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Applied anatomy to the Gurcu goat´s mandible in Kafkas and its clinical significance in regional anesthesia

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ABSTRACT

Objective: This study was designed to provide important clinical signs for tracking nerves in the mandible during regional anesthesia in Gurcu goats.

Materials and methods: The study was conducted on the mandible bones of ten adult Gurcu goats of both sex (five male and five female). The mandible bone samples of naturally dead Gurcu goat were collected from Kafkas University veterinary faculty education, research, and application farm and processed as per the standard maceration technique. Altogether, 16 measurements were taken in the mandible bones of Gurcu goats by using a digital caliper and the results were expressed as mean and standard deviation.

Results: The obtained parameters from the present study can be useful for an extraoral and intraoral approach for nerve block of mental and mandibular nerve in the mandibular regions of Gurcu goat. According to results the mandibular length and height were 158.86±10.37 mm, 89.38±5.81 mm, in females and 198.93±3.85 mm, 114.5±7.29 mm, in males of Gurcu goats, respectively. The distance between the first inferior incisor tooth and mental foramen and to the first premolar tooth was 19.72±2.3, 19.26±0.44 mm in females, and 29.41±6.10, 21.83±1.02 mm, in males, respectively. The present study revealed that all the obtained parameters related to regional anesthesia showed a significant statistical difference ($p<0.01^{**}$) between the males and females of Gurcu goat.

Conclusion: It can be concluded from the present study that the various applied parameters of the present study are thought to assist clinicians in the administration of regional anesthesia in the lower jaw area (mandibular region) of the Gurcu goat.

Keywords: *Anesthesia, Mandibular Region, Mental nerve, Gurcu goat*

INTRODUCTION

Gurcu goats, also known as Tbilisi goat or Caucasian goat, whose origins are Caucasian, are bred and raised in Northern Anatolia, especially in the province of Kars and Çıldır a district of Ardahan. The Gurcu goat, mostly in black, gray, and white colors, originates from the auger horned goat *Capra falconeri* (Batu, 1951; Yalçın et al., 1990; Sezgin et al., 2010). The long and upright horns of male Gurcu goats touch each other at the tip and sometimes seen to reach 50 cm as described from Batu (1951) (Figure 1).

The lower jaw is the only movable bone of the face which moves through the temporomandibular joint. Mandibles provide support for incisors, premolars, and molars as well as support for all oral base structures.

Under natural conditions, sheep often suffer from various problems such as abscesses in the jaw, damage, and loss of various teeth, fractures of the lower jaw bone, damage to the jaw joint, etc. which

seriously harm to the health and productivity of the animal through non-intake of the food, improper food processing and consequently inefficient conversion of it.

Figure 1. Male of the Gurcu goat.

According to Duncanson (2012), up to 25% of the small cattle herd have problems with incisors from trauma due to bare or uneven grazing in grazing, or the use of mineral and feed blocks. In addition, the study conducted by Erjavec and Crossley (2010) showed that up to 34% of the animals in the small herd had incisor problems.

In these conditions, preventive or even surgical interventions are necessary. The success of which depends on a very good knowledge of the clinical anatomy or applied anatomy of the mandible, which includes two holes: the mandibular and mental foramen, which are of fundamental importance in regional anesthesia of the mandible.

Regional anatomy is one of the most important foundations of clinical and surgical practice, as it enables the clinician or surgeon to visualize the details of the case-related structures. (Dyce et al., 1996). The knowledge of the regional anatomy of the head is crucial as it has to coordinate the body, deglutition, olfaction, and defense (Dyce et al., 1996). A great deal of research has been done on the regional anatomy of the head and jaw of domestic and wild animals such as ox, horse, sheep, goat, dog, pig, and camel. (Dyce et al., 1996; Hall et al., 2000; Onar et al., 2001; Olopade and Onwuka, 2005; Karimi et al., 2011a; Avdić et al., 2013; Gündemir et al., 2020; Yılmaz and Demircioğlu, 2020; Özkan et al., 2020; Yılmaz, 2020). It has been reported previously that the mental and mandibular nerve passes from the mental and mandibular foramen, respectively (Getty, 1975; Ghosh, 2012). In an emergency requiring surgical intervention for the mental and mandibular nerve, it is very easy to position this area as a topographic landmark for quick and easy anesthesia of the involved nerves.

