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Veterinary Sciences *and* Practices

ABOUT

Veterinary Sciences and Practices is a peer-reviewed, open access, online-only journal published by Atatürk University.

Veterinary Sciences and Practices is published triannually in Turkish and English, with issues released in April, August, and December. Beginning on October 17, 2023, Veterinary Sciences and Practices will exclusively consider articles in English. However, Turkish abstracts will still be included in English articles alongside the English abstracts. A language editor will be responsible for translating the English abstracts of accepted papers into Turkish.

Journal History
As of 2022, the journal has changed its title to Veterinary Sciences and Practices.

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Veterinary Sciences and Practices aims to publish studies of the highest scientific level in all fields of veterinary medicine.

Veterinary Sciences and Practices is a comprehensive journal dedicated to the field of Veterinary Medicine and relevant Departments, i.e., Basic Veterinary Sciences (Anatomy, Biochemistry, Pshysiology, Histology, Occupational/Professional Ethics and Deontology), Preclinical Veterinary Sciences (Pharmacology and Toxicology, Microbiology, Parasitology, Pathology, Virology), Clinical Veterinary Sciences (Surgery, Internal Medicine, Animal Obstetrics and Gynecology, Reproduction and Artificial Insemination), Animal Science and Nutritional Sciences (Biostatistics, Genetics, Animal Nutrition and Nutritional Disorders, Animal Enterprises Economy, Animal Science), Animal-Originated Food Hygiene and Technology, with exotic animal science and laboratory animals. The primary focus of the journal is to publish original research that addresses significant clinical inquiries and contributes to the advancement of knowledge and treatment of veterinary conditions. The scope of the journal includes studies on the efficacy of different treatment modalities, innovative diagnostic tools or techniques, and novel approaches to the prevention and management of diverse veterinary diseases and injuries. Veterinary Sciences and Practices aims to foster the dissemination of high-quality research that can enhance the well-being and healthcare of animals, promote animal welfare, and improve the overall practice of veterinary sciences.

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.1607091

Investigation of the Effect of Wheat and Corn Gluten on Inflammation, Transglutaminase, Gliadin and IgA Levels in Healthy Rat Intestines

Sağlıklı Rat Bağırsaklarında Buğday ve Mısır Gluteninin İnflamasyon, Transglutaminaz, Gliadin ve IgA Düzeyleri Üzerine Etkisinin Araştırılması

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ABSTRACT

The aim of this study was to evaluate the effects of wheat and corn gluten on some histopathologic parameters such as villus atrophy, crypt hyperplasia, lymphocyte plasma neutrophils and immunohistochemical parameters such as trans glutaminase, gliadin and IgA in the small intestine of healthy male rats without HLA-DQ2 and HLA-DQ8 genes. In the study, 21 healthy newborn male Sprague Dawley rats were fed wheat, corn and soy with the addition of 7 rats in each group from one-day age to 60 days of age. Histopathological (villous atrophy, lymphocyte plasma neutrophil, crypt hyperplasia) and immunohistochemical (transglutaminase, gliadin, IgA) parameter analyses were performed in small intestinal tissue samples. As a result of the study, it was found that the small intestinal villus lengths of the wheat gluten group were longer than the other groups ($P < .05$). Cryptic hyperplasia was detected most in the soybean group and the lowest in the wheat group ($P < .05$). Gliadin antibody levels were found to be in the soybean group with the highest and the lowest in the wheat group ($P < .05$). In healthy male rats lacking HLA-DQ2 and HLA-DQ8 genes, the effect of wheat gluten on crypt hyperplasia and gliadin levels in small intestinal tissue was significantly lower than in soy and corn gluten groups, while its effect on villous atrophy, lymphocyte plasma neutrophil and transglutaminase was limited. In addition, the intestinal villus lengths of the wheat gluten group were significantly higher than those of the corn and soybean groups.

Keywords: IgA, gliadin, gluten, tranglutaminase, villi.

ÖZ

Bu çalışmada HLA-DQ2 ve HLA-DQ8 genlerine sahip olmayan sağlıklı erkek ratlarda buğday ve mısır gluteninin ince bağırsaklarda villus atrofisi, kript hiperplazisi, lenfosit plazma nötrofil gibi bazı histopatolojik parametreler ile trans glutaminaz, gliadin, IgA gibi immünhistokimyasal parametrelere etkisinin değerlendirilmesi hedeflenmiştir. Çalışmada, 21 adet sağlıklı yeni doğmuş Sprague Dawley cinsi erkek rat bir günlük yaştan 60 günlük yaşa kadar her grupta 7 rat olmak üzere buğday, mısır ve soya eklenerek beslenmişlerdir. İnce bağırsak doku örneklerinde histopatolojik (villöz atrofi, lenfosit plazma nötrofil, kript hiperplazi) ve immunohistokimyasal (transglutaminaz, gliadin, IgA) parametre analizleri yapılmıştır. Çalışma sonucunda buğday gluteni grubunun ince bağırsak villus uzunluklarının diğer gruplardan daha uzun olduğu saptanmıştır ($P < ,05$). Kript hiperplazisi en fazla soya grubunda, en düşük buğday grubunda tespit edilmiştir ($P < ,05$). Gliadin antikor seviyesi en yüksek soya grubunda iken en düşük buğday grubunda olduğu tespit edilmiştir ($P < ,05$). HLA-DQ2 ve HLA-DQ8 genlerine sahip olmayan sağlıklı erkek ratlarda buğday gluteninin ince bağırsak dokusunda kript hiperplazisi ve gliadin değeri soya ve mısır gluteni verilen gruplardan önemli derecede düşük olduğu belirlenirken, villöz atrofisi, lenfosit plazma nötrofil ile transglutaminaz üzerine etkisi sınırlı düzeyde kalmıştır. Ayrıca buğday gluteni verilen grubun bağırsak villus uzunlukları mısır ve soya verilen gruplardan önemli oranda yüksek olduğu tespit edilmiştir.

Anahtar Kelimeler: IgA, gliadin, gluten, tranglutaminaz, villus.

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INTRODUCTION

Cereals are important plant-based foods and wheat, rice, corn are the most consumed cereals worldwide. It also contains protein, starch, vitamins and minerals in their structure. In cereals, proteins are classified as gluten-forming and non-gluten-forming proteins, and the proteins that make up gluten are glutelin and prolamine proteins. Glutelines are called glutenin in wheat and hordenine in barley whereas prolamins are called gliadin in wheat, hordein in barley, avenin in oats, secalin in rye and zein in corn. Gluten is the main storage form of wheat proteins, which makes up 85-90% of wheat proteins, and is basically a structure consisting of gliadin and glutenin complex. It is stated that 5-20 g/day of gluten is taken with the Western diet.[1] The increase in the production and consumption of gluten-containing products has led to the awareness of gluten-related diseases. Celiac disease is seen in at least 1% of the general adult population.[2] Gluten-related diseases are classified as immune, allergic and autoimmune according to pathogenesis. While celiac disease is in the autoimmune class, gluten sensitivity is in the autoimmune and non-allergic class.[3] Gastrointestinal symptoms such as celiac disease, diarrhea, steatorrhea, abdominal distention, abdominal pain and gas, and non-gastrointestinal symptoms such as abnormal liver function tests, iron deficiency anemia, bone and skin diseases can be seen.[4] Celiac disease is usually detected by serological tests and celiac specific antibodies and diagnosed by duodenal mucosal biopsies.[5] The primary treatment for celiac disease is a gluten-free diet.[6,7] In individuals with HLA-DQ2 and HLA-DQ8 genes in celiac disease, gluten intake may cause inflammatory response and villi damage. This leads to a new anti-inflammatory response in the intestine with the release of anti-gliadin, anti-endomysial antibodies and tissue transglutaminase. Although the general population prevalence of non-celiac gluten sensitivity is not clearly known, celiac-like symptoms may occur after gluten intake and may occur in the absence of celiac specific antibodies, villi atrophy and human leucocyte antigen (HLA) change. Therefore, anti-tissue transglutaminase and endomysial antibodies are negative and there is no change in the intestinal mucosa. However, an increase in interferon (IFN)-γ and CD3+ T cells can be seen.[8,9] In a study with rats, wheat gluten was found to increase the level of CD3 and CD8 in the intestines, although not statistically significant.[10] In another study with rats, it was found that the immunohistochemical parameters CD4, CD8, IgA, gliadin and transglutaminase in ovarian tissues were lower in the wheat group than in the soy group.[11] As a result, gluten can affect histopathological and immunohistochemical structures in many tissues, especially in the intestine. In this study, it was aimed to evaluate the effect of wheat and corn gluten on some histopathological parameters such as villus atrophy, crypt hyperplasia, lymphocyte plasma neutrophil in the small intestine and immunohistochemical parameters such as transglutaminase, gliadin, IgA in healthy male rats without HLA-DQ2 and HLA-DQ8 genes.

MATERIALS AND METHODS

Animal Working Groups

The animal supply used in the study, the care and feeding of the animals during the trial period were carried out at Atatürk University Medical and Experimental Application and Research Center (ATADEM). This study was approved by the Eastern Mediterranean University Scientific Research and Publication Ethics Board Health Ethics Sub-Committee with the decision dated 18.11.2020 and numbered 2020/07.

In the study, a total of 21 healthy Sprague Dawley male rats were fed soy, corn or wheat feeds isonitratically and isocalorically according to the experimental group in which they took part for a total of 60 days, with their mothers from one day old to 30 days of age, and separately from their mothers from 30 days to 60 days of age (Table 1). At the end of sixty days, rats in the soybean group (7 pieces), corn group (7 pieces) and wheat group (7 pieces) were sacrified under general anesthesia and tissue samples were taken from their small intestines for histopathological and immunohistochemical examinations.

Table 1. Feed given to rats.

Feed Additives %	Soy	Corn	Wheat
Wheat Bran	6	5	2
Oat, 11% Crude Protein	6	5	3
Sunflower Meal, 28% Crude Protein	6	4	1
Corn Gluten, 62% Crude Protein	5	3	1
Wheat Gluten, 75% Crude Protein	6	4	2
Soy Meal, 51% Crude Protein	5	4	1
Animal Fat	6	4	2
Vitamin-Mineral Combination			
Feed Nutritional Values			
Crude Protein,%	22.0	22.0	22.0
Metabolic Energy, ccal/kg	2598.0	2657.0	2599.0
Calcium%	0.14	0.11	0.15
Methionine + Cysteine,%	0.68	0.83	0.66
Lysine,%	1.15	0.63	1.17

Histopathological Examination

The eight animals from each group were sacrificed under anesthesia end of the sixty days. The tissue samples were fixed in 10% buffered formalin and routinely processed for histological examination by embedding in paraffin wax. Tissue sections were cut (thickness 4 µm), stained (Haematoxylin-Eosin) and observed under a light microscope.[12,13]

Villi Lengths

After the small intestine tissues of rats were stained with the above-mentioned method for histopathological analysis, the villi lengths were measured with the image analysis system (Leica Q Win Standard) from the tip of the villi to the villi crypt junction.[14]

Immunohistochemical Examinations

Tissue sections (thickness 4 µm) from all of the tissue samples were processed by a standard avidin-biotin-peroxidase method which is described by producer. Rabit policlonal antibodies that react with rat transglutaminase 2 (TG2) antibody (Catalog No:NB600-547), gliadin antibody (Catalog No: BS-13374-R), IgA antibody (Catalog No: BS-0648-R10491-R) were used for 60 minutes. A secondary antibody was used in compliance with protocol of the manufacturer (expose mouse and rabbit-specific HRP/DAB detection IHC Kit, Abcam Cat. No. ab80436). Then tissue sections was washed three times with 0.1% Tween 20 in PBS and were incubated with 3,3-diaminobenzidine (Dako Cytomation) and counterstained with Mayer's hematoxylin (Dako Cytomation).[12,15]

Image Analysis

High-power light microscopic examination (Olympus Bx51 with a DP72 camera system) was used for tissue section evaluation. Each specimens were examined in 10 randomly selected areas with an X40 objective. The scores were derived semi-quantitatively using light microscopy on the preparations from each rat and were reported as follows: Grade 0 = – (negative); Grade 1 = +1 (mild); Grade 2 = +2 (moderate); Grade 3 = +3 (severe); Grade 4 = +4 (most severe).[16]

Statistical Analysis

SPSS 10.01 program was used in the statistical evaluation of the obtained findings.[17] Histopathological parameters and immunohistochemical parameters were calculated in the statistical evaluation and median values and standard error (SE) values were calculated in the soy, corn and wheat groups. One Way ANOVA was used for small intestinal villus lengths and Duncan test was used for the difference between groups. Kruskal Wallis analysis was used for changes in histopathological and immunohistochemical parameters and Duncan test was used for the difference between the groups. Statistical significance was accepted as $P < .05$.

RESULTS

Histopathological Findings

At the end of sixty days, the mean villus length of wheat group was higher than soybean and maize groups ($P < .05$) (Figure 1).

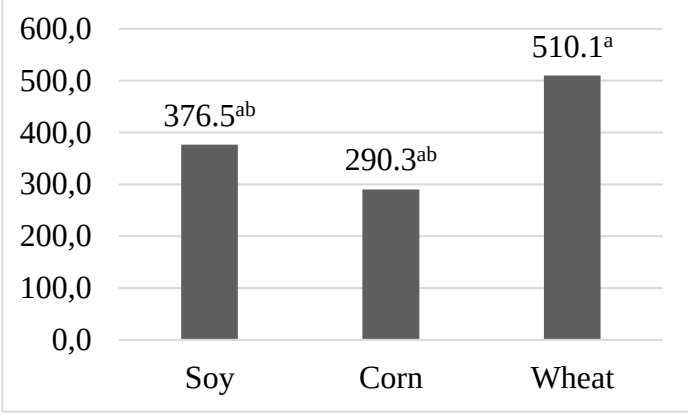

Figure1. Small intestine villus lengths (µm) [a,b] P < 0.05

Histopathological values are shown in Table 2 and the statistical results of these values are shown in Table 3. Villus atrophy was highest in the corn gluten group and lowest in the soybean group, and lymphocyte plasma neutrophil values were highest in the soybean group and lowest in the corn group ($P > .05$). Cryptic hyperplasia was highest in the soybean group and lowest in the wheat group ($P < .05$) (Table 3, Figure 2).

Immunohistochemical Findings

The immunohistochemical values of the groups are shown in Table 2 and the statistical results of these values are shown in Table 3. While there was no statistically significant difference between the transglutaminase and IgA values of the groups, the soy group with the highest transglutaminase value was determined in the wheat group with the lowest and the corn group with the highest IgA value was determined in the lowest soybean group. The highest gliadin level was found in the soybean group and the lowest in the wheat group ($P < .05$) (Table 3, Figure 3).

Table 2. Histopathological and immunochemical results of intestinal tissue.

	Histopathological			Immunochemical		
	Villous Atrophy	Lymphocyte Plasma Neutrophil	Crypt Hyperplasia	Transglutaminase	Gliadin	IgA
Soy						
1	++	+++	++	-	++	++
2	++	+++	++	+	++	-
3	+	++	++	-	++	++
4	+	+++	+	+	++	+
5	+	++	+	+	+	-
6	++	++	++	-	+++	+
7	+	+++	++	+	+	-
Corn						
1	+	++	+	-	++	++
2	++	+++	-	-	++	++
3	+	+	-	-	+	+
4	+++	+	+	-	+	+
5	+++	+	++	+	+	+
6	+++	++	-	+	+	++
7	++	+	+	+	++	++
Wheat						
1	-	+++	+	-	+	+
2	++	+	-	-	-	+++
3	+++	+++	+	-	+	+
4	+	+	-	-	+	+
5	-	+++	-	-	-	+
6	+	+	-	+	++	++
7	++	+	+	-	-	+

Table 3. Histopathological and immunohistochemical values of small intestine tissue

Parameter		Soy	Corn	Wheat	
Histopathological		$\bar{x}$±SH (median)	$\bar{x}$±SH (median)	$\bar{x}$±SH (median)	*P*
Villous Atrophy		1.42±0.202 (1.00)	2.14±0.340 (2.00)	1.50±0.428 (1.00)	.205
Lymphocyte Neutrophil	Plasma	2.57±0.202 (3.00)	1.57±0.297 (1.00)	1.66±0.421 (1.00)	.098
Crypt Hyperplasia		1.71±0.184^{a} (2.00)	0.71±0.286^{a}(1.00)	0.33 ± 0.202^{a} (0.00)	.008*
Immunohistochemical					
Transglutaminase		0.57±0.202 (1.00)	0.42±0.202 (0.00)	0.16±0.167 (0.00)	.345
Gliadin		1.85±0.261^{b} (2.00)	1.42±0.202^{bc}(1.00)	0.83±0.307^{c} (1.00)	.031*
IgA		0.85±0.340 (1.00)	1.57±0.202 (2.00)	1.50±0.341 (1.00)	.226

*,a,b,c, $P < .05$

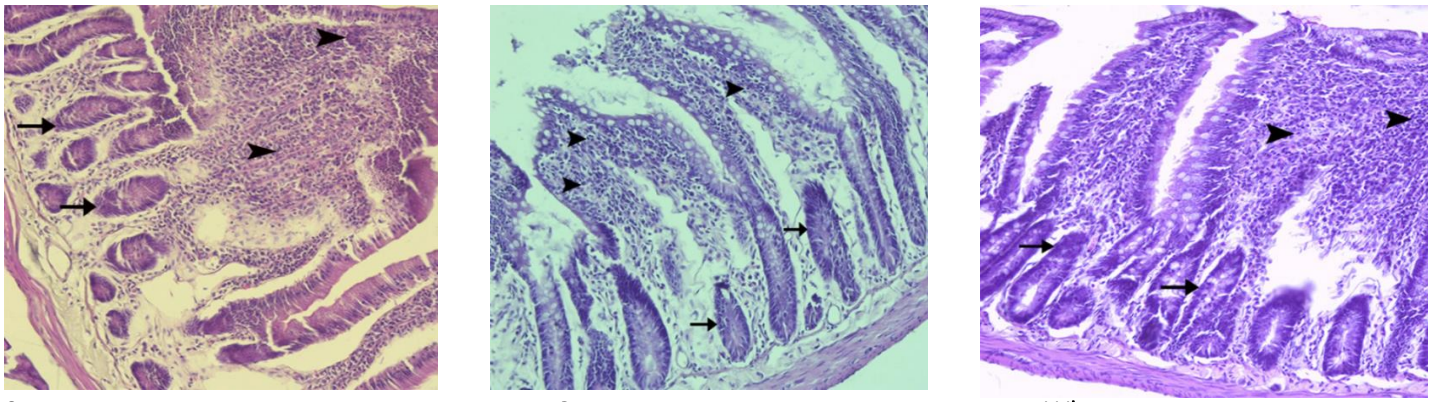

Figure 2. Hyperplasia of crypts in small intestine tissue (→)lymphocyte plasma neutrophil infiltration (➡)atrophy of villi (H&E, Bar: 100 µm)

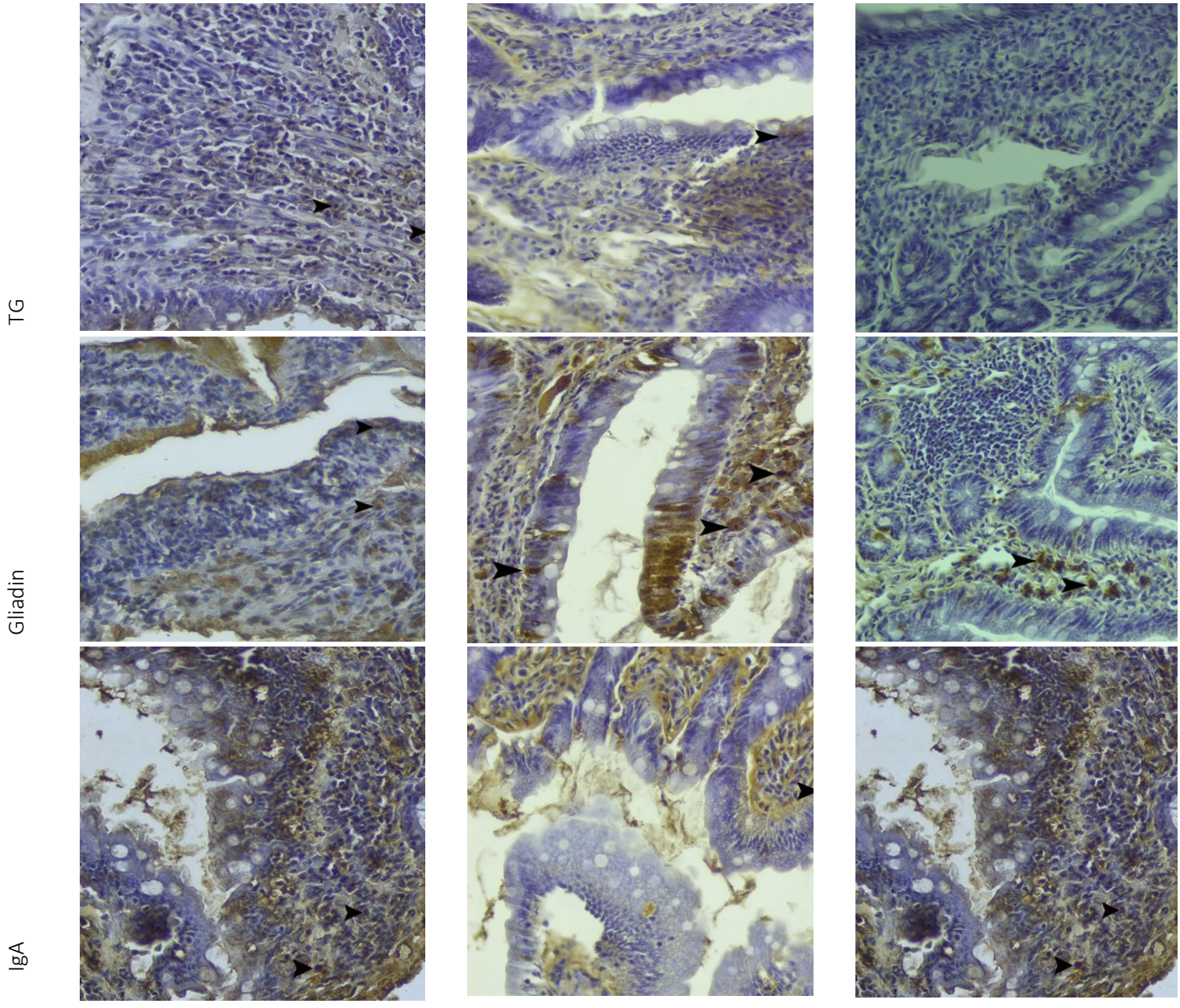

Figure 3. Transglutaminase formed in the small intestines (TG), gliadin and IgA immunohistochemistry (➡) (IHC, Bar: 50µm)

DISCUSSION

Gliadin peptides, which are formed by the breakdown of gluten, combine with HLA molecules to cause the initiation of clinical manifestations and a chain of immunological events. It is known that the peptides that make up the structure of gliadin initiate the cellular, humoral and inflammatory response in tissues.[18,19] The peptides found in the structure of gliadinin cannot be fully digested because they are resistant to proteases and proteolysis found in the gastrointestinal tract of celiac patients.[20] In this study, healthy male rats without HLA-DQ2 and HLA-DQ8 genes were given wheat and corn gluten and their effect on histopathological and immunohistochemical parameters in small intestine tissue was examined.

