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Contents

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In-vivo evaluation of an innovative feed additive formulation of *Pinus Brutia ten*. resin containing turpentine and colophony and the effects on milk production performance and somatic cell counts of Holstein dairy cows

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ABSTRACT

The aim of this study was to investigate a feed additive which is expected to be preventative, consist of new active ingredients for mastitis disease which is frequently seen in dairy cattle. For these purposes colophony which has antibacterial and turpentine which has antibacterial, antifungal effects have a potential that is researched in vitro previously. These herbal ingredients were suggested to use for prevention of mastitis in dairy cattle. Powdered herbal material have colophony and turpentine %96.5± 0.3 and % 3.5± 0.3, respectively. Animal experiment was studied with two group (experimental, control) which had 15 dairy cattle each and at the end of the study milk parameters, somatic cell count (SCC), average milk productions discussed. Although it was not statistically significant the experimental group had higher milk amount, milk fat and lower SCC than control group within two weeks.

Article History

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Introduction

Mastitis is the most common disease in dairy cattle all over the world, which significantly reduces the profitability of dairy farms (Bradley, 2002; Ruegg, 2017). Mastitis occurs when pathogenic microorganisms enter the nipple and cause inflammation in the breast tissue. It is quite easy to diagnose of acute clinical mastitis. Mastitis can be if the structure of the milk has changed and here is an abnormal appearance (watery appearance, flakes, or clots) in the milk (Adkins and Middleton, 2018). Although clinical mastitis is easy to diagnose in this way, subclinical mastitis is a condition that should be followed in the herd, where there is no visible change in the breast structure (redness, burning, pain and abnormal milk) when viewed from the outside, but somatic cell count (SCC) is higher than SCCs in milk from healthy animals' subclinical mastitis also decreases milk yield (Sharma et al., 2011).

Treating this condition creates a high cost per administration when conventional drug forms are used. In addition, excreted of antibiotics with milk prevents the use of milk during the treatment. Since antibiotics are the most preferred method in the treatment of mastitis, it is great importance for human health to prevent mastitis in order to prevent the development of resistance. Subclinical mastitis is a

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condition that there were no acute signs of clinical mastitis but SCC of milk sampled from breast are higher than a sampled milk from healthy breast. Decrease of milk production is most important economic effect of subclinical mastitis. As a result, milk industry always keeps searching for preventative solutions for mastitis. And various methods are used to detect subclinical and clinical mastitis. SCC is the most widely used mastitis detection method, and leukocyte concentration in one milliliter of milk (Moreira et al., 2019). It has been stated that SCC over 50.000 (Seegers et al., 2003), 100.000 (Koç, 2018), depending on the sources, is the diagnostic border between the infected breast and the healthy breast. Studies have shown that increased SCC cause decreases in the range of 1.4-2.7 liters of daily milk production (Dohoo and Meek, 1982).

Considering the above-mentioned mastitis is an important cattle disease and the needs to reduce its incidence, a mixture of colophony and turpentine was selected as a candidate to prepare an oral dosage formulation in order to prevent mastitis. The biggest reason why these active ingredients are preferred is that the first patent application in this field in the world was made in Turkey and remarkable results were obtained from field trials in cattle and many different animals subject to the patent application, apart from the properties examined in cattle.

In today's studies, in addition to antibacterial, antifungal (Savluchinske et al., 1999), antiviral (Savluchinske et al., 1999), anticancer (Tanaka et al., 2008), antiparasitic (Mercier et al., 2009) properties of colophony and turpentine separately or together, it can also prevent pathogens in the intestinal flora with oral use. It is understood that it supports feed efficiency and makes significant contributions to the veterinary field (Kettunen et al., 2015). In addition, it has been observed that abietic acid in colophony and turpentine containing a-pinene have a bactericidal effect against methane-producing bacteria that cause energy loss in cattle (Sierra-Alvarez and Lettinga, 1990). Methanogens uses feed in stomach of cattle to produce methane.

The aim of this study is to observe the effects of the formulation containing colophony and turpentine on milk production, milk protein and fat contents and somatic cell counts.

Materials

Pinus Brutia Ten. Resin and Bentonite gifted from Uğur Yem Katkı Maddeleri Ltd, Türkiye, Calcium Carbonate gifted from Ortaş Ltd, Türkiye, HPMC 10.000 Da, Gum Colophony purchased from Sigma Aldrich, Germany, and carboxy methyl cellulose (CMC 10.000) Da purchased from Zibo Hailan Chemical Co., Ltd, China.
Sonkaya bag closer device (smpy 402 40 CM) purchased from Sonkaya Machinery and Automation Technologies, Turkey.

Methods

The resin used in this study was obtained from the *Pinus Brutia Ten*. plant Gördes, Manisa, Turkey. Its components were used to make an oral reconstituted suspension formulation for dairy cows.
All prepared reconstituted suspension formulations were shown at Table 1.

While preparing the formulations, firstly the powders were weighed and mixed in the mixer according to the geometric dilution method. For easier wetting, CMC cp 10.000 with better water dispersion was preferred as wetting agent. The wetting agent was added to the mixer after mixing the powders. It was mixed until a homogeneous view was obtained, then the mixture transferred to a bottle and mixed with increments of dispersion medium by shaking.

Table 1. Prepared reconstituted suspension formulations

	Formulation code										
Contents (g)	**F7**	**F8**	**F9**	**F10**	**F11**	**F12**	**F13**	**F14**	**F15**	**F16**	**F17**
Colophony/Turpentine Mix.	15	15	15	15	**15**	15	15	15	15	15	15
CMC 10.000 cp				0.2	**0.35**	0.5	0.35	0.35	0.35	0.35	0.35
$CaCO_3$	4.5	6	7.5	6	**6**	6			6	6	6
Starch 1500							6				
Avicel Ph 102								6			
Sodium citrate									0.1	0.3	0.5
Methyl cellulose 4000 cp	0.35	0.35	0.35								
Glycerine											
Distilled water (mL)	50	50	50	50	50	50	50	50	50	50	50

CMC = carboxy methyl cellulose, cp = centipoise, Mix = mixture

The final formulation that is used in experiment was chosen with suspension properties F (sedimentation volume), degree of flocculation (b), redispersion time (s), number of turns (times).

The sedimentation volume (Equation 1), F, is the ratio of the final, or ultimate, volume of the sediment, Vu, to the original volume of the suspension, V0, before the settling.

$$F = \frac{V_u}{V_o} \qquad \text{Equation 1}$$

Degree of flocculation (Equation 2), b, is a better parameter for comparing flocculated systems is the degree of flocculation, b, which relates the sedimentation volume of the flocculated suspension, F, to the sedimentation volume of the suspension when deflocculated, F_∞.

$$\beta = \frac{F}{F_\infty} \qquad \text{Equation 2}$$

After the suspensions precipitated, waited until all particles were below the suspension, redispersion time and number of turns were measured. It was resuspended by turning it 180 degres in a 100 mL graduated cylinder to disperse it again. The number of spins and the elapsed time were recorded. 180 degree rotation was done once in 2 seconds. The second measurement was made until the time it looked like there would be no residue at the bottom. 30 cows used in this study were selected among 120 cows and 15 of cows were allocated as control and 15 of cows as experimental group. Animal characteristics that are used to choose these groups are listed in Table 2 and they had statistically same SCC at beginning of study (p>0,01).

Table 2. Animal characteristics of the groups

Animal breed	Holstein
Age (Month)	42-116
SCC	>50.000
Live weight	550-650 kg

SCC = somatic cell count

A reconstitute suspension formulation was chosen to apply the cattles orally. After adding 100 ml of water on the powder form of the combination and shaked well, then the formulation ready for applying orally. Density, Housner ratio, compressibility properties were performed on this powder combination (Jan, 2009).

In order to conduct the in-vivo experiment, the ethics committee approval dated 13/01/2017-17793 was obtained from the Istanbul University Rectorate Animal Experiments Local Ethics Committee.

The experimental period was designed as 30 days. Care was taken to ensure that both of the groups of animals included in the experiment were above 50.000 somatic cells/ml. The SCC of the 25 animals included in the experiment was above 100.000 cells/ml.

To avoid the effects of environmental differences, the animals were fed under the same conditions and on the same ration.

The dose is 2.1 mg/kg for 500 kg live weight dairy cattle used in the experiment. During trial, each milking cow in experimental group take orally 30 g Pinus Brutia Ten. resin which has colophony and turpentine 96.5% ± 0.3, 3.5% ± 0.3 respectively. Resin formulated as an oral reconstitute suspension formulation. Control group and experimental group had same feed ration and ad libitum water.

Somatic cell count fat, protein content in the milk samples taken on the same day of each week and average of weekly milk amount (L) of the experimental group and the control group were compared with the non-parametric Mann Whitney U test in SPSS (IBM Statistics Ver 23).

During the study, 40 ml milk samples were taken from each dairy cows once a week on the same day. During the transportation of milk samples, chemical tablets that stop microbial growth without affecting the somatic cell count (SCC) and milk components were used to prevent the deterioration of the structure of the milk by using “(Microtabs II)” and the milk samples were taken to the “Istanbul University Faculty of Veterinary Medicine Department of Animal Science” Laboratory within the same day in the cold chain. After the milk samples were heated in a water bath at 40°C and somatic cell count, fat, protein, lactose and dry matter values were determined. In the analysis, the "Combi 150" (Bentley), which was created with the integration of the cell counter (Somacount 150) and milk components measuring device (Bentley 150) and works with the "Flow cytometry analysis method" in the "Laboratory of the Department of Animal Science", together. The current device and the analysis methods it used were approved by “ICAR: The Global Standard For Livestock Data”.

Stability tests of final formulation were performed according to the accelerated 6-month test criteria in the International Council for Harmonisation (ICH) Q1a (R2) guideline.

Results

Results of in vitro studies: Properties of powder are listed in Table 3.

Table 3. Powder properties of the formulation (Mean ± SD)

Density ($r_{cluster}$)	0.444 g/ml ± 0.022
Density ($r_{compressed}$)	0.628 g/ml ± 0.043
Housner ratio	1.414 ± 0.028
Compressibility (%)	32.669 ± 3.449

All formulations properties of prepared reconstituted suspensions were shown in Table 4.

Table 4. Data of suspension controls of prepared reconstituted suspension formulations (* (Mean ± SD) The values of the formulations that do not use flocculating agents have not been calculated)

Formulation code	Flocculation volume (F)	Degree of Flocculation (*b*)	Redispertion time (sec)	Number of turns (time)
F7	0.589 ± 0.023	-*	67 ± 2.65	35 ± 1
F8	0.641 ± 0.003	-*	66 ± 3.61	32.6 + 3.06
F9	0.624 ±0.018	-*	77.6 ± 2.52	43.3 ± 1.53
F10	0.548 ± 0.014	-*	57.3 ± 2.52	30.3 ± 1.53
F11	0.853 ± 0.025	-*	48.3 ± 1.53	24.3 ± 2.31
F12	0.989 ± 0.009	-*	139 ± 3.61	70 ± 1
F15	0.974 ± 0.009	1.144 ± 0.036	59.3±0.58	32.3 ± 1.16
F16	0.979 ± 0.009	1.149 ± 0.036	60 ± 4.36	29.6 ± 2.52
F17	0.995 ± 0.009	1.168 ± 0.031	78 ± 2.65	43.3 ± 1.53

Viscosities of the formulations were examined by Brookfield (USA) rotating shaft viscometer. Viscosities of F10, F11, F12 formulations, which is advantageous for application and storage compared to other formulations in terms of redispersion time and settling volume (Table 5). The viscosity increases as the rpm increases, depending on the ratios of the suspension agent in these formulas.

Table 5. Viscosity for F10, F11, F12 formulations (n=3)

Spindle 2/ 30 rpm	F10 Mean ± SD	F11 Mean ± SD	F12 Mean ± SD
1' Cp	117.3 ± 9.7	624.7 ± 4.7	1701.3 ± 66.9
1' Torque	8.9 ± 0.7	46.7 ± 0.1	51 ± 2
5' Cp	84.3 ± 0.6	592 ± 12.5	1641.3 ± 43.3
5' Torque	6.3 ± 0.1	442 ± 0.6	49.2 ± 1.3

F11 formula was preferred as the formulation to be used in oral administration of active substances in animal experiments, due to its redispersion time, settling volume, a high active substance/filler ratio, and also closest F value to "1" (Table 4 and Table 5).

Results of some specifies of F11 Formula was shown in Table 6.

Table 6. Results of some specifies of F11 formula (n=3, Mean ± SD)

Density ($r_{cluster}$)	0.664 g/ml ± 0.004
Density ($r_{compressed}$)	0.843 g/ml ± 0.007
Housner Ratio	1.269 ± 0.002
Compressibility (%)	21.232 ± 0.158
Stack Angle (a)	37.251 ± 3.783
Weight Deviation (g) (packaged)	43.3783 ± 0.1149
Content Uniformity (g) (Amount of insoluble in organic solvent)	12.891 ± 0.079
Particle Size (d values of the semi-logarithmic results) (µm)	d_{10}= 45.236 d_{50}= 136.346 d_{90}=410.96

Results of animal experiments : Number of SCC in the milk, Logritmic SCC, milk fat percentage, milk protein percentage and average milk volume results are depicted in Figures 1-5.

The first week shows the results before the start of the trial and is marked as + in all Figures. Values from 2nd to 8th week of the trial shows the trial results. The results from 8th, 9th and 10th weeks show the results after the trial completed and pointed with "*" on figures.

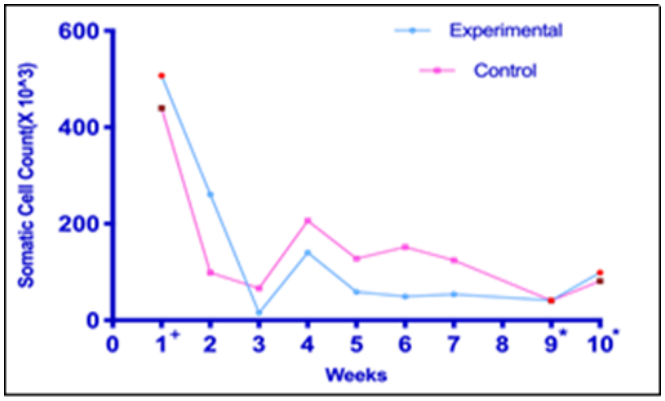

Figure 1. Weekly change in the number of SCC in the experimental and control groups.

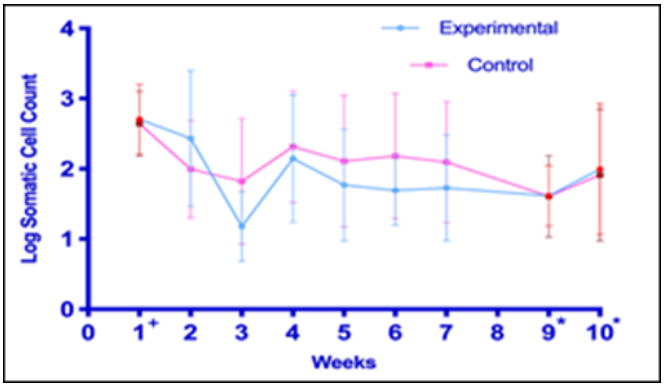

Figure 2. Weekly change in logarithmic SCC in the experimental and control groups.

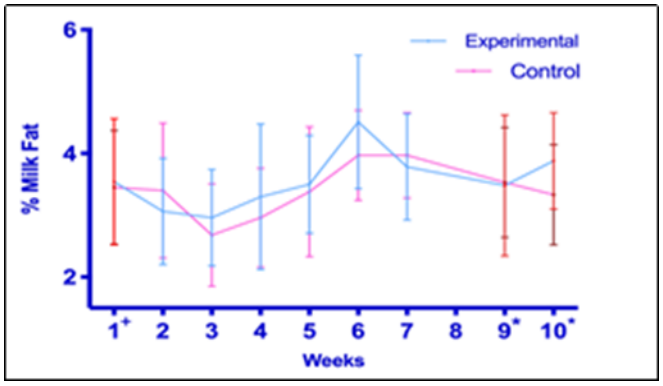

Figure 3. Weekly change in milk fat percentage in the experimental and control groups

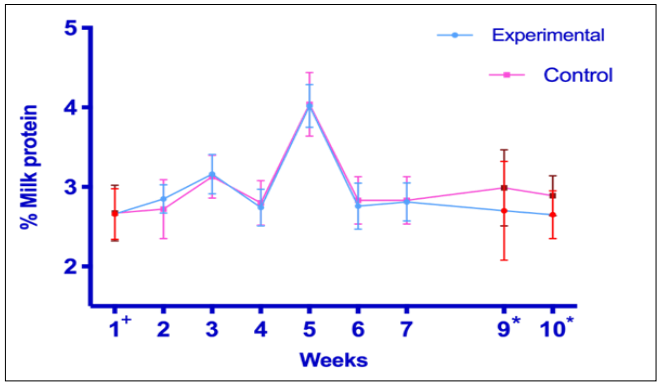

Figure 4. Weekly change in milk protein percentage in the experimental and control groups.

Furthermore, in Figure 1 showing the somatic cells, Figure 5 showing the weekly milking averages, it was seen that SCC decreased more in the experimental group than the control group within 2 weeks from the day the formulation started to given and the milk amount was in favor of the experimental group, and approximately 1 liter increase was observed. In the 7th week when the experiment ended, it was seen that SCC increased again in the experimental group compared to the control group, and the amount of milk decreased compared to the control group.

No statistically significant difference was found in % milk fat (Figure 3) ($p>0.05$). No difference was observed in weekly values between the two groups in milk protein (Figure 4). However, although there is no statistical difference in Figure 3, where milk fat % are shown, it was seen that the amount of milk fat increased in the experimental group in the 2nd week and remained higher than the control group until the 6th week.

In both groups, a decrease in the amount of milk in the summer period was expected, but the decrease in SCC was beyond the expected.

There were no significant difference between experimental and control groups for SCC, Log SCC, milk fat%, milk protein%, weekly average milk amount (L).

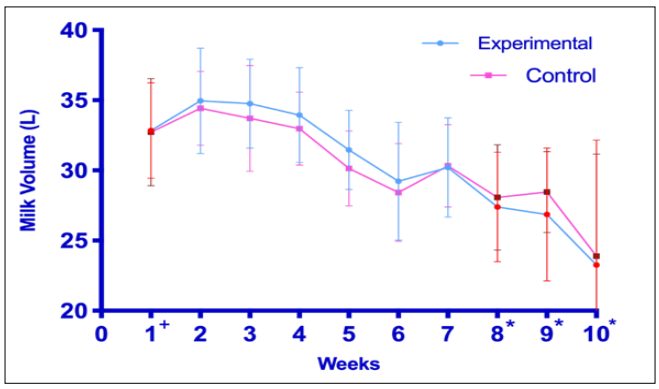

Figure 5. Weekly change in average milk volume in the experimental and control groups.

The selected formulation has successfully passed the ICH accelerated stability tests, and there has been no change in the major components of the herbal content above the criteria set by ICH Q1a(R2).

Discussion

There were no studies of the oral use of rosin (a resin from colophony) and turpentine before with cattle. Antibacterial (Dığrak et al., 1999; Roy, 2018) antifungal (Savluchinske et al., 1999) antiviral (Gigante et al., 2003) anticancer (Tanaka, 2008), antiparasitic (Mercier et al., 2009) properties have been demonstrated in vitro when rosin and turpentine are used alone or together. In addition to the antimicrobial properties of turpentine and rosin, it has been shown to suppress methanogenic bacteria in the rumen (rumen) at a concentration of 43-330 mg/L. Methanogens are bacteria that cause energy loss by producing methane gas in cattle (Sierra-Alvarez and Lettinga, 1990).

In studies of an invention made with a patent obtained in Finland (Patent number: FI124918), it was found to be effective against the mastitis agent S. Aureus in the study performed with the broth dilution method at a concentration of 0.1% (g/L) of resin acids. It was found to be effective against some strains of E. coli at a concentration of 0.5% (g/L). Inhibition was also detected against C. Perferinges, the causative agent of necrotic enteritis, which is a digestive system disease, even at a concentration of 0.01 (g/L). The subject matter of the patent and studies contains 8% resin acid. The effective concentration against S. aureus and E. coli is at 5 g/L (Roy et al., 2018; Kettunen et al., 2015).

When turpentine was used alone, the MIC value against S. aureus, E. coli and C. albicans was found to be 68 mg/ml (Ulukanlı et al., 2014). The volume of rumen, which is the largest compartment of the stomach in cattle, increses as the weight of the cattle increases up to 50-120 liters (Moran, 2005). The resin acids achieved the rate of 96.5% ± 0.3 (w/w) in 30 g

rosin turpentine. The desired MIC concentration, in order to suppress the pathogenic microorganisms and methanogens in the rumen, cannot be achieved by the turpentine at the rate of 3.5 ± 0.3 (w/w).