There is no reported data on the applied anatomy and clinical anatomy of the mandible region in Gurcu goats. Therefore, this study aimed to identify and evaluate some of the clinically important parameters and landmarks for regional anesthesia of the mandibular region in Gurcu goat.

MATERIALS and METHODS

The present study was carried out in the Department of Veterinary Anatomy at Kafkas University. The necessary permissions were obtained from the Kafkas University local ethical committee (KAÜ-HADYEK/2020-152). In this study, we have measured and evaluated ten mandibles of adult Gurcu goats (2-3 years) of both sexes (5 male and 5 female). It was collected from animals slaughtered for sacrifice, and consumption in the education, research and application farm of the Faculty of Veterinary Medicine of Kafkas University. After the samples were processed by using the boiling maceration techniques for skeleton preparation described by Simoens et al. (1994) and after cleaning it from all soft tissues, the mandibles were kept in 4% hydrogen peroxide for one day and further sundried for five days. Altogether, 16 measurements were taken in the mandible of Gurcu goats by using a digital caliper and the results were expressed as mean ± standard deviation (SD). The parameters taken into the mandible are described in Figures 2-4.

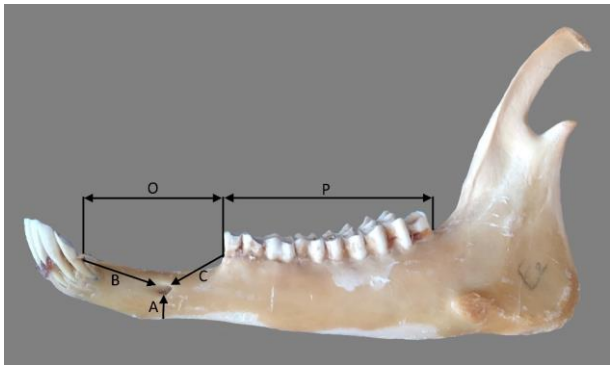

Figure 2. A. Space between the base of the mandible and mental foramen, **B.** Space between lateral incisor tooth and mental foramen, **C.** Distance between first premolar tooth and mental foramen, **O.** Diastema length, **P.** Distance between from first premolar tooth to the caudal border of last molar teeth.

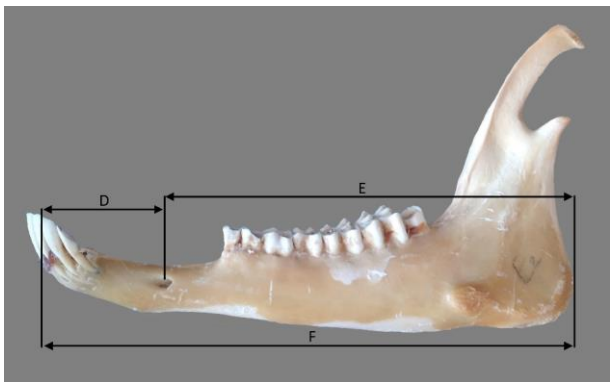

Figure 3. Space between inferior third incisor tooth and mental foramen **(D)**, Space between mental foramen and caudal mandibular border **(E)**, mandibular length **(F)**.

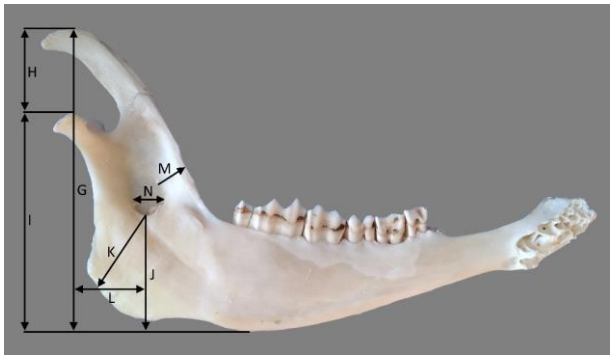

Figure 4. Maximum mandibular height **(G)**, height of mandible to the condylar process **(H)**, condylar process to the ventral margin of the mandible **(I)**, mandibular foramen to the horizontal plane at the level of the ventral margin of the mandible **(J)**, mandibular foramen to the border of mandibular angle **(K)**, below mandibular foramen to the caudal mandibular border **(L)**, mandibular foramen to the cranial border of the mandible **(M)**, and mandibular foramen length **(N)**.