Villus atrophy, crypt hyperplasia and lymphocyte plasma neutrophil parameters, which are indicators of histopathological structure, were examined. Villuses are structures that protrude towards the lumen in the small intestine tissue and increase the absorption area of the small intestine. The average small intestine length of rats is 110 cm.[21] In this study, the longest villi length was observed in the wheat gluten group ($P < .05$). Accordingly, it was observed that wheat gluten had no negative effect on the small intestine villi lengths of healthy rats. Villus atrophy, although not statistically significant, was observed to be the highest in the group given corn gluten. Lymphocyte plasma neutrophils distributed in the mucosal structure of performed as immunohistochemical parameters to determine the sensitivity of healthy male rats to gluten. Gliadins in the structure of grain proteins are perceived as antigens by the tissue and stimulate the development of T-cells to ensure the production of antibodies against it.[26-28] This leads to an increase in the transglutaminase antibody. The enzyme transglutaminase is intracellular in nature and stimulates the secretion of fibroblasts (from inflammatory and endothelial cells) after mechanical irritation or inflammation response. Gliadin is a protein that is resistant to proteolic enzymes that is deamidated by the enzyme transglutaminase in the digestive tract.[29] It is stated that Gliadin antibody is formed in 90% of untreated celiac patients. The presentation of gliadin protein by HLA-DQ2 and HLA-DQ8 to reactive CD4+T cells increases the level of pro-inflammatory cytokines that cause tissue damage, leading to the release of B lymphocytes and the formation of plasma cells. Plasma cells cause the release of gliadin and transglutaminase antibodies.[30,31]

In this study, transglutaminase ($P > .05$) and gliadin levels the entire intestinal tissue are known as defense barriers against viruses and bacteria.[22] The increase in antigenic agents in the digestive system leads to an increase in the number of lymphocyte plasma neutrophils.[23]

In this study, although the low number of lymphocyte plasma neutrophils in the corn and wheat group compared to the soy group was not statistically significant, it shows that the sensitivity of the small intestine tissue of healthy rats to wheat and corn gluten was less compared to the soy group. The most important task of the cells that settle in the crypts in the intestinal tissue is to help the intestinal tissue to perform its function more functionally by making secretions. Cryptic hyperplasias adversely affect the localization and secretion of these cells.[23,24] In this study, crypt hyperplasia was mostly observed in the soy group ($P <.05$). Accordingly, low crypt hyperplasia of wheat and corn gluten in healthy rats does not adversely affect the histopathological structure of intestinal tissue.

It is also very important to examine immunohistochemical parameters to support histopathological findings due to gluten sensitivity in tissues.[25] Therefore, antibodies are particularly used as immunohistochemical parameters for the detection of gluten sensitivity. In this study, transglutaminase, gliadin and IgA antibody analyzes were ($P < .05$) were observed in the wheat group with the lowest levels. This suggests that healthy rats lacking the HLA-DQ2 and HLA-DQ8 genes are not sensitive to wheat gluten. IgA is mainly present in many external secretions. Secretory IgA molecules are particularly effective against microbial agents on mucosal surfaces, preventing and neutralizing bacterial pathogens or their toxins on the mucosal surface by preventing them from adhering to epithelial cells.[32,33] In the study, IgA antibody parameters were found to be similar between the groups. However, it can be stated that the literature is not in agreement with the findings.[10,11,34] The most obvious reason for these differences can be attributed to the fact that the immune system metabolism has not been fully elucidated. It has done an extensive review of animal studies on gluten sensitivity and how to read their results, where he has provided invaluable information.[35]

As a conclusion in this study, the longest villus length, least crypt hyperplasia and lowest gliadin level were in the wheat gluten group and it was observed that wheat gluten had no negative effect on small bowel villus lengths. The fact that transglutaminase and gliadin levels, which are among the immunohistochemical parameters, were in the lowest

wheat group indicates that healthy male rats were not sensitive to wheat gluten. In addition, lymphocyte plasma neutrophil count and crypt hyperplasia are highest in the soy group, which means that wheat and corn gluten do not adversely affect the histopathological structure of small intestinal tissue. According to the findings obtained in this study, it was determined that wheat gluten added to the diet of healthy rats without HLA-DQ2 and HLA-DQ8 genes did not have a negative effect on the histopathological and immunohistochemical parameters of small intestinal tissue.

As a resort in the studies to be carried out in this regard, in rats with and without HLA-DQ2 and HLA-DQ8 genes, immunohistochemical parameters IgA, gliadin, tansglutaminase in addition to IgG, CD3, CD8 levels and gluten effect and inflammatory cytokines and serological parameters can be examined. Apart from intestinal cells, it may be useful to examine the histopathological and immunohistochemical effects of gluten on bone, skin, nerve cells. It can also be stated that it will shed light on new studies on the use of gluten as a protein source.

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The Research of Effectiveness of Parvulyte Gel® in Dogs with Parvoviral Enteritis

Parvoviral Enteritisli Köpeklerde Parvulyte Jelin® Etkinliğinin Araştırılması

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ABSTRACT

This study aimed to demonstrate the efficacy of Parvulyte® in dogs with parvoviral enteritis. The animal material of the study consisted of 14 dogs diagnosed with parvoviral enteritis due to clinical examination and immunochromatographic rapid test kits brought to XX University Veterinary Health Application and Research Center Internal Diseases Clinic and private veterinary clinics in Izmir. After the diagnosis of parvoviral enteritis, the dogs in the first group received fluid therapy along with vitamin-mineral-electrolyte-amino acid supplements, pantoprazole, cefazolin sodium and maropitant citrate (Group I, n=7). The dogs in the second group received Parvulyte® in addition to the same treatment protocol (Group II, n=7). Both groups were treated for 7 days. It was observed that the dogs in Group II had an increase in the lymphocyte count, a faster increase in antibody titers, and a faster clinical recovery compared to the stool scoring table created. As a result, Parvulyte® accelerated the clinical recovery and shortened the hospitalization time in dogs with parvoviral enteritis.

Keywords: CPV, Dog, Parvoviral enteritis, Parvulyte.

ÖZ

Bu çalışma Parvulyte®'nin parvoviral enteritisli köpeklerdeki etkinliğini göstermeyi amaçlamıştır. Çalışmanın hayvan materyalini, XX Üniversitesi Veteriner Sağlık Uygulama ve Araştırma Merkezi İç Hastalıkları Kliniği ve İzmir'deki özel veteriner kliniklerine getirilen, klinik muayene ve immunokromatografik hızlı test kitleri ile parvoviral enteritis tanısı konulan 14 köpek oluşturdu. Parvoviral enteritis tanısı konulduktan sonra birinci gruptaki köpeklere sıvı tedavisi ile birlikte vitamin-mineral-elektrolit-amino asit takviyesi, pantoprazol, sefazolin sodyum ve maropitant sitrat verildi (Grup I, n=7). İkinci gruptaki köpekler aynı tedavi protokolüne ek olarak Parvulyte® almıştır (Grup II, n=7). Her iki grup da 7 gün boyunca tedavi edilmiştir. Grup II'deki köpeklerin lenfosit sayısında artış, antikor titrelerinde daha hızlı bir artış ve oluşturulan dışkı skorlama tablosuna kıyasla daha hızlı bir klinik iyileşme olduğu gözlemlenmiştir. Sonuç olarak, Parvulyte® parvoviral enteritisli köpeklerde klinik iyileşmeyi hızlandırmış ve hastanede kalış süresini kısaltmıştır.

Anahtar Kelimeler: CPV, Köpek, Parvoviral enterit, Parvulyte.

INTRODUCTION

Parvoviral enteritis is considered one of the most important reasons for morbidity and mortality in puppies worldwide. Canine parvovirus is a single-stranded DNA virus that belongs to the *Protoparvovirus* genus, and *Parvoviridae* family and infects rapidly dividing cells of the gastrointestinal tract, bone marrow, lymphoid tissue and cardiac myocytes. Although the origin of canine parvovirus is not fully known, there is a theory that it may have emerged as a variant of the feline panleukopenia virus that can infect dogs, as it shares 98% structural homology.

** This study was accepted as a master's thesis.*

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Since the *Parvoviridae* family is also found in wild mammals, it is thought that genetic variations from wildlife may have played a role in the evolution of CPV-1 and CPV-2.[1,2] CPV-1, also known as canine minut virus, was first discovered as a cause of gastrointestinal and respiratory tract infections in dogs in the late 1960s. Mutation of CPV-1 resulted in a decade later in a markedly different variant, CPV-2, causing the first pandemic outbreaks in adult and young dogs not previously exposed to CPV. Since the first isolation of CPV-1 and CPV-2, three variants have emerged: CPV-2a, CPV-2b and CPV-2c. The disease still maintains its importance despite vaccines developed and administered against CPV-2 strains.[3,4] CPV-2 strains are highly resistant to infection strategies because their ability to infect mammalian hosts other than domestic dogs, such as raccoons, cats, coyotes, and wolves, can be found in many places in the environment, and can survive for more than a year under favorable conditions. Transmission of parvovirus occurs via the faecal-oral route after exposure to the virus in faeces, vomit, and fomites.[5,6] Since there is no specific antiviral drug, treatment of parvoviral enteritis largely involves supportive treatments. One of the primary challenges and burdens for patients in the treatment of parvoviral enteritis is the cost of hospitalization and treatment. A study conducted in Australia reported that most parvovirus cases occur in socioeconomically underprivileged areas. Possible lack of training and lack of financial opportunity for vaccination put dogs at higher risk of contracting the disease in disadvantaged areas. The decision whether to hospitalize an animal with parvoviral enteritis to receive standard treatment rather than outpatient treatment or euthanasia largely depends on the owner's ability and willingness to pay the costs of care.[5-9]

IgY can be found in birds, reptiles, amphibians, and lung-breathing fish. IgY is also the evolutionary precursor of IgG and IgE, which are found only in mammals.[10] Antibodies are protein molecules that are produced in response to an antigen. They are widely used in research, diagnosis and treatment due to their ability to bind to specific targets. Most antibodies available today are produced in mammals, especially in small rodents. However, antibody production in mammals can be challenging because some antigens can elicit weak immune responses or they are not even immunogenic. Additionally, the process of producing antibodies in mammals involves painful procedures such as immunization, blood sample collection, and sacrifice. The ongoing search for more efficient and economical techniques, as well as the reduction of animal use, has led to increased interest in egg yolk antibodies. Obtaining antibodies from egg yolk is a non-invasive method that eliminates the need for blood collection. The use of polyclonal IgY against infectious diseases minimizes the risk of antimicrobial resistance because it can target different antigens in the same microorganism. Therefore, specific IgY antibodies are a suitable alternative for antimicrobial use in human and veterinary medicine in the recent emergence of resistant bacteria. Due to its potential to prevent bacterial infections in animals, IgY technology is thought to be useful in strategies to reduce the use of antibiotics in animal husbandry, which has an important role in the emergence and spread of resistant bacterial strains.[11-13] Various studies have been conducted on the therapeutic effectiveness of IgY in viral, fungal and protozoon infections. It has been observed that the duration of diarrhea and hyperthermia was shortened in animals treated with oral administration of IgY-rich egg yolk powder against rotavirus infection in calves, and other findings such as calves with anorexia, dehydration and depression were not observed in calves without the application.[14-19]

The aim of this study was to evaluate the efficacy of Parvulyte®, a commercial product containing IgY, in dogs with parvoviral enteritis.

MATERIALS AND METHODS

Animal Material

Ethical committee approval was received from the Ethics Committee of Afyon Kocatepe University (Date: 24/02/2020, Decision No:49533702/219). The animal material of the study consisted of 14 dogs which are different breeds, 1-8 months of age, different genders, who applied to the Afyon Kocatepe University Veterinary Health Application and Research Center Internal Medicine Clinic with complaints of acute enteritis, and were diagnosed with parvoviral enteritis as a result of the clinical examination and immunochromatographic test, and also test results were excluded canine coronavirus and giardia infections (Asan Easy Test®, Korea). The dogs included in the study were numbered, then physical examination findings and laboratory findings were recorded.

Study Groups

14 dogs were randomly grouped in this study.

Group I

As part of the supportive treatment for the dogs in the first group, 0.9% NaCl solution (Bioflex®, Osel İlaç, Türkiye), which was clinically calculated according to the patient's dehydration status and maintenance needs; lactated ringer's solution (Bioflex®, Osel La., Türkiye); 5% dextrose solution (Bioflex®, Osel İlaç, Türkiye); solution (Duphalyte®, Zoetis, Türkiye) containing vitamins, minerals, electrolytes

and amino acids at a dose of 10 ml/kg; pantoprazole (Protaz®, MTA İlaç, Türkiye) at a dose of 1 mg/kg every 24 hours; cefazolin sodium (Cefozin®, Bilim İlaç, Türkiye) at a dose of 25 mg/kg every 12 hours intravenously and; and in cases where vomiting was observed, maropitant citrate (Cerenia®, Zoetis, Türkiye) was administered subcutaneously at a dose of 1 mg/kg every 24 hours were administered for 7 days.

Group II

For supportive treatment for the dogs in the second group, 0.9% NaCl solution (Bioflex®, Osel İlaç, Türkiye), which clinically calculated according to the patient's dehydration status and maintenance needs; lactated ringer's solution (Bioflex®, Osel İlaç, Türkiye); 5% dextrose solution (Bioflex®, Osel İlaç, Türkiye); solution (Duphalyte®, Zoetis, Türkiye) containing vitamins, minerals, electrolytes and amino acids at a dose of 10 ml/kg; pantoprazole (Protaz®) at a dose of 1 mg/kg every 24 hours; cefazolin sodium (Cefozin®, Bilim İlaç, Türkiye) at a dose of 25 mg/kg every 12 hours intravenously and; and in cases where vomiting was observed, maropitant citrate (Cerenia®, Zoetis, Türkiye) was administered subcutaneously at a dose of 1 mg/kg every 24 hours were administered for 7 days. In addition to these applications, the dose of 2.8 grams/dog of a product (Parvulyte®, Uranovet, Spain) containing IgY, essential fatty acids, starch, B-complex vitamins (B1, B2, B6, B12, D1), vitamin E, folic acid, biotin, niacin, oligoelements and amino acids were administered orally.

Samples and Measurements

Vascular access through the vena cephalic antebrachia using a 24G or 22G branul was obtained from the dogs in both study groups, and 4 ml blood samples were taken into EDTA-containing tubes using this vascular access on the 0^{th}, 3^{rd} and 7^{th} days of treatment. WBC, RBC, HGB, NEU, LYM, MCV, MCH, MCHC, HCT measurements were done using a fully automatic hemogram device and the results were recorded without waiting for the blood samples taken (HumaCount 80TS, Vet Mode, Germany).

4 ml blood samples were taken into EDTA-containing tubes using a 24G or 22G branul was obtained from the dogs in both study groups on the 0^{th}, 7^{th} and 14^{th} days of treatment. Canine parvovirus antibodies were detected at low titre (below 1:40), medium titre (1:80) and high titre (1:160) according to the intensity of the band lines in the result window was determined qualitatively and recorded using a commercial immunochromatographic test kit with 5 µL of the whole blood samples taken (Uranotest Parvo Immune Status®, Spain).

Faecal Scoring

A stool scoring table, which was based on the Nestle-Purina stool scoring system, was created and numbered 1-6 according to stool consistency, to be evaluated on days 0, 3 and 7 in dogs in both study groups (Table 1).

Table 1. Faecal scoring table.

Faeces score	Result
1	- Solid, not rigid, flexible - Segmented view - No residue on the ground when collected
2	- Log-shaped, moist surface - No visible segmentation - Leaves residue on the floor when removed but retains its shape
3	- Very moist, mud consistency - Log shaped - When removed, it leaves residue on the ground and loses its shape.
4	- Very moist but with a definite shape - Found in piles rather than logs - When removed, it leaves a residue and loses its shape, light brown.
5	- Has texture but no distinct shape - Brown in clumps or spots - Leaves residue on the floor when collected
6	- Aqueous - No texture - Puddle shaped, bloody

Statistical Analysis

The Kruskal-Wallis test at 5% significance level was used to test whether there was a significant difference in hematological findings and fecal scoring on days 0, 3 and 7 and antibody titers on days 0, 7 and 14. SPSS package program was used to analyze the data collected in the study.

RESULTS

In this study, the effects of standard treatment and an IgY-containing product (Parvulyte®) applied in addition to standard treatment were evaluated within the framework of hematological findings, stool scoring and antibody titres, and the findings are presented after applying the treatment protocols established for both groups. While a significant difference was observed between days in terms of stool score and antibody titre values in Group I, a significant difference was observed between days in terms of LYM, stool score and antibody titre values in Group II ($P<.05$).

Haematological Findings

Although an improvement parallel to the response to treatment was observed in all the WBC, NEU, RBC, HCT, PLT, LYM, MCV, MCH and HGB parameters measured from the blood samples taken from the dogs in Group I on days 0, 3 and 7, there were no statistically significant difference observed between days (*P*>.05).

An improvement parallel to the response to treatment was observed in all WBC, NEU, RBC, HCT, PLT, LYM, MCV, MCH and HGB parameters measured from the blood samples taken on days 0, 3 and 7 from the dogs in Group II. Unlike Group I, a statistically significant difference was observed in Group II in terms of LYM values between day 0 and day 3 and day 0 and day 7 (*P*<.05) (Table 2).

Table 2. Statistical analysis of lymphocyte data of dogs in group II.

Day	Significance (p value)
0 – 3	0.023
3 – 7	1.000
0 – 7	0.003

Faecal Score

The faecal score in the dogs included in the study was scored between 1 and 6. In dogs in both groups, the faecal score on day 0 was determined as 6 in five dogs and 5 in two dogs (Table 3, Table 4).

On the 3rd day of treatment, the faecal score in the dogs in Group I was determined as 5 in two dogs, 4 in four dogs and 3 in one dog. The faecal score in the dogs in Group II was determined as 3 in five dogs and 2 in two dogs (Table 3, Table 4).

On the 7th day of treatment, the faecal score in dogs in Group I was determined as 3 in one dog, 2 in three dogs, and 1 in three dogs. The faecal score in the dogs in Group II was determined as 2 in one dog, while it was determined as 1 in the other six dogs (Table 3, Table 4).

A significant difference was observed in the dogs in both groups in terms of stool score values between day 0 and day 7 (*P*<.05) (Table 5, Table 6). Although no statistically significant difference was detected, when evaluated clinically, it was observed that the stool scores in dogs in Group II improved faster compared to Group I (Table 3, Table 4).

A significant difference was observed in the dogs in both groups in terms of stool score values between day 0 and day 7 (*P*<.05) (Table 5, Table 6). Although no statistically significant difference was detected, when evaluated clinically, it was observed that the stool scores in dogs in Group II improved faster compared to Group I (Table 3, Table 4).

Table 3. Faecal scores of dogs in Group I on days 0, 3 and 7.

Number	Day 0	Day 3	Day 7
1	6	5	2
2	6	5	3
3	6	4	1
4	5	3	1
5	6	4	2
6	5	4	1
7	6	4	2

Table 4. Faecal scores of dogs in group II on days 0, 3 and 7.

Number	Day 0	Day 3	Day 7
1	6	3	1
2	6	3	1
3	6	2	1
4	5	2	1
5	6	3	1
6	5	3	1
7	6	3	2

Table 5. Statistical analysis of faecal score data of dogs in Group I.

Day	Significance (p value)
0 – 3	0.139
3 – 7	0.86
0 – 7	0.000

Table 6. Statistical analysis of faecal score data of dogs in Group II.

Day	Significance (p value)
0 – 3	0.081
3 – 7	0.113
0 – 7	0.000

Antibody Titres

Antibody titres were determined to be low (below 1:40) on day 0 in all dogs in both groups (Table 7, Table 8).

The antibody titres in dogs in Group I were determined as medium titre (1:80) in five dogs, and high titre (1:160) in two dogs on the 7th day of treatment. Antibody titres in the dogs in Group II were determined as medium titre (1:80) in two dogs and high titre (1:160) in five dogs (Table 7, Table 8).

Antibody titres were determined to be high (1:160) on the 14th day in all dogs in both groups (Table 7, Table 8).

A significant difference was detected in terms of antibody titre values between day 0 and day 7 and day 0 and day 14 for both Group I and Group II (*P*<.05) (Table 9, Table 10). However, antibodies in dogs in Group II reached high titres faster than in Group I (Table 7, Table 8).

Table 7. Antibody titres of dogs in Group I on days 0, 7 and 14.

Number	Day 0	Day 7	Day 14
1	<1:40	1:80	1:160
2	<1:40	1:80	1:160
3	<1:40	1:80	1:160
4	<1:40	1:160	1:160
5	<1:40	1:80	1:160
6	<1:40	1:160	1:160
7	<1:40	1:80	1:160

Table 8. Antibody titres of dogs in Group II on days 0, 7 and 14.

Number	Day 0	Day 7	Day 14
1	<1:40	1:80	1:160
2	<1:40	1:160	1:160
3	<1:40	1:160	1:160
4	<1:40	1:160	1:160
5	<1:40	1:160	1:160
6	<1:40	1:80	1:160
7	<1:40	1:160	1:160

Table 9. Significance level of antibody titres of dogs in Group I on days between 0, 7 and 14.

Day	Significance (p value)
0 – 7	0.029
7 – 10	0.320
10 – 14	0.000

Table 10. Significance level of antibody titres of dogs in Group II on days between 0, 7 and 14.

Day	Significance (p value)
0 – 7	0.003
7 – 10	1.000
10 – 14	0.000

The antibody titres in dogs in Group I were determined as medium titre (1:80) in five dogs, and high titre (1:160) in two dogs on the 7th day of treatment. Antibody titres in the dogs in Group II were determined as medium titre (1:80) in two dogs and high titre (1:160) in five dogs (Table 7, Table 8).

Antibody titres were determined to be high (1:160) on the 14th day in all dogs in both groups (Table 7, Table 8).

A significant difference was detected in terms of antibody titre values between day 0 and day 7 and day 0 and day 14 for both Group I and Group II (*P*<.05) (Table 9, Table 10). However, antibodies in dogs in Group II reached high titres faster than in Group I (Table 7, Table 8).

DISCUSSION

The most effective method of preventing CPV infection and disease is a careful and strategic vaccination program aimed at producing protective antibodies. Although dogs aged 6-16 weeks are more sensitive, CPV infection can be seen in dogs of all ages and breeds. Puppies born to vaccinated mothers who have received colostrum have passive immunity provided by maternal antibodies. Since circulating maternal antibody levels begin to decline at 8-12 weeks of age, the risk of infection is higher in offspring during this period. If the maternal antibody concentration from the mother is low, circulating maternal antibody levels may decline earlier than 8-12 weeks. Therefore, vaccination strategies are implemented to stimulate innate immunity with a series of vaccinations during the period when maternal antibodies decrease. Maternal antibodies, especially in puppies aged 49-69 days, can affect the antibodies produced by vaccine administration. For this reason, vaccination time has an important place in protocols for preventing infection in puppies.[5,6,20,21] All the dogs included in this study were between the ages of 2-6 months and no breed predisposition could be detected, but it was found that 57.14% (8/14) shared the same environment with at least one dog. According to the anamnesis information obtained from the owners of the dogs included in the study, it was determined that 35.7% (5/14) of the dogs were two-dose vaccinated, 21.42% (3/14) were single-dose vaccinated and 42.85% (6/14) were unvaccinated. Again, according to the anamnesis information obtained, it was determined that all vaccinated dogs were included in the vaccination program at the age of 8 weeks and the second application was made 14-21 days after the first application. These data obtained, consistent with other studies, have shown that the incidence of parvoviral enteritis may increase in crowded dogs, the effectiveness of early vaccinations may be impaired by maternal antibodies, and two doses of vaccination may be insufficient to prevent parvoviral enteritis. However, as a result of the information received

from dog owners, it is thought that the main reasons for not complying with appropriate vaccination programs are due to dog owners' concerns about vaccination costs and their insufficient knowledge about vaccination programs.

Because CPV attacks actively proliferating bone marrow, thymus and other lymphoid tissue cells, the total leukocyte count, and the presence of leukoneutropenia are considered important features in patients with CPV. Additionally, approximately 21% of dogs with parvoviral enteritis had anaemia, 31% had leukopenia, 28% had leucocytosis, 55% had neutropenia, 17% had neutrophilia, 27.6% had eosinopenia, 4% had eosinophilia, also lymphopenia was observed in 28%, lymphocytosis in 4%, monocytosis in 66%, and thrombocytopenia in 4%.[6,22] In this study, it was observed that 21.42% (3/14) of dogs with parvoviral enteritis had anaemia, 35.7% (5/14) had leukopenia, 14.2% (2/14) had thrombocytopenia, and 50% (7/14) had lymphopenia. The observation that the complete blood count parameters measured in the remaining dogs were within reference ranges suggests that the changes in the complete blood count associated with parvoviral enteritis occur mostly on lymphocytes.

The presence of cytopenia while the disease may be useful in predicting survival. It has been reported that there is no significant difference in survival in terms of neutropenia, but a total leukocyte count higher than 4500/ml and a lymphocyte count higher than 1000/ml at the time of admission and during the 48-hour hospitalization period strongly predict survival.[23,24] Lymphopenia was detected on day 0 in 50% of the fourteen dogs included in this study, and it was observed that the lymphocyte count was above 1000/ml on day 3 and later in all these dogs, consistent with the literature. However, the increase in the number of lymphocytes between days in dogs in Group II was also found to be statistically significant.