Toxicity studies of colophony and turpentine were also investigated. In particular, toxicity studies on oral administration are limited. Especially, studies for turpentine have focused on inhalation and skin exposure (Saeidnia, 2014). Although the toxic doses found for the turpentine part are lower than the doses found for rosin, the toxic doses determined for animals in the study are not reached for both substances. While the LD50 value for turpentine is 5760 mg/kg (Saeidnia, 2014; Vulava, 2005), and no harmful effect was not observed at value of rosin is 105-200 mg/kg (Golden, 2006). It was calculated as 57.9 mg/kg for cattle of the same characteristics.

In this study we decided to use SCC over 50.000 as an infected breast. Animal-based fluctuations in SCC were observed during the trial period, which was higher than previously predicted. And the increase in SCC expected with the effect of heat stress could not be seen by experimenting in the summer months. However, the experiment was continued and the differences in the experimental and control groups exposed to the same effects were tried to be observed. As a result of drinking the formulated oral suspension formulation for 33 days, weekly SCC were evaluated with the non-parametric Mann Whitney U test. However, no significant difference was observed in any of the weekly comparisons.

When the formulation applied in the light of the available data is analyzed statistically, it is noteworthy that although there is no significant difference in the amount of SCC and milk in the experimental group compared to the control group, the SCC, milk amounts and milk fat yield the desired results in favor of the experimental group only in the period when the formulation was applied.

In order to support the results obtained, it was expected that the formulation would suppress methanogens which causes energy loss by producing methane in the cattle digestive system according to the rumen volume at the dose used in the in vivo experiment (Sierra-Alvarez and Lettinga, 1990). It can be thought that the increase in the amount of milk in the experiment group compared to the control group in the period when the formulation applicated, decreased the energy loss and increased the amount of milk and supported the milk fat.

Conclusion

Lastly, an oral formulation that include of colophony and turpentine was developed. Although it was not statistically significant at the end of the presented study, it was observed that after the 2nd week, the amount of milk and the amount of fat in the milk increased in the experimental group. SCC decreased more in the experimental group compared to the control group within two weeks also.

It is remarkable as an antibiotic-free method to prevent mastitis by using the oral route and to increase milk yield, as an easy application and an economical solution. Further research is required to elucidate the mechanism of action of this combination based on experimental results. The formulation which has resin consistent has already started to attract attention as an active ingredient that we will see frequently in terms of animal health in the coming years.

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Prevalence of hemorrhagic septicemia in dromedary camel (*Camelus dromedarius*) of some selected farms at Benadir region, Somalia

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ABSTRACT

Pasteurellosis (Hemorrhagic septicemia) is common respiratory disease of camel that is an acute fatal disease caused by *Pasteurella multocida* type A or several serotypes of *Mannheimia hemolytica*, which also affect other animals. The disease had shown to spread between animals, across herds and to humans. Meaning that the disease is zoonosis. The study aimed at establishment of sero-prevalence of pasteurellosis in some selected Districts of camels rearing in Benadir Region. It was a cross-sectional study, where the study population was purposively chosen to consist animals taken within three sub-Districts of Benadir Region, namely Sub-District (Daynile Township), Sub-District (Yaaqshid) Sub-District (kaxda). This was because the normally handle many camels in a day, thus making it easy for the investigator to access the required number conveniently; it was also assumed that data collected from these for-slaughter camels was representative of the situation in the sub-District/county. A total of one hundred and sixty camels were tested using four serological tests: Rose Bengal Plate Test (RBPT),) and Complex Fixation Test (CFT). The serological tests were purposively chosen to increase the chances of picking positive cases and also to compare their sensitivities, with respect to camel serum, since they were originally meant for use on bovine serum. Blood samples (15 ml) were collected for serum harvesting from jugular veins of the animals as they were waiting to be examined. Rose Bengal plate test and CFT were run at a laboratory within the department of Veterinary Medicine, University of Horsed, 21 October campus; serum samples having been transported in a cool box. On average, out of an overall total of 300 serum samples tested, 180 samples were selected as sample procedure and were gives eleven (11) positive results, amounting to a prevalence of 6.67%. For the three districts, respective prevalence (averaged from the two (2) serological tests run) were: 7% (3/50) for Yaqshiid; 8% (3/60) for Deyniile and 10% (3/70) for Kaxda. When sensitivities of the two (2) serological tests were compared, there was no significant difference between them, with respect to picking of positive cases (p=0.05), The study has demonstrated presence of Pasterolosis in camels at Benadir Region and the authors are, recommending usage of RBPT and CFT as a screening test, since they are cheap, quick, and easy to carry-out. Any of the other three more involving tests can then be used if one wants to establish respective titers. Therefore, further detailed investigation needs to be conducted so as to understand specific etiological agents causing pasteurollosis in camel and can be instituted to optimize the benefit obtained from the camel sector.

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Introduction

Pasteurellosis are highly affecting livestock industry in the country. Pasteurellosis is a multifactorial disease caused by numerous etiologic agents (Hasso, 2016).

Mannheimia haemolytica, Bibersteinia trehalosi and Pasteurella multocida cause pasteurellosis in animals and humans. Pasteurella are commensal organisms of healthy animals which can be trigger with stress factors to cause fatal disease in farm animals. Pasteurellosis / Hemorrhagic septicemia is an acute fatal disease of camels caused by *Pasteurella multocida* type A or several serotypes of *Mannheimia haemolytica* is characterized by fever, edema of the throat region, dyspnea, and sudden death. P. multocida type A is considered to be a common inhabitant of the upper respiratory tract and it may cause disorders, in association with other microorganisms such as parainfluenza type 3 virus, in animals weakened by exposure to cold, malnutrition

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or gastro-intestinal parasitism (Jones et.al. 2013).

Infective agents acquired by inhalation of infected droplets or close contacts among susceptible animals. Pasteurellosis is responsible for huge mortality in feedlot animals worldwide. Haemorrhagic septicemia is an acute and characterized by sudden onset of fever, profuse salivation, severe dyspnea and death in about 24 hours whereas shipping fever causes severe broncho-pneumonia and pleurisy. The diagnosis of the disease is based on the clinical signs, gross pathological lesions, isolation of the pathogens and molecular characterization (Alemneh & Tewodros, 2016).

Pasteurellosis is complex multifactorial disease difficult to control however, good management, chemotherapy, chemoprophylaxis and early immunization are control and preventive measures. In Somalia, pasteurellosis is an endemic disease posing a serious threat to the animal productions. However, data on epidemiology, diagnosis, prevention and control is scarce (Gluecks, et.al. 2017).

Materials and Methods

Study area: The study carried out Benadir region Somalia. Benadir region consists of 17 districts. The region is one of the four regions in the south Somalia Benadir and it is the capital of Somalia. It covers the same area as the city of Mogadishu, which serves as the capital. It borders with middle Shebelle River in the north and the east, lower Shebelle River in the west and Indian Ocean in the south. The study was collected out in three (3) sub divisions for Benadir region district name: Daynile district, Yaaqshid district Kaxda district. However, the study was especially collected in dairy camel rearing farms which contributes the Daly life of capital Markets. Although by far the smallest administrative region in Somalia, it has the largest population, estimated at 3,650,227 (including 369,288 internally displaced persons) in 2014. Mogadishu, locally known as Benadir /Xamar or Hamar, is the capital and most populous city of Somalia. It is estimated to be about 2.3 million and covers an area approximately 637 km² and their Elevation is 9 m (Wikitravel, 2019). Therefore, the four districts study were selected purposively samples due to their Animal population. The Samples were collected randomly from the semi intensive and intensive dairy farms. Study design was a cross-sectional by collecting blood from sampled animals. The study was a cross-sectional on camel in some selected farm camels in three (3) districts of Benadir region in Somalia. The farms visited at one (1) months to collect suspect camels and samples were collected carefully. The camel brought to the farms (4 weeks) were collected for bacteriological examination. In addition the selected animals was examined clinical and bacteriological examination. The calculated information was include the size of the Animal in the herd, the Age of the animal, the hygiene of the farm production system and water sources of the farms.

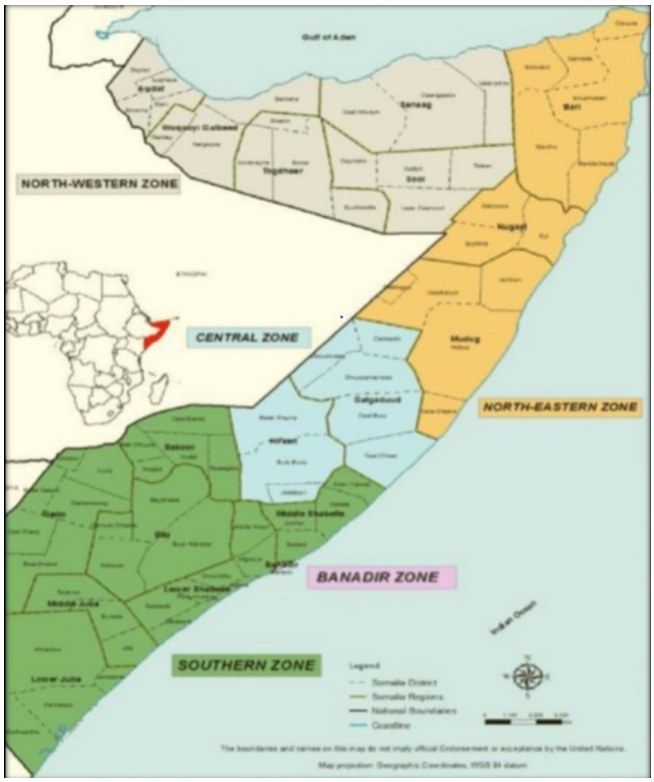

Figure 1. Map of Somalia showing the study sites of Benadir Region (National Bureau of Statistics, 2018).

Sample size determination: The sample size calculation was done using the equation of Dohoo et.al, (2016).

$$n = \frac{Z\alpha^2 pq}{L^2}$$

Where; N is required sample size
Zα= 1.96 the normal deviate at 5% level of significant
P = A priori estimation of prevalence for the disease
q =1-p and Lis allowable error of estimation
Slaughtered camel: using the highest prevalence estimation of 20% for hemorrhagic septicemia in camel (Arimi et.al, (2005) and L is at 5%. The required sample size was calculated as follows:

$$n = \frac{1.96^2(0.15)(0.69)}{(0.05)^2} \qquad n = \frac{3.8416\,(0.15)(0.69)}{(0.05)^2} \qquad n = \frac{3.8416(0.15)(0.69)}{0.0025} = 180$$

Therefore, sample size per sub-districts were calculated based on the number of camels per herd,

which was found to be in the ratio of 4:4:3 for the three sub selected districts in Benadir region respectively. The respective animals were recruited into the study on several visits to the camel farms until the required number was achieved. To be 95% confident that our estimate of Pasteurollosis seroprevalence is within 20% of the true population value (i.e., a relative error of 0.20) 300 camel should be sampled.

Sample collection procedure: The collected samples were from selected dairy farm camels. for (15ml) of blood samples were collected from jugular vein by using gauge 15 needle and 20 ml syringe. The blood samples were then placed in large test tubes, without anti-coagulant, taken to Veterinary Investigation Laboratory, at Horsed international University. The samples were then centrifuged at 4,500 xg, serum decanted into cryovials, which were labelled and stored in freezer (-20°C) at the laboratory of the university. The blood was centrifuged and harvested using the standard procedure of OIE and similarly done by (Belak et.al, (2016). Samples had cultured on casein/sucrose/yeast agar containing 5% blood. Conventional blood agar may also be used. Details, including biochemical methods for identification of the organisms. Serotyping methods include the rapid slide agglutination test, indirect haemagglutination test, somatic antigen agglutination tests, agargel immunodiffusion and counter immunoelectro-phoresis. Details were found in the OIE Terrestrial Manual.

For each serum sample, part were used to carry out RBPT at the Horsed laboratory, while part of it was transported in a cool box to Department of Veterinary medicine for carrying-out of c-ELISA and CFT.

Data collection and analysis: The data was collected through quantitative methods. The Information (data) was gathered through Descriptive examination from the investigation zone, revised, composed and organized. The data collected from the sub study area district, edited, collated and tabulated. Both MS Excel Windows® 2013 data base and Stata 17 statistical analysis Software by using descriptive statistics. The outcome between the variable (status of Hemorrhagic septicemia in the herds), blood serum and identification of the samples from selected district farms in Benadir region were the first screen in a university of hosed laboratory.

Results

Rose Bengal Plate Test (RBPT) results overall and with respect to the three study area of Benadir region Somalia are presented Table 1.

Table 1. Rose Bengal Plate Test (RBPT) results overall and with respect to the three study area of Benadir region Somalia.

Study sub-district	Number of examined camels	Number of positive	Number of negative	Positive %
Dayniile district	55	11	44	.020
Yaaqshiid district	45	12	33	0.26
Kaxda district	80	10	70	0.125
	Total: 180	Total: 33	Total: 147	0.585

The overall prevalence of hemorrhagic septicemia in camel different species were 58.6% positive which were indicated that examined camels having a higher number of positive results when compared to number of negative however there is no statistically significant connotation ($p < 0.05$).

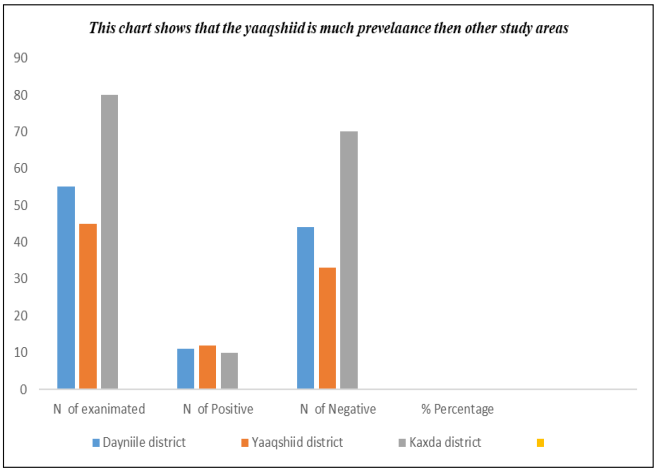

Figure 2. This figure were indicted that the three sub district had much near results at the prevalence level and Yaqsgiid sub district is much more positively charged camels due to farm animals.

Table 2. Overall sero-prevalence of hemorrhagic septicemia used by complex fixation test (CFT).

District	No of examined camels	No of Positive	No of Negative	Positive %
Dayniile District	55	11	14	0.20
Kaxda District	50	15	13	0.30
Yaaqshiid District	75	13	12	0.17
	Total: 180	Total: 39	Total: 39	Total: %0.67

In this table of seroprevallance of pastuerollosis for the three sub district Kaxda is indicating higher prevalence according to the other sub district due to camel farms were visited and they were much more examined camel farms.

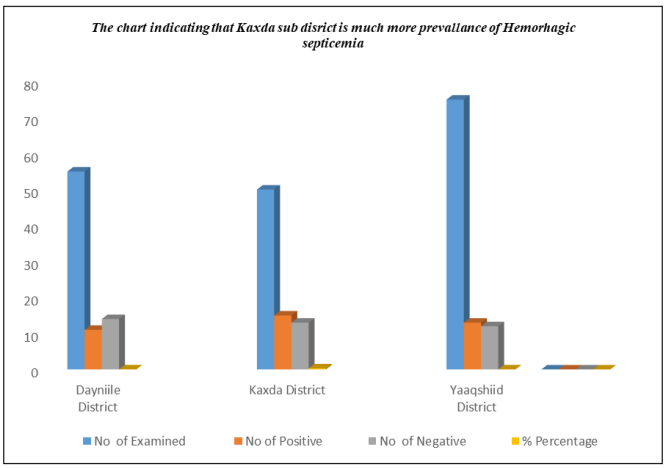

Figure 3. The above chart were used by state software for analysis of the date, the prevalence of the two study area were indicated that Kaxda sub count made overall prevalence result 0.3%.

Table 3. Prevalence of hemorrhagic septicemia in Kaxda and Yaqshiid

Districts	Number of examined camels	RBPT Positive	CFT Positive	Percentage %
Kaxda	162	7	6	0.08
Yaqshiid	138	5	4	0.06
Total	300	12	10	0.07

In this Table the researchers were selected the most common camel farms in Benadir region and their sub study area and we conformed that there was different prevalence values in the two higher study area both are very near as you see the result.

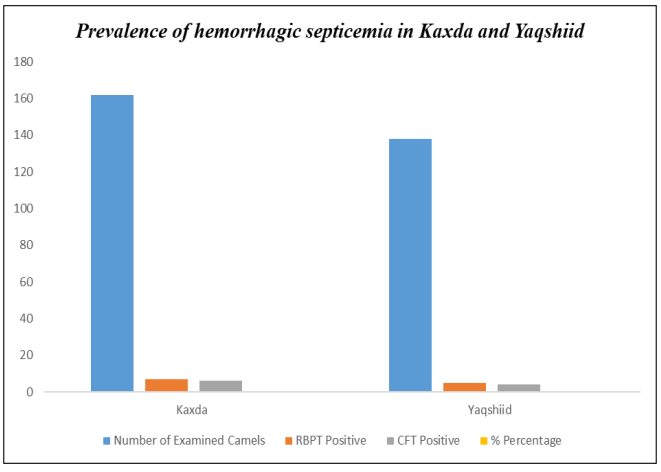

Figure 4. Prevalence of hemorrhagic septicemia in Kaxda and Yaqshiid.

In this chart indicates that both sex of camels examines for the two serological test with the selected sub study area (Kaxda &Yaqshid) which were totally different. However, there was no significant statistically analysis ratio (>P.005).

Table 4. Prevalence of hemorrhagic septicemia in male and female examines camels

Districts	Number of examined camels	RBPT Positive	CFT Positive	Percentage %
Male	152	8	7	0.09
Female	148	6	8	0.09
Total	300	14	15	0.09

This Table of the study concerns that the female and male camels examined used by RBPT and CFT. Therefore, both are near prevalence and percentages so female animals are most susceptible in Benadir region.

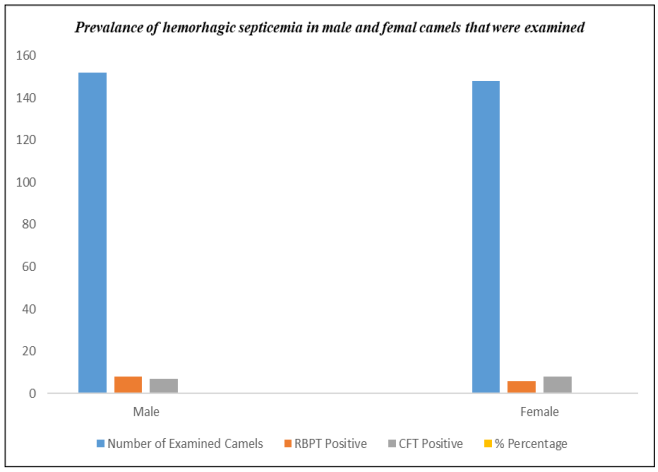

Figure 5. Prevalence of hemorrhagic septicemia in male and female examines camels.

In Figure 5 indicates that the female and male animals are so near, however females camels are more susceptible indicating that the sub study area of Benadir Somalia.

Table 5. Prevalence of hemorrhagic septicemia in herd level for examined camels at the three-study site.

Districts	Number of Tested	RBPT Positive	CFT Positive	% Percentage
Kaxda	104	9	7	0.15
Yaqshiid	107	8	6	0.13
Deynile	89	7	5	0.13
Total	300	24	18	0.14

In the above Table denotes that the herd level according to the study site therefore the most common positive (+) are Kaxda sub-district and it was estimated to 16 (0.15) and Yaqshiid was the second site for the herd prevalence and there was not statistically significant.

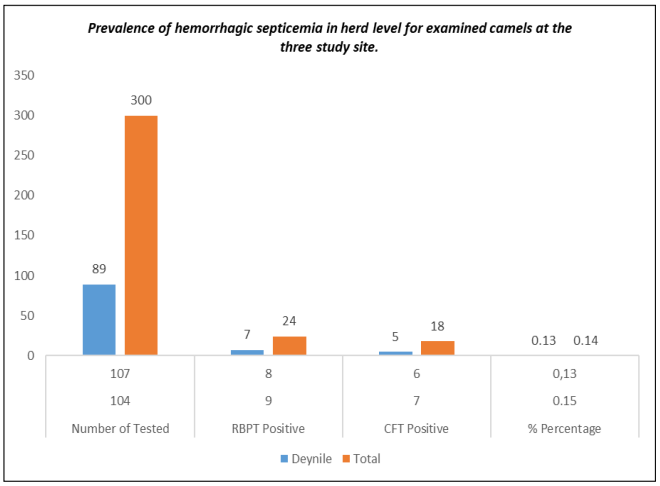

Figure 6. Prevalence of hemorrhagic septicemia in herd level for examined camels at the three-study site.