A. The distance between the base of the mandible (ventral border of the mandible) and mental foramen.

B. The distance from the lateral extent of the alveolar root of the lower incisor to the mental foramen.

C. The distance between the lateral alveolar border of the first premolar tooth and mental foramen.

D. It was measured from the lateral extent of the alveolar root of the third inferior incisor tooth to the mental foramen.

E. was measured from the mental foramen level to the caudal border of the mandible ramus.

F. Mandible length

G. It was measured from the highest level of the coronoid process perpendicular to the ventral mandibular edge of the mandible.

H. It was measured from the condylar process to the maximum height of the mandible.

I. Condylar process to the ventral margin of the mandible: It was measured from the highest level of the condylar process to the ventral mandibular margin.

J. Measured from the ventral border of the mandibular foramen to the horizontal plane at the level of the ventral border of the mandible.

K. was measured from the extreme caudal border of the mandible to the mandibular foramen.

L. Caudal border of the mandible, sub-mandibular foramen: measured from the caudal border of the mandible to the vertical line, created by a description of the measurement of the mandibular foramen to the ventral edge of the mandible.

M. Mandibular foramen to the cranial border of the mandible: It was measured from the mandibular foramen to the cranial border of the mandible.

N. The length of mandibular foramen

O: Diastema length

P: Distance between from first premolar tooth to the caudal border of the last molar teeth

Figure 5. The shape variation and numbers of mental foramen.

All the above measurement parameters of the mandible of Gurcu goats were obtained and analyzed by routine statistical analysis (IBM, SPSS, 20.0 version) program**.**

RESULTS

In general, the mandible of the "Gurcu" goat is similar to the mandibles of the other goats' breeds. In the body of the mandible, the mandibular symphysis is distinct, not ossified, and easily separable. The mandibular angle is quite pronounced and presents masseteric tuberosity. The articular surface of the condylar process is slightly concave and the coronoid processus is easily turned caudally.

All the parameter results of this study are presented in Table 1.

Table 1. The morphometric parameters of Gurcu goat´s mandible.

Parameters	Female		Male		P-value
	Mean	SD	Mean	SD	
A	9.16	0.6	10.63	1.17	-
B	19.26	0.4	21.83	1.02	-
C	19.72	2.3	29.41	6.1	-
D	33.2	2.3	44.34	1.93	p<0.01**
E	125.30	8.49	151.31	0.54	p<0.01**
F	158.86	10.37	198.93	3.85	p<0.01**
G	89.38	5.81	114.5	7.29	p<0.01**
H	26.25	1.70	37.69	4.94	p<0.01**
I	62.32	6.48	79.14	2.69	p<0.01**
J	32.22	2.80	42.79	2.36	p<0.01**
K	25.53	2.81	37.99	1.73	p<0.01**
L	19.56	2.54	28.55	1.90	-
M	14.30	1.25	19.80	1.43	-
N	9.04	0.24	8.78	0.81	-
O	33.58	2.54	41.08	0.7	-
P	60.53	2.87	80.27	3.70	-

DISCUSSION

The mandibular length and height were 158.86±10.37 mm, 89.38±5.81 mm, in females and 198.93±3.85 mm, 114.5±7.29 mm, in males of Gurcu goats, respectively. Whereas, the similar mandibular data were 12.00±0.89 cm, 6.90±1.09 cm for WAD goats (Olopade and Onwuka, 2005); 14.21±0.98 cm and 8.83±0.40 cm in black Bengal goat (Uddin et al., 2009); 14.1±1.03 cm and 8.69 ± 0.18 cm, respectively (Monfared et al., 2013); 17.6±0.32 cm and 9.96±0.25 cm in sheep, while in roe deer 15.6±1.22 cm and 8.43±0.15 cm (Avdić et al., 2013); 11.24±0.52 cm, 6.64±0.44 cm in GVD goat (Kataba et al., 2014); 16.53±0.12 cm, 10.69±0.02 cm in blackbuck (Choudhary and Singh 2015b) and 14.18±0.48 cm, 8.21±0.33 cm in males; 12.93±0.96 cm, 7.33±0.50 cm in females of chinkara (Din et al., 2020).