It was reported that niacin has a protective effect on intestinal health. Niacin increases intestinal immune function by regulating down-regulation of TNF-α, IL-1ß, IFN-γ and IL-8 expression and up-regulation of IL-10 and TGF-ß expression. In addition, niacin also reduces colonic myeloperoxidase activity. Niacin supplementation has been reported to increase the number of beneficial bacteria in the colon and alleviate the inflammatory response in the intestinal mucosa in piglets.[25] Biotin is a water-soluble vitamin and an essential micronutrient that must be obtained from exogenous sources and commensal bacteria. Biotin has a role in preventing the production of inflammatory cytokines and maintaining the integrity of the intestinal barrier.[26] Amylum escapes duodenal-ileal digestion and affects stool quality by preventing colonic bacterial fermentation.[27] In this study, Parvulyte® IgY applied to dogs in Group II contains niacin, biotin, and amylum in addition to oligoelements and amino acids. In Group II, where niacin, biotin and amylum supplementation were applied, the improvement in stool score was much faster compared to Group I considering the intestinal damage that occurs in parvoviral enteritis, we think that this improvement may be due to the anti-inflammatory and nutritional effects of niacin, biotin and amylum supplements on the intestinal mucosa and enterocytes.

IgY application can modulate the immune response at the mucosal level in viral diarrhoeas. It was also reported that IgY application is effective in the prophylaxis and treatment of both viral and bacterial diarrhoea.[28] It has been reported that intravenous administration of IgY is effective in the treatment of the severe clinical form of parvoviral enteritis without causing any side effects.[29] In this study, the number of lymphocytes in dogs in Group II, where IgY was applied, increased statistically significantly compared to Group I, and the improvement in the stool score was much faster, and the increase in antibody titres to high titres in a shorter period demonstrates the effectiveness of oral administration of IgY in parvoviral enteritis.

As a conclusion, parvoviral enteritis is one of the most important causes of mortality in puppies. The severity of damage caused by the virus in affected offspring and the application of appropriate supportive treatment have a significant impact on survival. This study revealed that oral IgY administration in addition to standard supportive treatment in dogs with parvoviral enteritis accelerates clinical recovery, shortens hospitalization time, and accelerates the immune response to the virus, without causing side effects compared to supportive treatment alone. Therefore, since IgY application is applied together with standard treatment, the study can be repeated by increasing the sample group and the effectiveness of IgY application alone can be investigated.

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Investigation of Oxidative Stress Parameters in Cattle Infected with *Mycobacterium avium subsp. Paratuberculosis*

Mycobacterium avium subsp. paratuberculosis ile Enfekte Sığırlarda Oksidatif Stres Parametrelerinin İncelenmesi

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ABSTRACT

Paratuberculosis is a zoonotic disease caused by Mycobacterium avium subsp. paratuberculosis (MAP) in cattle. MAP may cause the formation of reactive oxygen species (ROS) by increasing the release of proinflammatory cytokines in the host. Due to the increase in ROS, the oxidant-antioxidant balance may be disrupted and oxidative stress may occur. The aim of the study was to determine the oxidative stress parameters in cattle infected with paratuberculosis. For this purpose, 15 cattle sera that were positive for paratuberculosis and 15 clinically healthy 30 cattle sera were used as the control group. In the samples taken, oxidative stress parameters such as total antioxidant capacity (TAS), total oxidant capacity (TOS), oxidative stress index (OSI), native thiol, total thiol and disulphide levels were evaluated. According to our study results, TOS (21.911±11.80), OSI (37.99±21.40), total thiol (1836.671±877.06) and disulphide (715.420±395.32) values in the paratuberculosis group were significantly higher than TOS (8.538±5.18), OSI (10.24±7.23), total thiol (823.809±289.86) and disulphide (197.936±131.70) values in the control group (*P*<.001). The TAS (0.588±0.14) value in the paratuberculosis group was significantly lower than the TAS (0.952±0.26) value in the control group (*P*<.001). No significant difference was found between the two groups in terms of native thiol levels (*P*>.05). As a result, it was determined that the oxidant-antioxidant balance was disrupted and oxidative stress occurred in MAP infected cattle. Therefore, it was concluded that oxidative stress parameters can be used as biomarkers in the diagnosis and treatment of the disease.

Keywords: Cattle, oxidative stress, paratuberculosis.

ÖZ

Paratüberküloz sığırlarda Mycobacterium avium subsp. paratuberculosis (MAP) tarafından oluşturulan zoonotik bir hastalıktır. MAP konakçıda proinflamatuar sitokinlerin salımını artırarak reaktif oksijen türleri (ROS) oluşumuna neden olabilir. ROS artışına bağlı olarak da oksidan antioksidan denge bozulabilir ve oksidatif stres ortaya çıkabilir. Çalışmanın amacı paratüberküloz enfekte sığırlarda oksidatif stres parametrelerinin belirlenmesidir. Bu amaçla çalışmada paratüberküloz yönünden pozitif tespit edilmiş 15 adet sığır serumu ve kontrol grubu olarak 15 adet klinik açıdan sağlıklı toplam 30 adet sığır serumu kullanılmıştır. Alınan numunelerde oksidatif stres parametrelerinden total antioksidan kapasite (TAK), total oksidan kapasite (TOK), oksidatif stres indeksi (OSİ), native thiol, total thiol ve disülfid düzeyleri değerlendirilmiştir. Çalışma sonuçlarımıza göre paratüberküloz grupta TOK (21,911±11,80), OSİ (37,99±21,40), total thiol (1836,671±877,06) ve disülfid (715,420±395,32) değerleri kontrol grubundaki TOK (8,538±5,18), OSİ (10,24±7,23), total thiol (823,809±289,86) ve disülfid (197,936±131,70) değerlerine göre anlamlı düzeyde yüksek belirlenmiştir (*P*<,001). Paratüberküloz grubunda ki TAK (0,588±0,14) değeri ise kontrol grubunda ki TAK (0,952±0,26) değerine göre anlamlı düzeyde düşük belirlenmiştir (*P*<,001). Native thiol düzeyleri açısından her iki grup arasında anlamlı bir fark belirlenememiştir (*P*>,05). Sonuç olarak MAP enfekte sığırlarda oksidan antioksidan dengenin bozulduğu ve oksidatif stresin ortaya çıktığı belirlenmiştir. Bu nedenle hastalığın tanı ve tedavisinde oksidatif stres parametrelerinin biyobelirteç olarak kullanılabileceği kanaatine varılmıştır.

Anahtar Kelimeler: Oksidatif stres, paratüberküloz, sığır.

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INTRODUCTION

Paratuberculosis is a disease causing granulomatous enteritis, usually in cattle, sheep and goats, characterised by chronic diarrhea. The etiology of this disease is *Mycobacterium avium subsp. paratuberculosis* (MAP).[1] *Mycobacterium avium subsp. paratuberculosis* is an acid-fast bacillus that causes granulomatous, incurable enteritis progressing to severe cachexia and death. The disease is transmitted through infected drinking water, dry grasses, carrier animal droppings, and contaminated environments. In addition, housing calves together with the mother after birth and breastfeeding also play an important role in the transmission of infection. After ingestion, MAP settles in the lymphoid tissue associated with the intestine. *Mycobacterium avium subsp. Paratuberculosis* requires high concentrations of iron for development. There is a high amount of iron in tissue macrophages located in the ileocaecal intestinal passage.[2] In this way, MAP causes granulomatous enteritis and thickening in the ileocaecal duct.[3] In addition, hypertrophy and edema of the lymph nodes in the mesenteric region are observed. Microscopically, the disease is characterized by varying degrees of chronic granulomatous enterocolitis, regional lymphangitis, and lymphadenitis.[4] Even subclinical infection significantly reduces the performance of animals and leads to direct and indirect economic losses in the cattle industry.[5] The main economic losses of the disease are; decrease in milk yield and quality, decrease in live weight gain, infertility problems and increased susceptibility to other chronic diseases.[6] It has also been shown that MAP can be transmitted to humans and play a role in the etiology of a disease with clinical symptoms similar to paratuberculosis and has been named Crohn's disease (CD), which causes chronic inflammation of the intestine in humans.[7] Therefore, paratuberculosis is important in terms of public health as well as the economic losses it causes. The source of transmission of MAP bacteria to humans is meat, meat products, milk, dairy products, and drinking water contaminated with feces of paratuberculosis-infected animals. Since the transmission of MAP is foodborne, it is important for food safety. Crohn's disease is a chronic inflammatory disease that affects the gastrointestinal tract of humans from the mouth to the anus. The intestinal segment narrows and thickens and the accompanying mucosal deep ulcers and fissures give a cobblestone appearance.[8] The general symptoms of the disease are abdominal pain, diarrhea, and weight loss. Diarrhea is found in approximately 70% of patients with Crohn's disease. Weight loss may develop due to malabsorption and anorexia.[9] It is important to carry out detailed research due to reasons such as the fact that MAP is accepted as a pathogen in humans, it is more resistant to the temperature-time degrees of pasteurization than other pathogens, and the possibility of transmission with foods obtained from infected animals.[10] MAP is also present as an intracellular pateogen in macrophages of infected cattle. It decreases the phagocytosis ability of macrophages by preventing the pH in lysosomes from decreasing. It is also resistant to degradation even in lysosome.[11] In contrast, the body's primary defence mechanism is the induction of apoptosis of infected macrophages through a tumour necrosis factor-a (TNF-a)-dependent mechanism.[12,13] However, when apoptosis is not sufficient to clear infected cells, it may cause the cell to lose its membrane integrity and transform into secondary necrotic cells. This may result in systemic inflammation, oxidative stress and the formation of reactive oxygen species (ROS).[14,15] Reactive oxygen species are important defense substances involved in the immune response against pathogens.[16] However, excessive increases in ROS can cause oxidative stress (OS) by damaging the organism. Antioxidant enzymes (AE) are produced in the organism in order to neutralise OS. However, tissue damage may develop as a result of disruption of oxidant and antioxidant balance.[17] Since the response to OS is both an indicator of the activation of the immune system and an indicator of the ability to compensate for infection-induced damage, it is used as a biomarker in many studies. Considering all these situations, further research should be carried out for the rapid detection of MAP in meat and meat products, milk, and dairy products and new methods for diagnosis should be developed. Because the diagnosis of the disease is important for both economic, human, and food health. In this study, it was aimed to determine the levels of total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSI), native thiol, total thiol, and disulphide as biomarkers of oxidative stress parameters in cattle with serologically detected paratuberculosis.

MATERIALS AND METHODS

This study was carried out on the samples received for routine analyses from livestock farms in Amasya, Tokat, and Samsun provinces within the responsibility area of Samsun Veterinary Control Institute between 2023-2024. Paratuberculosis vaccination was not performed in the enterprises where the samples were received. In the study, 15 cattle blood samples that were positive for paratuberculosis and 15 clinically healthy cattle blood samples that were negative for paratuberculosis, totally of 30 cattle blood samples, were used as the control group. Serum samples received at the institute were stored at -20 °C until the analyses were performed.

Serum samples were tested for paratuberculosis using a

commercial antibody ELISA kit (IDEXX Mycobacterium paratuberculosis Antibody Test Kit). The test kit and samples were allowed to reach room temperature. The serum samples to be analysed, positive and negative control sera were diluted 1/20 using Dilution Buffer No12 in a sterile U-bottom microplate and pre-incubated for 15 min at 18-22 °C in a microplate shaker. After preincubation, 100 µl of all sera were added to the wells of the test kit plate. The plate was incubated in a shaker for 45 minutes at 18-22 °C. At the end of the incubation period, the plate was washed 3 times with washing solution. Anti-ruminant HRPO conjugate was diluted 1/100 in Dilution Buffer No1 and 100 µl was added to each well of the plate. It was incubated again at 18-22 °C for 30 minutes. At the end of the incubation period, the washing process was repeated. TMB-substrate solution was added 100 µl to each well of the plate and incubated at 18-22 °C in the dark for 10 minutes. At the end of incubation, 100 µl of stop solution was added to the wells and the reaction was terminated. Optical density (OD) values of the wells were read at 450 nm on an ELISA reader (Mindray MR-96A). The OD values obtained were placed in the formula below and the results were calculated.

$$\boldsymbol{Result}\ (\%) = \frac{\mathbf{ODsample} - \boldsymbol{ODnegative}}{\mathbf{ODpositive} - \mathbf{ODnegative}}\ \boldsymbol{x}\ \mathbf{100}$$

The results obtained according to the formula were evaluated as 55% and above positive, 45-55% suspect, and less than 45% negative. Results in the suspect range were not included in the study.

The study was conducted by the decision of the ethics committee numbered 2024/04, 19572899/031-85, taken by the "Ethics Committee Directive" of the Ministry of Agriculture and Forestry Samsun Veterinary Control Institute Animal Experiments Local Ethics Committee.

Biochemical Analyses

Total antioxidant status (Rel Assay Diagnostics, RL0017, Turkey), TOS (Rel Assay Diagnostics, RL0024, Turkey), native thiol (Rel Assay Diagnostics, RL0185, Turkey), and total thiol (Rel Assay Diagnostics, RL0192, Turkey) oxidative stress parameters were measured using colorimetric test kits according to the procedure recommended in the kit. The measurements of the kits used were performed on an ELISA plate reader (Tecan Infinite F50, Switzerland). Oxidative stress index was calculated using TAS and TOS data and disulphide was calculated using native thiol and total thiol parameters.

Total thiol and native thiol levels are determined using colorimetric test kits. The difference between the obtained values indicates the dynamic disulphide content. SS/SH+SS, SS/SH and SH/SH+SS ratios are calculated. These ratios show how much space disulphide bonds occupy. The methodology allows to analyze the balance of thiol and disulphide groups.

Statistical Analyses

Statistical analyses were performed using the SPSS Statistic 27 program. To determine the normality of the distribution, skewness, kurtosis values, and the Shapiro-Wilk test were used. For normally distributed groups, t-test was used to compare the groups. P values below 0.05 were considered significant. Since the data obtained showed normal distribution, the Pearson test was used to determine the correlation between the groups.

RESULTS

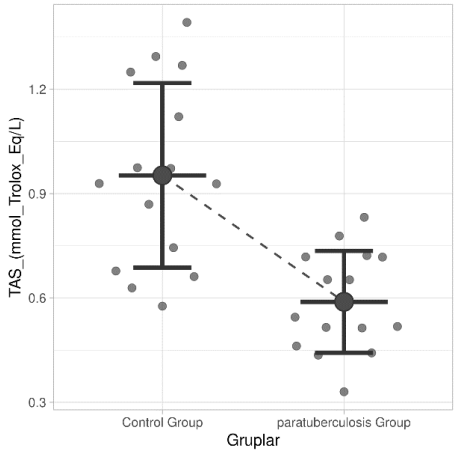
In the study, the values of some oxidative stress parameters were determined in blood samples taken from MAP-positive cattle and MAP-negative cattle. Oxidative stress values of control and MAP-infected cattle are given in Table 1.

Table 1. TAS, TOS, OSI, Total thiol, Native thiol, Disulphide, Native thiol/Total thiol (%), Disulphide/Native thiol (%), and Disulphide/Total thiol (%) levels (mean ± SD) in control and MAP-infected cattle.

Parameters	Control Group	Paratuberculosis Group	*P*
TAS (mmol Trolox Eq/L)	0.952±0.26	0.588±0.14	< .001
TOS (µmol H2O2 Eq/L)	8.538±5.18	21.911±11.80	< .001
OSI (AU)	10.24±7.23	37.99±21.40	< .001
Total thiol (µmoL / L)	823.809±289.86	1836.671±877.06	< .001
Native thiol (µmoL / L)	427.937±255.77	405.830±142.75	> .05
Disulphide (µmoL / L)	197.936±131.70	715.420±395.32	< .001
Native thiol/Total thiol (%)	51.94±26.00	22.09±15.81	< .01
Disulphide/Native thiol (%)	46.25±32.93	176.28±120.71	< .001
Disulphide/Total thiol (%)	24.02±13.00	38.95±7.90	< .01

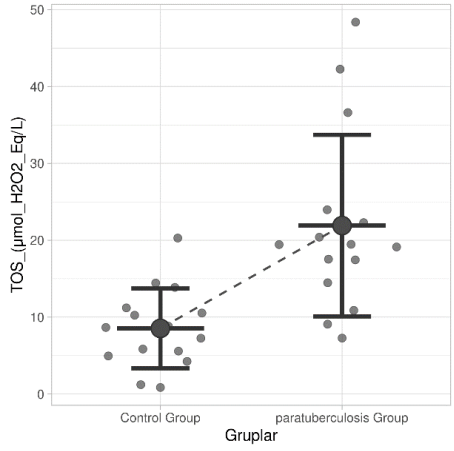
TAS, total antioxidant status; TOS, Total oxidant status; OSİ, Oxidative stress index.

Serum TAS, TOS, and OSI levels ($P < .001$) of the control group and paratuberculosis group are given in figure 1, figure 2, and figure 3.



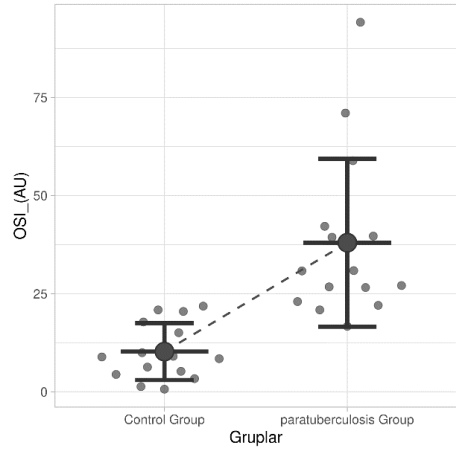
TAS: total antioxidant status

Figure 1. Serum TAS levels



TOS: total oxidant status

Figure 2. Serum TOS levels



OSI: oxidative stress index

Figure 3. Serum OSI levels

Serum total thiol levels ($P < .001$), native thiol ($P > .05$), and disulphide levels ($P < .001$) of the control group and paratuberculosis group are given in figure 4, figure 5, and figure 6.

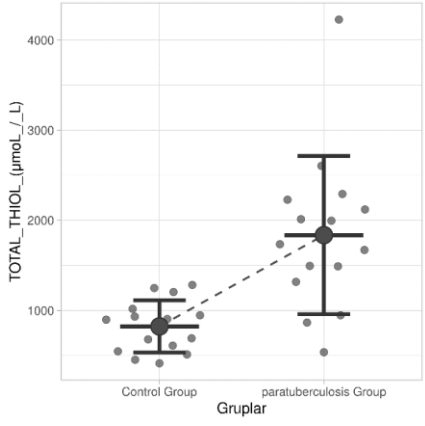


Figure 4. Serum total thiol levels

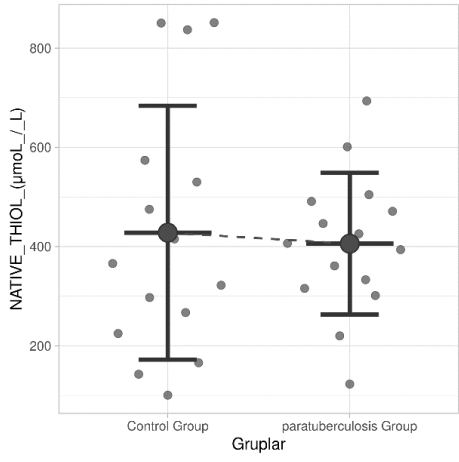


Figure 5. Serum native thiol levels

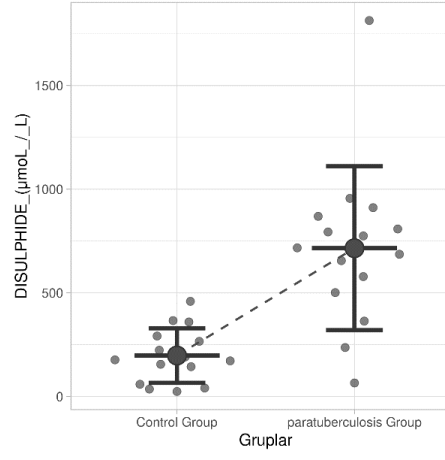


Figure 6. Serum disulphide levels

Serum native thiol/total thiol (%) levels ($P < .01$), disulphide/native thiol (%) levels ($P < .001$), disulphide/total thiol levels ($P < .01$) of the control group and paratuberculosis group are given in figure 7, figure 8, and figure 9.

According to Pearson correlation analysis, TAS was negatively correlated with TOS and OSI, while OSI and TOS were positively correlated ($P < .05$). There was also a positive correlation between total thiol, TOS, OSI, and disulphide ($P < .05$). Disulphide/native thiol (%) ratio was positively correlated with TOS, total thiol, disulphide, disulphide/total thiol (%) ratio and negatively correlated with native thiol/total thiol (%) ratio ($P < .05$).

Disulphide/total thiol (%) ratio was positively correlated with total thiol, disulphide, disulphide, native thiol and native thiol/total thiol (%) ratios ($P < .05$). Native thiol/total thiol (%) ratio was negatively correlated with total thiol, disulphide, disulphide/total thiol (%), disulphide/native thiol (%) ratio and positively correlated with native thiol ($P < .05$).

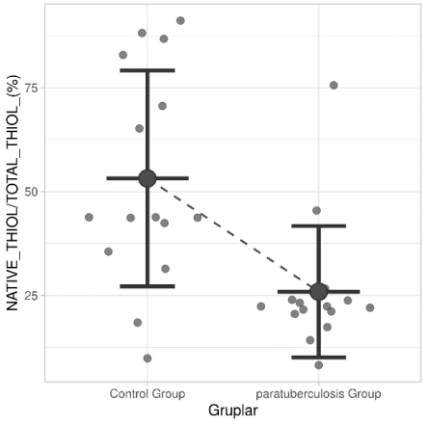


Figure 7. Serum Native thiol/total thiol (%) levels

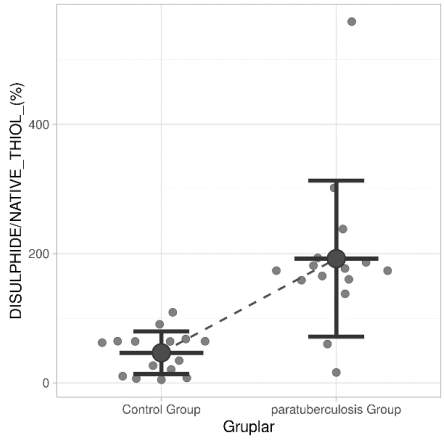


Figure 8. Serum Disulphide/Native thiol (%) levels

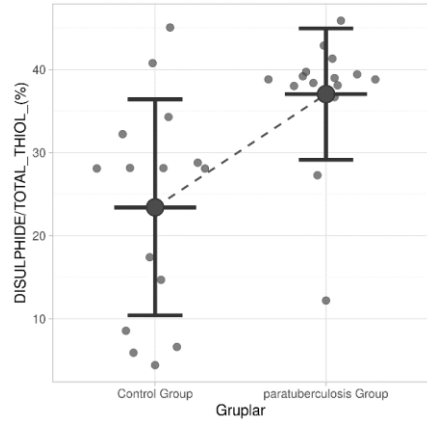


Figure 9. Serum Disulphide/total thiol (%) levels

DISCUSSION

Paratuberculosis is a granulomatous enteritis characterised by chronic diarrhea caused by *Mycobacterium avium subspecies paratuberculosis* infection. It mostly affects ruminants and animal welfare, raises public health concerns, and can cause direct and indirect economic losses.[18] Increased release of ROS is reported in bacterial diseases such as paratuberculosis. Free radicals or ROS are released from dendritic cells, neutrophils, and macrophages in response to an inflammatory stimulus.[19] The ROS produced must be kept in balance by the antioxidant system. If the oxidant-antioxidant balance is disrupted as a result of an increase in ROS levels in cells, oxidative stress, which has an important role in the pathophysiology of diseases, may occur.[20] Oxidative stress results from a disturbance of the balance between the production of reactive oxygen or nitrogen derivatives and the organism's defence capability.[21] During infection, defence cells produce large amounts of ROS and RNS in order to clear pathogens. However, these biochemical products may result in the triggering of a pathological process affecting healthy structures as well as pathogens.[22] Therefore, the oxidative response can be used as a biomarker indicating both the presence of the pathogen[23] and the onset of defence against pathogens.[24] For this purpose, we aimed to determine how TAS, TOS, native thiol, and total thiol parameters, which are oxidative stress markers, change and whether oxidative response occurs in MAP-infected cattle.