Table 6. Prevalence of hemorrhagic septicemia based on the site of the farms in three sub -district of the study area in Benadir Region Somalia.

Districts and Farm Names	Number of Tested	RBPT Positive	CFT Positive	Percentage %
Kaxda: Sheikh Adanley Farm	124	10	8	0.14
Yaqshiid: Jamhuriya Farm	84	8	9	0.20
Deynile: caliyaale Farm	92	11	7	0.19
Total:	300	29	24	0.17

In Table six (6) defined that the study has different sites and different Sero-prevalence and including the most susceptible site are Kaxda and Yaqshiid due to their farm animals (Camels) are more than other sites of the study area and further more identification will recommend.

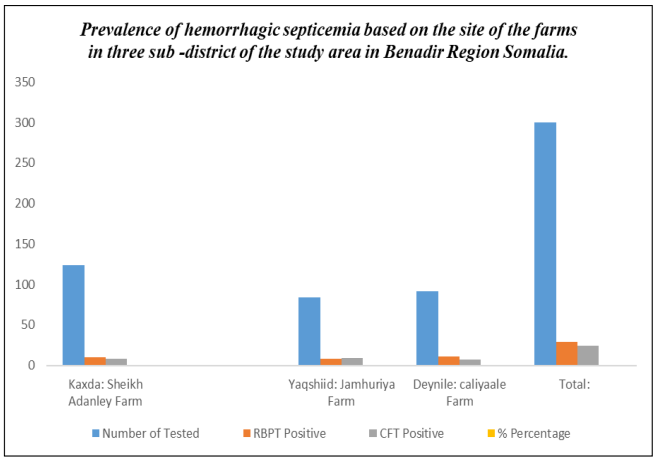

Figure 7. Prevalence of hemorrhagic septicemia based on the site of the farms in three sub -district of the study area in Benadir Region Somalia.

Discussion

In the study, the seroprevalence of Hemorrhagic Septicemia in camel, as well as the risky activities practiced by camel herders that may expose them to other disease from the camels, had been assessed. The study was conducted between February to Jun 2022, at the several villages of three (3) districts in Benadir region of Somalia so as to measure the prevalence of Pasteurellosis (Hemorrhagic septicemia), by using CFT and RBPT for the screening to evaluate the existent of the disease and other differential diagnosis of the disease. The overall prevalence of the disease hemorrhagic Septicemia in this study were estimated 7.5% therefore it indicates that there is susceptibility of the disease and other clinical manifestation were reported In camels infection associated with Pasteurella Multocida and Mannheimia haemolytica shows a that the wide range of pulmonary and septicaemic infections. P.multocida is associated with hemorrhagic septicaemia in adult camels and other enzootic camels were different in sings and had mostly shown pneumonia complex in young animals (Camels).however, Immune status and severity of infection depends on the predisposing factors like stress, climate change, Herd health status, deficient nutrition, concomitant infections, and virulence factors. According to the site of the study in Benadir region Somalia.

It also indicates that examined camels having a higher number of Negatives results compared to number of Positives tested in the three sub districts of the study area. However, there is no statistically significant association ($P < 0.05$). Therefore, our results strongly agreed with other studies conducted from Kenya and Ethiopia (Alemneh & Tewodros, (2016). Camel pasteurellosis: Isolation, identification have been confirmed the same prevalence of my study. There was other reports has been done hemorrhagic septicemia in the same prevalence but it is too old to mention in this study. Some study have been made in (Awad, et.al (1976a). Studies of prevalence with Pasteurella multocida types in camel published by Journal of Veterinary Science, 13(1), 53–56).

During the study, as a researchers we had been used tRBPT screening test due to its fast , easy, and susceptible to accept commonly (99.9%), and it allowed us processing for many of our samples per day by following the stander manual of (OIE, 2016). Therefore, The RBPT sera test positives were retested using by the Complex Fixation Test (CFT) that had having a specificity of 100% (Manish et al., 2017). İn order to maximize the specificity of their tests.

Animals were considered as positive if it was positive by both RBPT and CFT. Accordingly our overall Prevalence it doesn't mean that the disease is insignificant as it is a very serious disease responsible for reproduction failure of the dairy industry in the study area and its zoonosis disease so that it is very important to know and carefully .

Conclusion

This current study for cross-sectional Camel Pasteurollosis (Hemorrhagic Septicemia) at the Benadir region of Somalia especially the three sub district study targets were showed that very low Sero-prevalence. At the same time, the low prevalence of the disease was observed in different Camel sexes and different age groups of camel. However seroprevalence is low, it can still be a potential risk for both susceptible camels and also man. And imaginably in other areas of Somalia where nomadic pastoralism is practiced. There is a need for an felon try institute to control measures of this disease and related disease through vaccination, it is needed education, control to the public awareness, and conducting sero type surveys and those animals testing positive will be controlled . Also as we researchers recommend further research to be done in this area duet luck of awareness of the farms and society.

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Competing interests: Authors have declared that no competing interests exist.

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Determination of protein levels and amino acid composition of bee pollen collected from different geographical regions of Turkey

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ABSTRACT

In this study, determination of the amino acid profile of bee pollen produced in different parts of Turkey and performed to determine the protein level. The research was carried out with a total of 90 specimens from East-Southeast Anatolia, Central Anatolia, Black Sea, Marmara, Aegean, and Mediterranean. LC / MS-MS for bee pollen amino acid levels and Bradford method for protein level were preferred. According to the analysis results, the average on mg/g basis in bee pollen are alanine (143.40 ± 0.00), arginine (34.45 ± 0.00), histidine (32.55 ± 0.09), isoleucine (20.94 ± 1.24), leucine (23.75 ± 1.27), lysine (31.15 ± 0.43), methionine (39.10 ± 1.32), phenylalanine (20.09 ± 0.95), proline (309.05 ± 28.56), and valine (15.79 ± 0.88) amino acids were detected respectively. According to the analysis results, protein level of bee pollen in mg / g basis in Black Sea region (127.27 ± 0.31), Marmara region (117.56 ± 0.31), Mediterranean region (115.66 ± 0.31), Central Anatolia (115.09 ± 0.31), Aegean region (110.06 ± 0.31) and East-South East Anatolia (124.9 0 ± 0.31). The differences between the regions were found in protein and amino acid levels (P <0.01). In this study, the protein content and amino acid composition of bee pollen collected from plants growing in various regions were determined. We believe that this research may help with the manufacture, inspection, and standardization of healthy bee products.

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Introduction

Bee products have been used for centuries for the prevention and treatment of diseases and for a healthy life (Cherbuliez, 2013; Aker and Nisbet, 2020). With the introduction of new biological activities, these natural foodstuffs occupy an important place among research topics in recent years. Honeybee pollen, a product of apitherapy, is the main food source used for colony development, consisting of a mixture of flower pollen and glucose oxidase enzyme from bee secretion (Gerigelmez, 2003). In many studies and research based on clinical experience, bee pollen has been found to be effective on antioxidant (LeBlanc et al., 2009), antibacterial (Proestos et al., 2005), antifungal (Garcia et al., 2001), stress reducer (Seven et al., 2011), stomach disorders (Wang et al., 2007), immunomodulatory (Oliveira et al., 2013) and anti-inflammatory (Xiaozhi et al., 2018) properties. However, it is not correct to rely on these properties for all bee pollen (Nisbet et al., 2018). The reason is that the chemical content of pollen varies depending on the plant source. Honeybees not only collect pollen from different kinds of flowers, but also add different

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chemicals found in this plant to their pollen content (Nisbet et al., 2009; Denisow and Wrzesien, 2015). The chemical composition and biochemical properties of pollen depend on the plant species from which the pollen was collected (Szczęsna, 2006), its geographical origin (Morgano et al., 2012), by season (Morgano et al., 2012; Denisow and Wrzesien, 2015), soil type, and storage method (Feás et al., 2012; Siuda et al., 2012. These factors also change the composition of the active substances of the pollen. Therefore, the protein, carbohydrate, mineral, oil, vitamin and phenolic compounds contained in bee pollen are different. In other words, the food quality and use of each bee pollen produced in apitherapy should be revealed with laboratory data. In this study, which we think may contribute to the production, inspection and standardization of healthy bee products, the protein levels and amino acid components of bee pollen obtained from plants grown in different regions were determined.

Materials and Methods

Sampling: A total of 90 pollen samples from different regions of Turkey, including Central and Eastern Black Sea (23), Marmara (14), Central Anatolia (12), Mediterranean (12), Aegean (17), East-South Anatolia (12), were used. Pollen samples were dried in the laboratory by baking at 40°C for 48 hours. The dried samples were ground.

Extraction: A solution was prepared by weighing 1 g of the ground samples and using 10 ml of 0.2 mmol acetic acid. The prepared solutions were vortexed for 2 minutes. After the vortex application, it was centrifuged at 5000 rpm at -4°C for 10 minutes. After centrifugation, the supernatant part of the solution under the oil layer was taken and filtered with disposable filters of 0.45 nmol, and 5 ml solutions were prepared (Ulusoy, 2010).

Biochemical analysis: Amino acids to be analyzed in pollen samples were determined as aspartic acid, glutamic acid, proline, alanine, cysteine, methionine, valine, leucine, isoleucine, tyrosine, phenylalanine, lysine and arginine, glutamine, tryptophan, glycine, histidine, serine, threonine and asparagine. The method reported by Szczesna (2006) was preferred for the analysis of the identified amino acids. High Performance Liquid Chromatography-Tandem Mass Spectrometer (LC/MS-MS 8040, SHIMADZU) device was used for amino acid analyses. In this study, ready-made commercial standard solution was used for qualitative and quantitative analyses of amino acids (Amino acids Mix Solution., Product No: 79248-BCBS9675V). A modified Bradford method was used to determine the protein concentration in pollen (Lu et al., 2010).

Statistical analysis: The data obtained from the study were evaluated with the analysis of variance (ANOVA) technique in factorial order and the differences between the means were determined by Duncan multiple comparison test. Statistical evaluations were made using the SPSS statistical program (SPSS,2004).

Results

Amino acid analysis results: Amino acid types and levels determined in pollen samples were presented in Table 1 and 2. As a result of the analysis of variance performed according to the randomized plot design, a possibility difference was determined between the regions ($P<0.01$).

Table 1. Mean, minimum and maximum free amino acid levels compared to Turkey's average (mg/g pollen) (n=90)

Amino acid	Mean (mg/g pollen)	Minimum (mg/g pollen)	Maximum (mg/g pollen)
Alanine	143.40 ± 0.0 0	143.40	143.40
Arginine	34.45 ± 0.0 0	34.45	34.45
Histidine	32.55 ± 0.09	32.22	33.73
Isoleucine	20.94 ± 1.24	12.58	62.76
Leucine	23.75 ± 1.27	16.30	58.50
Lysine	31.15 ± 0.43	30.04	31.87
Methionine	39.10 ± 1.32	37.78	40.43
Phenylalanine	20.09 ± 0.95	13.27	48.11
Proline	309.05 ± 28.56	9.50	1503.41
Valine	15.79 ± 0.88	10.60	21.83

Table 2. Free amino acid mean values by region (mg/g pollen)

	Mediterranean	East-Southeast	Central Anatolia	Black Sea	Marmara	Aegean
Alanine	143.40±0.00	-	-	-	-	-
Arginine	34.45±0.00	-	-	-	-	-
Histidine	32.46±0.17	32.45±0.09	32.64±0.24	32.53±0.11	32.53±0.30	32.66±0.35
Isoleucine	18.01±0.94	22.98±3.28	19.66±1.19	21.22±4.65	21.52±3.89	22.12±1.71
Leucine	20.59±1.06	23.95±2.15	22.47±1.24	29.33±7.81	24.15±4.04	23.04±1.34
Lysine	30.04±0.00	-	-	31.85±0.00	-	31.35±0.51
Methionine	-	-	-	37.78±0.00	40.43±0.00	-
Phenylalanine	30.04±0.00	20.81±2.01	20.75±1.29	20.66±4.16	20.05±3.45	18.83±0.95
Proline	374.88±74.20	424.69±91.64	408.78±60.03	207.00±66.88	293.99±47.57	254.65±55.20
Valine	16.02±1.31	18.76±3.07	-	14.13±1.61	16.61±2.10	14.45±2.45

Table 3. The total protein and total amino acid amounts by regions (mg/g)

Regions	Total protein (mg/g)	Total amino acid (mg/g)	Total essential amino acid (mg/g)	Total non-essential amino acid (mg/g)
Marmara	117.56 ± 0.31	103.36 ± 22.96	22.47 ± 1.93	310.26 ± 53.93
Aegean	110.06 ± 0.31	92.19 ± 22.19	22.56 ± 1.04	253.55 ± 65.47
Mediterrenian	115.66 ± 0.31	113.8 ± 29.18	20.81 ± 1.14	374.88 ± 74.2 0
Central Anatolia	115.09 ± 0.31	135.18 ± 32.63	21.96 ± 1.95	408.78 ± 60.03
Black Sea	127.27 ± 0.31	100.29 ± 30.25	24.57 ± 2.56	119.40 ± 45.68
East-Southeast Anatolia	124.90 ± 0.31	123.51 ± 33.70	22.32 ± 1.53	424.69 ± 91.64

Table 4. Differences between regions according to protein analysis results (mg/g)

Regions	Mean (mg/g)	Minimum (mg/g)	Maksimum (mg/g)
Black Sea	127,27 ± 0.31^{a}	120.97	133.57
Marmara	117.56 ± 0.31^{bc}	111.25	123.86
East-Southeast Anatolia	124,90 ± 0.31^{ab}	118.60	131.20
Aegean	110.06 ± 0.31^{c}	103.75	116.36
Central Anatolia	115.09 ± 0.31^{c}	108.78	121.39
Mediterrenian	115.66 ± 0.31^{bc}	109.35	121.96

Table 5. Differences in protein content between regions (mg/g)

Aegean region	110.06c		
Central Anatolia region	115.09c		
Mediterranean region	115.66c	115.66b	
Marmara region	117.56c	117.56b	
East-Southeast region		124.90b	124.90a
Black Sea region			127.27a
Sig.	1.27	0.53	5.93

Table 6. Comparison of the amino acid profile of Turkey with the amino acid profiles of other countries

Amino Acid	Turkey	China (Yang, 2013)	Spain (Gonzalez, 2006)	South Africa (Nicolson, 2013)	Brazil (Negrao, 2018)
Alanine	143.40 ± 0.0	12.5 ± 0.6	10.68 ± 1.10	53.7 ± 4.60	11.54 ± 0.50
Arginine	34.45 ± 0.0	14.00 ± 0.9	5.03 ± 1.50	41.8 ± 2.60	8.75 ± 1.70
Histidine	32.55 ± 0.10	8.10 ± 0.5	6.84 ± 7.20	56.6 ± 5.50	4.58 ± 0.40
Isoleucine	20.94 ± 1.20	11.10 ± 0.7	9.22 ± 2.00	38.8 ± 2.50	7.17 ± 0.70
Leucine	23.75 ± 1.3	170.00 ± 1.1	10.81 ± 1.70	63.2 ± 3.80	12.59 ± 9.50
Lysine	31.15 ± 0.40	15.30 ± 0.8	10.97 ± 1.97	62.9 ± 2.70	11.69 ± 0.10
Methionine	39.10 ± 1.3	4.20 ± 0.1	4.10 ± 1.57	-	5.18 ± 0.30
Phenylalanine	20.09 ± 10	1.80 ± 0.6	9.65 ± 2.2	39.0 ± 2.60	7.15 ± 0.60
Proline	309.05 ± 28.60	15.70 ± 0.8	22.88 ± 3.5	61.9 ± 5.00	21.24 ± 2.90
Valine	15.79 ± 0.80	12.9 0 ± 0.8	7.26 ± 1.90	43.4 ± 3.20	8.42 ± 1.20

Protein Analysis Results: The verage of protein concentration determined in pollen samples in Turkey was presented in Table 4. As a result of the analysis of variance performed according to the randomized plot design, a possibility difference was determined between the regions (P<0.01).

Discussion and Conclusion

Today, pollen and pollen products are used in the fields of health and cosmetics as well as nutrition (Morais et al., 2011; Denisowand and Denisow-Pietrzyk, 2016). The high nutritional value of pollen is completely dependent on the chemical structure and biochemical properties of bee pollen. The therapeutic activity of bee pollen is not equally valid for all pollen. In other words, the chemical structure of pollen differs from region to region and country to country. The plant composition shaped by each flora offers honey bees a source of pollen from different species (Aker and Nisbet, 2020). On the other hand, it is known that honey bees do not use all flowering plant species as pollen source in the natural flora where they work, there is a preference, and the number of flowering species preferred for bees in the flora in general has a very low share among all flowering plant species (Baydar and Gurel, 1998).

According to the results we obtained, alanine, arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, and valine amino acids were found in the amino acid profile of the pollen samples included in the study. On the other hand, threonine, glycine, aspartate, cysteine, glutamate, tyrosine and serine amino acids could not be detected because they were at very low levels. Studies conducted in different countries (Poland, Belgium, Korea, South Africa, Spain, China) differ in terms of amino acid diversity in pollen (Gonzalez et al., 2006; Yang et al., 2013). In the obtained data, the amino acid concentration in the samples is Proline > alanine > methionine > arginine > histidine > lysine > leucine > isoleucine > phenylalanine > valine , respectively. It originates from lysine, histidine, threonine, phenylalanine, leucine, isoleucine and valine, which are very important essential amino acids for the honey bee (Cook et al., 2003). In the present study, except for threonine and tryptophan, other essential amino acids were detected in pollen. When the minimal essential levels required for honey bees are compared with data obtained, Turkish pollen seems to be in a good position (Nicolson et al., 2013). In this study, proline and alanine are the predominant amino acids that make up 45% of the total amino acids in Turkey.

The results of the analysis reveal that Turkish pollen (689.18 mg/g) has a high nutritional value as a food substance compared to countries such as China (238.96 mg/g), Poland (201.52 mg/g) and Korea (181.10 mg/g) in terms of total amino acid concentration. When compared between regions in this study, it seems that the amino acid content and concentration of pollen obtained from different regions are different from each other

According to the results obtained, the protein level in the pollen is 127.27±0.31 mg/g in the Black Sea region, 117.56±0.31 mg/g in the Marmara region, 115.66±0.31 mg/g in the Mediterranean region, 115.09±0.31 mg/g in the Central Anatolia region, 110.06±0.31 mg/g in the Aegean region, 124.9±0.31 mg/g in the East-South East Anatolia region. In the study conducted by DeGrandi-Hoffman et al. (2018) in the Sonoran region of Arizona, the total protein level was determined as 425 ± 30 mg/g.

In a study conducted in eastern Saudi Arabia, the total protein level was reported as 202.3 mg/g (Taha et al., 2019). Ketkar et al. (2014) looked at the potential values of bee pollen obtained from Indian mustard monoflorally, they determined the amount of protein is 182.2±5.9 mg/g. When compared with the data of other countries, it is thought that the difference in the amount of protein in the results obtained from the bee pollen in Turkey is due to the multifloral plant diversity. At this point, it has been confirmed by the results of the study that the chemical composition of pollen is variable due to the fact that it is obtained from flower products, it differs according to the plant species and the geographical structure of the plant, season and soil structure. The emergence of different data is an indication that it is not considered possible for the pollen samples to be standard.

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Conflict of Interest

The authors declared that there is no conflict of interest.

