is Stress Important?

Stress affects animal growth rate, offspring mortality, disease resistance, meat and milk production, and reproductive ability by reducing the animals' ability to cope with the environment. Animals under stress get sick more easily, as a result, more drugs are used, so drug residues in animal products increase and this threatens public health.[1]

Stress factors such as transport and pre-slaughter conditions cause a decrease in muscle glycogen level and an increase in meat pH. Thus, dark, hard, dry meat and economic losses take place.[21] Animal welfare standards ensure animal health. Only healthy products are obtained from healthy animals.

Why is Stress Measured?

The glucocorticoid (cortisol) secreted by the adrenal glands is the frontline of the battle for both overcoming and measuring the stress. Cortisol levels can provide information about a stressed organism and can help to protect the animal from stressors and manage the environmental issues.

Cortisol, which is frequently used as a stress indicator in animal behavior researchs, is a defense hormone that protects the organism against any changes in physiological balances by affecting electrolyte, carbohydrate, protein, and lipid metabolism. Cortisol is a two-carbon steroid glucocorticoid found in plasma-bound to protein.[22]

Cortisol is commonly measured in plasma, saliva, feces, eggs, hair, and feathers. The welfare level of animals can be increased by environmental improvement with the information obtained from the animal.

How is Stress Measured?

Stress can be measured by invasive and non-invasive methods. Many of the invasive techniques can induce a stress response and this may affect the results. Recently, non-invasive methods have become increasingly popular for the determination of glucocorticoids themselves or their metabolites in the stressed animal. They offer a reliable measurement of stress without interfering with the animal.

Some of the studies, searching GCM (GlycoCorticoidMetabolites) usage potential in stress animals by non-invasive methods are summarized as folllows:

In a study conducted in New Zealand, fecal GCM was measured after dairy cattle exposed to stress factors (transport and adaptation to a new an environment). GCM was found to be significantly higher in the animal exposed to the transport than the control group ones; and peaked two days after they were moved to the new environment.[23] In Canadian deer and wolves, the fecal GCM values were significantly increased after sledding activity on the snow.[24]

The fecal GCM of the rabbits which smelt fox feces was found to be significantly higher than the control group and males had more GCM levels than females.[25]

In another study, red-footed partridges were caught, captived and exposed to various stress series. It was determined that the amount of GCM observed in feathers, was significantly correlated with plasma corticosteroid level.[26]

Laying hens were exposed to high environmental temperatures and moved to new cages, then GCM accumulation in egg albümin and plasma corticosterone levels were investigated. As a result of this study indicated that GCM levels in eggs were useful indicators of stress in laying hens.[27]

In a research performed in wild-living orangutans showed that, fecal GCM levels increased significantly after tourist visits when compared to the pre-visit.[28]

Fecal GCM levels were measured in captive bengal and sumatran tigers at Dreamworld Theme Park and Zoo in Australia. As a result of the study, no significant difference was found between bengal and sumatran tigers. However, the mean fecal GCM level was higher in females and sick animals than males and healthy ones, respectively.[29]

It was concluded that cats living in the crowd had significantly higher fecal GCM levels than those singles. Besides, older cats showed higher fecal GCM levels than youngers.[30]

In a study carried out in broilers which were exposed to heat stress for two hours, fecal GCM levels increased significantly when compared to control animals.[31]

Guinea pigs were intermittently brought together in a new social environment by applying regroubing. Stress levels were measured by looking at fecal and salivary GCM; then a significantly higher increase in fecal and salivary GCM levels was detected.[32]

A significant increase in both plasma cortisol and fecal GCM levels was observed during the inflammatory disease outbreak in Atlantic Salmon.[33]

In a previous study, male green lizards were exposed to hierarchy and regrouping stressors. Fecal GCM levels were found to be high in stressed groups.[34]

Fecal GCM levels of otters were investigated both in a man-made environment and natural environment. As a result; fecal GCM levels of otters which were living in a man-made environment were found to be significantly higher than the others.[35]

In a study conducted in African wild dogs, fecal GCM levels of captive dogs were found to be higher than those of free dogs.[36]

It was observed that hair GCM levels of the snowshoe rabbits increased when they encountered their predators, the lynx.[37]

Fecal GCM levels were investigated in polar bears before and after transportation of them to the zoos. The transportation process effected the levels of GCM significantly.[38]

In a study conducted on a farm in South America, cows were divided into 3 groups. The cows in the 1st group were constantly listened to classical music. The cows in the second group were listened to classical music for a limited time. The cows in the 3rd group were not allowed to listen to any music. Faecal glucocorticoid metabolites of cows were examined and milk yields were measured. As a result, it was observed that the cows in the 1st group that constantly listened to classical music had higher milk yield and lower fecal glucocorticoid metabolites than the others. These findings showed that auditory stimuli such as classical music have a positive effect on cow welfare and milk yield. [39]
In a study, it was seen that the fecal GCM levels of whales showed a significant positive correlation between ship traffic and underwater ambient noise levels. This suggests that noise generated by ship traffic may be a causal factor for the increased fecal GCM. [40]

In another study in which stress experienced by weaning foals was determined through fecal GCM, foals showed a marked stress response to the weaning process through increased fecal GCM levels. [41]

CONCLUSION

Stress is defined as the biological response to any effect that disrupts the internal balance of the organisms. Although the animals have mechanisms to cope with stress, the long exposure to stress puts them on irreversible paths. The stress response can be detected by abnormal behavioral pattens, also biochemical changes due to the extent of the stress. Aggression, loss of appetite, decrease in growth and reproductive activities together with biochemical hormone secretions can be measured objectively. Glucocorticoid (cortisol), one of the most important hormone among other stress hormones, increases when the animal faced a stress factor. This incline in cortisol level shows us that the animal is under stress. Invasive applications to measure the stress level can cause extra stress and increase cortisol levels. This makes it difficult for the researchers to make a healthy measure of stress. Using non-invasive tools to measure stress can help alleviate this problem. Noninvasive methods for measuring stress can help and improve our understanding of stress biology and animal welfare. In the scientific literature, there are limited studies on the measurement of glucocorticoid metabolites in plasma, saliva, feces, hair and/or feathers. The application of this method in many animal species and biological materials will not only present accurate results, but also support animal welfare by adapting the environment.

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