In this study, TOS, OSI, total thiol, and disulphide levels were significantly higher in MAP-infected cattle, while TAS level was significantly lower in MAP-infected cattle compared to the control group. There was no significant difference in native thiol levels between both groups. Merhan et al.[25] determined that MDA and NO levels, which are oxidative stress parameters, were significantly increased in the MAP-infected group in a study conducted in 15 cattle infected with MAP and 15 clinically healthy cattle.[25] Cenesiz et al.[26] evaluated oxidative stress parameters such as MDA, SOD, and GSH-Px in another study and determined that while MDA level increased significantly in the MAP-infected group, there was no significant change in SOD and GSH-Px enzyme activity levels.[26] Akyuz et al.[27] reported that oxidative stress developed in cattle with subclinical paratuberculosis and serum MDA level was significantly higher compared to the control group.[27] Balıkcı and Gurdogan[28] found that SOD, GSH-Px, and GSH levels decreased significantly in clinical and subclinical groups compared to the control group in a study conducted in sheep with paratuberculosis.[28] El-Deeb et al.[29] in a study conducted in paratuberculosis-infected dromedary camels, MDA level of the paratuberculosis-infected group increased while SOD, CAT, and reduced glutathione enzyme activities decreased. As a result of this situation, it was determined that oxidative stress developed.[29] In the presented study was similar to other studies and it was determined that TOS and OSI levels increased while TAS level decreased in MAP-infected cattle. This situation suggests that free radical formation increases

as a result of infection and accordingly, oxidative stress develops by disrupting the oxidant-antioxidant balance. Because tissue damage, inflammation, and infections cause free radical formation by activating phagocytic cells which have an important role in host defense.[30] It has been reported that oxidative stress occurs by disruption of oxidant-antioxidant balance especially in bacterial and viral diseases. The decrease in TAS activity in the study indicates that the antioxidant defense system is insufficient due to increased oxidative stress due to free radical formation. In addition, it was determined that disulphide level increased in the MAP-infected group. As a result of the literature review, no studies evaluating native total thiol levels in cattle with paratuberculosis were found. However, it was determined that native thiol, total thiol, and disulphide levels changed in some infected diseases in cattle.[31–34] The reason for this situation is that the increase in proinflammatory cytokines due to the increase in infection may cause an increase in ROS, which may cause an increase in disulphide levels by binding native and total thiols with reversible disulphide bonds to increasing oxidants. To maintain oxidative balance in the organism, the conversion of thiol groups into disulphide bonds increases. Thiols can be converted into disulphide bond structure by oxidation by oxidant molecules. This transformation is an early indicator of protein oxidation under oxidative stress. Disulphide formation reflects antioxidant status and redox status, indicating oxidative stress. The resulting disulphide bond is converted back to thiol groups, thus ensuring thiol-disulphide balance. In biological organisms, thiol-disulphide balance can be used as a biomarker to evaluate OS response. Evaluating disulphide formation is important to understand the effects of OS and the functioning of antioxidant defense systems.[35] Therefore, thiol-disulphide balance is a critical parameter in the evaluation of OS balance.[36]

In conclusion, it is known that paratuberculosis is a worldwide widespread disease affecting a large proportion of dairy cattle herds. If it is not combated with effective control measures, its prevalence and effect will increase gradually. Since it is a disease that causes economic losses by affecting both human and animal health, studies for the prevention, diagnosis, and treatment of the disease are important. In this study, cattle with MAP infection were evaluated in terms of oxidative stress parameters and as a result, it was observed that oxidant-antioxidant balance was disrupted and oxidative stress developed. Therefore, it is thought that oxidative stress markers may be important as biomarkers in the diagnosis and treatment of the disease. In addition, we believe that studies in which the course of the disease can be followed by giving antioxidant preparations to the cattle externally to balance the oxidative stress for therapeutic purposes during the disease process can be tried and this study may be a pioneer in such studies.

Ethics Committee Approval: Ethical approval was obtained from "Ethics Committee Directive" of the Ministry of Agriculture and Forestry Samsun Veterinary Control Institute Animal Experiments Local Ethics Committee. (Date: 03.05.2024, Number:19572899/031-85)

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The Relationship Between Lipid Profile, Oxidative Stress, and Thiol-Disulfide Levels in Healthy, Naturally Overweight and Obese Cats

Doğal Olarak Kilo Alan veya Obezite Gelişen Kedilerde Lipid Profili, Oksidatif Stres ve Tiyol-Disülfür Düzeyleri Arasındaki İlişkiler

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ABSTRACT

This study aimed to evaluate systemic inflammation, oxidative stress and lipid profile in cats that had either naturally gained excess weight or had developed obesity. The following groups were examined in the study: ten obese cats with a body condition score of (BCS) >8 (the obesity group), ten overweight cats with a BCS score of >6 (the overweight group) and ten ideal weight cats with a BCS score of 4-5 (the control group). In the cats that had either gained too much weight or had become obese, the serum AST ($P < .001$), albumin ($P = .002$) and total protein (TP) ($P < .001$) levels were found to be significantly higher than the values determined in the control group cats. Furthermore, blood serum high-density lipoprotein (HDL) ($P = .009$) and triglyceride (TG) ($P < .001$) levels in cats that had developed obesity were found to be significantly higher than the values defined in the control group cats. In the obese cats, serum procalcitonin (PCT), paraoxonase-1 (PON-1), total thiol, native thiol and MDA levels were found to be significantly higher than in overweight cats ($P < .001$). As a result, it was concluded that it would be useful for veterinarians to consider significant changes in parameters related to liver function and lipid metabolism, as well as to emphasize systemic inflammation and oxidative stress in their clinical evaluations in cats that had either naturally gained excess weight or had developed obesity.

Keywords: Cat, lipid metabolism, liver enzymes, obesity, oxidative stress.

ÖZ

Bu çalışmada, doğal olarak fazla kilo almış olan veya obezitenin geliştiği kedilerde sistemik inflamasyonun, oksidatif stresin ve lipid profilinin değerlendirilmesi amaçlandı. Çalışmada, vücut kondisyon skoru (BCS) >8 olan on obez kedi (obezite geliştiren grup), BCS skoru >6 olan on fazla kilolu kedi (fazla kilolu grup) ve BCS skoru 4-5 olan on ideal kilolu kedi (kontrol grubu) incelendi. Aşırı kilo almış olan ve obezitenin geliştiği kedilerde, serum AST ($P <$,001), albumin ($P =$,002) ve toplam protein (TP) ($P <$,001) düzeylerinin kontrol grubundaki kedilerde tanımlanan değerlerden anlamlı derecede yüksek olduğu belirlendi. Ayrıca, obezitenin geliştiği, kedilerde kan serumu yüksek yoğunluklu lipoprotein (HDL) (P = ,009) ve trigliserit (TG) ($P <$,001) düzeyleri kontrol grubundaki kedilerde belirtilen değerlerden anlamlı derecede yüksek bulundu. Obezitenin geliştiği kedilerde, serum prokalsitonin (PCT), paraoksonaz-1 (PON-1), toplam tiyol, doğal tiyol ve MDA düzeylerinin aşırı kilolu kedilere göre anlamlı derecede yüksek olduğu saptandı ($P <$,001). Sonuç olarak veteriner hekimlerin klinik değerlendirmelerinde, doğal olarak fazla kilo almış olan veya obezitenin geliştiği kedilerde, karaciğer fonksiyonları ve lipid metabolizması ile ilgili olan ve sistemik inflamasyon ve oksidatif stresi gösteren parametrelerdeki anlamlı düzeydeki değişiklikleri dikkate almalarının yararlı olacağı kanısına varıldı.

Anahtar Kelimeler: Karaciğer enzimleri, kedi, lipit metabolizması, obezite, oksidatif stress.

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INTRODUCTION

The most common health problems of domestic cats brought to the vets of many countries are energy metabolism issues that are related to excessive weight gain or obesity. Excess weight or obesity as a result of increased accumulation of fat tissue often give rise to mechanical (increased stress on joints and muscles) issues, as well as having other metabolic consequences. It is also reported that excess weight creates various other health problems in a cat's later life.[1-3]

As the body condition score (BCS) increases, the balance of antioxidants and oxidants in the body is disrupted in favor of oxidants, which means the body is under the intense influence of oxidative stress. This, in turn, means that various metabolic and organic disorders may occur in the organism, or there is increased risk of their occurrence. The process means that an animal's quality of life is reduced. It has been shown that elevated liver enzymes in overweight cats are associated with lipid metabolism disorders and oxidative stress potentiation.[4,5]

Since total thiol and native thiol molecules contain sulfhydryl groups with antioxidant/prooxidant properties in their structures, it is crucial to evaluate the thiol and disulfide status in order to determine the level of oxidative stress in patients. If oxidative stress increases and persists for a sufficient length of time, the lipid matrix of the biological membranes become inflamed or are destroyed due to oxidation, and so there is clearly a strong link between oxidative stress and systemic inflammation in the body.[6] Medical monitoring of serum CRP and procalcitonin levels are important biomarkers in the evaluation of systemic inflammation in feline sepsis patients.[7,8] The severity of oxidation caused by reactive oxygen species in the lipid matrix of biological membranes can be assessed by monitoring the changes in serum malondialdehyde (MDA) levels.[9] It has been shown that excessive weight gain in cats is associated with hepatic lipidosis and oxidative stress, and that reactive oxygen species can oxidize the lipoid matrix of biological membranes. Oxidative damage in membrane phospholipids begins with lipid peroxidation. The hydrolyzing capabilities of phospholipids after peroxidation suggest that paraoxonase-1 (PON-1) protects lipids from oxidation.[4,9] PON-1 is an extracellular hydrolase produced in the liver and bound to high-density lipoprotein (HDL). In animals with a high BCS score, it is important to monitor the serum PON-1 level in order to evaluate difficulties in the metabolic process.[10-12]

This study examines blood serum PCT and CRP levels to determine whether systemic inflammation has developed. The degree of oxidative stress is also evaluated by considering blood serum PON-1, total thiol, natural thiol and MDA levels. Blood serum HDL, TG, and CHOL levels are also considered to evaluate levels of lipid profile and the blood serum AST, ALT, albumin and the total protein. The overall aim is to examine the metabolic processes related to liver functions in naturally overweight or obese cats.

MATERIALS AND METHODS

Ethical approval was obtained from the Animal Experiments Local Ethics Committee (Date:14.12.2022 Number:2022-22-197) of Ankara University.

The animals examined in this study consisted of 30 neutered cats, aged 7-11 years, from different breeds and of both sexes. The animals were being kept as domestic pets in the homes of owners aged over 18 years and living in Ankara, Turkey. According to the information received from the owners of all of the cats used in this study, the animals were fed with commercial food in the amount recommended by the manufacturer. It was also stated that the cats began to be more than the ideal weight from the age of 2–3 years.

A general health check-up of the cats in the study was performed, and no medical issue was detected in 20 cats besides them being overweight (ten cats had a BCS >6 and ten a BCS >8). No medical condition was detected in clinical examination of the ten cats (BCS = 4–5) used as the control group.

In scoring the BCS of the animals, a system ranging from 0 to 9 points was used which took into account the physical appearance of the body and breed-specific body measurements. In this assessment system, cats with a BCS of >6 were considered overweight, and those with a BCS of >8 were considered to have developed obesity.[13]

Blood samples were collected from the vena cephalica antebrachii of all of the cats used in the study. Serum was extracted from blood samples from tubes without an anticoagulant, and CBC analysis was conducted from tubes with anticoagulant. CBC parameters were determined in the blood samples with anticoagulant using an automatic blood count device (Mindray BC 5000, Nanshan, China). The serum samples were placed in godets and kept at –20°C until biochemical analysis. ALT, AST, TP, albumin, urea, and creatinine were determined spectrophotometrically with an autoanalyzer (Mindray BS-120, Nanshan, China) placed in the blood serum samples, which were brought to

room temperature for melting before analysis. CRP level measurements were performed using the "Fuji film Nx 500" analyzer. Procalcitonin levels was measured spectrophotometrically by the ELISA method using relevant ELISA kits (BT Lab, Bioassay Technology Laboratory, Cat No: E0065Cat, Zhenjiang, China).

The blood serum MDA level was calculated according to the method specified by Draper and Hadley[14]. For this analysis, trichloroacetic was prepared at 10%, 2.5 mL of which was mixed in a test tube with 0.5 mL of the serum sample during the first stage of measurement. The next stage was for the mixture to be placed in a bath of boiling water, left to cool for 15 minutes, and then centrifuged at 400 g for 10 minutes. Two milliliters were taken from the supernatant and placed in a separate tube; precisely 1.0 mL of 0.67% thiobarbituric acid was added, gently mixed, and the resulting mixture incubated in a bath of boiling water for 15 minutes. To evaluate the reaction when the solution reached room temperature, absorbance values were measured at 532 nm in the spectrophotometer device (Tecan Sunrise RS 232, Grödig, Austria).

The method developed by Eckerson et al.[15] was used to measure PON-1 activity. This method is based on the formation of diethyl phosphate and p-nitrophenol metabolites caused by the action of a paraoxon (diethyl p-nitrophenyl phosphate) (1 mM) and $CaCl_2$ (1 mM) in 0,05 M glycine buıffer molecules as a result of the enzyme activity of paraoxonase in the serum. Measurement and calculation of the resulting p-nitrophenol was determined using a spectrophotometer with an absorbance of 412 nm.

During the determination of the thiol-disulfide levels, the thiol [-SH] and disulfide [-S-S] levels were determined. Measurements were made spectrophotometrically according to the method described by Erel and Neselioglu.[16] NaBH4 was used to reduce sulfide bonds in the serum samples and form free functional thiol groups, while formaldehyde was used to remove the unused portion of NaBH4 from the reaction medium. The DNTB and thiol groups were determined spectrophotometrically at 415 nm as total thiol and total thiol/native thiol.

Disulfide levels were calculated using the formula: (µmol/L) = (total thiol-native thiol)/2.

Statistical Analysis

Before proceeding to hypothesis testing, the data was analyzed in terms of normal distribution and homogeneity of variance for assumption controls. After the Shapiro-Wilk and Levene tests; control, overweight and obese groups were compared using One-way ANOVA test or Kruskal-Wallis test, as appropriate. The results were summarized as mean±standard error (Mean±SE). In order to determine significance difference, Tukey and Dunn's tests were used as for multiple comparisons. The differences between the groups were expressed as superscripts (a b c). In all analyses, P value of < .05 was accepted as significance criterion. The data was analyzed using IBM SPSS Statistics 26.0 (SPSS®, IL,USA).

RESULTS

Among the cats used in the study, the mean age of the animals in the control group was 9.3 ± 1.3 years, the overweight group 9.1 ± 1.4 years, and the obese group 9.2 ± 1.3 years.

The hematological and biochemical data of the cats in the control, overweight, and obesity groups are given in Tables 1, 2, and 3.

Table 1. Hematological parameters.

Groups	WBC (10^9/L)	NEU (10^9/L)	LYM (10^9/L)	MONO (10^9/L)	EOS (10^9/L)	BAS (10^9/L)	PLT (10^9/L)
Control (10)	9.78 ± 0.39	6.88 ± 0.48	2.33 ± 0.11	0.155 ± 0.03	0.497 ± 0.13	0.007 ± 0.001	251 ± 27.9
Overweight (10)	9.92 ± 0.35	6.54 ± 0.48	2.56 ± 0.1	0.205 ± 0.03	0.604 ± 0.14	0.019 ± 0.01	310 ± 45.4
Obesity (10)	9.81 ± 0.22	6.91 ± 0.37	2.36 ± 0.35	0.201 ± 0.02	0.283 ± 0.07	0.012 ± 0.001	228 ± 27.1
P	.949	.809	.371	.398	.125	.419	.243

Values in the table are given as arithmetic mean ± standard deviation (X ± SD).***** denotes differences between groups in the same column.

Table 2. Biochemical parameters.

Groups	PCT (mL/L)	PON-1 (U/L)	Total thiol (µmol/L)	Native thiol (µmol/L)	CRP (mg/L)	MDA (nmol/g protein)
Control (10)	9.78 ± 0.39	6.88 ± 0.48	2.33 ± 0.11	0.155 ± 0.03	0.497 ± 0.13	0.007 ± 0.001
Overweight (10)	9.92 ± 0.35	6.54 ± 0.48	2.56 ± 0.1	0.205 ± 0.03	0.604 ± 0.14	0.019 ± 0.01
Obesity (10)	9.81 ± 0.22	6.91 ± 0.37	2.36 ± 0.35	0.201 ± 0.02	0.283 ± 0.07	0.012 ± 0.001
P	.949	.809	.371	.398	.125	.419

The values in the table are given as arithmetic mean ± standard deviation (X±SD); ^{a,b,c} indicate differences between groups in the same column.

Table 3. Biochemical parameters.

Groups	AST (U/L)	ALT (U/L)	ALB (g/dL)	TP (g/dL)	UREA (mg/dL)	CREA (mg/dL)	HDL (mg/dL)	TG (mg/dL)	CHOL (mg/dL)
Control (10)	106±3.06^{c}	46.8±6.73	3.05±0.15^{b}	6.44±0.33^{b}	28.1±1.41	0.805±0.04	45.7±6.12^{a}	49.8±1.92^{b}	59.4±3.32
Overweight (10)	121±2.37^{b}	47.6±4.43	3.5±0.06^{a}	7.66±0.12^{a}	25.8±1.01	0.717±0.01	58.2±1.71^{ab}	53.2±1.4^{b}	63.3±1.51
Obesity (10)	132±1.31^{a}	52.7±2.9	3.62±0.06^{a}	8.06±0.28^{a}	27.4±1.26	0.755±0.02	65.5±2.01^{b}	62 ± 1.29^{a}	62.8±1.81
P	< .001	.325	.002	< .001	.403	.071	.009	< .001	0.452

The values in the table are given as arithmetic mean ± standard deviation (X ± SD); ^{a,b,c} indicate differences between groups in the same column.

There were no statistically significant differences in the levels of hematological parameters between the cats with an ideal BCS score, the cats with naturally gained excess weight and the cats with naturally developed obesity.

It was determined that the PCT and MDA, total thiol, and native thiol levels were higher in both overweight cats compared to the cats with ideal BCS score, and in cats with obesity compared to the cats with excess weight ($P < .001$). The PON-1 and CRP levels were lower in overweight cats compared to cats with an ideal BCS score cats, as well as in obese cats compared to overweight cats ($P < .001$).

DISCUSSION

A BCS value increase above the ideal score in cats is caused by a decrease in daily activity, neutering, aging, living in a domestic environment and being fed high-calorie food. [2,17] If the BCS score exceeds the ideal values, lipid metabolism deterioration, systemic inflammation and increases in oxidative stress lead to long-term organ function deterioration. In order to detect and monitor the damage that an increase in BCS may cause in the body, changes in the levels of various biochemical parameters related to metabolic processes are evaluated. For this purpose, firstly, serum triglyceride, cholesterol, HDL, AST, ALT, total protein, albumin, CRP, urea and creatinine levels are measured. Blood serum paraoxanase-1 (PON-1) level is also determined to evaluate the metabolic interaction of HDL and low-density lipoprotein levels.[18]

In this study, serum AST, TG and HDL levels measured for the purpose of evaluating liver function in overweight and obese cats were found to be statistically significantly higher than those reported in cats with ideal BCS (control group), which is consistent with the findings of various authors. [6,19,20] Okada et al.[21] found that serum triglyceride levels in obese cats were statistically significantly higher than those determined in the control group. Other studies on obese cats have shown that serum HDL and triglyceride levels were significantly higher than those determined in control cats. [6,22] In studies conducted by Bauer[23] on cats with ideal BCS, and Hoenig et al.[24] on obese cats, it was stated that the high HDL levels in both groups of cats may be due to cholesteryl ester transfer protein deficiency. Similarly, it was noted in this study that serum HDL and triglyceride levels in cats with obesity were significantly higher than the values determined in the control group cats. In addition, it was noted that in cats with increased BCS values, serum total protein and albumin values were higher than the values determined in the control group cats. This result shows that metabolic pathways related to protein production and degradation were affected in cats that gained excessive weight and developed obesity. Considering that oxidative stress and body fluid distribution are also effective in protein production and degradation, it is obvious that changes in serum total protein and albumin levels should be investigated in more detail on a pathway basis.

PON-1 is synthesized in the liver[25] and is directly related to serum HDL and triglyceride levels.[26] The level of blood serum PON-1 is strongly correlated with the degree of oxidative stress and inflammation states in the body.[9] In this study, serum PCT, PON-1, total thiol, native thiol and MDA levels were found to be significantly higher in overweight cats, as compared to the values determined in the control group, and in obese cats compared to the values determined in overweight cats ($P < .001$). According to these results, it can be seen that in cats exceeding the ideal BCS score, primarily fat metabolism is impaired in the body leading to gradual increases in systemic inflammation and oxidative stress. It has also been reported that as PON-1 protects lipids from peroxidation, it is an important parameter in evaluating the level of MDA, one of the lipid peroxidation metabolites of PON-1. Okada et al.[21] found that serum MDA levels were statistically significantly higher in obese cats compared to the control group. Rossi et al.[8] indicated that the serum PON-1 level in healthy cats is 58–154 U/L. Dağ and Şahinduran[27] reported that the PON-1 values in overweight patients were statistically lower than in the control group. In their study conducted on obese cats that then lost weight, and those that did not, Tvarijonaviciute et al.[28] determined that the PON-1 levels in the group that could not lose weight were significantly lower than the values determined in the other group.

The molecules in the plasma thiol pool have antioxidant

effects. The status of the plasma thiol pool, which reflects thiol/native thiol homeostasis, is evaluated by looking at the levels of serum albumin and thiol, which are low molecular weight proteins and compounds.[29,30] Mengen et al.[31] determined that the disulfide/total thiol level in obese individuals was significantly higher than the value determined in healthy individuals. Tursun et al.[29] stated that the antioxidant thiol level determined in obese rats was lower than the value in the control group. Giannuzzi et al.[30] found that the measurement of the level of thiol groups was significant in evaluating the liver functions of dairy cattle. In our study, it was determined that total thiol and native thiol levels were significantly higher in overweight cats and cats with developed obesity, as compared to the control group. This was interpreted as an increase in oxidative stress due to weight gain.

Various studies have shown that obesity causes a proinflammatory state in humans[32,33], dogs[34], and cats[35] due to an increase in inflammatory cytokines. It has been shown that neutrophil and monocyte counts are high in both obese children[32,33] and in obese dogs.[34] In our study, no statistically significant difference was found in terms of WBC values in overweight and obese cats compared to the values in the cats in the control group.

This study provides an insight into the metabolic pathways on which organic and metabolic disorders that occur in cats due to excessive weight gain or obesity are based, as well as on the pathogenesis of the disorders.

As a result of this study, statistically significant differences were found between the changes in the levels of parameters related to liver function and lipid metabolism, as well as in the levels of parameters indicating systemic inflammation and oxidative stress in overweight cats and obese cats compared to the values in the control group. These results both indicate that health problems begin in cats which exceed the ideal BCS values. It also demonstrates that it would be useful for veterinarians to take into account both significant changes in parameters related to liver function and lipid metabolism, and to indicate systemic inflammation and oxidative stress in the control and treatment of the health of such cats.