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Changes in phosphorus and some biochemical parameters in cats with open and closed cervix pyometra

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ABSTRACT

Pyometra is a disease defined as a purulent inflammation of the uterus and may have multisystemic effects. In this study, the investigation of the changes in leukocytes, calcium, phosphorus, and some biochemical parameters in cats with open or closed cervix pyometra was aimed. A total number of 48 queens were enrolled in the study. Twenty three healthy queens presented in diestrus phase were classified as Group C that revelaed to the clinic for elective ovariohysterectomy. Twenty five queens constituted the pyometra group (Group PYO) which were divided into 2 subgroups whether the presence of vaginal discharge (open cervix pyometra; Group OP; n=15 and closed cervix pyometra; Group CP; n=10). The mean white blood cell (WBC) level in the Group PYO was significantly higher than in Group C ($P<0.001$). The highest WBC level was detected in the Group CP. Serum alanine aminotransferase (ALT) and total protein (TP) levels in the Group CP were higher than those detected in the Group OP ($P<0.01$) and Group C ($P<0.05$). The highest albumin (ALB) ($P<0.05$) and albumin/globulin (ALB/GLOB) ($P<0.01$) levels were determined in the Group C. Serum globulin (GLOB) levels in the Group CP and Group OP were significantly higher than those measured in the Group C ($P<0.01$). A significant rise in alkaline phosphatase (ALKP) levels in the Group PYO were detected ($P<0.01$). The highest phosphorus (PHOS) levels were identified in the Group CP ($P<0.01$). In conclusion, to the best of our knowledge, this is the first report which is detected hyperphosphatemia in the closed cervix pyometra in the queens. Besides, pyometra is a serious disease that needs to be well evaluated with laboratory tests in order to reveal its multisystemic effects.

Keywords: biochemistry, cat, phosphorus, pyometra

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Introduction

Pyometra is an acute or chronic suppurative inflammation of the uterine wall in queens. It is characterized by endometrial hyperplasia with cystic dilation of endometrial glands and accumulation of purulent exudate in the uterine lumen (Hollinshead and Krekeler, 2016). Cystic endometrial hyperplasia-pyometra complex is not very common in queens (Agudelo 2005). The disease usually develops during the luteal phase, and progesterone plays a key role for the establishment of infection with ascending opportunistic bacteria (Hagman et al., 2014). The incidence of the feline pyometra increases over five years of age (Johnston et al., 2001). However, pyometra occurrence has also been reported after the first oestrus in a five-month-old mixed breed queen (Günay Uçmak et al., 2019). Hemorrhagic and/or mucopurulent vaginal discharge is noticeable in open cervix pyometra. Besides, more severe clinical signs and abdominal enlargement frequently occur in closed cervix pyometra cases (Hagman, 2022). The common systemic signs are depression, anorexia or inappetence, vomiting, diarrhoea, listlessness, abdominal distention, polyuria and polydipsia. Pyometra affects the function of several organs,

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including marrow, liver and kidneys (Johnston et al., 2001). It usually develops a normocytic, normochromic non-regenerative anaemia and leukocytosis. In addition, serum chemistry abnormalities including hyperproteinemia, hyperglobulinemia, hypokalemia, and azotemia are presented in cases of pyometra (Nak et al., (2005).

The aim of this study is to evaluate the changes in leukocytes, calcium, phosphorus and some biochemical parameters in cats with open and closed cervix pyometra.

Materials and methods

Animals and study design: All animal procedures were carried out in accordance with the approval of the Istanbul University-Cerrahpaşa Animal Experiments Unit Ethical Committee (Approval number: 2022/42). A total number of 48 queens were enrolled in the study. Gross clinical (hearth rate, respiratory rate, body temperature and dehydration status) and gynaecological (vaginal cytology and transabdominal ultrasonography with 6.6 MHz convex transducer (Esaote Pie Medical MyLab Five Vet., Genova, GE, Italy)) examinations were revealed. Twenty three healthy queens in diestrus that presented to the clinic for ovariohysterectomy were included in control group (Group C). Twenty five queens constituted the pyometra group (Group PYO). The pyometra was treated with ovariohysterectomy. The queens in group PYO were divided into 2 groups with regard to the presence of vaginal discharge in which consisted of open cervix pyometra (Group OP; n=15) and closed cervix pyometra (Group CP; n=10). Before all queens had surgical intervention, hematological analyses were performed.

Hematology and biochemistry: Blood was drawn by the puncture of jugular vein and collected into one clot separator tube and one EDTA containing tube prior to surgery. Blood biochemistry (DRI-CHEM NX600, Fujifilm, Japan) and total blood count tests (Procyte Dx Hematology Analyzer, Idexx, USA) were performed. White blood cell (WBC), glucose (GLU), creatinin (CREA), blood urea nitrogen (BUN), BUN/CREA, total protein (TP), globulin (GLOB), albumin (ALB), ALB/GLOB, Alanine aminotransferase (ALT), Alkaline Phosphatase (ALKP), calcium (Ca) and inorganic phosphorus (PHOS) measurements were incorporated into the study.

Vaginal cytology: Vaginal smear was obtained for cytological examination of the vagina for both groups. The smears were stained with Diff-Quick stain set (ADR Group, Mediko Kimya, Istanbul, Turkey) according to the manufacturer's instructions. Slides were examined by a light microscope (Olympus CX41, Tokyo, Japan) at x 400 magnification.

Statistical analysis: Statistical analyses were performed with SPSS 13.0 (SPSS Inc, Chicago, Illinois, USA). Normality of the data was analyzed by Shapiro Wilk test. The normally distributed data were tested by One-way ANOVA for the comparison of the groups with regard to the hematological parameters. The Pearson correlation was used to determine the relationships among the parameters evaluated. Values were given as mean ± standard error of the mean (SEM). The significance level was accepted as $P<0.05$.

Results

The mean ages and SEM in group C and group PYO were 4±0.45 years and 5±0.68 years; respectively ($P>0.05$). Vaginal cytology smears of all queens included into the study had intermediate cells on the slide while polymorphonuclear neutrophils were dense in group PYO. Also, background of the smear slides were not clean in queens with open cervix pyometra. In group PYO, enlarged uterine horns and uterine wall were observed in abdominal ultrasonography. Also, uterine lumen was visualized as fulfilled with pus in pyometra affected queens. The corpora lutea were observed in the ovaries of the queens during ovariohysterectomy. The mean values and SEM of hematological parameters and their significances related to the groups were presented in Table 1, whereas the relationships among the evaluated parameters were given in Table 2. Accordingly, positive correlations were observed between PHOS and ALKP ($P<0.01$, $r=0.423$), CA and CREA ($P<0.05$, $r=0.341$), GLOB and TP ($P<0.001$, $r=0.872$), BUN and CREA ($P<0.001$, $r= 0.511$).

Table 1. The concentration of white blood cell and biochemistry profile of healthy (Group H), open (Group OP) and closed-cervix pyometra (Group CP) groups.

	Group C (n=23)	Group OP (n=15)	Group CP (n=10)	Sig.
WBC (10^9/L)	8.96±0.75^a	23.42±4.34^b	41.95±8.40^c	P<0.001
GLU (mg/dL)	120.69±7.00	120.75±7.63	151.60±21.19	ns
CREA (mg/dL)	1.40±0.07	1.34±0.07	1.34±0.31	ns
BUN (mg/dL)	20.58±1.28	20.62±2.25	18.80±3.01	ns
BUN/CREA (mg/dL)	15.95±0.84	16.37±2.39	15.60±1.60	ns
TP (g/dL)	7.50±0.12^a	7.98±0.18^{ab}	8.03±0.22^b	P<0.05
ALB (g/dL)	3.23±0.06^a	3.00±0.06^b	2.98±0.07^b	P<0.05
GLOB (g/dL)	4.27±0.11^a	4.95±0.20^b	4.88±0.23^b	P<0.01
ALB/GLOB	0.76±0.03^a	0.62±0.02^b	0.62±0.03^b	P<0.01
ALT (U/L)	63.86±7.36^a	78.37±21.90^a	154.10±35.83^b	P<0.01
ALKP (U/L)	30.56±2.99^b	50.39±5.10^a	58.10±10.69^a	P<0.01
Ca (mg/dL)	9.87±0.18	9.75±0.12	9.92±0.24	ns
PHOS (mg/dL)	4.95±0.18^a	4.66±0.20^a	5.95±0.39^b	P<0.01

a, b, c : Different letters indicate the significance. ALB: Albumin, ALKP: Alkaline Phosphatase, ALT: Alanine aminotransferase, BUN: Blood urea nitrogen, Ca: Calcium, CREA: Creatinin, GLOB: Globulin, GLU: Glucose, PHOS: Phosphorus, TP: Total protein, WBC: White blood cell. ns: P>0.05

Table 2. Pearson correlation assessment of the parameters related to the haematology and biochemistry both in open and closed cervix pyometra.

	WBC	GLU	CREA	BUN	BUN/ CREA	TP	ALB	GLOB	ALB/ GLOB	ALT	ALKP	CA	PHOS
WBC	1	0.240 ns	0.150 ns	0.189 ns	0.120 ns	0.144 ns	-0.394 **	0.249 ns	-0.353 *	0.088 ns	-0.177 ns	0.048 ns	0.177 ns
GLU	0.240 ns	1	0.245 ns	0.199 ns	0.011 ns	0.184 ns	-0.020 ns	0.234 ns	-0.187 ns	-0.04 ns	-0.238 ns	0.035 ns	-0.249 ns
CREA	0.150 ns	0.245 ns	1	0.511 ***	-0.391 **	0.022 ns	0.004 ns	0.042 ns	-0.083 ns	-0.173 ns	-0.126 ns	0.341 *	0.037 ns
BUN	0.189 ns	0.199 ns	0.511 ***	1	0.403 **	-0.069 ns	0.048 ns	-0.068 ns	0.036 ns	-0.187 ns	-0.269 ns	0.167 ns	-0.094 ns
BUN/ CREA	0.120 ns	0.011 ns	-0.391 **	0.403 **	1	-0.131 ns	0.078 ns	-0.091 ns	0.022 ns	-0.052 ns	-0.051 ns	-0.176 ns	-0.066 ns
TP	0.144 ns	0.184 ns	0.022 ns	-0.069 ns	-0.131 ns	1	0.033 ns	0.872 ***	-0.616 ***	0.044 ns	0.153 ns	0.140 ns	0.199 ns
ALB	-0.394 **	-0.02 ns	0.004 ns	0.048 ns	-0.078 ns	0.033 ns	1	-0.352 *	0.676 ***	0.041 ns	0.184 ns	0.196 ns	-0.136 ns
GLOB	0.249 ns	0.234 ns	0.042 ns	-0.068 ns	-0.091 ns	0.872 ***	-0.352 *	1	-0.866 ***	0.004 ns	0.79 ns	0.065 ns	0.181 ns
ALB/ GLOB	-0.353 *	-0.187 ns	-0.083 ns	0.036 ns	0.022 ns	-0.616 ***	0.676 ***	-0.866 ***	1	0.001 ns	0.010 ns	0.014 ns	-0.270 ns
ALT	0.088 ns	-0.040 ns	-0.173 ns	-0.187 ns	-0.052 ns	0.044 ns	0.041 ns	0.004 ns	0.001 ns	1	0,236 ns	0,005 ns	0,184 ns
ALKP	-0.177 ns	-0.238 ns	-0.126 ns	-0.269 ns	-0.051 ns	0.153 ns	0.184 ns	0.079 ns	0.010 ns	0.236 ns	1	0.036 ns	0.423 **
CA	0.048 ns	0.035 ns	0.341 *	0.167 ns	-0.176 ns	0.140 ns	0.196 ns	0.065 ns	0.014 ns	0.005 ns	0.036 ns	1	-0.189 ns
PHOS	0.177 ns	-0.249 ns	0.037 ns	-0.094 ns	-0.066 ns	0.199 ns	-0.136 ns	0.181 ns	-0.270 ns	0.184 ns	0.423 **	-0.189 ns	1

ALB: Albumin, ALKP: Alkaline Phosphatase, ALT: Alanine aminotransferase, BUN: Blood urea nitrogen, Ca: Calcium, CREA: Creatinin, GLOB: Globulin, GLU: Glucose, PHOS: Phosphorus, TP: Total protein, WBC: White blood cell. ns: P>0.05 , *: P<0.05 , **: P<0.01 , ***: P<0.001

Discussion

Pyometra which is an inflammatory disorder of the uterus, usually occurs over 5 years of age in queens (Hagman et al., 2014). Although the significant differences were not observed related to the ages of the queens in either group, the mean age of the group PYO was at the verge age which was reported before (Hagman et al., 2014).

Hormones and bacteria are involved in the disease development (Hagman 2022). The diagnosis is based on case history, clinical signs and findings on physical examination, hematology and biochemistry laboratory tests, and diagnostic imaging techniques (especially B-mode ultrasonography) (Keshavprasad et al., 2023). The same diagnostic methods with the previous report (Keshavprasad et al., 2023) were used to determine the pyometra in this study.

Most of the queens with pyometra show abnormal WBC which is frequently characterized by leukocytosis (Agudelo, 2005). As previously reported, the mean WBC was significantly higher in group PYO than in healthy queens in this study. While increased total leukocyte counts may be found in the cat with open-cervix pyometra, normal leukocyte counts may also be encountered (Hollinstead and Krekeler, 2016). In contrast with the researchers' report, definite rise in WBC was also observed in group OP as compared to the Group C, in this study. The total leukocyte count is generally increased in the cat with closed-cervix pyometra, usually exceeding 30x109/L (Kenney et al., 1987). Similarly, the highest WBC levels were detected in Group CP in the study. Absolute leukocytosis in closed cervix pyometra is thought to be depended on the severity of the disease and septicemia.

The renal dysfunction may secondarily be developed due to the bacterial endotoxemia, dehydration and azotemia (Alaçam, 1998). Unlike bitches, queens have significantly less pyometra-related kidney damage (Hollinstead and Krekeler, 2016). It was stated that a high CREA concentration was determined in 12% of the cats with pyometra (Kenney et al., 1987). Nak et al. (2005) reported that there was no difference between pyometra and healthy queens in terms of BUN and CREA values ($P>0.05$). In this study, GLU, BUN and CREA levels were not significantly different related to the presence of pyometra ($P>0.05$). It is thought that the similar results have been obtained in accordance with the previous studies, since the lower incidence of renal failure is more common due to pyometra the in female cats.

Enginler et al. (2014) detected that TP concentrations showed a significant increase in pyometra group than those measured in healthy controls. In this study, the mean TP level of the queens with closed cervix pyometra was significantly higher as compared to the queens with the open cervix pyometra and the control group. The finding might be explained by the suggestion of Borresen and Skreden (1980) who found the similar results and indicated that plasma protein levels in pyometra cases has changed as a result of acute phase reactions.

Albumin is considered to be a negative acute phase protein as the serum concentrations decrease in inflammation and/or infection (Hagman 2012). Fantoni et al. (1999) stated that vascular permeability increase due to the sepsis and endotoxemia which subsequently led to loss of protein. Vilhena et al. (2018) have detected the decreased ALB concentrations in queens with pyometra. In accordance with Vilhena et al. (2018), serum ALB concentration was significantly higher in healthy queens than those detected in the Group PYO. The similar results may have been obtained due to the decreased production and increased loss of this protein during systemic inflammation (Fantoni et al., 1999; Werner and Turnwald, 1999; Hagman, 2012).

In feline pyometra, hyperglobulinemia is usually one of the mild changes in serum biochemistry (Hollinstead and Krekeler 2016). Johnson (1994) reported that hyperglobulinaemia can be present in 30 -60% of the pyometra cases in cats. In consistent with the previous reports, the mean levels of GLOB in both pyometra groups (group CP and group OP) were significantly higher than group C in this study. Alpha-2 or beta globulins increase in the acute inflammation, and gamma globulins increase in the chronic inflammation (Comazzi et al., 2004). This may explain why hyperglobulinemia is detected in both open and closed cervix pyometra.

Pyometra can cause liver disarrangement (Nak et al., 2005). In a group of 183 cats with pyometra, only 7% with high ALT activity was reported (Kenney et al., 1987). Although the ALT obtained in this study was not at a low percentage as in Kenney's report, the ALT level was also found to be high in the closed cervix pyometra group. Increase of ALT levels may be due to the hepatocellular damage caused by septicemia, diminished hepatic circulation and cellular hypoxia in the dehydrated cats as Nak (1999) reported.

The most sensitive tests for hepatic diseases are ALT and ALKP which rise especially in the presence of sepsis and dehydration (Hollinstead and Krekeler, 2016). Increase in ALKP is often associated with hepatobiliary obstruction, with a very high sensitivity in the dog in comparison to the cat (Comazzi et al., 2004). In both open and closed cervix pyometra cases, elevated ALKP levels were observed in this study. Also, the queens with closed cervix pyometra had the highest ALKP level. These changes reflect hepato-cellular damage in response to toxemia, sepsis or decreased hepatic perfusion due to the dehydration as Verstegen et al. (2008) stated.

It was reported that significant differences were not observed, when the serum calcium levels of bitches suffered from pyometra were compared with the healthy ones (Asheim, 1964). Similar to the previous report (Asheim, 1964), the mean Ca level in Group PYO was not significantly different than those measured in the Group C in this study. King et al. (2007) detected mild correlation between plasma creatinine concentration and plasma calcium levels in cats with chronic kidney disease. Similarly, creatinine concentration was significantly but not highly correlated with calcium in this study ($P<0.05$ and $r=0.341$). Pyometra can cause polyuria which shows the reduction of renal concentrating ability. A similar renal dysfunction occurs in conjunction with potassium depletion and with hypercalcemia (Asheim, 1964). Considering the results of our study, it is thought that not only calcium and creatinine but also potassium should be evaluated for kidney damage/ dysfunction.

Verstegen et al. (2008) observed elevated serum ALKP levels in approximately 50–75% of pyometra cases in bitches. Also, high circulating PHOS levels lead to hepatic dysfunction by considerably lowered initial blood sugar and the glycogen content of the liver (Althausen and Thoenes, 1932). In this study, serum ALKP levels were strongly correlated with serum PHOS levels ($P<0.01$; $r=0.423$). This positive relationship

could be explained by the effect of both parameters on liver function as the researchers previously reported (Althausen and Thoenes, 1932; Verstegen et al., 2008).

Chung et al. (2003) suggested that lower serum phosphorus levels in patients who recovered from hepatic failure may be associated with recovery of hepatic function. Also, hyperphosphatemia had been associated with the lower survival rate and increased morbidity in cats with chronic kidney disease (King et al., 2007). Koo et al. (2011) reported the serum PHOS levels within the reference ranges in two young dogs with closed-cervix pyometra. Unlike the above mentioned research (Koo et al. 2011), the highest inorganic PHOS levels were detected in Group CP in this study. Particularly in cases of closed cervix pyometra, severe systemic clinical signs are often observed as renal and hepatic dysfunction (Kurtzler et al., 2012). The hyperphosphatemia in queens with closed cervix pyometra could be explained by the more frequent occurrence of renal and hepatic dysfunction in pyometra cases (Kurtzler et al, 2012) and negative impact of serum PHOS levels on the hepatic and renal function (Chung et al., 2003; King et al., 2007). To the best of our knowledge, this is the first report which is detected hyperphosphatemia in closed cervix pyometra in queens.

In conclusion, pyometra is a serious disease that needs to be well evaluated with laboratory tests in order to reveal its multi-systemic effects. Although transabdominal ultrasonography is a golden standart to diagnose the pyometra, hemogram and biochemistry tests are essential to diagnose the multi-systemic effects of this illness. Besides, the presence of hyperphosphatemia could be predictive to reflect the hepatorenal dysfunction in closed cervix pyometra cases of queens.

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Morphological characteristics of Şebap pigeons (*Columba Livia Domestica*)

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ABSTRACT

Şebap pigeon is unique to Şanlıurfa region, which has a deep pigeon breeding culture in Turkey. In this region pigeon breeding is a tradition. In Şanlıurfa there are 5 different colours varieties of Şebap, including Miski, Kürenk, Çakmaklı, Gök and Arap, which were determined by Şebap Pigeon Association and Federation. The objective of this study is to determine the phenotypic characteristics of the Şebap pigeon. Animal material (n=132) of the study consists of stated varieties, which were analyzed according to age and as well as gender (n=66 male, n=66 female). Age groups were formed on the basis of 4 development periods, including 06-12 months (Group 1, n=28), 13-24 months (Group 2, n=35), 25-36 months (Group 3, n=37) and >36 months (Group 4, n=32). The distribution of varieties was as follows: Miski (57.5%), Kürenk (16.7%), Çakmaklı (11.4%), Gök (11.4%) and Arap (3.0%). According to morphological characteristics analyzed in the study, the difference between the gender groups in terms of body length was found to be statistically significant ($p < 0.01$). The Şebap pigeon is represented by a federation of 28 associations in Turkey. Since the beginning of the 20th century. Şebap pigeon has a large head and thick neck. The beak is bluish grey in the Çakmaklı and Gök varieties and vibrant pink in the Miski, Kürenk and Arap varieties. The findings of this study support the view that Şebap pigeon is breed. However, it would be meaningful to support the results obtained for morphometric characterization with future genetic characterization studies.