The morphometric parameters related to the mandibular and mental foramen as the most two important clinical parameters of the mandible situated on the medial and lateral surface of the mandible bone respectively have been shown in Figure 2-4. The mental foramen at the Gurcu goats looks oval in shape (Figure 5). Mandible incisure was found to be narrower in females than in male Gurcu goats. In two cases, it was found double mental foramen on mandibles belonging to female goats.

The distance between the first inferior incisor tooth and mental foramen and to the first premolar tooth was in females 19.72±2.3, 19.26±0.44 mm and 29.41±6.10, 21.83±1.02 mm, in males, respectively (Figure 2). The space between the lateral alveolar roots of the third inferior incisor tooth to the mental foramen was 33.2±2.3 mm in females, 44.34±1.93 mm in males of Gurcu goats (Figure 3), which was an important landmark for achieving the location of the mental foramen nerve for the regional nerve block in Gurcu goats. In contrast, it was 1.60±0.22 cm in WAD goat (Olopade and Onwuka, 2005); 2.00±0.3 cm in red Sokoto (Maradi) goats (Olopade and Onwuka, 2007); 2.45 cm in blackbuck (Choudhary and Singh, 2015a); 1.25±0.19 cm in females of chinkara (Din et al., 2020). The space from the lateral alveolar first inferior incisor tooth to the mental foramen was 2.40±0.26 cm, which is the study made by Monfared et al. (2013).

Nervus mentalis is a branch of the lower alveolar nerve that arises through the foramen mentale and divides into three branches below the musculus depressor anguli oris. (Farag et al., 2017; NAV, 2017). These branches are distributed to the skin of the chin as well as the skin and mucous membrane of the lower lip (Farag et al., 2017). In Gurcu goat mandibles, the blockage of the extraoral can be obtained by injecting the mental nerve approximately 3-4 cm of the anesthetic drug into the mental foramen from the lateral extension of the alveolar root of the lower third incisor. However, a similar nerve block can be successful by injecting approximately 2.80 cm of the anesthetic drug into the barking deer and approximately 3.00 cm into the sambar deer (Keneisenuo et al., 2020). The blockade of the nervus mentalis is beneficial in numbing the lower lip during surgical procedures.

The maximum distance from the mental foramen to the caudal border of the ramus of the mandible was 125.30±8.49 mm in females, 151.31±0.5 mm in males of Gurcu goats, while the same parameters were 13.43±0.08 cm in blackbuck (Choudhary and Singh, 2015b); 11.69±0.40 cm in black Bengal goat (Uddin et al., 2009); 13.74±0.18 cm in Mehraban sheep (Karimi et al., 2011b); 9.26±0.49 cm in GVD goat (Kataba et al., 2014); 15.23±1.46 cm in Barbados black belly sheep (Mohamed et al., 2016); 11.8±0.89 cm in black Bengal goat (Poddar et al., 2018) and 12.38±1.52 cm in Abaza goats (Dalga, 2020).

The mandibular foramen looks quite large, 9.04±0.24 mm in females and 8.78±0.81mm in males. It is located at about one-third of the maximum height of the mandible and is positioned almost in the middle of the pterygoid fossa, which is not very pronounced.

The distances from the mandibular foramen to the ventral margin of the mandible was 32.22±2.80 mm in females and 42.79±2.36 mm in males, whereas the distance from mandibular foramen to the border of mandibular angle was 19.56±2.54 mm in females and 28.55±1.90 mm in males of Gurcu goat. These two parameters of the mandibular foramen had statistically significant differences between females and males of Gurcu goats. The distance from the caudal border of the mandible to the level of the mandibular foramen was 25.53±2.81 mm in females and 37.99±1.73 mm in males (Figure 3). Whereas the same parameters were recorded as; 4.18±0.01 cm, 1.36±0.01 cm, 3.07 cm in blackbuck (Choudhary and Singh, 2015a); 2.88±0.93 cm, 2.50±0.73 cm, and 1.29±0.12 cm respectively about the study made by Monfared et al., (2013). The same distances for WAD goats of Nigeria were 1.57±0.44 cm, 2.58±0.34 cm for the caudal border of the mandible to below mandibular foramen and the mandibular foramen to the ventral margin of the mandible (Olopade and Onwuka, 2005).