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The Effects of a Diet Containing Yoghurt with Krill Oil Consumed by Rats During Their Pregnancy on Long Bones of Their Offspring

Ratların Gebelik Döneminde Tükettikleri Krill Yağlı Yoğurt İçeren Diyetin Yavrularının Uzun Kemikleri Üzerindeki Etkisi

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ABSTRACT

The use of calcium, vitamins, minerals, and omega 3 fatty acids during pregnancy are recommended to support the bone development of infants. The aim of this study is to feed pregnant rats with the probiotic yoghurt mixed with krill oil, which is rich in these features, and examine the morphometric development of long bones in their offspring. For this purpose, a total of twelve 2-month-old offspring including 6 in the experimental group (offspring of pregnant rats fed with yoghurt mixed with krill oil) and 6 in the control group (offspring of pregnant rats fed a standard feed), were used in the study. When they became 2 months old, their biometric measurements were taken. After euthanasia, long bones of the offspring (Humerus, antebrachium, femur, ossa cruris) were cleaned by maceration. These bones were photographed. Morphometric measurements of the length and width of these bones were made using the Image J program. In the SPSS (20.0 Version) program, the parameters were compared between the right and left leg bones and between the control and experimental groups by running the Independent Samples T test. Additionally, Pearson's correlation test was applied between the parameters. The results of the study indicated that this diet with krill oil and yogurt consumed by pregnant rats had positive effects on the length parameters of the bones and biometric parameters of their offspring.

Keywords: Krill oil, morphometry, rat, yoghurt.

ÖZ

Gebelikte yavruların kemik gelişimini desteklemek için kalsiyum, vitamin, mineral ve omega 3 yağ asitlerinin kullanımı önerilmektedir. Bu çalışmanın amacı, bu özelliklerden zengin olan krill yağı ile karıştırılmış probiyotik yoğurt ile gebe ratları beslemek ve yavrularında uzun kemiklerin morfometrik gelişimini incelemektir. Bu amaçla deney grubunda 6 adet (krill yağı ile karıştırılmış yoğurtla beslenen gebe ratların yavruları) ve kontrol grubunda 6 adet (standart yemle beslenen gebe ratların yavruları) olmak üzere toplam 12 adet 2 aylık yavru rat çalışmada kullanıldı. Yavru ratlar 2 aylık olduklarında biyometrik ölçümleri alındı. Ötanazi sonrasında yavruların uzun kemikleri (Humerus, antebrachium, femur, ossa cruris) maserasyonla temizlendi. Bu kemikler fotoğraflandı. Bu kemiklerin uzunluk ve genişliklerinin morfometrik ölçümleri Image J programı kullanılarak yapıldı. SPSS (20.0 Versiyonu) programında sağ ve sol bacak kemikleri arasında ve kontrol ve deney grupları arasında Independent Samples T testi ile parametreler karşılaştırıldı. Ayrıca parametreler arasında Pearson korelasyon testi uygulandı. Çalışmanın sonuçları, gebe ratların tükettiği krill yağı ilaveli yoğurt diyetinin kemik uzunluk parametreleri ve yavrularının biyometrik parametreleri üzerinde olumlu etkileri olduğunu gösterdi.

Anahtar Kelimeler: Krill yağı, morfometri, rat, yoğurt.

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INTRODUCTION

Krill oil is a substance obtained from a sea creature called "*Euphausia Superba*" living in the oceans. It contains high rates of omega 3 fatty acids in the form of phospholipids. It is also a nutritional supplement that contains astaxanthin, vitamin A and vitamin E. Astaxanthin has a strong antioxidant activity. In recent years, Krill oil has become more and more important than fish oil. It is safe to use it during pregnancy. However, the high content of docosahexaenoic acid and eicosapentaenoic acid has increased the importance of krill oil.[1, 2] Furthermore, this oil has recently become the focal point of the researchers in terms of high absorption.[3-7]

Omega 3 fatty acid supplements are known to be important in mental development, hyperlipidemia, premenstrual syndromes, and inflammatory[8] and cardiological diseases.[9] However, multiple unsaturated fatty acids are effective in stimulation of growth. In fact, Omega-6 fatty acids elevate prostaglandin E2 levels and this hormone also suppresses bone development. For this reason, it is suggested that feeding with a ration enriched by omega-3 fatty acids suppresses the release of prostaglandin E2 and promotes bone development.[10-12]

Yoghurt is a fermented dairy product that is rich in protein, calcium, phosphorus, and especially B-group vitamins.[13, 14] Probiotics in yoghurt positively affect the absorption of minerals such as calcium, phosphorus, and zinc. By means of minerals it contains, yoghurt has significant effects on bone development.[15] However, some components are added to increase the nutritional value of yoghurt. Yoghurt enriched with omega-3 fatty acids has a positive effect on consumption in terms of food quality.[16] Probiotic yoghurt added with krill oil has been reported to have very good physical, chemical, microbiological, and sensory properties and a high nutritional value.[17]

It is recommended to consume calcium-rich foods, vitamins, mineral supplements and omega-3 foods to support the bone development of infants during pregnancy.[18] Yoghurt with krill oil used in the study is rich in these features. For this reason, this study aims to investigate morphometric development of the long bones in the offspring of rats fed yoghurt with krill oil during their pregnancy.

MATERIALS AND METHODS

Animals

In the study, a total of 24 rats including 12 pregnant rats and their 12 offspring were used. The pregnant rats were divided into two groups as experimental and control groups including 6 rats in each.

Experimental Group

This study was approved by the Burdur Mehmet Akif Ersoy University Animal Experiments Local Ethics Committee (No: 1046, Date: 07.03.2023).

The experimental group consisted of male offspring (2 months old, n=6) of rats that fed probiotic yoghurt with 2 %krill oil during their pregnancy. These pregnant rats were given standard feeding for 21 days and during this period, yoghurt with krill oil (daily dose: 1 ml) was given them via gavage once a day.

It is known that gender has an effect on bone morfometria. In the study, the gender factor was taken into account in case the morphometric difference that would affect the results could be gender-related. For this reason, only male rats were used as an experimental material in the study.

Control Group

The control group consisted of male offspring (2 months old, n=6) of rats that fed the standard feed during their pregnancy.

The offspring, which were included the experimental and control groups, were reared in similar care units for 2 months. They were fed the standard feed in this process. They were euthanized under the anesthesia with xylazine-ketamine at the end of 2 months.

First of all, the weight, head-tail length and tail length parameters of these rats were determined. Then, their long bones (Humerus, Antebrachium, Femur, Ossa Cruris) were cleaned from rough meats via maceration. Cleaned bones were photographed. In the Image J program, morphometric measurements were carried out over the photographs (Figures 1, 2, 3, and 4) and the results were recorded. Morphometric parameters were determined upon the literature review. [19]

Statistical Analyses

The parameters were analyzed using the SPSS (20.0 version) packaged software. These parameters were compared in terms of groups (control group- experimental group) and direction (right-left) by independent samples t test. Moreover, Pearson's correlation test was performed between these parameters.

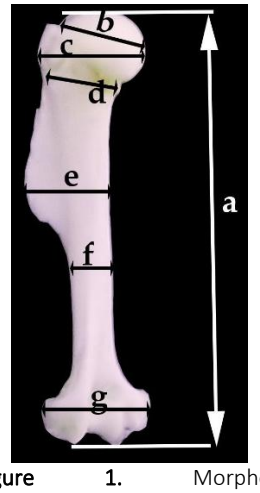

Figure 1. Morphometric measurements determined on humerus (Caudal view of the left humerus): a. Humerus length, b. caput humeri width, c. proximal width, d. collum humeri width, e. largest width of the corpus, f. smallest width of the corpus, g. distal width

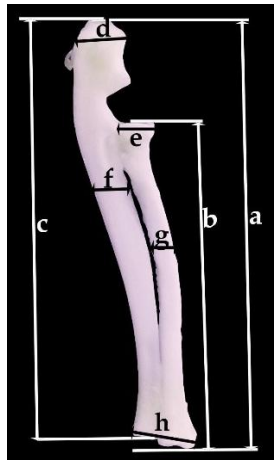

Figure 2. Morphometric measurements determined on antebrachium (lateral view of the left antebrachium (radius and ulna)) a. Antebrachium length, b. radius length, c. ulna length, d. proximal width of ulna, e. proximal width of radius, f. largest corpus width of the ulna, g. largest corpus width of the radius, h. distal width

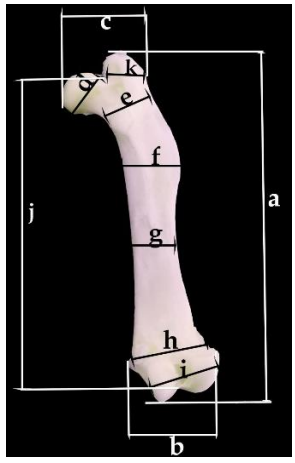

Figure 3. Morphometric measurements determined on femur (Caudal view of the right femur) a. Femur length, b. distal width, c. proximal width, d. caput femoris width, e. collum femoris width, f. largest width of the corpus, g. smallest width of the corpus, h. largest distal width (above the condylus level), i. condylus width, j. smallest length of corpus, k. trochanter major width

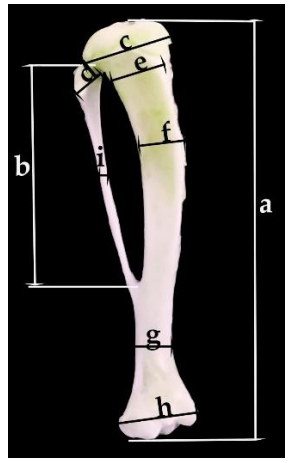

Figure 4. Morphometric measurements determined on ossa cruris (Caudal view of the left ossa cruris (tibia and fibula): a. tibia length, b. fibula length, c. tibia's proximal width, d. fibula's proximal width, e. collum tibia width, f. largest width of the corpus, g. smallest width of corpus, h. distal width, i. corpus fibula width

Probiotic Yoghurt Mixed With 2% Krill Oil

Krill oil is rich in omega 3 fatty acids, astaxanthin, vitamin A and vitamin E.[20] Tables 1, 2, and 3 show the nutritional values of the probiotic yoghurt with 2% krill oil used in the study.

RESULTS

Table 4 shows biometric parameters of the rats. In the comparison made in terms of length (Head - tail length and tail length), the rats in the experimental group had statistically significantly longer values ($P < .05$). The parameter of weight was greater in the experimental group, but it was not statistically significant ($P > .05$).

Table 1. Nutritional values of probiotic yoghurt mixed with 2% Krill oil.

Main components		Minerals	
Serum	31.08%	Ca (Calsium)	3894.40 ppm
Protein	4.30%	Mg (magnesium)	1323.15 ppm
Oil	4.18 %	P (phosphor)	1245.30 ppm
dry matter	14.45%	K (potassium)	1932.68 ppm
Ash	0.89 %	Na (sodium)	2169.45 ppm

Table 2. Amounts of volatile aroma compounds contained in probiotic yoghurt mixed with 2% Krill oil.

Matter	Amount (ppm)
Acetaldehyde	27.06
Ethanol	2039.12
Diacetyl	22.03
Acetoin	5.81

Table 3. Microbiology of probiotic yoghurt mixed with 2% Krill oil.

Microbiological analysis	Amount (log10 KOB/g)
Lactobacillus delbrueckii subsp. bulgaricus	5.68
Aerobic mesophilic bacteria count	8.52
Lactobacillus acidophilus	7.02
yeast numbers	3.85
Mold	(<1 log10 KOB/g
Coliform	(<1 log10 KOB/g)
Streptococcus salivarius subsp. thermophilus	9.24

Table 4. Biometric parameters of the experimental group and control group in offspring.

Biometric value	Control group	Experimental group
Weight (gr)	201.83 ±18.91	220.16±10.18
Head-tail length (mm)	193.53± 6.92*	211.83 ±13.20*
Tail length (mm)	158.00± 14.11*	181.46 ±17.68*

*: Comparison between control and experimental groups, $P < .05$.

Table 5 shows morphometric parameters of humerus. Accordingly, the humerus was statistically significantly longer in the experimental group; whereas, the proximal and distal width of humerus was statistically significantly greater in the control group ($P < .05$). Except for width of the collum humeri, the other parameters related to width were larger in the control group, but they were not statistically significant ($P > .05$).

Table 5. Morphometric parameters of humerus in offspring (Average value ± standard deviation) (mm).

Humerus	Control – right leg	Control- left leg	Control	Experimental right leg	Experimental- left leg	Experimental
Humerus length	23.99 ± 0.29	24.18 ± 0.25	24.08 ± 0.27*	24.90 ± 0.87	24.70 ± 1.04	24.80 ± 0.92*
Caput humeri width	4.18 ± 0.14	4.06 ± 0.25	4.12 ± 0.20	4.05 ± 0.18	3.79 ± 0.43	3.92 ± 0.34
Proximal width	6.06 ± 0.25*	6.12 ± 0.40	6.09 ± 0.32*	5.76 ± 0.15*	5.68 ± 0.24	5.72 ± 0.19*
Distal width	6.65 ± 0.36[b]	6.03 ± 0.52[b*]	6.34 ± 0.54*	6.05 ± 0.55[a]	5.30 ± 0.32[a*]	5.67 ± 0.58*
Largest width of the corpus	4.04 ± 0.29[b]	4.58 ± 0.31[b]	4.31 ± 0.40	3.76 ± 0.27[a]	4.31 ± 0.24[a]	4.04 ± 0.37
Smallest width of the corpus	2.15 ± 0.16	2.20 ± 0.18	2.18 ± 0.16	2.00 ± 0.17	2.17 ± 0.23	2.09 ± 0.21
Collum humeri width	3.11 ± 0.12[b]	3.67 ± 0.39[b]	3.39 ± 0.40	3.25 ± 0.33	3.60 ± 0.22	3.43 ± 0.32
Condylus humeri width	3.53 ± 0.14[b]	2.79 ± 0.51[b]	3.16 ± 0.52	3.36 ± 0.28	2.88 ± 0.50	3.12 ± 0.46

* : shows the difference between control and experimental groups ($P < .05$). [a,]: shows the difference between right and left humerus in the experimental group ($P < .05$). [b] **:** shows the difference between right and left humerus in the control group ($P < .05$).

Table 6 shows the correlation values between parameters of humerus. Based on this table, no significant correlation was observed between humerus length and the other parameters. In both groups, there was a significant negative correlation between the largest width of corpus and the distal width and between the width of condylus humeri and the collum humeri ($P < .001$)

Table 6. The correlation values between morphometric parameters of humerus in offspring (c: control group, e: experimental group).

TOP-c DOWN e	HL	CHW	PW	DW	LWC	SWC	Collum	Condylus	W	HTL	TL
HL		-.522	.229	-.215	.024	.441	.391	-.421	.245	.416	.162
CHW	.210		-.329	.197	-.444	-.146	-.634*	.261	-.449	.451	.487
PW	-.086	.220		-.142	.283	.291	.480	-.462	-.541	-.042	-.162
DW	.419	.182	.007		-.817**	-.437	-.793**	.666*	.426	-.201	-.114
LWC	.023	-.382	-.096	-.745**		.221	.889**	-.584*	-.111	-.237	-.126
SWC	-.164	-.839**	.169	-.182	.369		.366	-.568	.103	.771	.200
Collum	.223	-.274	-.490	-.267	.634*	.093		-.752**	-.041	-.566	-.654
Condylus	-.050	.443	.276	.386	-.592*	-.271	-.577*		.687	-.329	-.486
W	-.032	.828*	.022	-.166	-.483	-.877*	-.025	-.117		-.077	.180
HTL	-.637	.534	-.041	-.780	-.020	-.672	.036	.-487	.642		.642
TL	-.583	.452	-.141	-.819*	.187	-.661	.244	-.555	.631	.959**	

HL: Humerus length, CHW: caput humeri width, PW: Proximal width, DW: Distal width, LWC: Largest width of the corpus, SWC: Smallest width of the corpus, Collum: Collum humeri width, Condylus: Condylus humeri width, W: Weight of offspring, HTL: Head-tail length, TL: Tail length.*: $P < .05$, **: $P < .01$

Table 7 shows morphometric parameters of antebrachium. The length values of radius, ulna and total antebrachium were greater in the experimental group. However, the length of the radius was statistically significantly greater in the experimental group ($P < .05$). Distal width of the left radius was statistically significantly larger in the experimental group ($P < .05$). The parameters of ulna and radius were greater in the control group, while the corpus width was larger in the experimental group. However, these differences were not statistically significant ($P > .05$).

Table 7. The morphometric parameters of antebrachium in offspring (mm).

Antebrachium	Control – right leg	Control – left leg	Control	Experimental – right leg	Experimental – left leg	Experimental
Antebrachium Length	28.03 ± 0.22	28.34 ± 0.58	28.18 ± 0.45	28.36 ± 0.72	28.18 ± 1.74	28.27 ± 0.97
Ulna length	27.00 ± 0.20	27.43 ± 0.64	27.21 ± 0.51	27.10 ± 0.84	26.96 ± 1.24	27.03 ± 1.01
Radius length	20.84 ± 0.25*	21.38 ± 0.54	21.11 ± 0.49*	21.78 ± 0.53*	21.92 ± 0.76	21.85 ± 0.63*
Proximal width of ulna	3.25 ± 0.44	3.27 ± 0.44	3.26 ± 0.42	3.29 ± 0.36	3.04 ± 0.25	3.16 ± 0.32
Proximal width of radius	2.17 ± 0.17	2.19 ± 0.25	2.18 ± 0.20	2.27 ± 0.41	2.03 ± 0.20	2.15 ± 0.33
Distal width	3.85 ± 0.47	4.14 ± 0.60*	4.00 ± 0.54	3.98 ± 0.51[a]	4.83 ± 0.31[a*]	4.40 ± 0.60
Largest corpus width of the ulna	1.87 ± 0.27	2.07 ± 0.11	1.97 ± 0.22	2.02 ± 0.30	2.18 ± 0.22	2.10 ± 0.26
Largest corpus width of the radius	1.23 ± 0.12	1.36 ± 0.20	1.30 ± 0.17	1.39 ± 0.09	1.41 ± 0.18	1.38 ± 0.04

Statistical analysis between control and experimental groups, [a]: shows the difference between right and left antebrachium in the experimental group * : $P < .05$.

Table 8 shows correlation values between the morphometric parameters of antebrachium. Based on this table, a significant positive correlation was observed between total length and length of ulna and radius in both groups ($P < .01$). Moreover, there was a significant positive correlation between the head-tail length and the length of the antebrachium in the experimental group ($P < .05$).

Table 8. The correlation values between morphometric parameters of antebrachium in offspring (c: control group, e: experimental group).

TOP-c DOWN -e	AL	UL	RL	PWU	PWR	DW	LCWU	LCWR	W	HTL	TL
AL		.780**	.789**	.408	.082	-.234	.289	.785**	-.849*	.173	-.324
UL	.956**		.703*	.275	-.262	-.008	.457	.769**	-.552	-.098	.203
RL	.946**	.884**		.526	.192	-.283	.379	.646*	-.097	.059	.324
PWU	.547	.555	.453		-.199	-.393	-.194	.084	-.041	.662	.395
PWR	.351	.286	.298	.670*		-.124	.098	-.106	-.490	-.362	-.541
DW	.131	.225	.254	-.119	-.092		.583*	-.111	-.305	.012	.433
LCWU	.487	.472	.526	.246	.495	.624*		.477	-.363	-.307	.344
LCWR	-.150	-.225	-.229	-.099	-.361	-.125	-.235		-.196	-.125	.221
W	.722	.808	.493	.344	.132	.645	.258	.073		-.077	.180
HTL	.831*	.841	.794	.458	.115	.002	-.048	.045	.642		.642
TL	.683	.717	.630	.295	.162	-.015	.051	.052	.631	.959**	

AL: Antebrachium length, UL: Ulna length, RL: Radius length, PWU: Proximal width of ulna, PWR: Proximal width of radius, DW: Distal width of antebrachium, LCWU: Largest corpus width of the ulna, LCWR: Largest corpus width of the radius, W: Weight of offspring, HTL: Head-tail length, TL: Tail length *: $P < .05$, **: $P < .01$.

Table 9 shows morphometric parameters of femur. Accordingly, the femoral length was greater in experimental group, but the difference between groups was not statistically significant ($P > .05$). Width of caput femoris and the largest width of corpus were larger in the control group and this difference between the groups was statistically significant ($P < .05$). The parameters related to the width of distal femur (Condylus width, distal width) were larger in the experimental group, but this difference between groups was not statistically significant ($P > .05$)

Table 10 shows correlation values between morphometric parameters of femur. While a significant negative correlation was observed between the head - tail length and length of the femur ($P < .05$) in the control group, there was a weak positive correlation (not significant) between them in the experimental group ($P > .05$).

Table 9. Morphometric parameters of femur in offspring (mm).

Femur	Control – right	Control - left	Control	Experimental - right	Experimental - left	Experimental
Femur length	33.05 ± 0.42	32.60 ± 0.74	32.82 ± 0.63	33.23 ± 1.31	33.73 ± 0.96	33.48 ± 0.32
Caput femoris width	4.00 ± 0.13*	3.81 ± 0.16	3.90 ± 0.17*	3.69 ± 0.14*	3.58 ± 0.24	3.63 ± 0.19*
Trochanter major width	3.00 ± 0.19	3.15 ± 0.07	3.07 ± 0.15	3.29 ± 0.42	3.30 ± 0.33	3.29 ± 0.36
Smallest width of the corpus	4.09 ± 0.27[b]	3.76 ± 0.19[b]	3.93 ± 0.28	3.83 ± 0.17	3.63 ± 0.16	3.73 ± 0.14
Largest width of the corpus	5.30 ± 0.29*	5.00 ± 0.38	5.15 ± 0.36*	4.94 ± 0.14*	4.82 ± 0.08	4.88 ± 0.12*
Proximal width	7.68 ± 0.11	7.71 ± 01.7	7.69 ± 0.14	7.69 ± 0.43	7.76 ± 0.41	7.72 ± 0.40
Largest distal width (above the condylus level)	6.33 ± 0.21[b]	6.64 ± 0.17[b]	6.49 ± 0.24	6.51 ± 0.24	6.57 ± 0.29	6.54 ± 0.25
Condylus width	6.79 ± 0.23	6.61 ± 0.27	6.70 ± 0.25	6.77 ± 0.10	6.91 ± 0.20	6.84 ± 0.50
Smallest length of corpus	29.66 ± 0.23	29.19 ± 0.73	29.42 ± 0.57	29.41 ± 0.70	29.41 ± 0.96	29.41 ± 0.80
Collum femoris width	4.38 ± 0.13	4.68 ± 0.47	4.53 ± 0.36	4.32 ± 0.17[a]	4.73 ± 0.12[a]	4.53 ± 0.25

* : shows the difference between control and experimental groups ($P < .05$). [a] : shows the difference between right and left femur in the experimental group ($P < .05$). [b]: shows the difference between right and left femur in the control group ($P < .05$).

Table 10. The correlation values between morphometric parameters of femur in offspring (c: control group, e: experimental group).

TOP-c DOWN -e	FL	Caput	TMW	SWC	LWC	PW	LDW	condylus	SLC	collum	W	HTL	TL
FL		.332	.051	.100	.331	-.204	-.050	-.163	.832**	.248	.358	-.345	-.839*
Caput	.430		.223	.467	.465	.079	-.392	.160	.427	-.132	.278	-.249	-.771
TMW	-.119	.372		-.329	-.282	-.189	.175	-.377	-.048	.003	.230	-.362	-.866*
SWC	-.407	-.129	-.493		.852**	.516	-.195	.837**	.333	.021	-.503	-.350	-.314
LWC	.272	.394	-.444	.429		.615*	-.002	.743**	.536	.443	-.095	-.344	-.386
PW	.622*	-.015	-.320	-.225	.368		.315	.594*	.185	.567	-.664	-.071	.375
LDW	.385	.228	.436	-.439	-.177	.526		-.244	-.029	.622*	-.480	-.138	.028
Condylus	.450	-.105	-.127	-.197	-.285	.479	.341		-.007	.170	-.486	-.307	-.142
SLC	.799**	.371	-.221	-.221	.250	.356	-.044	.434		.232	.507	.056	-.571
Collum	.192	-.298	.030	-.276	-.590*	-.042	.090	.463	-.024		-.419	-.733	-.205
W	.290	.488	-.325	.341	.405	.200	.148	.582	.245	-.199		.180	-.077
HTL	.173	.115	.093	.206	-.149	-.303	.308	.177	.145	.026	.631		.642
TL	.275	.240	.187	-.049	-.056	-.072	.554	.224	.175	-.240	.642	.959**	

FL: Femur length, Caput: Caput femoris width, TMW: Trochanter major width, SWC: Smallest width of the corpus, LWC: Largest width of the corpus, PW: Proximal width, LDW: Largest distal width (above the condylus level), Condylus: Condylus width, SLC: Smallest length of corpus, Collum: Collum femoris width, W: Weight of offspring, HTL: Head-tail length, TL: Tail length. *: $P < .05$, **: $P < .01$.