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Introduction

As a member of Columba genus and Columbidae family, apart from few exceptions the rock pigeon constitutes of morphologically uniform family. The Columbidae family has 5 sub-families, including Columbinae, Otidiphabinae, Treroninae, Gourinae and Didunculinae. Among these sub-families Columba is the largest genus of this family. Columba genus is used to refer rock pigeons generally represented by the livia (Biray, 2019) The monogamous nature of pigeons provides convenience to breeders and makes it possible to develop the breed in the same dovecote and to obtain new breeds for enthusiasts (Darwin, 1976). The common pigeon and feral pigeon, that we see in our daily lives, were the members of the Columba. The domestication of the pigeon dates back around 10 000 years ago (Blasco et al., 2014; Çelik, 2022). Throughout human history pigeon fulfilled the needs of human beings as a food source. It was used for the purposes of communication and racing animal. More importantly, the pigeon satisfied the enthusiasts thanks to the aesthetic appearance of different breeds. They were accepted as a religious symbol, and today they are the symbol of peace (Atasoy et al., 2013). Mesopotamian figurines, mosaics, coins and tablets portraying pigeon's dates back 5000 years ago (Biray, 2019; Çelik, 2022).

In Şanlıurfa, the number of individual bird keepers referred as "curious fancier people" is high

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(Kürkçüoğlu, 2011). There are many special places where pigeon shows, and trades are held. As such, pigeon breeding stands out not only as a branch hobby breeding, but also as a common commercial activity.

The Şebap pigeon breed is represented by a federation of 28 associations in Turkey. Since the beginning of the 20th century (Şebap Güvercin Federasyonu, 2021), Şebap has widely been bred in the south of Turkey. Although it has similar color and physical characteristics of the Turkish tumbler pigeon (Taklacı), Şebap has been differentiated from the Tumbler breed and has preserved its current characteristics for a century. Today it is used as a costume bird. General appearance of Şebap can be described as medium in size, having fluffy and soft plumage, with a large head and thick neck, and plump legs. The color of chest fur and eye color varies according to body fur. The distinguishing feature of Şebap is that it has white middle tail feather (Şebap Güvercin Federasyonu, 2021).

Although there are studies focusing on the characteristics of different native pigeon breeds, such as Bursa Oynarı (Balcı et al., 2018), Cakal, Mulakat (Özbaşer et al., 2020), Muradiye Dönek (Özbaşer et al., 2021), Edremit Kelebek Roller Pigeon (Erdem et al., 2018), Thrace roller (Soysal et al., 2011), Squadron flyer (Özbaşer et al., 2016), Scandaroon pigeons (Yıldırım et al., 2018), Klasik Manisa Hünkârisi (Türkeş and Gündüz 2021) and Alabadem (Erdem et al., 2021) of Turkey, the characteristic of Şebap pigeon have not been described in detail elsewhere. Considering the gap in the literature, this study was carried out to define the phenotypic characteristics of the Şebap pigeon. It aims to contribute existing literature by giving a direction to breed contradictions of Şebap pigeon native to Şanlıurfa and Turkey.

Material and Methods

Pigeon: This research was carried out on Şebap pigeons raised by Şanlıurfa Şebap Pigeon Association and local breeders of Şanlıurfa province. The animal material of the study consisted of a total of 132 Şebap pigeons from 5 different varieties, whose gender distributions as male (n=66) and female (n=66). The animal material was also taken from different age groups. The pigeons were classified according to their ages: 06-12 months (Group 1, n=28), 13-24 months (Group 2, n=35), 25-36 months (Group 3, n=37), and >36 months (Group 4, n=32).

Data of Şebap pigeons were collected from association and 5 independent breeders. In addition to measurements and observations to determine the distinctive features of the Şebap pigeon, such as plumage, markings, trotters, head structure, eye color, tail and wing feathers, specifications and local terms used by the federation and pigeon breeders were used.

Morphological characterization: Characteristics of beak type, beak tip color, nail color, head type and eye color were determined with the naked eyes qualitatively. Photographs were also taken (Canon EOS 650D and canon EF 50 mmF1.8 lens). Photographs of the pigeons were taken in 60×50 cm special boxes which are illuminated from the right, left and above, and covered with a black cloth of 500x300 cm in order to distract the pigeons from the outside stimuli. Measurements were taken by the same people throughout the study.

The number of wing and tail feathers was counted; The wing feathers were followed in the order of primary axial and secondary feathers (Table 1).

Body length, wingspan, wing length, tail length, chest circumference, chest width, head length, head width, beak length and beak depth were measured. The pigeons were weighed with the aid of a scale sensitive to 1 g (Gamry). Body length, wingspan, wing length, body length, tail length, and chest circumference were measured using a measuring tape, and chest width, head length and width, beak length and depth, and body diameter were measured with digital calipers (Atasoy et al., 2013; Özbaşer et al., 2016; Erdem et al., 2021; Çelik , 2021).

Table 1. Number of wing and tail feathers in Şebap pigeon.

Morphological characteristics	Ratio (%)
Number of wing feather	
10-1-10	80.3
10-1-11	13.6
10-1-9	6.1
Number of tail feather	
12 feathers	78.8
13 feathers	12.9
14 feathers	5.3
15 feathers	3.0

Statistical analysis: All statistical analyzes were carried out by using the IBM SPSS 22 program package. One-Way ANOVA procedure was used to analyze the difference between groups for measured characteristics. The methods of Independent-Samples T-tests analyzes were used between genders (Özdamar, 2001; Soysal, 2000). The conformity of the data to the normal distribution was examined using the homogeneity of variance test. The “Descriptive” method was adopted for explicative statistics subsequently. The factors that reveal significant effects were compared in Duncan test (Duncan, 1955).

Results

Şebap pigeons are divided into varieties according to the color of the feathers covering the body. In the Arap Variety, all the feathers covering the body are black. In other varieties, the main color covering the body is light shiny cream or bluish white. However, they are separated according to black or brown markings in the chest and wing regions. These marks in the wing area are called stamps. Accordingly, in the Miski variety, patterns on wings are dark vibrant brown in color, the shapes are clear, coarse-grained, and cover the wing symmetrically. in the Çakmaklı variety, patterns on wings are black. In the Kürenk and Gök varieties, the patterns on the wing are not in the form of scales, but rather in the form of columns. The color of these columns is brown in the Kürenk variety and black in the Gök variety. (Figure 1, Figure 2).

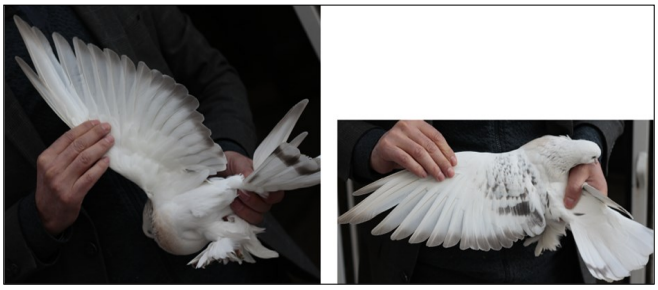

Figure 1. Examples of Wings in Şebap pigeons.

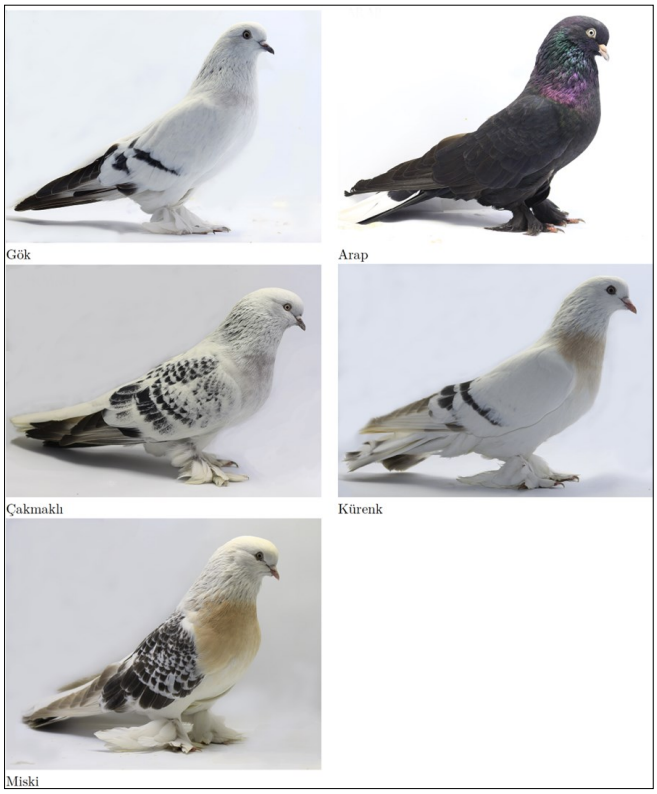

Figure 2. Color variants of Şebap pigeons; Gök, Arap, Çakmaklı, Kürenk, Miski

Taking into consideration the measurements, observations and evaluations previously determined by the breeders and Şanlıurfa Şebap Pigeon Federation it was found that 5 different color variations were determined among the 132 pigeon samples, which are Miski 57.5 % (n=76), Kürenk 16.7 % (n=22), Çakmaklı 11.4 % (n=15), Gök 11.3 % (n=15) and Arap 3.0 % (n=4) (Figure 2).

Statistical analyzis displayed significant variations between different age groups in terms of wing length and tail length characteristics ($P<0.05$). Significant differences in wingspan, chest circumference and chest width ($P<0.01$) were also determined. Mean values for body weight, body length, head length, head width, beak depth, and beak length in different groups were found as statistically significant ($P<0.001$).Comparative differences of these measurements in terms of genders; body weight, head length, beak depth and shank length ($P<0.001$); body length, wingspan, head width, beak length and tail length ($P<0.01$) and wing length ($P<0.05$) (Table 2) were significant.

Three different eye colors were determined in the varieties of Şebap. In the Miski and Çakmaklı variety, pearl eye color, in which the iris turns a yellow straw color from the pupila to the outer rim, has been observed. In the Kürenk and Gök varieties, the iris turns into slightly orange (amber) colour from the pupil to the outer rim and lastly in the Arap variety, iris is pearly white (Figure 3).

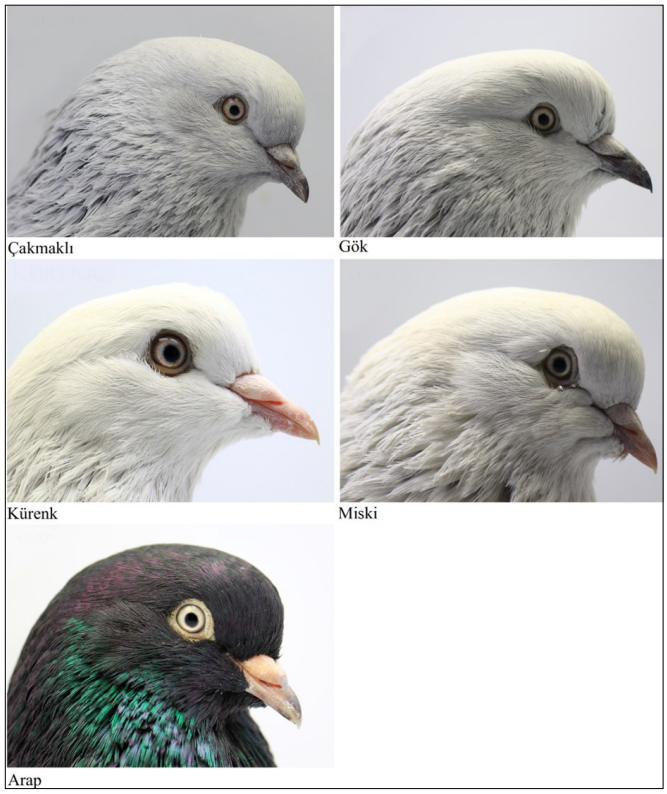

Figure 3. Head structure, eye colors and beak types of Şebap pigeons. Çakmaklı, Gök, Kürenk, Miski and Arap

Table 2. The statistical values of the morphometric characteristics (X±Sx) in Şebap pigeon

Şebap Age Group	n	Body Weight (g)	Beak length (mm)	Beak depth (mm)	Head length (mm)	Head width (mm)	Shank length (mm)
1	28	339.82±3.38^{a}	17.58±0.05^{a}	8.18±0.05^{a}	34.55± 0.11^{a}	23.19± 0.11^{a}	42.57±0.28
2	35	354.86±2.64^{b}	17.73±0.06^{a}	8.35±0.05^{b}	34.83±0.11^{ab}	23.37± 0.11^{a}	42.90±0.26
3	37	359.59±2.60^{bc}	17.92±0.06^{b}	8.40±0.04^{bc}	34.91±0.10^{b}	23.88±0.11^{b}	43.11±0.24
4	32	365.16±2.51^{c}	17.93±0.06^{b}	8.52±0.05^{c}	35.24±0.09^{c}	24.28±0.11^{c}	43.53±0.25
Total	132	355.49±1.57	17.80±0.03	8.37±0.03	34.89±0.05	23.70±0.07	43.04±0.13
Age		***	***	***	***	***	-
Female		350.00±2.33	17.72±0.05	8.26±0.04	34.63±0.07	23.51±0.08	42.36±0.17
Male		360.98±1.90	17.88± 0.04	8.47± 0.03	35.15±0.07	23.88±0.10	43.73±0.16
Gender		***	**	***	***	**	***
Şebap Age Group	n	Wing length (cm)	Wingspan (cm)	Tail length (cm)	Body length (cm)	Chest perimeter (cm)	Chest Width (cm)
1	28	32.04±0.29^{a}	71.71±0.46^{a}	14.88±0.13^{a}	36.27±0.23^{a}	21.64±0.17^{a}	5.41±0.04^{a}
2	35	32.74±0.22^{b}	73.47±0.44^{b}	15.11±0.12^{ab}	37.00±0.16^{b}	22.18±0.14^{b}	5.54±0.03^{b}
3	37	32.80± 0.19^{b}	73.49±0.34^{b}	15.28±0.11^{b}	37.11±0.16^{b}	22.19±0.13^{b}	5.55±0.03^{b}
4	32	33.03± 0.18^{b}	73.84±0.39^{b}	15.33±0.10^{b}	37.47±0.17^{b}	22.41±0.14^{b}	5.60±0.03^{b}
Total	132	32.68±0.11	73.19±0.21	15.16±0.06	36.99±0.09	22.12±0.07	5.53±0.02
Age		*	**	*	***	**	**
Female		32.40±0.16	72.52±0.29	14.99±0.07	36.70±0.13	22.04±0.11	5.51±0.03
Male		32.95±0.15	73.87±0.29	15.33±0.09	37.28±0.13	22.21±0.10	5.55±0.03
Gender		***	***	***	***	-	-

-: P>0.05; *: P<0.05; **: P<0.01; ***: P<0.001, a-c means within a column with different letters are significantly different (P<0.05).

The feathers growing sideways covering the tarsal area are called trotter. In Şebap breed the trotters are sword type and all the Şebap pigeons have trotters. The color of the trotter feathers is the same color as the body feathers, it is black in Arap variety and white in other varieties. These feathers are soft, curved and overlapping. They give the foot a thick appearance. The leg pads above the heel are curved in and out, not very hard and long feathers are arranged under the heel in a symmetrical form that supports each other (Figure 4).

In the Şebap pigeon breed, the dark part near the ends of the light-colored tail feathers is called Toka (Clip) The tail clip was determined to be vibrant dark brown in the Miski variety, light brown in the Kürenk variety, and black in the Çakmaklı and Gök variety. The total number of tails in the Şebap pigeon is between 12 and 14. In the Miski, Çakmaklı, Kürenk and Gök variety, two to four long tail feathers are not white symmetrically on the sides, the side feathers are equal on the right and left, and up to four tail feathers in the middle are white. The entire or part of the tail cannot consist of only white tail feathers. In the Arap variety the tail is completely black. (Fig. 5).

Figure 4. Examples of trotters in Şebap pigeons.

Figure 5. Example of tails feather in Şebap pigeon.

Discussion

The topic of domestication mainly focuses on the mammals and oversees the importance of other species like birds (Blasco et al., 2014). Domestic pigeon is derived from wild rock dove, and it is the oldest domesticated bird species (Cobo-Simón et al., 2020; Price, 2002; Rose et al., 2006). As being a small prey, domestication process of birds can't be proved by the bone evidence, therefore cuneiform tablets can be considered as tell-tale physical evidence which were found in Mesopotamia.

Adult Rock pigeon is at 29-37 cm body length. It has 62 to 72 cm wingspan. According to the standard measurements, the wing is usually ranges from 22.3 cm, whereas the tail 9.5 to 11 cm, the beak is around 1.8 cm, and the shank length of 2.6 to 3.5 cm (Rock dove, 2022). In comparison with their wild ancestors and common feral pigeon, domestic pigeons show great differences in morphological structure, especially in the color, length and distribution of the feathers, as well as the anatomy of the head, differences in the beak and claws. These features significantly affect the appearance (Özbaşer et al., 2021; Parés-Casanova and Kabir, 2019; Shapiro et al., 2013; Vickrey et al., 2018). The intense selective breeding lead by enthusiasts resulted in the emergence of many breeds and varieties around the world (Johnston, 1990; Murton et al., 1972; Price, 2002; Stringham et al., 2012). This is the reason why breeders take utmost care to preserve the pedigree of pigeons and to breed birds selectively (Balcı et al., 2018; Baptista et al., 2019; Bartels, 2003).
Si et al (2021) claimed that the domestic pigeon basically displays three main iris colors, including yellow to orange "pebble", white "pearl" and black "bull's" eyes. The Bull's eye is due to the complete absence of stromal pigment cells, whereas pebble and pearl irises in pigeons contain bright pigment cells with birefringent crystals in the anterior stromal tissue. Although the bull's eyes feature was associated with white feathers in some studies (Bond, 1919; Hollander and Owen, 1939), it was not observed in Şebap varieties.

The average of body length, which was determined as 36.99 cm in Şebap pigeons, is similar to Squadron flyer pigeons (Özbaşer et al., 2016). It was found as 36.48 cm. This was higher than the values obtained from Klasik Manisa Hünkârisi (Türkeş and Gündüz, 2021) which is 31.40 cm, Alabadem pigeons (Erdem et al., 2021) which is 31.56 cm, Tumbler pigeons (Atasoy et al., 2013) in the province of Ankara, which is 34.95 cm, and Trakya Makaracısı pigeons (Soysal et al., 2011) which is 34.42 cm. The results confirm the reports of the federation of the Şebap pigeon as a medium-sized breed.

Soysal, et al. (2011) determined the morphological characteristics of the Trakya Makaracı pigeon breed and reported that the chest width was higher in females than males. However, in Sebap pigeons, the overall mean chest width was found to be higher in males. Similar findings were reported by Atasoy et al. (2013), Özbaşer et al. (2021) and Yıldırım, et al. (2018) The overall mean chest width was found to be 55.30 mm in Şebap pigeons. This finding is similar to Bursa Oynarı 56.00 mm (Balcı et al., 2018), Scandaroon Pigeon 55.75 mm (Yıldırım et al., 2018) and Alabadem 56.86 mm pigeons (Erdem et al., 2021). However, higher numbers were reported in Tumbler pigeons (Atasoy et al., 2013) which are found in the city of Ankara and Squadron flyer pigeons (Özbaşer et al. 2016). They are 62.98 mm and 65.03 mm respectively. Moreover, in terms of chest width, the difference between age groups was significant ($p < 0.01$), but the difference between genders was not statistically significant.

When compared with different breeds in Turkey in terms of body weight. The average body weight of Şebap pigeons was determined as 355.49 g. This body weight was close to the average values of Bursa Oynarı pigeons 341.95 g (Balcı et al., 2018). Şebap pigeons determined to be lighter than Squadron flyer pigeons, which is 428.85 g (Özbaşer et al., 2016) and Cakal pigeon which is 374.02 g (Özbaşer et al., 2020); heavier than Alabadem which is 321.17g (Erdem et al., 2021), Tumbler pigeons (Atasoy et al. 2013) in province of Ankara which is 321.62 g, Mülekat pigeon (Özbaşer et al., 2020) which is 328.96 g and Trakya Makaracısı (Soysay et al., 2011) which is 335.58 g. Body weight values of Şebap pigeon were found to be higher in males than females in the gender groups. These findings are similar to the results reported in the studies above (Atasoy et al., 2013; Özbaşer et al. 2020, Yıldırım et al., 2018).

When the body weight, wingspan and length values of the Şebap pigeons are considered together, it is seen that they generally have heavier and longer wings than the game bird pigeon breeds and genotypes. In fact, it has a wingspan value close to the size of a large Scandaroon pigeon. It is possible to say that the medium size of the Şebap pigeons restricts its game behavior and maneuvering movements and therefore it is more often bred as costume pigeons.