Table 11 shows morphometric parameters of ossa cruris. As shown in this table, tibia and fibula lengths were greater in the experimental group, but this difference was not statistically significant ($P > .05$). Tibia's proximal width and the narrowest diameter of the corpus were larger in the control group ($P < .05$). The parameters related to width, except for the largest diameter of corpus tibia and the corpus fibula, were larger in the control group, but this difference was not statistically significant ($P > .05$).

Table 11. Morphometric parameters of ossa cruris in offspring (mm).

Ossa cruris	Control – right leg	Control – left leg	Control	Experimental – right leg	Experimental – left leg	Experimental
Tibia length	36.93 ± 1.15	36.55 ± 0.45	36.74 ± 0.86	37.45 ± 0.86	37.04 ± 0.93	37.25 ± 0.88
Fibula length	20.85 ± 0.85	20.64 ± 0.83	20.74 ± 0.81	21.48 ± 0.42	20.76 ± 0.82	21.12 ± 0.72
Tibia's proximal width	7.65 ± 0.45	8.02 ± 0.30	7.83 ± 0.41*	7.44 ± 0.28	7.48 ± 0.57	7.46 ± 0.43*
Fibula's proximal width	2.12 ± 0.37[b]	2.62 ± 0.35[b]	2.37 ± 0.43	1.79 ± 0.19[a]	2.39 ± 0.26[a]	2.09 ± 0.38
Distal width	6.38 ± 0.94	6.60 ± 0.42	6.49 ± 0.71	5.92 ± 0.27[a]	6.34 ± 0.26[a]	6.13 ± 0.33
Largest width of the corpus	3.83 ± 0.31	3.45 ± 0.35	3.64 ± 0.37	4.09 ± 0.23[a]	3.46 ± 0.23[a]	3.77 ± 0.40
Smallest width of corpus	2.93 ± 0.17*	2.89 ± 0.24	2.91 ± 0.20*	2.58 ± 0.17*	2.59 ± 0.24	2.58 ± 0.20*
Collum tibia width	6.04 ± 0.30	5.69 ± 0.33	5.86 ± 0.35	5.95 ± 0.40	5.68 ± 0.49	5.81 ± 0.45
Corpus fibula width	0.98 ± 0.04	0.91 ± 0.06	0.95 ± 0.06	1.08 ± 0.24	0.92 ± 0.01	1.00 ± 0.18

* : shows the difference between control and experimental groups ($P < .05$). [a] : shows the difference between right and left crus in the experimental group ($P < .05$). [b]: shows the difference between right and left crus in the control group ($P < .05$).

Table 12 shows the correlation values between morphometric parameters of ossa cruris. A significant positive correlation was observed between both groups in terms of fibula and tibia length values ($P > .05$). A significant positive correlation was determined between tibia length and largest width of the corpus tibia and between tibia length and collum tibia width in the experimental group ($P < .05$).

Table 12. The correlation values between morphometric parameters of ossa cruris in offspring (c: control group, e: experimental group)

TOP-c DOWN -e	TL	FL	TPW	FPW	DW	LWC	SWC	CTW	CFW	W	HTL	TL
TL		.749**	.236	.319	.276	.380	.488	.533	.361	-.012	.748	.648
FL	.612*		.149	.247	.280	.235	.578*	.502	-.027	-.564	.637	.498
TPW	.453	-.037		.567	.656*	-.222	.399	.236	-.108	-.228	.061	.423
FPW	-.045	-.333	.001		.374	-.627*	-.158	-.346	.036	-.475	.531	.311
DW	.506	-.062	.456	.536		-.223	.465	.299	.213	-.535	-.021	-.051
LWC	.589*	.534	.211	-.537	-.256		.591*	.700*	-.044	.877*	.338	.372
SWC	-.124	-.216	-.070	-.136	.143	.025		.690*	-.159	-.153	.257	.379
CTW	.709**	.669*	-.093	-.129	.155	.534	-.303		.104	-.039	.413	.674
CFW	.569	.416	.316	-.559	.161	.478	-.114	.337		-.112	.004	.162
W	-.747	-.716	-.614	.809	-.792	-.289	-.194	-.786	-.441		-.077	.180
HTL	-.419	-.027	-.110	.248	-.594	-.383	-.832*	-.172	-.245	.642		.642
TL	-.316	.049	.003	.142	-.446	-.336	-.860*	-.193	.003	.631	.959**	

TL: Tibia length, FL: Fibula length, TPW: Tibia's proximal width, FPW: Fibula's proximal width, DW: Distal width, LWC: Largest width of the corpus, SWC: Smallest width of c
CTW: Collum tibia width, CWF: Corpus fibula width, W: Weight of offspring, HTL: Head-tail length, TL: Tail length,*: $P < .05$, **: $P < .01$.

When the biometric parameters were examined in the correlation tables, a significant positive correlation was observed between the tail length and head-tail length in the experimental group ($P < .05$), and this correlation was not statistically significant in the control group ($P > .05$).

DISCUSSION

In the study, the long bones of the 2-month-old offspring of rats fed yoghurt with krill oil during their pregnancy were compared morphometrically with those of the control group. Results of the study indicated that the length values of humerus, antebrachium, femur and ossa cruris were greater in the experimental group. However, only length of the radius was statistically significantly greater in the experimental group ($P < .05$). The proximal widths of the bones were higher in the control group. However, the proximal width values of humerus and tibia were statistically significantly larger in the control group ($P < .05$). In the study, it was noteworthy that bone width was larger in the control group and bone length was larger in the experimental group.

A cartilage area called the epiphysis plaque is located between the primary and secondary ossification centers serving the prolongation of the bone. Until the end of the ossification, the cartilage cells in the epiphysis plaques grow by being divided into diaphysis and constantly make cartilage tissue. This cartilage tissue is replaced by bone tissue.[21] When the epiphysial plaque is closed, it can continue to grow transversely while the longitudinal growth of the bone stops.[22] In the mice, epiphysial plaque is not closed exactly due to age and genotype properties

and the lack of haversian system. Rats do not have a haversian system as in mice and hamster. In adult rats, especially in some male ones, the growth of the epiphysis is not completed until the age of one year.[21, 23] However, in the literature, it is reported that the degree of elongation and development in each part of the long bones is not the same, and the epiphysis has more elongation than the diaphysis. In the study, the differences in bone length detected between the two groups show the extent to which the growth plaques at different levels are affected.

A study was conducted in which the effects of nutrition on bone development during pregnancy were examined in rat offspring.[24] In the literature,[24] bone elongation rate has been evaluated in offspring of rats fed organic dried apricots during their pregnancy and at different stages of development. Although some elongation was observed in the femur and tibia in the groups fed with organic dried apricots compared to the control group, it was determined that this elongation was not significant ($P > .05$). This is thought to be associated with the fact that hereditary factors have a more significant effect on bone growth than environmental factors.[24] In the study, similar to the literature,[24] the effects of nutrition during pregnancy on bone morphometry were investigated in rat offspring. Unlike the literature,[24] in this study, probiotic yoghurt with krill oil was given to pregnant rats in the experimental group and it was observed that there was no statistical difference between the groups in the length of the femur and tibia ($P > .05$), but there was some growth in these bones in the experimental group, which is compatible with the literature[24]

In the literature,[15] the effect of yoghurt on bone mineralization and the bioavailability of Ca, P and Zn was evaluated in rats. In this study,[15] yoghurt and probiotic yoghurt were given to the rats in the experimental group. As a result of the study, it was revealed that feeding with yoghurt did not have a significant effect on weight. Serum Ca, P, and Zn rates, femur density, and femur calcium rate were also significantly higher in rats fed probiotic yoghurt. Feeding with probiotic yoghurt had beneficial effects in terms of providing significant mineral absorption.[15] In the present study, it was determined that feeding with probiotic yoghurt with krill oil during pregnancy did not have a significant effect on weight, similar to the literature.[15] However, there was a statistically significant difference in radius and humerus length ($P < .05$). On the other hand, a statistically insignificant increase was detected in the lengths of the femur and tibia ($P > .05$).

This study has some limitations in terms of the number of experimental groups. In the study, the possible effects of only krill oil and only yoghurt on bone development could not be evaluated. It may be a hypothesis of another topic.

In conclusion, it was determined that feeding rats with yoghurt with krill oil during their pregnancy had positive effects on the biometric parameters and the length parameters of the bones in their offspring. The average value of bone length was greater in the experimental group. Among morphometric parameters of bone, only radius and humerus length were statistically significant ($P < .05$). Accordingly, it is thought that the increase in length of femur and ossa cruris may be significantly affected when the feeding dose of yoghurt with krill oil is increased.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of the Animal Experiments Local Ethics Committee of Burdur Mehmet Akif Ersoy University University (Date: 07.03.2023, Number: 1046)

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Evaluation of Animal Welfare in Dairy farms in Kars Province for Barn and Breeding Conditions

Kars İli Süt Sığırcılığı İşletmelerinde Hayvan Refahının Barınak ve Yetiştirme Şartları Açısından Değerlendirilmesi

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ABSTRACT

This study was conducted in 54 dairy farms, including 48 tie-stall (TS) barns and 6 closed free-stall (CFS) barns, in two different types of farms registered to the TURKVET system in the city center and districts of Kars. The welfare level was determined based on the Animal Needs Index (ANI) 35L Model. Use of yard or pasture (days/year) among the criteria of the freedom of movement category according to barn types; space per animal (m^2/500 kg), management of young and use of yard or pasture (days/year) among the criteria of the social interaction category; softness and cleanliness of the bedding space among the criteria of the floor condition category; the use of open space among the criteria of the light and air conditions category; and cleanliness of stables among the criteria of the stockmanship category; the condition of equipment, condition of integument, cleanliness of animals, and condition of hooves were found to be statistically significant ($P < .05$). The in-barn mean temperature, humidity and temperature humidity index (THI) were 23.76 °C, 37.83% and 68.73, respectively in the tie-stall barns, while the mean temperature, humidity and temperature humidity index (THI) were 22.20 °C, 38.13% and 66.98, respectively, in the closed free-stall barns and no statistical difference was found ($P > .05$). As a result of the research, 2.1% of the closed-tie barns were determined to be borderline suitable, 33.3% partially suitable, 37.5% largely suitable, 27.1% suitable in terms of animal welfare, while no unsuitable or very suitable enterprises were determined. While 16.7% of the closed free-stall barns were suitable and 83.3% were very suitable, no unsuitable, rarely, little, and fairly suitable barns were determined. The breeders and personnel working in relevant units should be trained on animal welfare to increase awareness on welfare.

Keywords: ANI 35/L, animal welfare, barn, dairy farming.

ÖZ

Bu araştırma, Kars merkez ve ilçelerinde TÜRKVET sistemine kayıtlı, farklı iki tip işletmede 48 adeti kapalı bağlamalı ve 6 adeti ise kapalı serbest dolaşımlı olmak üzere 54 adet süt sığırı işletmesinde gerçekleştirilmiştir. Refah düzeyini belirlemede, Animal Needs Indeks (ANI) 35L yöntemi temel alınarak yapılmıştır. Ahır tiplerine göre hareket özgürlüğü kategorisi kriterlerden, avlu ya da mera kullanımı (gün/yıl); sosyal etkileşim kategorisi kriterlerinden, hayvan başına alan (m^2/500 kg), gençlerin yönetimi ve avlu ya da mera kullanımı (gün/yıl); zemin durumu kategorisi kriterlerinden, yatma alan yumuşaklığı ve yatma alan temizliği; ahır içi iklim koşulları kategorisi kriterlerinden, açık alan kullanımı ve bakım kategorisi kriterlerinden, bölme, yemlik ve suluk temizliği, teknik ekipman durumu, deri durumu, hayvanların temizliği ve tırnakların durumunun istatiksel olarak önemli olduğu belirlenmiştir ($P < ,05$). Kapalı bağlamalı ahırlarda, barınak içi sıcaklık, nem ve sıcaklık nem indeksi (THI) ortalamaları sırası ile 23,76 ^{0}C, %37,83 ve 68,73 belirlenirken, kapalı serbest dolaşımlı ahırlarda aynı sıra ile 22,20 ^{0}C, %38,13 ve 66,98 olarak belirlenmiş ve istatiksel bir fark tespit edilmemiştir ($P > ,05$). Araştırma sonucunda, kapalı bağlamalı ahırların hayvan refahı açısından %2,1'nin sınırda uygun, %33,3'ünün kısmen uygun, %37,5'inin büyük ölçüde uygun, %27,1'nin uygun olarak belirlenirken, uygun olmayan ve çok uygun olan işletme belirlenmemiştir. Kapalı serbest dolaşımlı ahırların %16,7'sinin uygun ve %83,3'ünün çok uygun olduğu belirlenirken, uygun olmayan, nadiren uygun olan, kısmen uygun ve oldukça uygun ahır belirlenmemiştir. Hayvan refahı konusunda ilgili birimlerdeki yetiştiriciler ve personelin, bilinçlendirilmesi için eğitim almaları sağlanmalıdır. Hayvan refahı konusunda ilgili birimlerde çalışan yetiştiricilerin ve personelin eğitilmesi sağlanarak refah konusunda farkındalık artırılmalıdır.

Anahtar Kelimeler: ANI 35/L, barınak, hayvan refahı, süt sığırcılığı.

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

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INTRODUCTION

In recent years, there has been a deficit in the production of animal products as the agricultural lands have gradually reduced and the need for food supply has grown, and it has become necessary to switch to intensive stock farming in order to raise the yield per animal in order to satisfy the demand for these products. Consequently, animals have been removed from their habitats and have come across some problems in terms of welfare.[1,2]

Due to the raising of animals away from their habitats, animal research has been focused on animal welfare in recent years and importance has been placed on conducting studies in this field. It is important to restore the habitual order of cattle removed from their habitats in raising and make every effort to eliminate all kinds of problems that may occur at this point, to create better welfare conditions, and to rear animals in healthy conditions.[2-5]

Improving animal welfare in livestock raising will enhance access to animal food, boost economic returns, provide food safety, and protect animal health. It would also play an important role in improving people's welfare as it would reduce risks to human health.[4,5]

Animal welfare has a multidimensional structure. Therefore, the assessment of animal welfare is based on complementary measures covering all dimensions.[6,7] Currently, three different methods are followed to assess animal welfare: assessment of animal welfare using the four basic principles, good barn conditions for animals, and the animal needs index (ANI) method.[6-10]

The method followed should include a combination of physiological, health and behavioral indicators to assess animal welfare at the farm level in order to be comprehensive, valid, and reliable. There is a wide variation among the welfare assessment methods used in terms of welfare indicators. Several studies following the animal welfare criteria method developed by Bartussek et al.,[11], and named ANI 35L/2000-cattle, have reported that this method is a sensitive and reliable approach for welfare assessment at the farm level.[6,12]

The animal needs index is one of the most widely used methods for assessing animal welfare in cattle. The researcher developed this method as an appropriate and comprehensive assessment tool to meet the need for evaluating animal welfare on farms.[11,13-15]

This study aimed to evaluate animal welfare in dairy farms in Kars province based on ANI 35L criteria for barn and breeding conditions.

MATERIALS AND METHODS

Material

This study was approved by the Animal Ethics Committee of Animal Experiments of the Veterinary Faculty at Kafkas University (Date: 27.05.2021, Number: 2021-096). The material of this study consisted of data obtained from 54 dairy farms registered in the TURKVET system in the city center and districts of Kars in the spring of 2021.

The researcher personally visited these farms and assessed welfare using the ANI 35L/2000-cattle method developed by Bartussek et al.,[11]. The farms included in the study were divided into two groups: tie-stall (TS) and closed free-stall (CFS). The animal welfare in these farms was assessed and compared using the ANI 35L/2000-cattle model.

Method

In the study, the researcher visited the farms, collected data and information through face-to-face interviews with the farmers, and filled out the questionnaires. The theoretical data on the physical structures of the barns on the farms was recorded in the forms as a first stage in the questionnaires. The number of lame cattle in the barns, the state of the animals' superficial wounds, and their body condition scores were determined. The second stage involved the assessment of animal welfare in the farms using the ANI score developed by Bartussek et al.,[11] by assigning scores according to 5 categories and criteria.

Statistical Analysis

The data were analyzed using the SPSS 22.0 statistical software (IBM Company, USA). Mann-Whitney U test was run to analyze whether or not the obtained scores differed statistically between the barn types according to the criteria of the animal welfare category. According to the ANI 35L assessment, the chi-square test was run to compare the barn types in terms of the proportion of farms in different welfare categories.

The researcher measured the temperature (T) and relative humidity (RH) inside the barns to identify temperature stress. Using the formula below, the temperature-humidity index (THI) was calculated based on the temperature values. [16,17]

$$THI = 0.8T + [(RH/100) (T-14.3)] + 46.4$$

RESULTS

The animal welfare was scored according to the categories and criteria specified in the ANI 35L model according to the barn type in the study. The results were evaluated separately according to the welfare measurement categories.

It was determined that the difference between the barn types was statistically significant *(P* < .05) in terms of freedom of movement category, number of days in the yard or pasture criterion. No statistically significant difference was found between the barn types in terms of stall area value (*P* > .05). It was determined that CFS barn types had higher welfare scores than TS barn types in terms of the number of days in the year according to the criterion of yard or pasture use (Table 1).

While there was a statistically significant difference in the criteria scores of the social interaction category, space per animal, the management of young, and the number of days spent in the yard or pasture according to the barn types, there was no statistically difference in the score of the social structure criterion of the herd (*P* > .05). All criteria evaluated in the social interaction category according to the barn type had higher mean values in the CFS barn type than in the TS barn type (Table 2).

Table 1. Evaluation of locomotion category criteria according to barn types.

Criteria	Barn types	n	Mean±SEM	Z	*P*
Available floor area (m^2 /AWU)	Tie-stall	48	-[a]	-	-
	Closed Free-stall	6	1.2±0.45		
	Total	54	1.2±0.45		
Lying down-rising	Tie-stall	48	-[a]	-	-
	Closed Free-stall	6	2.3±0.33		
	Total	54	2.3±0.33		
Stall size and boundaries	Tie-stall	48	1.23±0.17	-	-
	Closed Free-stall	6	-[a]		
	Total	54	1.23±0.17		
Movement of tether (m)	Tie-stall	48	0.0±0.00	-	-
	Closed Free-stall	6	-[a]		
	Total	54	0.0±0.00		
Outdoor areas (yards or pasture) (days/year)	Tie-stall	48	1.0±0.01	6.573	.001
	Closed Free-stall	6	2.8±0.20		
	Total	54	1.7±0.03		

n: sample size; SEM: standard error of the mean; Z: Z-score: p: probability; -[a]: The Mann-Whitney test could not be run for empty groups m^2=square meters; AWU: animal weight unit; m: meter

Table 2. Evaluation of social interaction category criteria according to barn types.

Criteria	Barn types	n	Mean±SEM	Z	*P*
Available floor area (m^2/AWU)	Tie-stall	48	1.22±0.17	2.213	.027
	Closed Free-stall	6	2.33±0.49		
	Total	54	1.35±0.17		
Herd structure	Tie-stall	48	0.98±0.02	0.944	.345
	Closed Free-stall	6	1.08±0.30		
	Total	54	0.99±0.03		
Management of young	Tie-stall	48	0.53±0.02	4.964	.001
	Closed Free-stall	6	0.92±0.08		
	Total	54	0.57±0.02		
Outdoor areas (yards or pasture) (days/year)	Tie-stall	48	1.50±0.01	5.821	.001
	Closed Free-stall	6	2.00±0.22		
	Total	54	1.56±0.03		

n: sample size; SEM: standard error of the mean; Z: Z-score: p: probability; m^2=square meters; AWU: animal weight unit

A statistically significant difference was found between the barn types in terms of the softness and cleanness of the bedding space among the criteria of the floor condition category ($P < .05$). No statistically significant difference was found between barn types in terms of the slipperiness of the bedding space and the activity areas (service roads) among the criteria of the floor condition category ($P > .05$). The CFS barns had the highest rank mean values for the criteria of softness and cleanliness of the bedding space (Table 3).

Table 3. Evaluation of floor condition category criteria according to barn types.

Criteria	Barn types	n	Mean±SEM	Z	*P*
Softness	Tie-stall	48	1.00±0.01	4.038	.001
	Closed Free-stall	6	1.17±0.11		
	Total	54	1.02±0.01		
Cleanliness	Tie-stall	48	0.52±0.05	3.630	.001
	Closed Free-stall	6	1.33±0.11		
	Total	54	0.61±0.06		
Slipperiness	Tie-stall	48	1.02±0.04	0.525	.599
	Closed Free-stall	6	1.08±0.08		
	Total	54	1.03±0.03		
Activity areas	Tie-stall	48	1.27±0.07	1.558	.112
	Closed Free-stall	6	1.17±0.11		
	Total	54	1.26±0.06		
Outdoor yards	Tie-stall	48	-[a]	-	-
	Closed Free-stall	6	1.33±0.11		
	Total	54	1.33±0.11		

n: sample size; SEM: standard error of the mean; Z: Z-score: p: probability; -[a]: The Mann-Whitney test could not be run for empty groups

The difference between the barn types in terms of the use of open space criterion for the in-barn climate conditions category was found to be statistically significant ($P < .05$). No statistically significant differences were determined between barn types in terms of other criteria for the in-barn climate conditions category ($P > .05$). Except for the use of open space (day/hour) in terms of the criteria for the in-barn climate category, the other criteria had similar welfare scores according to the barn type (Table 4).

Table 4. Evaluation of light and air category criteria according to barn types.

Criteria	Barn types	n	Mean±SEM	Z	*P*
Daylight in animal house	Tie-stall	48	1.16±0.08	0.504	.614
	Closed Free-stall	6	1.17±0.21		
	Total	54	1.07±0.07		
Air quality and air flow	Tie-stall	48	0.66±0.06	1.588	.112
	Closed Free-stall	6	0.92±0.08		
	Total	54	0.69±0.05		
Draught in lying area	Tie-stall	48	0.76±0.04	0.669	.503
	Closed Free-stall	6	0.83±0.11		
	Total	54	0.77±0.03		
Noise	Tie-stall	48	0.97±0.02	1.213	.225
	Closed Free-stall	6	0.92±0.08		
	Total	54	0.96±0.02		
Outdoor areas (days/year)	Tie-stall	48	1.47±0.02	1.560	.119
	Closed Free-stall	6	1.75±0.28		
	Total	54	1.50±0.03		
Outdoor areas (hours/day)	Tie-stall	48	1.97±0.02	3.332	.001
	Closed Free-stall	6	1.58±0.20		
	Total	54	1.93±0.04		

n: sample size; SEM: standard error of the mean; Z: Z-score: p: probability

While a statistically significant difference was determined between the barn types in the criteria for cleanliness of stables within the maintenance category, the condition of equipment, cleanliness of animals and condition of hooves ($P < .05$), no statistically significant difference was determined in the criteria of technopathies, condition of integument and animal health ($P > .05$). The CFS barn type had a better condition for animal welfare than the TS barn type in terms of the criteria of the maintenance category (Table 5).

Table 5. Evaluation of stockmanship category criteria according to barn types.

Criteria	Barn types	n	Mean±SEM	Z	*P*
Cleanliness of stables	Tie-stall	48	0.46±0.05	3.013	.003
	Closed Free-stall	6	0.92±0.08		
	Total	54	0.51±0.05		
Condition of equipment	Tie-stall	48	0.43±0.06	3.711	.001
	Closed Free-stall	6	1.00±0.01		
	Total	54	0.49±0.05		
Condition of integument	Tie-stall	48	0.88±0.03	1.376	.169
	Closed Free-stall	6	1.00±0.01		
	Total	54	0.89±0.02		
Cleanliness of animal	Tie-stall	48	-0.29±0.04	2.998	.003
	Closed Free-stall	6	0.25±0.17		
	Total	4	-0.23±0.04		
Condition of hooves	Tie-stall	48	0.33±0.04	2.981	.003
	Closed Free-stall	6	0.75±0.11		
	Total	54	0.38±0.04		
Technopathies	Tie-stall	48	1.49±0.01	1.767	.077
	Closed Free-stall	6	1.42±0.08		
	Total	54	1.48±0.01		
Animal health	Tie-stall	48	0.54±0.07	0.458	.647
	Closed Free-stall	6	0.66±0.25		
	Total	54	0.56±0.07		

n: sample size; SEM: standard error of the mean; Z: Z-score: p: probability

No statistically significant differences were found between barn types for temperature values ($P > .05$). According to the barn types, the average humidity, temperature, and THI values were 37.83%, 23.76 °C, and 68.73 in TS barns and 38.13%, 22.20 °C, and 66.98 in CFS barns, respectively (Table 6).

Table 6. Average temperature, humidity and THI values by barn types.

Parameter	Barn types	n	Mean±SEM	Z	*P*
Humidity (%)	Tie-stall	48	37.83±1.48	0.193	.847
	Closed Free-stall	6	38.13±5.51		
	Total	54	37.87±1.43		
Temperature (℃)	Tie-stall	48	23.76±0.42	1.240	.215
	Closed Free-stall	6	22.20±1.13		
	Total	54	23.59±0.39		
THI	Tie-stall	48	68.73±0.45	0.078	.078
	Closed Free-stall	6	66.98±0.66		
	Total	54	68.54±0.41		

THI: temperature humidity index; n: sample size; SEM: Standard error of the mean; Z: Z-score: p: probability; %: percentage: ℃: Centigrade

A statistically significant difference was determined in terms of scores of the ANI 35L welfare assessment according to barn types ($P < .05$). The scores obtained from the ANI 35/L welfare categories showed that 2.1% of the TS barns were suitable at margin for animal welfare, but there were none in the CFS barns. 37.5% of the TS barns were largely suitable for animal welfare, while 33.3% were only partially suitable. The total scores showed that 27.1% of the TS barns and 16.7% of the CFS barns were suitable for animal welfare. 83.3% of the CFS barns were highly suitable for animal welfare, but none of the TS barns were. The total scores showed that 1.9% of the barns were suitable at margin for animal welfare, 29.6% were partially suitable, 25.9% were suitable, and 9.3% were highly suitable (Table 7).

Table 7. Distribution of different barn types according to their scores in the ANI 35L evaluation.

Total ANI scores	Tie-stall		Closed Free-stall		Total		χ2	*P*
	n	%	n	%	n	%		
<11 (Not suitable with respect to welfare)	-	-	-	-	-	-		
11-16 (Scarcely suitable with respect to welfare)	1	2.1	-	-	1	1.9		
16,5-21 (Little suitable with respect to welfare)	16	33.3	-	-	16	29.6	44.598	.001
21,5-24 (Fairly suitable with respect to welfare)	18	37.5	-	-	18	33.3		
24,5-28 (Suitable with respect to welfare)	13	27.1	1	16.7	14	25.9		
>28 (Very suitable with respect to welfare)	-	-	5	83.3	5	9.3		
Total	48	100.0	6	100.0	54	100.0		

ANI: animal needs index; χ^2: chi-square; p: probability

DISCUSSION

Tethering animals in TS barns imposes severe restrictions and negatively affects animal welfare. Rousing et al.,[18] and Bowell et al., [19] stated that barn types and designs affect animal welfare. The number of tie-stall barn types in dairy farms in Kars province and its districts was quite high and they were not suitable for animal welfare.

It was determined that CFS barn types had higher welfare scores than TS barns in terms of the number of days in the year according to the criterion of yard or pasture use. CFS barns were more suitable for animal welfare than TS barns due to allowing animals freedom of movement and having more space per animal (Table 1). The results of this study are supported by the results of the studies by Seo et al.[20] and Armbrecht et al.[21]. According to the ANI 35L method, TS barns showed insufficient scores in terms of suitability for animal welfare compared to CFS barns. The results obtained in previous studies support the fndings obtained in the current study about having higher points if the farm has a closed free-stall system.[22,23]

The farm owners prefer to use long chains for the animals to be more comfortable in terms of chain length. Although this provides comfort for the movement of animals, 48 out of 54 farms were not suitable for animal welfare according to the ANI 35L assessment due to continuous or seasonal tethering of animals.

Barn comfort has been reported to have an effect on the social interaction behaviors of animals.[24,25] In this study, it was concluded that the CFS barns were suitable for animal welfare according to the ANI 35L since they met the social needs of animals in terms of barn type, space per animal, the management of young, and the number of days spent in the yard or pasture according to the space per animal criterion for social interaction category (Table 2). This is considered to be effective due to the differences in the capacity and herd size of the farms where the study was

carried out and the wide usage spaces in the CFS barns.

According to the ANI 35L model, the highest score should be 10.0 for good animal welfare in the social interaction category. A similar study conducted by Akbay[26] reported that the animals could neither sufficiently meet their social needs nor have a suitable structure for animal welfare in the farms (type 1, type 2, and type 3 in tie-stall systems) where social interaction was researched. In their study, Keçici et al.,[27] reported that social interaction scores ranged between 5.71 and 6.30 in the summer months. This study determined that the score in the social interaction category was 4.47. The different farm sizes and raising methods may have contributed to the low value of the study.

Since floor cleanliness also affects the cleanliness of the animal, it is important for animal welfare. Floor cleanliness is also important for hoof and udder health. According to the criteria of softness and cleanliness of the bedding space, the highest ANI score was determined in CFS barns.

As a result of the animal welfare score evaluation in the study, the score of the floor category was 5.25 points (Table 3) in different farms in the study. This value was higher than the value between 2.92 and 3.9 reported in the study by Keçici et al.,[27], the value between 0.42-2.19 reported in the study by Koçak et al.,[17] in fattening cattle farms with different barn systems, and the value between 3.46-4.56 reported in the study by Keskin[28] in different types of dairy farms. The floor category value found in this study meets animal welfare at a medium level.

Total score of animal welfare for different barn types in the category of in-barn climate conditions was found to be 6.23 in the study (Table 4). This value was lower than the values between 6.57-8.89 reported in the studies conducted by Keskin[28] and Sakar et al.,[29] and, lower than the value of 8.00 reported in the studies conducted by Koçak et al.,[17] in loose housing farms and higher than the value of 2.86 reported in the studies conducted in family type tether systems. Differences were found between barn types in terms of the criteria of use of open space in the category of in-barn climate conditions. Except for the use of open space (day/hour) in terms of the criteria for the in-barn climate category, the other criteria had similar welfare scores according to the barn type.

The total score of animal welfare in different barn types in the stockmanship category was determined to be 4.08 (Table 5). This value was found to be lower than the value of 6.46 reported by Stuoge et al.[30] in fattening and dairy farms in organic farms, lower than the value of 5.21–5.83 reported by Keçici et al.,[27], and lower than the value of 4.53-6.56 reported by Keskin[28]. The cleanliness of stables had a mean score of 0.51 points for animal welfare, and the condition of equipment of 0.49 points among different barn types (Table 5). These two values represent a mean value for welfare conditions. The total value for the animal cleanliness variable was determined to be -0.25, and this value shows that the cleanliness of the animals is insufficient according to the scores of the ANI 35L welfare assessment model.

When the barns in this study were evaluated according to their overall health conditions, it was observed that the TS barns had lower ANI points compared to the CFS barns. A similar study by Koçak et al.,[17] reported that the free-stall barns had a higher score in the animal health criterion than the tie-stall ones. The study is similar in terms of results.

Heat stress affects key behaviors, impacting animal welfare and production. Lacetera[31] and Islam et al.,[32] found that heat stress leads to metabolic dysfunctions, oxidative stress, and immune suppression, causing infections and deteriorating welfare and performance. Additionally, THI trends correlate directly with animal behaviors used to assess health and predict production losses.There was a direct correlation between THI trends and the behaviors of animals, which are commonly used to monitor health status and predict production losses.[33] The temperature-humidity index (THI) is the most widely used environmental indicator of heat stress effects in scientific literature.[34,35]

In the study, it was found that the THI value was 68.73 in TS barns and 66.98 in CFS barns (Table 6). These values were lower than the value (74.59) reported by Koçak et al.,[17] in the TS barns and higher than the value of 61.00 in the CFS barns. These values were between the valuse (48,45-71,80) determmined at different temperatures (thermoneutral, hot and cold seasons) reported by Lovarelli et al.,[36]. This value is similar to the value of 67.43 reported by Sakar et al.,[29]. When the THI inside the barn surpassed that outside, the environmental conditions within the barn were inadequate for ensuring animal welfare, indicating the need for structural improvements.[34]

This study showed differences between total welfare score and barn type (Table 7). While 2.1% of the TS barns were suitable at margin for animal welfare, none of the CFS barns were suitable at margin. It was found that 37.5% of the TS barns were largely suitable for animal welfare, while 33.3% were only partially suitable. According to the total scores,

it was found that 27.1% of the TS barns and 16.7% of the CFS barns were suitable for animal welfare. It was found that 83.3% of the CFS barns were highly suitable for animal welfare, but none of the TS barns were. The total scores showed that 1.9% of the barns were suitable at margin for animal welfare, 29.6% were partially suitable, 25.9% were suitable, and 9.3% were highly suitable. In their study, Keskin[28] reported that 77.3% of the welfare levels of the farms were highly suitable, 13.6% were suitable, 5% were quite suitable, 4.5% were suitable at margin, and none of the assessed farms were partially suitable or unsuitable and showed that the lowest ANI score was 12.5 and the highest ANI score was 38.5, which is different from the results of this study. This is attributed to the season and duration of the study.

In conclusion, free-stall barn type was better for animal welfare than the closed tie-stall barn type. As a result of the ANI assessment, animal welfare and yield can be maximized in these types of barns by eliminating the problems identified in the unsuitable closed tie-stall barns and raising the awareness of the workers about animal welfare and health issues. This study suggests that the use of the ANI 35L method can be recommended for the successful application of the ANI 35L method in farms with different barn types and for the assessment of small family farms that produce using traditional methods for animal welfare. Given the importance of animal husbandry for the future of humankind, increasing the number and quality of similar studies in the region and, consequently, identifying the problems in more detail and introducing effective and feasible solutions to these problems would significantly contribute to both the literature and the farms. Both the environmental conditions and the technical equipment within the housing environment are fundamental components of animal production systems. Consequently, their inclusion in research efforts is pivotal for advancing animal welfare. The ANI 35L method can be suggested for assessing the welfare levels of farms that operate using traditional methods and where it is not feasible to examine many animal-based parameters. Breeders and staff in related units should receive training on animal welfare to raise awareness on the subject. It is important to educate consumers, not just focus on the parameters, to ensure compliance with welfare standards. Multidisciplinary studies conducted at regional and national levels should more effectively must be executed the sustainability of animal welfare and its economic connections. National or international projects developed on animal welfare are expected to contribute positively to educational efforts.

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Biochemical Processes During Cheese Ripening

Peynir Olgunlaşmasında Biyokimyasal Olaylar

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ABSTRACT

Cheese ripening entails specific biochemical changes that occur under certain conditions during storage. These changes allow different cheese varieties to develop their unique characteristics. The ripening process is influenced by both primary and secondary biochemical events. These are driven by coagulating enzymes, milk's natural enzymes, and the enzymes of both starter and non-starter microflora. The main biochemical events during cheese ripening include proteolysis, lipolysis, and the metabolism of citrate and lactate. Secondary biochemical reactions then process the primary metabolic products, such as lactic acid, fatty acids, and amino acids. This leads to the formation of volatile compounds like alcohols, aldehydes, ketones, acids, lactones, phenols, esters, and sulfur compounds, which play a crucial role in determining the cheese's quality. These processes give each cheese type its distinctive features, like aroma, taste, color, texture, and pore structure, influencing consumer preferences. This review provides insights into the biochemical events that occur during the cheese ripening period.

Keywords: Cheese biochemistry, enzyme activity in cheese, glycolysis, lipolysis, proteolysis, volatile compounds.

ÖZ

Peynir olgunlaşması, depolama sırasında belirli koşullar altında gerçekleşen spesifik biyokimyasal değişimlerdir. Olgunlaşmadaki değişimler, farklı peynir çeşitlerinin kendine özgü özelliklerini geliştirmesine olanak tanımaktadır. Olgunlaşma süreci, birincil ve ikincil biyokimyasal olaylar tarafından şekillendirilmektedir. Bu olaylar, pıhtılaştırıcı enzimler, sütün doğal enzimleri ile starter ve starter olmayan mikrofloranın enzimleri tarafından yönlendirilmektedir. Peynir olgunlaşması sırasında meydana gelen temel biyokimyasal olaylar arasında proteoliz, lipoliz, sitrat ve laktat metabolizması bulunmaktadır. Birincil metabolik ürünler (örn., laktik asit, yağ asitleri ve amino asitler) ikincil biyokimyasal reaksiyonlarla işlenmekte ve bu süreçte alkol, aldehit, keton, asit, lakton, fenol, ester ve sülfür bileşikleri gibi uçucu bileşikler oluşmaktadır. Bu uçucu bileşikler, peynirin kalitesini belirlemede kritik bir rol oynamaktadır. Bu süreçler, her peynir türüne özgü aroma, tat, renk, doku ve gözenek yapısı gibi ayırt edici özellikler kazandırarak tüketici tercihlerini etkilemektedir. Bu derleme, peynir olgunlaşma dönemi boyunca gerçekleşen biyokimyasal olaylara ilişkin bir bakış sunmaktadır.

Anahtar Kelimeler: Glikoliz, lipoliz, proteoliz, peynir biyokimyası, peynirde enzim aktivitesi, uçucu bileşikler.

INTRODUCTION

Milk is frequently transformed into long-lasting dairy products to extend its shelf life and enhance its flavor, aroma, and texture. Chief among these is cheese—one of the oldest foods crafted by humans.[1,2] Globally, cheese stands out as one of the most widely consumed dairy items, offering a staggering variety exceeding 2000 types. Each type possesses distinctive shapes, textures, and flavors.[3] The nuanced differences between these cheese varieties are primarily attributed to their production methods and the composition of the raw milk utilized.[2,4]

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Cheese is best described as a bio-complex ecosystem, populated by diverse microorganisms sourced from raw milk, starter cultures, and adjunct cultures.[5,6] The cheese-making process involves pressing, shaping, and salting curd, which can subsequently be consumed either fresh or after undergoing ripening.[7] What often drives consumer preference for cheese is its taste, texture, and overall visual appeal. These defining characteristics, which dictate the organoleptic quality of cheese, undergo development through biochemical transformations during the ripening phase.[6]

Cheese production, a multifaceted procedure punctuated by various stages and unique biochemical events, ensures the cheese's biochemical structure remains in flux throughout its ripening. This dynamism is steered by both primary and secondary biochemical reactions, influenced by coagulating enzymes, the inherent enzymes of milk, and the combined action of starter and non-starter microflora and their respective microbial enzymes.[3]

Within the ripening landscape of cheese, we observe primary biochemical events like proteolysis, lipolysis, and the metabolism of citrate and lactate.[1,8] The secondary events primarily focus on the catabolism of lactic acid, fatty acids, and amino acids which emerge from the primary metabolic activities. These events culminate in the creation of a range of volatile compounds—alcohols, aldehydes, ketones, acids, lactones, phenols, esters, and sulfur compounds—to name a few. It's these compounds that play a decisive role in determining cheese quality and shaping consumer inclinations, endowing each cheese variety with its characteristic aroma, taste, color, and texture.[3,4,9,10] In this review, we discuss the biochemical changes that occur during the cheese ripening process.

1. Ripening in Cheese

Cheese ripening is a crucial technological process rooted in microbiological and biochemical principles. It defines the organoleptic quality of cheese.[5] Ripening involves biochemical transformations, such as glycolysis, proteolysis, and lipolysis, occurring under specific storage conditions, which allows cheese varieties to develop their distinct characteristics, including aroma, taste, color, texture, and porosity.[3,11,12] The biochemical events during cheese ripening are illustrated in Figure 1. Owing to various enzymes derived from milk (like plasmin and lipoprotein lipase), coagulating enzymes (like chymosin), and microorganisms (like lactase and proteinase) active during this period, each cheese type establishes its unique properties.[1,5,13,14]

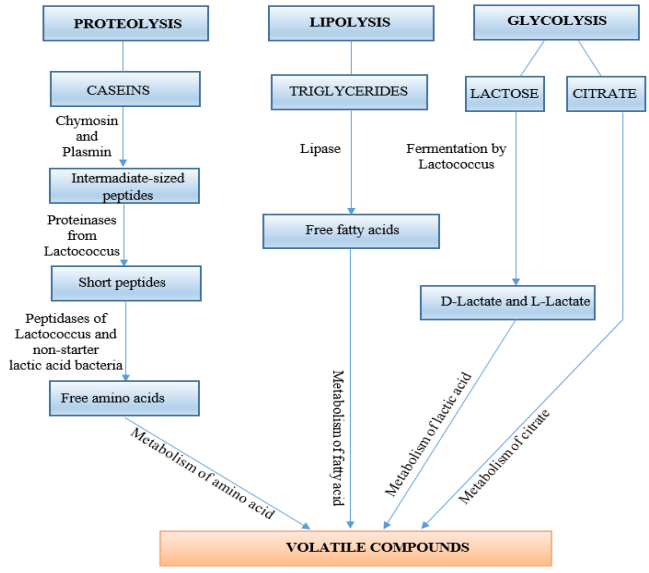

Figure 1. Illustration of primary and secondary biochemical processes during cheese ripening.[10]

The microflora of cheese significantly influences its ripening. The combined metabolic activities of microbial flora on cheese's fat, protein, and carbohydrates refine its organoleptic properties, especially its flavor.[15] This is achieved through a myriad of enzymatic and chemical changes within the cheese matrix. Cheese microorganisms are categorized as starter lactic acid bacteria (primary microflora) and non-starter microorganisms (secondary microflora). In the early cheese production stages, primary microflora, particularly starter lactic acid bacteria, intensifies the acidity by metabolizing lactose.[16-18] This lactic acid production reduces the pH, facilitating clot formation and inhibiting pathogen growth. Yet, during ripening, these bacteria diminish due to autolysis, releasing intracellular enzymes and generating compounds conducive for non-starter lactic acid bacteria (NSLAB) growth.[6,15,18,19]

Frequently utilized starter lactic acid bacteria in cheese-making include species from the genera *Lactococcus*, *Streptococcus*, *Lactobacillus*, *Leuconostoc*, and *Enterococcus*.[20] These bacteria can be either mesophilic (optimal growth at 25-30 °C) or thermophilic (optimal growth at 40-45 °C).[15] Essential starter cultures in cheese biochemistry are detailed in Table 1.[21]

Table 1. Starter cultures utilized in the production of selected cheeses.[21]

Microorganisms	Types of Cheese Used
Mesophilic group	
Lc. lactis subsp. lactis Lc. lactis subsp. cremoris Lc. lactis subsp. diacetylactis Leu. cremoris	Brie, Camembert, Cheddar, Cottage, Cream Cheese, Danish Blue, Edam, Turkish White Cheese, Gouda, Stilton
Propionobacterium spp.	Emmental, Gruyere
Thermophilic group	
Str. thermophilus Lb. delbrueckii subsp. bulgaricus Lb. helveticus, Lb. lactis	Emmental, Gruyere, Parmesan, Romano
Molds	
P. camemberti	Brie, Camembert
P. roqueforti	Danish Blue, Roquefort, Stilton

Lc: Lactococcus, Leu: Leuconostoc, Str: Streptococcus, Lb: Lactobacillus, P: Penicillium

Contrarily, secondary microflora contribute minimally to acid production. These microorganisms significantly influence the emergence of various volatile compounds (like organic acids and aldehydes) during cheese ripening. Comprising NSLAB, propionic acid bacteria, yeasts, and molds, the secondary microflora predominantly originates either from cheese components or environmental exposure during manufacturing.[16,22] NSLAB significantly enhance the flavor, texture, nutritional value, and microbial safety of many ripened cheeses.[23,24] Nonetheless, some quality issues, particularly off-flavors in later ripening stages, can be attributed to these bacteria.[6] While mesophilic lactobacilli dominate NSLAB in cheese, species from *Pediococcus*, *Enterococcus*, and *Leuconostoc* genera are also present. Predominant mesophilic lactobacilli species in cheese reportedly include *Lb. casei*, *Lb. rhamnosus*, *Lb. plantarum*, *Lb. paracasei*, and *Lb. curvatus*.[20,22]

During the ripening process of cheese, biochemical reactions can be broadly categorized into primary and secondary types. Primary reactions involve the metabolism of milk casein into peptides and amino acids, the conversion of lactose to lactic acid, and the breakdown of fats (triglycerides) into fatty acids. Secondary reactions result in the formation of amines, aldehydes, sulfur compounds, and CO_2 from amino acids and organic acids, with fatty acids further metabolized into secondary alcohols.[8,25,26] Proteolysis, lipolysis, and glycolysis are fundamental biochemical reactions during ripening, playing pivotal roles in producing volatile compounds that influence the cheese's quality, especially its flavor development.[3,5]

2. Primary Biochemical Events

2.1. Glycolysis in Cheese

During cheese production, approximately 96% of the milk's lactose remains in the whey.[1] As cheese undergoes fermentation, the residual lactose in the curd is converted to lactate by lactic acid bacteria. This conversion is fundamental to the creation of all cheese varieties.[13,18,27] Initially, starter lactic acid bacteria metabolize the lactose to lactate through glycolysis. This lactate is subsequently transformed into volatile compounds by NSLAB.[5,6] In certain cheese types, like cheddar, lactic acid formation predominantly occurs in the vat before molding the curds. However, for the majority of cheese varieties, this process transpires after the curds have been set in the molds. Typically, the pH of the curd drops to a range of 5.0–5.3 within approximately 12 hours from the onset of cheesemaking.[28]

Glycolysis, driven by the breakdown of lactate and citrate, is completed during the early phases of the ripening period, typically within the first one or two weeks.[26] The metabolism of the residual lactose in the curd from the cheese production process elevates the acidity, influencing the pH of the cheese.[6,18] The lactic acid produced not only inhibits the growth of undesirable microflora, enhancing cheese quality, but also impacts the texture by affecting the demineralization and solubility of caseins. As a result, cheeses with a higher pH tend to be softer than those with pronounced acidity.[1,6,27,28] Pyruvate, a by-product of lactose metabolism, serves as a precursor for the synthesis of short-chain flavor compounds like acetate, acetoin, diacetyl, ethanol, and acetaldehyde.[5] The final stage of lactose glycolysis involves converting pyruvate to lactate, a reaction facilitated by lactate dehydrogenase.[29]

2.2. Lipolysis in Cheese

Lipolysis plays a crucial role in shaping the flavor and texture of cheese. The fat involved in cheese lipolysis directly adds to its flavor through components like fatty acids or indirectly via the transformation of these acids into volatile compounds, such as methyl ketones and esters.[3,9] Although lipids in foods can degrade through hydrolytic or oxidative processes, cheese experiences limited oxidative changes due to its low oxidation/reduction potential (around -250 mV) and the presence of antioxidants.[6,30] In every cheese variety, triglycerides undergo decomposition into free fatty acids during ripening, courtesy of bacterial and native milk enzymes, including lipases.[5,27,31] These free fatty acids act as precursors for catabolic reactions, resulting in the creation of compounds like methyl ketones,

lactones, esters, alkanes, and secondary alcohols.[30,32]

The sources of lipolytic enzymes in cheese are primarily milk, coagulant (rennet), and the microflora of the cheese, both starter and non-starter types.[1,27] Their action on triglycerides leads to the generation of both medium-chain (with a carbon chain length ≤10) and long-chain (carbon chain length >10) free fatty acids.[30,33] Cheeses derived from pasteurized milk typically lack potent lipolytic enzymes compared to their raw milk counterparts. However, lipolysis still occurs during the ripening phase due to the influence of enzymes from both starter and non-starter microflora.[29]

Lactic acid bacteria, used as a starter culture, generally exhibit a mild lipolytic effect. Most fatty acids result from the breakdown of triglycerides by molds.[1,6,9] The extent of lipolysis varies across cheese varieties. In certain cheeses like cheddar, gouda, and Swiss types, even a moderate presence of free fatty acids can introduce a bitterness, which consumers might interpret as spoilage.[5] Conversely, lipolysis is both necessary and desired for the flavor development in hard Italian cheeses, as well as in blue, camembert, and feta cheeses.[27,32] The flavor contributions from free fatty acids in cheese are largely influenced by pH. At elevated pH levels, free fatty acids, perceived as less aromatic, can often come across as "soapy" since they transform into non-volatile salts. In contrast, at lower pH levels, the free fatty acids remain unbound and high concentrations evoke a sour taste.[12,33,34]

2.3. Proteolysis in Cheese

Proteolysis stands out as the most intricate and pivotal primary biochemical event during cheese ripening. The pH, during cheese ripening, plays a pivotal role in shaping its texture and flavor.[1,25,35] Texture development in cheese due to proteolysis arises from the hydrolysis of its protein matrix, increased water-binding capacity by newly formed carboxylic acid and amino groups through peptide bond hydrolysis, and a decrease in water activity (aw). Furthermore, ammonia, a by-product of amino acid catabolism, elevates the cheese's pH and aids texture development.[36,37] Casein metabolization leads to textural transformations in cheese, turning a rubbery hard curd into a creamy, smooth texture.[5]

Various enzymes, like proteinases and peptidases, facilitate proteolysis during cheese ripening. They have multiple origins: coagulants (e.g., chymosin/rennin), milk (e.g., plasmin, cathepsin D), starter lactic acid bacteria, NSLAB, and secondary starters. For instance, *P. roqueforti* is found in blue cheese, while *P. camemberti* is present in camembert cheese. In some instances, to hasten ripening, exogenous proteinases or intracellular peptidases may be added to milk or curd.[1,8,36] Two primary sources of proteolytic enzymes in cheese are chymosin (rennin), a residual coagulant left in curd post-whey filtration, and plasmin, which transfers from blood to milk.[5,31]

Proteolysis in cheese is twofold:

i. In primary proteolysis, chymosin and plasmin hydrolyze caseins, yielding medium-sized peptides.[35]

ii. Secondary proteolysis results in further breakdown into smaller peptides and amino acids due to hydrolysis by proteinases and peptidases released from the breakdown of starter lactic acid bacteria and NSLAB.[8,25,37,38]

The peptides and amino acids derived from the cheese matrix via proteolysis serve as critical substrates for various catabolic reactions, producing significant flavor and aroma compounds, such as amines, aldehydes, alcohols, acids, phenols, and sulfur compounds.[3,39] Since each cheese variety has distinct production and ripening conditions, their proteolysis methods can differ. Factors influencing proteolysis in cheese include ripening temperature and duration, pH, moisture content, residual coagulant activity, the transformation of plasminogen to plasmin, and the growth of secondary microflora.[3,29]

3. Secondary Biochemical Events

3.1. Lactate and Citrate Catabolism

Lactose is initially converted into glucose and galactose by the lactase enzyme (β-galactosidase) produced by lactic acid bacteria (LAB). The homofermentative LAB species, such as *Lb. acidophilus*, *Lb. bulgaricus*, and *Lb. helveticus*, possess aldolase and hexoisomerase enzymes but lack phosphoketolase. These homofermentative LAB utilize the Embden-Meyerhof Parnas (EMP) fructose-1,6 diphosphate metabolic pathway, also known as the glycolytic pathway. Here, glucose is first transformed into pyruvic acid and then predominantly (about 90%) into lactic acid.[6,18,21,40,41]

On the other hand, heterofermentative LAB like *Leuconostoc* spp., *Lb. casei* group, and *Lb. plantarum* are equipped with the enzyme phosphoketolase. These LAB species produce not only lactic acid (around 50%) but also

acetic acid, ethyl alcohol, and CO_2 from glucose, using the phosphoketolase (phospholytic) pathway.[6,21,40,41]

Lactate metabolism plays a vital role in shaping the organoleptic properties of aged cheeses, such as camembert and brie.[21,29] Figure 2 provides a schematic illustration of the biochemical pathways of lactose and citrate catabolism, which lead to the generation of flavor compounds by LAB in cheese.