Although it requires knowledge, expense and organizational skills, there are economic, scientific, cultural and ecological benefits for the protection of local genetic resources of a country. Animals, as a local genetic resources and an element of biodiversity, are frequently investigated in terms of genetics and various morphological characteristics for not only to shed light on phylogenetic studies, but also to develop more efficient production systems and the studies of genetic improvement (Casanova, 2013). For this reason, studies started to recognize Şebap pigeon breed as a local genetic resource. On this basis, morphological measurements followed by the genetic association and characterization studies are important for breed description.

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Usage areas of nanoparticles in veterinary dermatology

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ABSTRACT

Şebap Elements such as gold and silver have been used to treat various diseases since ancient times. These elements, which are used today, have been diversified and sized through research.Recently, it is seen that nanoparticles are frequently used in the medical field. Nanoparticles can be 1-1000 nanometers in size and gain biological, physical and chemical functionality due to their nano-size. The type and size of these nanoparticles are chosen according to the area in which they will be used. These prepared drugs are used for purposes such as biosensor imaging, transporting drugs to the target organ, protecting the transported substance against denaturation, increasing the immunological response, and transporting chemotherapeutic drugs. Today, with the increase in the number of dermatological cases in clinics, different treatment methods and systems are being developed. There are various nanoparticles used in dermatological cases to increase the bioavailability of topical, oral or injectable drugs and to increase the effect in the targeted area. These drugs have been used in conditions such as antimicrobial, antiparasitic, antifungal, allergen-specific immunotherapy, wound healing, tumors and atopic dermatitis. Many studies have also been carried out in the field of dermatology and it has been shown that nanoparticles used for follicular application provide advantages in dermal drug delivery, including improved skin bioavailability, increased depth of penetration, prolonged residence time, rapid transport to the skin and tissue targeting, in dermal drug delivery by using the appropriate nanoparticles in the right sizes. Particles can collect in the follicular opening and penetrate through the follicular canal when applied to the skin surface. This review has been prepared to investigate the usability of nanoparticle-derived drugs used in human medicine in veterinary applications.

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Introduction

Background: Today, Nanoparticles (NP) and nanostructured materials (NSM) represent an active research area and techno-economic sector with a full expansion in many application areas. NPS and NSMs have gained importance in technological advances due to their adjustable physicochemical properties such as melting point, wettability, electrical and thermal conductivity, catalytic activity, light absorption, and scattering. A nanometer (nm) is a unit of the International System of Units (Système international d'unités, SI), representing a length of 10-9 meters. In principle, the diameters of NSMs are usually defined as 1- to 100 nm. Today, several legislation in the European Union (EU) and the USA specifically refer to

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Nanomaterials. The use of different definitions in different jurisdictions is a major obstacle to regulatory efforts, as it creates legal hesitations in applying regulatory approaches to the same names. Therefore, there is no single internationally accepted definition for NMs (Jeevanandam et al.,2018; Karaoğlan,2017).

History of nanomaterials

More than 4500 years ago, humans benefited from the reinforcement of ceramic matrices containing natural asbestos nanofibers. Medically, gold nanoparticles have been used in various medicines from ancient times to the present day. In 2500 BC, China used nanoparticles to treat diseases. Also, many ancient cultures, such as those in India and Egypt, used gold-based medicinal preparations (Huaizhi and Yuantao, 2001). In recent years, gold nanoparticles have been used frequently as catalysts, carriers, and biosensor applications thanks to their properties (Dağlar, 2009).

Scientific terms about nanoparticles

The British Standards Institute has recently proposed definitions for scientific terms used in nanotechnology. If we give an example of definitions; Nanoscience is the science and study of matter at the nanoscale, understanding its size and structure-related properties, and comparing the emergence of individual atoms or molecules or differences in the bulk material. Nanotechnology is the manipulation and control of matter at the nanoscale using scientific knowledge of various industrial and biomedical applications. A nanomaterial is a material that has any internal or external structure at the nanoscale. A nanoparticle is a nano-sized nano-object (PAS 71:2011, Nanoparticles. Vocabulary. British Standards Institution: London, United Kingdom, 2011).

Classification of nanoparticles

Most existing NPS and NSMs can be organized into four item-based categories:

Carbon-based nanoparticles: Generally, these NMs contain carbon and exist in morphologies such as hollow tubes, ellipsoids, or spheres. Fullerenes (C60), carbon nanotubes (CNTs), carbon nanofibers, carbon black, graphene (Gr), and carbon bulbs are included in the carbon-based NM category. Laser ablation, arc discharge, and chemical vapor deposition (CVD) are important production methods in the manufacture of these carbon-based materials (excluding carbon black) (Kumar and Kumbhat, 2016).

Inorganic-based nanoparticles: These NMs include metal and metal oxide NPs and NSMs. Metals such as Au or Ag NPs can be synthesized into metal oxides such as TiO2 and ZnO NPs, and semiconductors such as silicon and ceramics (Jeevanandam et al., 2018).

3- Organic-based nanomaterials: These include NMs mostly made of organic matter, excluding carbon-based or inorganic-based NMs. The use of non-covalent (weak) interactions for the self-assembly and design of molecules helps convert organic NMs into desirable structures such as dendrimers, micelles, liposomes, and polymer NPs (Jeevanandam et al., 2018).

Composite-based nanomaterials: Composite NMs are nanoscale-sized one-phase multiphase NPs that can combine NPs with other NPs or NPs combined with larger or bulk type materials (hybrid nanofibers) and are NSMs. Composites can be any combination of carbon-based, metal-based, or organic-based NM with any form of metal, ceramic, or polymer bulk material (Jeevanandam et al., 2018).

Nanoparticle formulations

Polymeric nanoparticles: Polymeric nanoparticles are prepared by combining the active substance/drug with a polymer. The active ingredients are dissolved, held, or adsorbed onto the polymer nanoparticle surface. It also finds widespread application in veterinary medicine (Underwood and Van Eps, 2012). Studies have stated that nanoparticles carrying molecules with antigenic properties provide promising results in immunology. In veterinary medicine, nanoparticles, which are given in drinking water and released into the intestine, are used to protect animals against parasites (Alonso, 1996; Derman et al., 2013).

Solid lipid nanoparticles consist of lipids that are solid at room temperature, equilibrated with a surfactant, and suspended in an aqueous solution. The pharmaceutical is dissolved or dispersed in the lipid (Underwood and Van Eps, 2012). Solid lipid nanoparticles show several advantages over polymeric nanoparticles. For example, they have relatively higher drug retention efficacy and can be administered by multiple routes (oral, topical, and Intravenous). Moreover, hydrophobic drugs are stable in lipid matrices, protect sensitive drugs from the external environment, have minimal toxicity, and do not require the use of organic solvents in production (Mishra et al.,2010). In addition, SLNs can provide controlled-release formulations lasting up to several weeks, where they conform to mucosal surfaces, promote absorption of orally administered drugs, and are likely to cross the blood-brain barrier as they can transport drugs in the blood (Blasi et al., 2009).

Liposomes: Liposomes are highly flexible delivery systems that can transport both hydrophobic and hydrophilic substances. They can be conjugated into antibodies or ligands. They are suitable for topical, intravenous, and intramuscular use, but are rarely suitable for oral use because they are susceptible to degradation in the gastrointestinal tract. Promising

studies have been conducted on targeted drugs, imaging agents, and vaccines (Dams et al., 2000; Underwood and Eps, 2012)

Nanoemulsions consist of oil droplets dispersed in an aqueous solution. Drugs are loaded into the dispersed phase where the droplet size is typically 20-200 nm. Low-cost, solvent-free nanoemulsions have been produced for use in animal species (Vandamme and Anton, 2010), and promising results have been obtained using nanoemulsions, especially for oral and transdermal drug administration (Kang et al., 2004; Ke et al., 2005).

Micelles: Hydrophobic substances are stored in the micelle core, where they are dissolved and protected against enzymatic degradation. Micelles can be easily prepared, have low toxicity, and have the potential to be a versatile system for the effective delivery of different classes of therapeutic agents (Kim et al., 2010; Yokoyama, 2019).

Inorganic Nanoparticles have shown great potential as nanocarriers for therapeutic agents, vaccines, and imaging agents. However, their clinical use is limited by concerns of toxicity, lack of biodegradability, and permanent tissue deposition (Underwood and Eps, 2012; Fadeel and Garcia-Bennet, 2010).

Ceramic nanomaterials: Ceramic nanoparticles made of materials such as silica, alümina, and titania have several advantages over polymeric nanoparticle systems; they are easy to prepare and have the desired shape, size, and porosity, are biocompatible, and have large surface/volume ratios. In addition, they protect the absorbed particles they carry against denaturation induced by extreme pH and temperature (Underwood and Eps, 2012; Fadeel and Garcia-Bennet, 2010).

Carbon nanomaterials: Fullerenes, carbon nanotubes, and carbon nanomaterials are found and used as drug carriers. In addition, vaccines have the potential to be used as carriers because they increase the immunological response (Pantarotto et al. ,2003).

Metallic Nanomaterials: Gold, silver, and copper are the most widely used nanomaterials, most often working with gold nanoparticles (Huaizhi and Yuanto,2001; Ghosh et al., 2008). Metal nanoparticles are used in drug delivery, imaging, and cancer thermotherapy. Metal nanoparticles can be easily synthesized in various sizes (1-150 nm), are stable, and can be modified by conjugation with various functional groups (Jain et al., 2007).

Quantum dots: They are nanoparticles that are about 2-10 nm in size. The main focus is on the use of quantum dots as imaging tools in biomedical applications (Bentolila et al., 2009).

Nanotechnology & veterinary dermatology

Nanoparticle research in veterinary medicine has primarily focused on the development of therapeutic agents, vaccines, and targeting new diagnostic methods. In some of these areas, nanoparticles offer effective and scientifically validated solutions, leading to their incorporation into already marketable products (Underwood and Eps, 2012).

In many studies, nanoparticles have shown remarkable efficacy in targeting the delivery of anticancer agents, antimicrobials, analgesics, and anti-inflammatory agents (Patwekar et al., 2021).In addition to these areas of use, many studies have been carried out in the field of dermatology in recent years, and it has been concluded that nanoparticles are the future of dermal drug delivery by using appropriate nanoparticles in the right sizes (Patzelt et al. ,2016). Nanoparticles used for follicular delivery have been shown to offer some advantages over traditional routes, including improved skin bioavailability, increased depth of penetration, prolonged residence time, rapid transport to the skin, and tissue targeting (Fang et al., 2014). One of the properties that make nanoparticles interesting for topical application is their tendency to disperse and accumulate in the hair follicles. Nanoparticles have the potential to deliver drugs through follicles (Patzelt and Lademann, 2020; Mathes et al.,2016). When applied to the skin surface, particles can collect in the follicular opening and penetrate through the follicular canal. It has also been reported that nanocarriers can introduce active ingredients deep into the skin and into the systemic circulation for therapeutic purposes (Fang et al., 2014)

Nanotechnology is used for antimicrobial, antiparasitic, antifungal purposes and in allergen-specific immunotherapy (AIT), wound healing, and the treatment of skin diseases such as melanoma and atopic dermatitis.

Antimicrobial usage: The increase in antibiotic resistance among microbial pathogens has paved the way for the search for new antimicrobial techniques that will not be affected or show resistance (Wang et al., 2017). Photo-inactivation technique is one of them. Many of these photo-inactivation techniques rely on the use of various nanoparticles and nanostructures that have dimensions very similar to the wavelength of light. In a study conducted on captive penguins, lesions treated with photoinactivation or antibiotics in Bumblefoot disease, one of the most important clinical complications, were compared. There was a significant difference in the recovery rate and mean recovery time between the photoinactivation and antibiotic

groups (Nascimento et al., 2015).

In a study on mice, silver-chitosan acetate or non-silver chitosan acetate bandages were applied to infected burns after bacteria were applied to the burned areas. In conclusion, it has been shown that combining chitosan acetate with nanoparticle silver has a significant synergistic effect and that silver-chitosan acetate on bandages can treat P. aeruginosa burn infection in mice (Huang et al., 2011).

Antiparasitic-acaricide usage: Recently, metal, metal oxide, and carbon nanoparticles are highly effective against a wide variety of arthropod insects and vectors. In the study on the toxicity of nanoparticles against tick vectors of medical and veterinary importance, it was emphasized that nanoparticles are effective, but research should be expanded (Benelli et al., 2017).

Solid lipid nanoparticles (SLNs) were used for transdermal ivermectin (IVM) delivery to prevent the potential systemic toxicity of ivermectin, thus providing prolonged release without blast release. It has been shown that it can be considered an effective carrier for (Guo et al. ,2018).

Antifungal usage: In a human and dog scraping study, Malassezia was isolated and silver synthesized by green synthesis using Azadirachta indica leaf extract and characterized by UV-Visible spectrophotometer, Transmission electron microscopy (TEM), X-ray diffraction spectrophotometer (XRD), and Fourier transform infrared. The antifungal activity of nanoparticles was evaluated. The characteristic silver nanoparticles inhibit the growth of Malassezia species by forming a scavenging zone. It has been reported that silver nanoparticles can be an alternative to treat fungal infections (Saranya et al., 2016).

In a study on plants, it was observed that colloidal silver inhibited the mycelium growth of various fungal species (Venat et al.,2018).

Allergen-specific immunotherapy (asit) usage: Encapsulation of allergens or DNA vaccines with nanostructures in rodents has made it possible to achieve higher local concentrations, the protein/DNA molecules can be protected from degradation, and targeted access to the site of action. It has been stated that the encapsulated allergen can be prevented from being recognized by the immune system, especially by IgE antibodies, and that agents containing nanoparticles can offer a safer and potentially more effective treatment method for allergic diseases (Pohlit et al., 2017).

Use of nanoparticles in wound healing: It has been proven by studies that gelatin-silver nanoparticles have an excellent film-forming ability that strengthens wound healing properties . Gold nanoparticles have antibacterial and biocidal properties that help prevent infection in burns, traumatic wounds, and diabetic ulcers. It has been reported to have properties (Leu et al., 2012).

In another study, faster healing was observed in treatment with silver in wound treatment trials with turmeric and silver nanoparticles in rabbits (Islam et al. ,2015).

In the treatment of melanoma usage: It has been emphasized that nanoparticles are effective in the absorption and distribution of drugs in the chemotherapy phase in melanoma and that more studies on cell targeting and distribution are needed (Naves et al., 2017).

Atopic dermatitis usage: In human medical research, nanocarriers have been investigated for atopic dermatitis, acne vulgaris, and hyperpigmented skin lesions. In the use of various nanoparticles in atopic dermatitis in humans, with the aid of versatile nanocarriers, it is possible to explore new strategies, together with new routes of administration, for the optimization of skin-specific nanoparticulate systems to treat atopic dermatitis. It has been predicted that methodologies based on nanotechnology will revolutionize aspects of clinical dermatology (Weber et al., 2018).

In a study on atopic dermatitis in dogs, the clinical and immunological effects of gelatin nanoparticle (GNP) bound CpG ODN (CpG GNP) on patients were evaluated. A significant reduction in lesions and pruritus was observed (Wagner et al., 2017).

Filling gold nanoparticles with drugs such as Ruxolitinib (jack 2 inhibitors) in the treatment of alopecia in humans has been indicated as an ideal method for triggered localized drug release to the hair follicle, and it has been observed that they can be targeted to stem cells, immune cells or various key elements in skin physiology. GNPs are hydrophobic with skin lipids. It has been stated that it interacts and causes the deterioration of lipids, and as a result, it contributes to the increase of skin porosity and permeability to drugs (Boca et al., 2017).

Conclusion

It has been concluded that in addition to the studies in veterinary medicine, studies in human medicine can be adapted to veterinary medicine, and the use of nanoparticles in veterinary dermatology can be increased with new and larger studies and can be used as an alternative method in the treatment of dermatological diseases.

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Alternative clinical approaches to the treatment of pruritus related with canine atopic dermatitis

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Review Article

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ABSTRACT

Canine atopic dermatitis (CAD) is a genetically inheritable, inflammatory and pruritic skin disease with characteristic clinical features, most commonly associated with immunoglobulin E (IgE) antibodies to environmental allergens. Itching is the most prominent clinical finding. Depending on the allergens involved, seasonal or non-seasonal pruritus may occur. In the first active phase of pruritus treatment, which consists of two stages, acute exacerbations should be controlled by drugs with active ingredients such as corticosteroids, oclacitinib, lokivetmab, etc. In the proactive pruritus treatment, it is aimed to prevent exacerbations and prolong the pruritus-free period with maintenance treatment. For this purpose, in addition to active phase of the therapy, different treatment options such as cyclosporine, tacrolimus, antihistamines, essential fatty acids, Palmitoylethanolamide (PEA), topical drugs and shampoos can be used to repair the skin barrier. Due to the side effects and costs of the drugs used in the treatment of pruritus in atopic dermatitis, researches on alternative treatment methods are still continuing. Applications such as mesenchymal stem cell therapy, recombinant canine gamma-interferon, luteolin, vitamin D, vitamin E, lactoferricin/verbascoside, mastinib, cannabidiol (CBD), probiotics and vaccination against interleukin-31 (IL-31) are the alternative treatment options for atopic dermatitis in dogs. However, more studies are needed before their inclusion in our routine clinical practices and added to the guidelines. In this review, it is aimed to provide information about new treatments used for pruritus in CAD and to encourage their use in routine veterinary clinical practice.

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Introduction

Canine atopic dermatitis (CAD) is a genetically inheritable, inflammatory and pruritic skin disease with characteristic clinical features, most commonly associated with IgE antibodies to environmental allergens (Noli et al., 2014). Itching is the most prominent clinical finding associated with this disease. Depending on the allergens involved, seasonal or non-seasonal pruritus may occur. The face, pinna, abdomen, inguinal region, perineal area and distal extremities are the most affected areas in CAD (Hensel et al., 2015). The treatment of atopic dermatitis in dogs varies depending on the clinical findings of the patient and should be planned individually. In general, treatment principles for CAD include: reduction of

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itching and inflammation with symptomatic treatments, allergen-specific immunotherapy, treatment and prevention of secondary bacterial and yeast infections, improvement of skin barrier function, and identification and prevention of exacerbation factors, including environmental allergens (Gortel, 2018). This review focuses on treatments aimed at reducing itching and inflammation with symptom-relieving treatments.

The main therapeutic goal when treating CAD is to stop itching quickly and safely to minimize skin damage and improve the patient's quality of life (Cosgrove et al., 2015). In the first active phase of pruritus treatment, which consists of two stages, acute exacerbations should be controlled by using drugs with active ingredients such as corticosteroids, oclacitinib, lokivetmab. In the proactive phase of pruritus treatment, it is aimed to prevent exacerbations and prolong the pruritus-free period with maintenance treatment (Olivry et al., 2015). In this review, routinely drugs used for the treatment of CAD and alternative treatment methods that can be applied in cases where there is no response to conventional treatment are included. In addition, it is aimed to provide information about new treatments used for pruritus in CAD and to encourage their use in routine veterinary clinical practice.

Drugs Routinely Used for The Treatment of Pruritus in CAD

Corticosteroids:

Systemic Corticosteroids: Systemic glucocorticoid therapy in CAD reduces the number of inflammatory cells. In addition, glucocorticoid therapy also reduces the production of inflammatory mediators that effectively control both acute and chronic cutaneous inflammation and itching (Saridomichelakis and Olivry, 2016). One of the main ways glucocorticoids affect inflammatory responses is through the effect on cytokine production. Generally, glucocorticoids suppress the production of cytokines, particularly interferon-gamma, IL-2, IL-3, IL-4, IL-5, IL-6 and IL-13 (Olivry et al., 2001).

Oral prednisolone, prednisone or methylprednisolone at 0.5-1.0 mg/kg/day divided into one or two dosages provides improvement of clinical manifestations in dogs with severe or diffuse atopic dermatitis (AD) (Olivry et al., 2015). Systemic glucocorticoid therapy is known to have side effects such as susceptibility to infection, immunosuppression, decreased urinary osmolality and increased risk of urinary tract infection. These side effects are directly proportional to the dosage and duration of administration of the drug (Olivry et al., 2015; Elkholly et al., 2020).

Long-acting injectable glucocorticoids (dexamethasone and methylprednisolone acetate) are not recommended in dogs with atopic dermatitis due to risks such as hepatopathy and hyperadrenocorticism (Olivry et al., 2001; Elkholly et al., 2020).