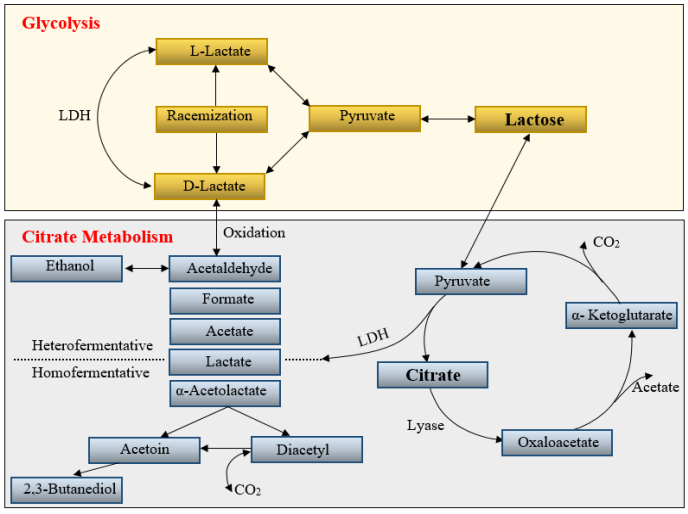

Figure 2. Diagram illustrating the catabolism of lactose and citrate by lactic acid bacteria in cheese.[15,41]

Lactate and citrate serve as significant substrates for various reactions that take place during cheese ripening and the formation of flavor. The metabolic pathways of lactate in cheese include:[1,5,28]

i. In most cheeses, NSLAB racemizes L-lactate to D-lactate (see Fig. 2). Additionally, *Lactobacillus* spp. can produce D-lactate from any residual lactose. While racemization does not influence flavor, calcium-D-lactate, being less soluble than L-lactate, results in calcium lactate pentahydrate crystals that appear as white spots on cheese surfaces.

ii. In Swiss-type cheeses, *Propionibacterium freudenreichii* catabolizes lactate, producing by-products like H_2O, CO_2, propionate, and acetate, leading to the formation of characteristic pores.

iii. *P. camemberti* breaks down lactate into CO_2 and H_2O, crucial for tissue development in surface-ripened cheeses such as camembert and brie.

iv. Some NSLAB (notably *Pediococcus* spp.) can oxidize lactate to formate, ethanol, and acetate in the presence of O_2. This lactate oxidation depends on NSLAB population and the available O_2, determined by the packaging material's oxygen permeability.

v. Anaerobic lactate metabolism by *Clostridium tyrobutyricum*, leading to the production of butyrate, CO_2, and H_2, can result in the “late swelling” defect in cheese-making.

Around 90% of milk's citrate content is soluble and is largely lost with the whey. Metabolizing the minor amounts of citrate remaining post cheese production often results in the formation of organoleptic properties. This process enhances aroma compounds and the creation of cheese pores.[42] Initially, citrate lyase hydrolyzes citrate into oxaloacetate and acetate (refer to Figure 2). Subsequently, oxaloacetate decarboxylates to pyruvate, giving rise to compounds like diacetyl, acetoin, and 2,3-butanediol.[41]

While *Lc. lactis* ssp. *lactis* and *Lc. lactis* ssp. *cremoris* do not metabolize citrate, *Lc. lactis* ssp. biovar *diacetylactis* and *Leuconostoc* spp. do so, producing favorable flavor compounds like diacetyl, acetoin, and 2,3-butanediol.[27,34,43] Some facultative heterofermentative *Lactobacillus* species, such as *Lb. casei* and *Lb. plantarum*, synthesize citrate into acetate, diacetyl, and acetoin. *Lactococcus* spp. and *Leuconostoc* spp. exhibit similar catabolism.[15,28] Moreover, both *Enterococcus* spp. and *Weissella* spp. can metabolize citrate. The acetoin/diacetyl catabolism pathway of citrate, depicted in Fig. 2, is essential to produce the volatile compounds granting cheese its distinctive buttery taste.[42] The CO_2 emerging from the metabolism of lactate and citrate forms the signature pores in cheeses like emmental and cottage Dutch types. However, in solid cheeses, this feature is undesirable.[5,34]

3.2. Catabolism of Free Fatty Acids

Free Fatty Acids (FFA), particularly acetic, octanoic, and decanoic acids, directly infuse flavor into cheese. Yet, the volatile by-products of their catabolism, such as methyl ketones, secondary alcohols, straight-chain aldehydes, lactones, and esters, have a profound impact on cheese flavor.[3,27] Figure 3 schematically illustrates the biochemical pathways of fatty acid catabolism leading to flavor compound formation in cheese.

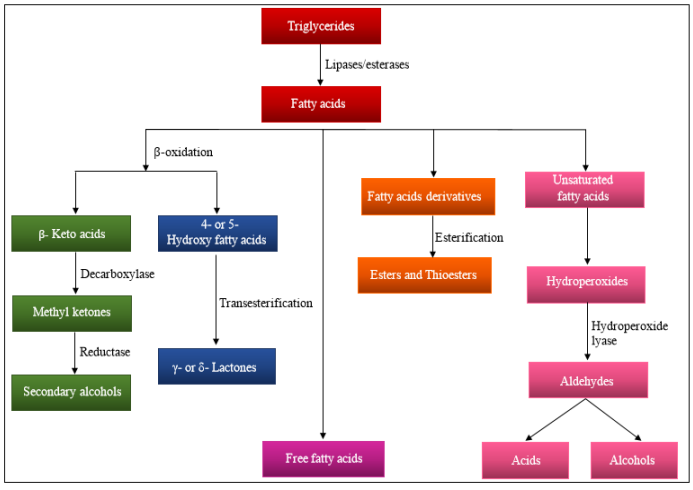

Figure 3. Biochemical pathways of fatty acid catabolism in cheese: A schematic representation.[6,34]

Esters in many cheese varieties result from the reaction between a fatty acid and an alcohol. In cheese, the most prevalent esters are ethyl esters of straight-chain free fatty acids (C2:0–C10:0), with ethanol—derived from lactose or amino acid catabolism—being the most common alcohol forming methyl, propyl, and butyl esters.[30] Lactones, cyclic compounds, originate from hydroxyacids via intramolecular esterification. Cheese contains both γ- and δ-lactones, with their production during ripening constrained by the availability of hydroxyacids, their precursors. Moreover, free fatty acids can be transformed into methyl ketones, crucial to the flavor of blue-veined cheeses, particularly those surface-ripened by molds like *Penicillium* spp., through β-oxidation.[27,29,33,34]

Typically, secondary alcohols and even-numbered methyl ketones arise from the autoxidation of unsaturated fatty acids. Singular chain methyl ketones and secondary alcohols form from the β-oxidation of free fatty acids (as depicted in Figure 3). In cheeses, enzymes such as lipoxygenase and hydroperoxide lyase from *P. camemberti* can also generate secondary alcohols by reducing methyl ketones.[30] Although numerous aldehydes emerge from amino acid catabolism, straight-chain aldehydes, like butanal and heptanal, might arise from the oxidation of unsaturated fatty acids. However, the oxidation extent in cheese remains minimal due to its low redox potential and inherent antioxidants.[34]

3.3. Catabolism of Free Amino Acids

Free amino acid catabolism significantly affects the development of cheese's flavor and textural attributes.[27] These amino acids undergo various reactions—such as deamination, amination, and decarboxylation—resulting in volatile and non-volatile compounds.[3,44] Primary products of this catabolism include aldehydes, alcohols, carboxylic acids, amines, and sulfur compounds.[6,45] A schematic showcasing the biochemical pathways of amino acid catabolism that leads to flavor compounds in cheese can be seen in Figure 4.

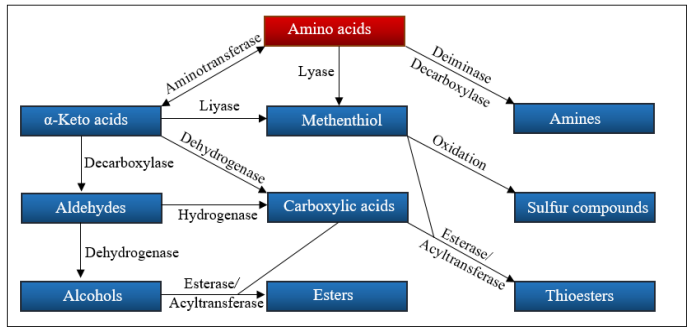

Figure 4. Schematic representation of the biochemical pathways of amino acid catabolism in cheese.[34,44]

Sulfur-containing aromatic compounds, such as dimethyl sulfide, primarily arise from methionine degradation, contributing to the characteristic "garlic" flavor found in cheeses like cheddar and camembert.[3,4,15,46,47] Another pivotal pathway in free amino acid catabolism involves the transamination reaction, central to the breakdown of all amino acids by lactic acid bacteria.[27,46] This reaction transforms aromatic amino acids into α-ketoacids.[5,6,33,34] Aminotransferases, intracellular enzymes present in starter cultures, facilitate this reaction, needing pyridoxal-5-phosphate for their function.[1,44] Resulting α-ketoacids, acting as intermediaries, are reduced by the cheese microflora into various compounds in the ongoing biochemical process.[5,44,45]

There is a symbiotic relationship between starter lactic acid bacteria and NSLAB in the aroma formation of certain cheeses, such as cheddar. For instance, *Lactobacillus* spp. initiates the conversion of amino acids to keto- and hydroxyl acids, whereas *Lactococcus* spp. transforms these by-products into carboxylic acids.[27]

Amino acids undergo deamination and decarboxylation, leading to the production of α-keto acids, ammonia, and amines.[3] These are further transformed into volatile compounds such as alcohols, esters, acids, and aldehydes. Decarboxylation involves the transition of an amino acid to an amine (primarily tyramine) accompanied by a CO_2 loss.[1] Branched-chain amino acids, including leucine, isoleucine, and valine, can be decarboxylated to produce amines with off-putting flavors, for instance, ketoisocaproate, α-keto isovalerate, and α-keto-L-methyl valerate.[5] The deamination reaction produces NH_3, a vital component in certain cheeses like camembert and gruyere. Additionally, ammonia can arise from the oxidative deamination of

aldehyde-producing amines.[1] Ammonium compounds, pivotal for the flavor in mold-ripened cheeses such as camembert and brie, are linked to the pronounced proteolytic activities of the *Penicillium* genus.[6]

CONCLUSION

As cheeses ripening, they develop distinct flavor (taste and aroma), appearance, and texture characteristics due to enzyme-driven biochemical reactions from diverse sources, like milk, coagulants, and microflora. These biochemical transformations can be categorized into primary events (like glycolysis, proteolysis, and lipolysis) and secondary ones (such as the catabolism of lactate, citrate, fatty acids, and amino acids). It is evident that volatile compounds, stemming from secondary biochemical processes, are crucial in establishing the unique organoleptic properties of cheeses. A deeper grasp of cheese biochemistry can greatly influence production and storage conditions, enhancing cheese quality standards. Moreover, fully understanding the mechanisms behind the formation of volatile compounds and aroma identification can offer the cheese industry substantial benefits, paving the way for standardized quality cheese production and innovative manufacturing processes.

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Case Report *Olgu Sunumu* DOI: 10.17094/vetsci.1465264

Contracaecum rudolphii Hartwich, 1964 (Nematoda: Anisakidae) in a white pelican (*Pelecanus onocrotalus*) in Türkiye

Türkiye'de bir beyaz pelikanda (*Pelecanus onocrotalus*) *Contracaecum rudolphii* Hartwich, 1964 (Nematoda: Anisakidae)

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ABSTRACT

Two injured and exhausted white pelicans were brought to Afyon Kocatepe University Animal Hospital Surgery Clinic by Nature Conservation and National Parks officials. An open wound and a fracture of the right-wing radius-ulna bones were detected due to gunshot wound in one of the pelicans. The fracture was operated with intramedullary pin fixation. In the post-mortem examination of the pelican that died one day after the operation, 249 nematodes were found in the proventriculus. On parasitological examination, the nematodes were identified as *Contracaecum rudolphii* and in pelicans for the first time in Türkiye. In the coprological examination of the second pelican, *Contracaecum* sp. eggs were detected. Complete recovery was achieved with anthelmintic and supportive treatment.

Keywords: *Contracaecum rudolphii,* pelican, Türkiye.

ÖZ

Doğa koruma ve Milli Parklar yetkilileri tarafından Afyon Kocatepe Üniversitesi Hayvan Hastanesi Cerrahi Kliniği'ne yaralı ve bitkin iki beyaz pelikan getirildi. Pelikanlardan birinde ateşli silah yaralanmasına bağlı açık yara ve sağ kanat radius-ulna kemiklerinde kırık tespit edildi. Operasyona alınan pelikan intramedüller pin fiksasyonu ile tedavi edildi. Operasyondan bir gün sonra ölen pelikanın post-mortem incelemesinde proventrikulusta 249 adet nematoda rastlandı. Parazitolojik muayenede nematodların *Contracaecum rudolphii* olduğu ve Türkiye'de ilk kez pelikanlarda görüldüğü belirlendi. İkinci pelikanın koprolojik muayenesinde ise *Contracaecum* sp. yumurtaları tespit edildi. Antelmentik ve destekleyici tedavi ile tam iyileşme sağlandı.

Anahtar Kelimeler: *Contracaecum rudolphii,* pelican, Türkiye.

INTRODUCTION

Anisakid nematodes of the genus Contracaecum (Railliet and Henry, 1912) are parasites of piscivorous birds, penguins, marine mammals and some species are zoonotic. It is seen mostly in pelicans, cormorants, penguins and seals in wetlands, freshwater and marine ecosystems around the world.[1,2] Contracaecum is found usually in the proventriculus of piscivorous and pinnipeds, the definitive hosts and has a heteroxeneous life cycle. The worm eggs are released into the water with the definitive host's feces, and the developing larvae are ingested by copepods, the intermediate host. Fish that eat copepods carrying *Contracaecum* larvae serve as paratenic hosts. The larvae remain in capsules in the intestinal wall, mesentery, liver and internal organs without developing in the paratenic host. When infected fish are consumed by piscivorous birds and marine mammals, 4th instar larvae develop and subsequently reach

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the mature stage.[3,4] It has been reported that the parasite can cause pathological disorders in fish, birds and mammals and economic losses in commercial fisheries. In piscivorous birds, these parasites cause bleeding, serious ulcerative eosinophilic granulomas in proventricular mucosa, weight loss and even death. People who consume raw or undercooked infected fish experience symptoms of vomiting, diarrhoea and abdominal pain.[3,5]

CASE PRESENTATIONS

Two male young white pelicans (*Pelecanus onocrotalus*) were brought to Afyon Kocatepe University Animal Hospital Surgery Clinic by Natur Conservation and National Parks officials (Figure1a). Initial examination of the pelicans, revealed that their body weight was 4.7 and 5.6 kg, respectively, they were hypothermic (35.6°C and 36.5°C), and lethargic with weak vital signs. During the radiographic and orthopedic clinical examination, in one pelican an open wound, a gunshot-related fracture in the right-wing radius-ulna bones, and a radio-opaque foreign body thought to be pellets were detected (Figure1b).

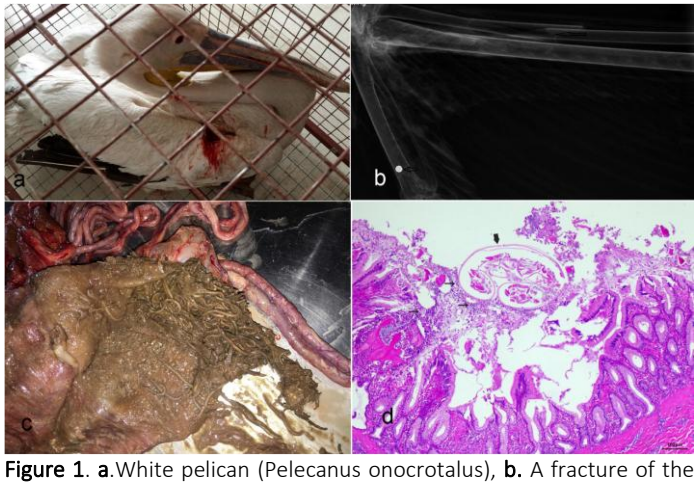

Figure 1. **a**.White pelican (Pelecanus onocrotalus), **b.** A fracture of the right-wing radius-ulna bones and a pellet (arrows), **c.** Nematodes (C. rudolphii) in proventriculus, **d.** Histopathological findings; heterophile leukocyte infiltration (thin arrows) and mouthparts of C. rudolphii (Thick arrow) (HE staining).

Both pelicans were kept under observation for 3 days, with intravenous fluid replacement, as well as antiparasitic and antibacterial treatments (levamisole at 20mg/ml orally and enrofloxacin at 15 mg/ml intramuscularly). The bone fracture was repaired with 3.0 and 4.0 Steinmann intramedullary pin fixation, the wing was bandaged, and fluid replacement was continued in the postoperative period.

However, the bird died for an unknown reason the day following the operation. Post-mortem examination revealed that the nutritional status was poor, the mucous membranes were pale and dull. Moreover, a severe decrease in body fat, especially the fat layers around the heart was noted. The chest muscles and extremity muscles were atrophic. On cut section of proventriculus, 249 nematodes were found (Figure 1c). The proventriculus mucosa was hyperemic with small hemorrhages. Representative tissue samples were taken from all organs, fixed in buffered neutral formaldehyde solution, and processed routinely for histopathological examination. Microscopically section of proventriculus showed disruption in the integrity of mucosa along with heterophile leukocyte infiltration. Cross section of parasites along with cellular infiltration was also observed in the proventricular mucosa (Figure 1d).

For the specific identification of adult nematodes collected from the proventriculus, worms were examined under a stereo-microscope. The worms were then dehydrated, coated with carbon, examined under a scanning electron microscope and photographed (Figure 2a,b,c). The examined nematodes were identified as *Contracaecum rudolphii* Hartwich, 1964 according to the morphology of the labia/interlabia, the distribution of the distal papillae and the morphology of the tip of the spicules and the diagnostic key.[6-9]

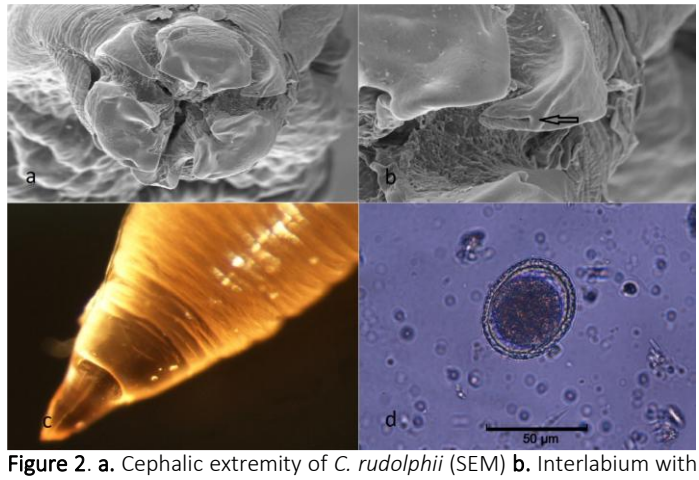

Figure 2. **a.** Cephalic extremity of *C. rudolphii* (SEM) **b.** Interlabium with bifid tip (Arrow) (SEM) **c.** Female tail (Stereo-Microscopy) **d.** *Contracaecum* sp. egg (55X45 µm).

No signs of trauma were observed in the clinical and radiographic examination of the second pelican. However, on faecal examination, large number *Contracaecum* sp. eggs were identified (Figure 2d). The pelican regained full health after antiparasitic and antibiotic treatment and rehabilitation.

DISCUSSION

Although *Contracaecum* sp. larvae were reported in previous studies[10,11] from fish in Türkiye, *C. rudolphii* larvae were detected in only one study.[12] Similarly *Contracaecum* spp. were detected in the digestive tract of a Dalmatian pelican brought to Bursa Uludağ University Animal Hospital, but species identification could not be made.[13]

In this study, *C. rudolphii* was detected in the proventriculus of a white pelican for the first time from Türkiye. Similar to the present obsevation the previous histo-pathological studies on proventriculus of infected piscivorus birds revealed attachment sites of adult nematodes, deep mucosal ulcers covering areas of necrosis, and inflammations of varying severity and depth. Histopathological examinations have reported severe granulomatous inflammations that expand and destroy the lamina propria and gastric glands. Additionally, small ulcerated areas and eosinophilic granulomas have been reported. Section of the parasites were surrounded by desquamations and mononuclear inflammatory cell infiltrations were detected in some areas of the proventriculus surface epithelium and submucosal glandular epithelium.[5,9,13-15]

In conclusion, the anisakid nematode *C. rudolphii* was detected from a white pelican for the first time in Türkiye, that caused severe pathological disorders and even death when in large number.

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