Topical corticosteroids: Topical glucocorticoids are useful for short-term use, particularly in localized skin lesions. However, the long-term application of these products can cause steroid-induced skin atrophy (Olivry et al., 2015). Hydrocortisone aceponate (HCA) spray, which is a topical corticosteroid, contributes to the repair of the skin barrier by showing antipruritic and anti-inflammatory properties, and has fewer side effects when compared to other topical corticosteroids (Nam et al., 2012). In a randomized, double-blind, placebo-controlled study evaluating the efficacy of 0.0584% HCA, it was administered to dogs with atopic dermatitis once daily for 28 days, as two sprays from 10 cm distance to treat an area of 100 cm. According to the improvement, two applications per week were continued for 42 days. As a result of this study, a significant decrease in canine atopic dermatitis extent and severity index (CADESI) and pruritus visual analog scale (PVAS) scores in the HCA group were compared to placebo (Nuttall et al., 2009). As a result of a study comparing the efficacy of 0.0584% HCA spray and oral cyclosporine (5 mg/kg), no significant difference was found in the efficacy, tolerance, and ease of administration scores of the drugs (Nuttall et al., 2012). As a result of a study about CAD, dogs were administered HCA spray for 260 days, no side effects were seen. Therefore, it has been shown to be effective and well tolerated in the proactive treatment of CAD (Lourenço et al., 2016).

Another topical glucocorticoid, 0.015% triamcinolone spray, is also known to be well tolerated and effective in the treatment of atopic dermatitis in dogs for 28 days (Olivry et al., 2001; DeBoer et al., 2002). In addition, it has been shown that the application of another topical glucocorticoid, 0.025% budesonide cream, once a week for 3 times at 1 g/kg, is an effective treatment for the control of clinical manifestations of AD in dogs (Ahlstrom et al., 2010).

Oclacitinib: Oclacitinib is a janus kinase inhibitor that inhibits the cytokine IL-31, which causes pruritus in dogs (Denti et al., 2022). In addition to its antipruritic effect, it also shows an anti-inflammatory effect by inhibiting pro-inflammatory and pro-allergic cytokines such as IL-2, IL-4, IL-6 and IL-13 (Cosgrove et al., 2013). In active treatment, 0.4-0.6 mg/kg orally twice a day is prescribed for 14 days to reduce skin lesions and itching (De Caro Martins et al., 2022; Denti et al., 2022). In proactive treatment, it has been found that it can be used for up to 630 days with once a day

application following active treatment is safe and improves the quality of life of dogs (Cosgrove et al., 2015). The efficacy of oclacitinib treatment is limited when severe inflammation, lichenification, otitis, and pododermatitis are present. In addition, oclacitinib is contraindicated in the presence of neoplasia or severe infection and in dogs younger than 1 years old (Gortel, 2018).

Although it has not been licensed in cats yet, it has been found to be effective and safe in cats with feline atopy syndrome, using a dosage of approximately 1 mg/kg q12h. However, more studies are needed whether oclacitinib is a suitable alternative for the treatment of pruritus in feline atopy syndrome (Lopes et al., 2019; Noli et al., 2019; Mueller et al., 2021).

Lokivetmab: Lokivetmab is a canine-specific monoclonal antibody (mAb) that selectively binds to and neutralizes IL-31 (Souza et al., 2018). Since it binds only to IL-31 and does not affect other cytokines, its spectrum of action is more limited than oclacitinib (Gortel, 2018). Lokivetmab is administered at a dosage of 2.0 mg/kg by subcutaneous injection to dogs with atopic dermatitis and its efficacy is expected to last at least one month. The advantages of this drug compared to other antipruritic agents are its rapid onset of action, less frequent dosing, no age restriction, and compatibility with other drugs (Souza et al., 2018). Its spectrum of action is limited which could be a disadvantage (Gortel, 2018).

In a placebo-controlled study in which the safety of lokivetmab was investigated, side effects such as vomiting, diarrhea and anorexia were observed in both groups, but these effects were reported as temporary and disappeared with supportive treatment (Michels et al., 2016).

In another study evaluating its safety, no abnormal health findings associated with lokivetmab were observed as a result of its use for 6 months (Moyaert et al., 2017). In a study comparing lokivetmab and oclacitinib, no significant difference was found between their antipruritic effects (Lee et al., 2021).

Essential Fatty Acids

It is known that essential fatty acids, especially omega-6 (gammalinolenic acid, GLA) and omega-3 (eicosapentaenoic acid, EPA-docosahexaenoic acid, DHA) have anti-inflammatory effects and immunomodulatory properties on the skin (Abba et al., 2005).

Oral administration of essential fatty acids as supplements or enriched diets has been found to be beneficial in reducing the clinical manifestations of CAD. In addition, essential fatty acids are known to affect superficial skin lipids, improving coat shine and quality (Marchegiani et al., 2020). However, they are not effective for acute flares as they must be used for at least two months to be effective. Topical lipid formulations act by helping to heal stratum corneum lipid barrier damage in dogs with AD (Olivry et al., 2015).

Palmitoylethanolamide (PEA): PEA is a naturally occurring bioactive lipid compound which is produced on demand in response to stress and tissue damage. It has also an important role in the regulation of cutaneous inflammation and immunity (Noli et al., 2015). It is thought that PEA may be a promising treatment in dogs with atopic dermatitis, as it improves their quality of life by reducing skin lesions and itching scores (Marchegiani et al., 2020; Noli et al., 2015).

In an 8-week, open-label, multicenter study evaluating the efficacy and safety of PEA, oral administration of PEA at a dosage of 10 mg/kg in CAD was shown to significantly reduce itching and skin lesions in approximately 80% of dogs (Noli et al., 2015).

Shampoos: In dogs with atopic dermatitis, non-irritating shampoos containing agents such as chlorhexidine, lactoferrin, piroctone olamine, chitosan and essential fatty acids can be a part of the treatment (Schilling et al., 2012; Olivry et al., 2015). Their advantages are soothing effects on the skin, increasing skin moisture, and physically removing surface allergens and microorganisms (Olivry et al., 2015). A study was conducted to evaluate the effectiveness of a shampoo containing piroctone olamine and lipid complexes. In this study, dogs were bathed with this shampoo once in 3 days for 3 weeks, and as a result of the study, a decrease in pruritus and lesion indexes was detected in almost half of the dogs (Reme et al., 2004).

The type of shampoo should be tailored to each situation: emollient shampoos are the ones with the highest soothing effect, but anti-seborrheic and antiseptic shampoos are preferred in case of oiliness, crusting and infection on the skin (Olivry et al.,2010).

Antihistamines: Antihistamines act as reverse agonists on histamine receptors, stabilizing the negative structure of the receptor and exerting an effect by stopping signal transduction (Eichenseer et al., 2013). Although type I antihistamines have relatively good safety, their effectiveness in canine AD is limited. Due to its mechanism of action, it should be given before an acute exacerbation to block the effects of histamine (Saridomichelakis and Olivry, 2016). Cetirizine, dimetinden, fexofenadine, hydroxyzine, clemastine, oxatomid, trimeprazine, diphenhydramine, chlorpheniramine and hydroxyzine-chlorpheniramine maleate combinations are the antihistamines that can

be prescribed to dogs with AD (Olivry and Mueller, 2003). Instead of injections; syrup and tablet forms are usually used in CAD.

Although their efficacy is limited, type I antihistamines can be used in dogs with mild atopic dermatitis because of their sedative effects (Saridomichelakis and Olivry, 2016). It can be used as part of combined therapy in the long-term management of AD to reduce the dosage of other drugs such as corticosteroids (Olivry et al., 2010).

Cyclosporine: Cyclosporine is a calcineurin inhibitor that reduces cytokine synthesis by binding to cyclophilin in the cytoplasm of lymphocytes. Compared to glucocorticoids, cyclosporine is similarly effective and has fewer side effects. However, onset of action is slower (usually 2-3 weeks) and more expensive than glucocorticoids (Saridomichelakis and Olivry, 2016). Cyclosporine is used in atopic dermatitis, especially in cases of chronic otitis externa, severe inflammation and conditions such as lichenification. In addition, it is preferred as an alternative to the long-term use of corticosteroids (Gortel, 2018).

Cyclosporine should be administered to dogs with AD at 5 mg/kg once a day. This dosage should be continued until control of clinical signs is achieved, which will usually last 4 to 6 weeks. Afterwards, the dosage or frequency of treatment required to maintain remission should be reduced and the drug should be discontinued (Olivry et al., 2015).

In dogs with atopic dermatitis, ketoconazole inhibits cyclosporine metabolism and prolongs its half-life when administered orally at a dosage of 2.5 mg/kg once daily with cyclosporine. In this way, the dosage and cost of the treatment can be reduced by approximately 50%, but the possibility of increased side effects such as hepatotoxicity should also be considered (Saridomichelakis and Olivry, 2016).

Tacrolimus: Tacrolimus is a topical calcineurin inhibitor that can be used instead of systemic immunosuppressive therapy to treat localized inflammation and pruritus (Kaya et al., 2020; Santoro et al., 2019). It has been shown to be effective as an alternative to topical glucocorticoids, particularly in dogs with localized atopic dermatitis. Although it is not suitable for the treatment of acute AD exacerbations in dogs due to its slow onset of clinical benefit, it is used in proactive therapy (Olivry et al., 2010). It has been determined that the use of tacrolimus ointment (0.1-0.3%) twice a day for 4-6 weeks is safe and especially effective in proactive treatment (Bensignor and Olivry, 2005).

In cases where the itching cannot be stopped with conventional treatment methods in dogs with atopic dermatitis, alternative treatment methods can be used.

Alternative Treatment Methods for Pruritus in CAD

Recombinant Canine Gamma-Interferon: Interferons (IFNs) are cytokines that have important roles in the immune response of animals and humans (Mueller and Hartmann, 2021). In dogs, interferons are used in the treatment of viral diseases, neoplasms, and immune-mediated disorders due to their potent antiviral, antiproliferative and immunomodulatory properties (Klotz et al., 2017).

As a result of studies evaluating the efficacy of recombinant canine interferon-gamma in dogs with atopic dermatitis, subcutaneous administration of 5,000–10,000 IU/kg three times a week for 4 weeks and then once a week for 4 weeks was found to be effective for the treatment of CAD (Olivry et al., 2015; Yasukawa et al., 2010).

A study was conducted in Japan in which dogs with atopic dermatitis were administered recombinant canine interferon-gamma (KT-100) subcutaneously at a dosage of 10,000 IU/kg three times a week for 4 weeks or a topical antihistamine (diphenhydramine) twice daily topically. The efficacy of the two drugs was compared using pruritus, excoriation, erythema and alopecia as evaluation criteria. As a result of the study, the effectiveness rates of the KT-100 group; it was found 72.1% for pruritus, 73.8% for excoriation, 75.4% for erythema and 60.7% for alopecia (Iwasaki and Hasegawa, 2006).

Studies using subcutaneous injections of recombinant feline interferon omega (rFeIFN-ω) suggest that it may have clinical efficacy for treatment in dogs with AD. Dosages of 1-4 million units per injection for 6 months have been shown to be well tolerated (Carlotti et al., 2009).

In a study comparing oral and subcutaneous use of recombinant rFeIFN-ω, oral IFN treatment was found to have higher efficacy than SC treatment. However, it is thought that the efficacy of oral IFNs as a treatment for CAD should be confirmed by larger, randomized, double-blind clinical trials (Litzlbauer et al., 2014).

Mesenchymal stem cell: Mesenchymal stem cells (MSCs) have tissue repair potential due to their self-renewal and differentiation abilities (Daltro et al., 2020). After the discovery of the immunosuppressive effect of MSCs on T cells, studies have been conducted on their use as a therapeutic agent for diseases such as CAD. In these studies, allogeneic MSC (cAd-MSC) produced from adipose tissue was administered intravenously and intramuscularly to dogs that did not

respond to conventional therapy. As a result of these studies, an improvement in the clinical signs of dogs with AD was observed, and no side effects were found in dogs (Oliveira Ramos et al., 2020; Reis et al., 2021).

In a study of 26 dogs who had suffered from CAD for at least 1 year and were not responding to conventional therapy, the dogs were administered a single dosage of 1.5×10^6 cAd-MSCs per kg intravenously (IV) and then followed for 6 months. From the 26 animals involved, 22 completed the study within six months of treatment without the need for a systemic immunosuppressant and showed a significant improvement in PVAS and CADESI-04 scoring. No systemic or local side effects were observed during this study (Villatoro et al., 2018).

In another study, 0.5×10^6 cAd-MSC per kg was administered intramuscularly (IM) in the pelvic femoral muscle region once a week for 6 weeks to 12 dogs previously diagnosed with CAD. As a result of the study, a significant reduction in pruritus scores was observed in patients, while no systemic or local adverse reactions developed (Enciso et al., 2019).

A double-blind, placebo-controlled evaluation of adipose-derived mesenchymal stem cells was conducted in the treatment of canine atopic dermatitis. In this study, dogs with atopic dermatitis were randomly assigned to placebo (PBS saline), low-dosage (5×10^5 cells/kg), and high-dosage (5×10^6 cells/kg) treatment groups. Each patient received three subcutaneous injections of MSC treatments or PBS saline at four-week intervals from five sites. At the end of the study, the PVAS and CADESI-4 scores of the high-dosage group were found to be significantly lower than the placebo group, and no serious side effects were observed in any patient. High-dosage MSC treatment has been found to be effective in alleviating clinical manifestations of CAD up to 30 days after the last subcutaneous administration of MSCs (Kaur, G., 2022). Although there is no standardization for the use of MSC in CAD yet, it can be used as an alternative treatment method in patients who do not respond to conventional treatment (Reis et al., 2021).

Mastinib: Masitinib mesylate is a potent and selective tyrosine kinase inhibitor of the cKIT receptor. It is approved for the treatment of mast cell tumors in dogs. Canine mast cells are known to produce a variety of inflammatory mediators that are partially responsible for the complex inflammatory processes associated with allergic disease and cause the development of clinical symptoms of CAD. Therefore, it is thought that molecules that can inhibit the survival or activation of mast cells can be used for the treatment of CAD (Daigle et al., 2010).

Stem cell factor, the ligand of the c-Kit receptor, is a critical growth factor for mast cells. Therefore, there is a strong link between c-Kit and the pathogenesis of CAD (Cadot et al., 2011). It was assumed that dermatological diseases such as CAD could be controlled by disrupting this link with the inhibitory effect of masitinib on c-KIT tyrosine kinase activity, and studies were conducted on this subject (Daigle et al., 2010; Cadot et al., 2011). In a pilot study with 11 dogs, mastinib was administered orally at a mean dosage of 11.0 ± 1.83 mg/kg/day for 28 days. As a result of the study, a decrease was observed in the pruritus scores and surface area of the lesions in dogs (Daigle et al., 2010).

A 12-week, prospective, multicenter, randomized, double-blind, placebo-controlled, pivotal phase 3 study was conducted to evaluate the efficacy and safety of 12.5 mg⁄kg per day macitinib in the treatment of CAD. In this study, 61% of dogs treated with masitinib and 35% of the control group ($P < 0.001$) 12. a decrease of ~50% was observed in the CADESI-02 score at week 12 ($P < 0.001$). However, serious side effects were detected in a total of 13.2% of dogs during the study. A risk of reversible protein loss from mastinib has been observed in dogs. As a result of this study, it was found that masitinib can be an effective and mostly well-tolerated treatment of CAD, including severe and resistant cases with medically manageable side effects (Cadot et al., 2011).

Luteolin: Luteolin is one of the most powerful and potent polyphenols found in vegetables, fruits and herbs. Luteolin is known to have various biological properties such as anticancer, antioxidant, neuroprotective and anti-inflammatory effects in both in vitro and in vivo models. Luteolin modulates many inflammatory processes in the skin by suppressing proinflammatory mediators and regulating various signaling pathways (Gendrisch et al., 2021). In a study investigating the effect of luteolin on IL-33, which causes itching in CAD, it was found that it significantly reduced the expression levels of IL-33, IL-1β, IL-6 and IL-8 (Gugliandolo et al., 2020).

Lactoferricin/Verbascoside: Lactoferricin is a natural antimicrobial peptide that shows a wide spectrum of activity against bacterial, fungal, viral and parasitic pathogens. Lactoferricin/Verbascoside is known to promote skin repair and improve skin inflammation due to its antioxidant, iron chelator, glutathione transferase activity inducer, antibacterial and anti-inflammatory effects (Sijbrandij et al., 2017; Belvedere et al., 2021).

In dogs, AD causes damage to the skin barrier, making the skin vulnerable to secondary infections. Therefore, local antibacterial agents are used to reduce the most relevant symptoms and limit skin infection. In a study of thirty-eight dogs with atopic dermatitis and secondary bacterial or yeast overgrowth, a lotion containing lactoferricin, verbascoside (a caffeoyl phenylethanoid glycoside) and glycerophosphoinositol lysine (derived from sunflower lecithin) was obtained and applied to lesions in dogs with atopic dermatitis. As a result of the study, clinical signs improved and no side effects were reported in any of the dogs (Biasibetti et al., 2018).

Vaccination against IL-31: In dogs, IL-31 is an important cytokine that triggers pruritus. A study showed that a virus-like particle-based developed vaccine against canine IL-31 induced a potent IgG response that essentially eliminated pruritus symptoms in dogs sensitized to house dust mites. In this study, dogs were vaccinated at a dosage of 100 µg followed by two dosages of 300 µg, and the vaccinated dogs were sensitized to house dust mites. As a result of the study, it was determined that a strong IgG response was formed in dogs, which eliminated the symptoms of itching. The vaccine is thought to be an alternative treatment option for CAD as it induces a longer-lasting antibody response compared to other treatments (Bachmann et al., 2018).

Cannabidiol : CBD is a non-psychoactive component in cannabis plants that has multiple beneficial effects on the body (Samara et al., 1988). CBD can be used in the management of pain, seizures and anxiety in dogs (Gamble et al., 2018; McGrath et al., 2019; Kogan et al., 2019).

Studies have shown that cannabinoid receptors (CB1 and CB2) are highly expressed in the skin of dogs with CAD compared to healthy dogs. It is involved in the regulation of the endogenous cannabinoid system of CBD. Therefore, CBD is expected to improve skin symptoms in dogs with atopic dermatitis. In a study of 8 dogs with atopic dermatitis, broad-spectrum cannabis oil containing 10% CBD and free of delta-9-tetrahydrocannabinol (THC) was administered orally every 12 hours at a dosage of 0.14 to 1.43 mg/kg/day for at least 8 weeks. As a result of this study, a decrease in the itching levels of the dogs has been observed (Mogi et al., 2022).

Cannabidiolic acid (CBDA) is the precursor carboxylic acid form of CBD. In a recent study, it was shown that CBDA in dogs is similar to CBD but has better absorption (Wakshlag et al., 2020). Immunomodulatory and anti-inflammatory effects of CBD/CBDA have been reported in mammals. To determine whether CBD/CBDA is an effective treatment for canine atopic dermatitis, in a study of 32 dogs, either 2 mg/kg of CBD/CBDA mixture was administered for 4 weeks. The results of the study showed that CBD/CBDA did not affect lesion severity, but had a positive effect on pruritus as adjunctive therapy in some dogs with CAD. Although side effects such as behavioral changes, loss of energy, and changes in appetite were observed in some of the dogs in the study, it was well tolerated (Loewinger et al., 2022).

Probiotics: In humans with atopic dermatitis, probiotics and prebiotics are known to modulate T helper cytokine activation, upregulate regulatory T cells and accelerate recovery of barrier function (Nole et al., 2014). Based on this, studies have been conducted in dogs with atopic dermatitis evaluating the role of probiotics for preventing the disease and their effectiveness in treatment (Marsella, 2009; Kim et al., 2015; Yamazaki et al., 2019). An experimental study of 2 adults and 16 puppies genetically predisposed to atopic dermatitis evaluated the efficacy of Lactobacillus rhamnosus GG strain for alleviating or preventing clinical manifestations. As a result of this study, it was determined that the administration of L. rhamnosus GG to puppies reduced the immunological indicators of AD but did not provide a significant reduction in clinical signs (Marsella, 2009). Another study with 42 experimental dogs with atopic dermatitis found that administration of the L.sakei probio-65 strain for 2 months significantly reduced the disease severity index compared to the placebo group (Kim et al., 2015). A study was conducted to evaluate the effect of Enterococcus faecium SF68 in reducing the dosage of oclacitinib as adjunctive therapy for dogs with atopic dermatitis responsive to oclacitinib. In this placebo-controlled study of 21 dogs, the SF68 group was administered at a dosage of 1× 108 colony forming units/g orally twice daily for 12 weeks. After 8 weeks of supplementation the dosage of oclacitinib was reduced by approximately 25%. As a result of the study, no significant difference was observed in dogs that received SF68 supplementation compared to placebo (Yamazaki et al., 2019). In a study conducted with dogs with mild or moderate atopic dermatitis, the efficacy of cetirizine and L. paracasei K71 in addition to AD treatment was compared. As a result of this 12-week study, CADES and pruritus scores in the K71 group were slightly lower than in the control group, and the reduction in drug scores in the K71 group was significantly lower ($P < 0.05$) compared to the control group (Ohshima Terada et al., 2015).

These studies suggest that the administration of probiotics may be beneficial in preventing or reducing the clinical manifestations of AD in dogs. However, larger studies are needed to standardize probiotic use in CAD.

Psychogenic therapy: Psychogenic factors may also contribute to the clinical manifestations of AD in some dogs. Anxiolytic agents such as tricyclic antidepressants and specific serotonin reuptake inhibitors and N-methyl-D-aspartate can be used in such patients (Saridomichelakis and Olivry, 2016). Tricyclic antidepressants act as both serotonin and norepinephrine inhibitors. They also have antihistamine and anticholinergic effects and are a1-adrenergic antagonists (Crowell Davis and Murray, 2006). Doxepin (1-2 mg/kg, orally twice a day) and amitriptyline (1-2 mg/kg, orally twice a day) can be used in dogs with atopic dermatitis (Saridomichelakis and Olivry, 2016). However, their efficacy is limited and variable (Olivry and Mueller, 2003).

In a randomized, double-blind, placebo-controlled study evaluating the efficacy of fluoxetine (1mg/kg), one of the selective serotonin reuptake inhibitors, no statistically significant difference in CADESI-03 and PVAS scores was observed between fluoxetine and placebo (Fujimura et al., 2014). Dextromethorphan is an N-methyl-D-aspartate receptor antagonist with some non-specific serotonin reuptake inhibition properties. In a study of 12 dogs with allergic dermatitis, 2 mg/kg orally administered twice daily resulted in a mild to moderate improvement in clinical signs (Dodman et al.,2004).

Vitamins: The effect of vitamin D in canine atopic dermatitis was evaluated in a placebo-controlled, double-blind, randomized clinical trial. As a result of this study, oral vitamin D reduced both itching and acute and chronic skin lesions in dogs with AD. The reduction in pruritus scores was significantly associated with an increase in serum 25-hydroxycholecalciferol levels (Klinger et al., 2018; Kotnik, 2018).

As a result of a study investigating the effects of vitamin E supplementation on clinical manifestations improvement and oxidative stress markers in canine atopic dermatitis, it was shown that vitamin E supplementation was highly effective in reducing clinical manifestations in moderate AD cases after 8 weeks of treatment (Plevnik Kapun et al., 2014).

Additional treatments: Pentoxifylline is a phosphodiesterase inhibitor that down-regulates activation of inflammatory cells and TNF-α production (Saridomichelakis and Olivry, 2016). Its effectiveness is moderate, but it appears to be relatively safe. Although it has not yet been officially approved in dogs with atopic dermatitis, it is used at 10-20 mg/kg two or three times daily to reduce glucocorticoid use (Singh et al., 2010; Saridomichelakis and Olivry, 2016).

Misoprostol is a synthetic prostaglandin E1 analog that reduces the production of IL-1, tumor necrosis factor -a and leukotriene B4 (Saridomichelakis and Olivry, 2016). A randomized controlled trial of misoprostol monotherapy for canine atopic dermatitis found that the use of 3-6 µg/kg three times daily for 3 weeks resulted in a moderate reduction in pruritus scores (Olivry et al., 2003b). Some drugs such as leukotriene inhibitors, dextromethorphan, and capsaicin are not recommended for use in the treatment of CAD, as some evidence has been confirmed that they have no or very low efficacy (Olivry et al., 2010; Olivry et al., 2010b).

In a study to evaluate the clinical efficacy of neural therapy (NT), procaine was used intravenously and intradermally (in the affected areas). As a result of this study, a significant reduction in clinical signs and pruritus score was observed (Bravo-Monsalvo et al., 2008). The purpose of using topical neuromodulators in atopic dermatitis is to control the itch by overriding the itch sensation with another. Examples of these drugs include menthol, capsaicin, and pramoxine (Paterson, 2019). The mode of action of such treatments is not always clear, but it is thought that they can normalize the defective epidermal barrier, remove allergens and irritants from the skin surface, and reduce inflammation and itching (Saridomichelakis and Olivry, 2016).

A study was conducted to evaluate the effectiveness of shampoo treatment with ultrapure soft water. In this study, dogs with moderate atopic dermatitis were divided into two groups, and both groups were bathed with the same shampoo, while ultrapure soft water was used in one group and tap water in the other group. As a result of the study, a significant decrease was found in the clinical symptoms and itching scores in the pure water group compared to the control group (Ohmori et al., 2010).

A double-blind, randomized, controlled, cross-over evaluation of zinc methionine supplementation as an adjunctive treatment for canine atopic dermatitis found that zinc supplementation reduced clinical signs and pruritus scores, especially in dogs using glucocorticoids (McFadden et al., 2017).

Conclusion

Canine atopic dermatitis is a disease that reduces patients' quality of life and is difficult to control. Due to the side effects and costs of the drugs used in the treatment of pruritus in CAD, researches on alternative treatment methods are still continuing. Different treatment techniques such as mesenchymal stem cell therapy, recombinant canine gamma-interferon, luteolin, vitamin D, vitamin E, lactoferricin/ verbascoside, mastinib, CBD, probiotics and vaccination against IL-31 can be among alternative methods in the treatment of atopic dermatitis in dogs. More scientific studies are still needed for these protocols to be included in our routine practices and added to the definite guidelines. Treatment responses may vary due to individual differences. However, based on up-to-date information, we believe that these methods can be beneficial, especially in patients who do not respond to conventional treatment.

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Investigation of the effect of water temperature on water consumption of cats

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ABSTRACT

Cats can naturally obtain their water requirements on the water content of their food. However, if the water level of the food is less than 60%, cats need additional drinking water. Otherwise, they will be easily dehydrated and chronic dehydration can lead some health problems such as bladder and renal diseases and circulatory problems. Any practical method that could increase cats water consumption, would have a reducing effect on the before mentioned diseases. Regarding the water consumption, the taste of water has been found as effective as the other physiological stimulants such as mouth dryness, plasma osmolality and blood volume. Temperature is considered to be very important for the taste perception of animals. The preference for the water temperature varies among the animal species. So we hypothesized that, cooling the drinking water can encourage cats to drink more water and we aimed to investigate the effect of the water temperature on water consumption of cats. This research has conducted with 8 domestic, mature and healthy pet cats (Felis domesticus) that live indoor. We measured the water consumption of cats for two weeks. During the first week, temperature of water has not been intervened, and the cats' normal water consumption were measured. On the 1st day, 500 ml water, measured with graduated cylinder, was provided in a standard water bowl. After 24 hours, the remained water has been measured and noted. After each measurement, cat owners refreshed the drinking water. In the 2nd week, we started to add four ice cubes to the water bowl, three times in a day. First week, cat's average normal water consumption has found 142.26±8.09 ml/kg/day. ($p<0.05$) In the second week, water consumption increased to 203.97±12.52 ml/kg/day after water cooling. In conclusion, cooling the water resulted with an increase in the water consumption in cats. These results will contribute to develop new water cooling practices to help increasing water consumption in cats and minimizing the clinical risks caused from inadequate water consumption.

Keywords: cat, water consumption, cold water, urinary system

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Introduction

Water is the most important source of nutrients required for the normal functioning of all living cells, with many important functions such as its leading role in the circulatory system and thermoregulation (Brown, 1998; Noyan, 2011; Rogers et al., 1986). Water can be obtained from drinking water and from food, and is also produced during protein, carbohydrate and fat metabolism (metabolic water, oxidation water). This exogenous or produced water is also lost from the body through the lungs, urine, skin, milk and feces. The body water content is thus maintained at a certain level through a balance between water gain and water loss (Reece, 2012; Zanghi, 2017).

Cats have evolved to meet their water needs from the food they eat; in the wild, cats generally do not need to take in water from outside, as they utilize the body fluids of the creatures they hunt and the bodies of these creatures contain 70-75% water (Hall et al., 2003; Myrcha and Pinowski, 1970). Although cats are slightly more resistant to acute dehydration than dogs and other omnivores with water losses of up to 20% of

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their body weight, they must maintain body water balance in the long term like all living things (Debra, 2002 ; Rogers et al., 1986).

A cat needs approximately 50-60 ml of water for one kilogram of body weight (National Research Council, 2006). If sufficient water is not taken, dehydration may occur (Houpt, 1991). When dehydration is mild, the thirst mechanism is activated to restore water balance (Reece, 2012). If dehydration becomes chronic, health problems such as stone formation in the bladder, kidney failure and circulatory problems may occur (Houpt, 1991). Feline Lower Urinary Tract Disease (FLUTD) is one of the most common health problems related to water intake among domestic cats (Little, 2012).

Chronic kidney disease (CKD) is the most common kidney disease in cats. Normally, waste products are filtered from the blood by the kidneys and excreted in the urine, but in cats with CKD, these waste products begin to accumulate in the bloodstream as the filtering function of the kidneys deteriorates (Bartlett et al., 2010). Establishing the habit of adequate fluid intake or increasing water intake (by encouraging the cat to drink more water than it normally consumes) will increase urination and minimize the risk of FLUTD and CKD (Alipourmazandarani, 2021).

There are many environmental factors that affect drinking water intake. Since the way water is presented can affect the amount of water consumption in cats, methods that can encourage water intake are being investigated to reduce the risks of these diseases (Handl and Fritz, 2018).

Feeding with canned foods or moistened dry foods to increase the moisture content of food in the diet to improve water intake can increase the accumulation of dental tartar and resulting periodontal disease (Debra, 2002).

Another method to increase the water intake and decrease the possibilities of urinary system risks was considered as modifying the diet (Chew and Buffington, 2007). Although one study showed that cats' water intake can be increased by adding sodium to the diet, it is questionable whether such a procedure is healthy in the long term, as high amounts of sodium in the diet can cause different diseases such as hypertension (Forrester and Roudebush, 2007; Hawthorne and Markwell, 2004 ; Nguyen et al., 2017). In order to increase water intake voluntarily, water fountains are commercially advocated in recent years (Turner and Bateson, 2013). A study by Grant, (2010) investigated the effect of different water sources (water bowl or water fountain) on water intake and urine concentration in cats and the results showed that the fountain caused an increase in water intake compared to the water bowl. On the other hand, 1 in 12 cats was recorded to experience stress due to the dynamic water source, with symptoms of excessive cleaning, vomiting and aggression. In another survey that performed with 549 cat owners, more than 80% of cats who had access to both a static/standard water bowl and a moving/dynamic water bowl at the same time preferred the static water bowl, so currently, this is a contradictory solution (Handl and Fritz, 2018 ; Pachel and Neilson, 2010). In studies conducted in different animal species, it has been understood that temperature may be an important factor in the formation of taste sensation in animals, as in humans (Nesheim et al., 1993; Turner and Bateson, 2013). For example, it has been observed that chickens prefer to suffer from acute thirst rather than drink water warmer than their body temperature, whereas they are happy to consume water close to freezing (Reece, 2009). In another study, it was found that chickens preferred cooled water in both seasons (summer and winter), but the reason was not known (Degen and Kam, 1998). Based on this information, it is suggested that cooling of drinking water may be one of the methods that can encourage water intake in cats and reduce the risks of urinary system diseases which are mentioned above. In our literature review, no scientific studies on the subject was found. The aim of this project is to investigate the effect of cooling the drinking water on water consumption in cats.

Materials and Methods

Before the start of the study, approval was obtained from the Local Ethics Committee for Animal Experiments. 8 adult cats with no known health problems and with a certain level of activity due to living in a stabilized home environment, were included in this study. Age, gender, body weight and body condition scores (9-point scale) for each cat were noted and given in Table 1 (Bjornvad, 2011 ; Teng et al., 2018).

Table 1. Age, gender, body weight and body condition scores of the cats

	Age	Gender	Body weight (kg)	Body condition score
Patron	5	Male	4.39	5
Miyu	1.5	Female	4.6	6
Dennis	5	Male	4.49	5
Eda	4	Female	8.54	8
Sassy	3	Female	4.79	6
Prenses	3	Female	2.9	3
Aslan	2	Male	6.19	7
Maya	1	Female	3.2	3

All of the cats were feeding with dry commercial pet food. Ad libitum feeding was applied and, instead of tap water, drinking water was served as their normal, daily practices. The cats observed in the study were selected as their owners are veterinary students who were familiar with the research and measurement processes.

Since performing daily water consumption measurements in a different environment may cause stress on the cats and accordingly, differences in feeding and water intake behaviors may be observed, the measurements were performed by the owners in their own living environment without any change in the daily routine of the cats.

In order to minimize measurement errors, the measurement procedure was simplified, a standard water bowl was provided for all cats, and owners were provided with detailed and applied information about the measurement. The consumption of water at room temperature and the consumption of cooled water were measured and compared on 8 adult cats.

Water consumption measurements lasted for 2 weeks and performed between same dates for each cat. Consumption of the water at the room temperature was measured in the first week and cooled water consumption was measured in the second week. For the first week, a standard water container that could hold 900 mL of water, a 500 mL graduated cylinder that could be used to measure water volume, a funnel to be used to empty the water from the standard water container into the graduated cylinder, a standard chart to note the amount of consumed water, and 12 standard ice cubes for each 24 hours were provided to each cat owner. In the first week, all of the cat owners served to cat 500 mL of fresh water at room temperature in a standard water container once every 24 hours, while measuring and taking notes of the volume of water left from the previous day.

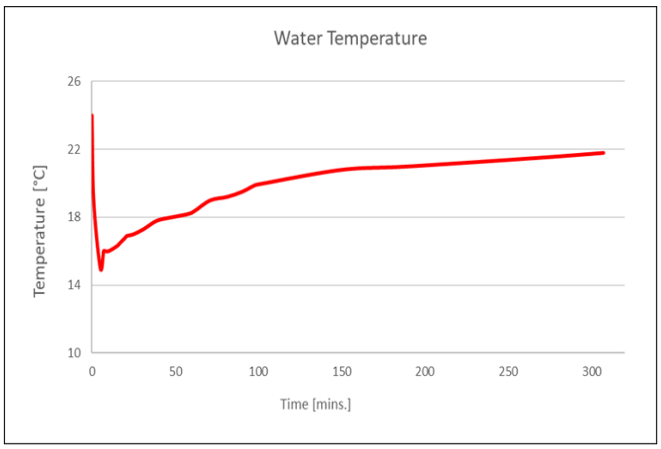

Figure 1. The changes in water temperature after 4 ice cubes added.

In the second week, all of the cat owners served 500 mL of fresh water at room temperature with 4 ice cubes added, in a standard water container three times a day. After 4 standard ice cubes were added to the water at room temperature served in a standard water container, the changes in water temperature were measured and given in Figure 1.

Measurements performed once every 24 hours, while the volume of water left from the previous day was measured with a graduated cylinder and noted. When evaluating the measurement results, it was taken into account that each of the standard ice cubes provided 12.96 mL of water volume when they are completely defrosted.

"The Paired Samples T-test", which is developed to compare the mean of two matched groups or cases, or compares the mean of a single group, examined at two different points in time, was used to compare normal and cooled water consumptions of 8 cats on the first and second week (Ross and Willson, 2017). Statistical analysis has been done with the SPSS program (Ver. 17.0).

Results

In the first week, the average water (at room temperature) consumption of the cats was 142.26±8.09 mL/kg/day. In the second week, the average water consumption (cooled water) increased to 203.97±12.52 mL/kg/day.

The average daily water consumption of the cats is given in Table 2. When the water consumption at room temperature in the first week was compared with the cooled water consumption in the second week, it was observed that the increase in the water consumption of 4 out of 8 cats was statistically significant.

Table 2. Average daily water consumption

	First Week			Second Week			Dif.	*P*
Patron	113.3	±	5.29	113.4	±	12.55	0.14	0.99
Miyu	8.6	±	3.15	91.3	±	9.47	9.71	0.46
Dennis	99.7	±	11.70	187.9	±	18.77	88.14	0.01
Eda	478.6	±	9.99	575.6	±	23.38	97.00	0.00
Sassy	80.4	±	14.00	170.6	±	3.83	90.20	0.00
Prenses	104.6	±	6.40	149.9	±	15.14	45.29	0.06
Aslan	101.7	±	6.22	243.0	±	3.55	141.3	0.00
Maya	78.3	±	7.97	100.1	±	13.44	21.86	0.31

Dif = Difference

Discussion

In a study conducted with broiler chickens, it was concluded that broilers preferred cooled water more

regardless of the season, but the reason for this preference was not fully understood (Degen and Kam, 1998). Our findings suggest that cats preferred to consume cooled water in parallel with the role of temperature in the differentiation of taste sensation. Warm water saturates with dissolved oxygen faster, while cold water carries more dissolved oxygen (Michaud, 1991). This may be why cold water is perceived by many of us as fresher than warm water. In the light of the discussion that the freshness of water is one of the water-related factors that can affect water intake preferences in cats, it is conceivable that cats may also have a tendency towards cold water for this very reason (Turner and Bateson, 2013). Although some pet owners provide information based on observation that cooling drinking water increases water consumption, however there are not enough studies on this subject.

Conclusions

In the 2-weeks study, it was observed that serving the water that cooled at a certain level, increased the voluntarily water consumption of indoor cats, and this increase in the consumed water volume, was found to be statistically significant in 4 out of 8 cats. We believe that the results of a more detailed study with more cats, using a more advanced method that will allow more controlled processes of cooling the water and keeping the cold constant within a certain temperature will contribute to increasing water consumption in cats and minimizing the disease risks that may be encountered in inadequate water consumption.

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Covid-19 and China, history could repeat itself!

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Letter to the Editor
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Following the draconian measures already put in place throughout the SARS-CoV-2 pandemic, a very relaxed approach has been recently adopted by Chinese health authorities in order to stop the people's angry protests against the "zero Covid" strategy. This has resulted, in turn, in a dramatic surge of CoViD-19-associated hospitalization and death rates especially among elderly patients, due to the low level of anti-SARS-CoV-2 immunization with vaccines less effective than those based upon the mRNA technology.

During the first three years of the pandemic, we have gained crucial knowledge about the complex viral-(human and animal) host interaction dynamics. Noteworthy, the acquirement of "non-silent" mutations in the SARS-CoV-2 genome, consisting of approximately 30,000 nucleobases, appears to be tightly connected with viral multiplication kinetics, with each replication cycle implying the occurrence of one mutational event every 10,000 nucleotides (Di Guardo, 2022a). This clearly justifies the progressive development, in the course of the pandemic, of pathogenic "variants of concern" (VOCs) like "alfa", "beta", "gamma" and, overall, "delta", or highly transmissible and immune-evasive VOCs like "omicron" and its numerous subvariants, including "Centaurus", "Chiron", "Gryphon" and "Cerberus" alongside the newly emerged "Kraken".

Based upon the above, the emergence of additional, highly pathogenic and/or contagious SARS-CoV-2 VOCs, capable of bypassing the immunity conferred either by vaccination or previous infection, is a matter of concern in the current China's epidemiologic situation. Still of interest, while SARS-CoV-2 - in a similar fashion to the vast majority of the pathogens causing emerging infectious diseases - most likely originated from an animal (Rinolophus spp. bat) source, with the possible intervention of an "intermediate" host (Casalone and Di Guardo, 2020), at least thirty domestic and wild animal species have been hitherto deemed sensitive (albeit with different susceptibility levels) to SARS-CoV-2 infection, either spontaneously or experimentally. Furthermore, beside acquiring the virus from infected human hosts, intensely reared mink from Denmark and The Netherlands, along with white-tailed deer (Odocoileus virginianus) from Ontario (Canada), were also able to "return" the virus in a mutated form ("cluster 5" and "B.1.641" VOCs, respectively) to mankind (Di Guardo, 2022b).

These are very important lessons we have learned throughout the dramatic CoViD-19 pandemic, which has thus far killed almost 7 million people worldwide, according to the official data released by the World Health Organization.

And, sic stantibus rebus in China, the history could repeat itself (at least partially, in view of the mass vaccination campaigns put in place worldwide), considering the "global village" we all live in!

In conclusion, a holistic, multidisciplinary and scientific evidence-based approach, permanently inspired by the "One Health" principle, is what we need in order to adequately cope with this as well as with all the future epidemics and pandemics, while firmly keeping in mind that human, animal and environmental health are tightly and mutually linked to each other.

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