Mandibular nerve blockage is used to anesthetize the mandibular nerve during clinical examinations and surgical procedures involving the alveoli and teeth of the lower jaw in animals (Lahunta and Habel, 1986).

The mandibular nerve is beneficial in the treatments to be obtained with all operational intervention related to the lower jaw such as lower incisors, molars, and premolars, tooth extraction, tumor. Mandibular nerve block can be achieved by injecting anesthetic drugs approximately 32.22±2.80 mm to 42.79±2.36 mm in Gurcu goat from the horizontal plane at the level of the ventral margin of the mandible to the ventral limit of the mandibular foramen. However, the same nerve block can be achieved by injecting anesthetic drugs approximately 2.5 cm and 5 cm in barking deer and sambar deer, respectively at the level given above (Keneisenuo et al., 2020) or, 3.43±0.25, 2.58 ± 0.34 cm to Markhoz goat WAD goat and 2.88±0.93 cm to Iranian native goat (Goodarzi and Hosseini, 2013).

The distance from the mandibular foramen to the cranial border of the mandible was 14.30±1.25 mm in females and 19.80±1.43 mm in males of Gurcu goats; however, the same parameter was recorded as 5±55 cm in cattle (Nazih and El-Sherif, 2018). An intraoral mandibular nerve block can be achieved by injecting anesthetic drugs approximately 1.4-1.9 cm in Gurcu goat from the cranial border of the mandible to the mandibular foramen.

CONCLUSION

The applied anatomical data created from the mentioned parameters described in the present investigation were not reported previously in the Gurcu goat, which has great surgery significance and may be used as a landmark for tracing the mental and mandibular nerve desirable for their desensitization during any type of operative procedure at the level of the specific foramen.

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Author's Contributions: SD, KA designed the study. SD collected the materials and made the maceration operations. SD created certain parameters based on samples and took measurements. SD made the statistical analysis of the measurements taken. SD and KA did the writing of the study (SD:Semine Dalga, KA:Kadir Aslan)

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Researching some mineral substance and vitamin levels in the cattle with indigestion

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ABSTRACT

Objective: This study; It was aimed to compare serum and rumen content, trace element and serum vitamin levels and rumen content in indigestion cattle with healthy animals.

Materials and Methods: The study was conducted on a total of 30 cattle, 10 healthy (control group) and 20 indigestion group. Indigestion diagnosis in animals was determined by anamnesis information, clinical and rumen content examination.

Results: Hematologically, there was no statistical difference between indigestion and control groups. Serum magnesium (Mg), calcium (Ca), zinc (Zn) levels of the measured trace elements decreased in the group with indigestion ($p<0.05$), while the levels of cobalt (Co) increased significantly ($p<0.01$). While there was a significant increase ($p<0.5$) in cattle with indigestion in rumen content Ca values compared to healthy cattle, serum levels were decreased ($p<0.05$). A positive correlation ($p<0.01$) was determined between rumen content levels of indigestion cattle and serum Mg levels of control group and rumen content of control group. In addition, a negative correlation was found between serum Ca and rumen content values of cattle with indigestion. In serum vitamin levels, Vitamin B_1 (Vit B_1) decreased statistically ($p<0.05$), while Vitamin B_{12} (Vit B_{12}) was found to increase non-statistically ($p>0.05$).

Conclusion: As a result, it was concluded that the decrease in serum Mg, Ca and Zn values in indigestion animals is important and these trace elements should be used in treatment.

Keywords: *Cattle, Indigestion, Rumen Content, Trace Elements*

INTRODUCTION

Indigestion, is a state of disruption of stomach movements and functions (İmren, 2003). Simple indigestion is a small-scale abnormality observed in forestomach motility or fermentative activity due to a sudden change in the quality and content of the diet used in feeding or other factors (Gül, 2016; Shah et al., 2017; Arora et al., 2018). Simple indigestion is of acute onset and does not lead to significant pathological consequences (Stefańska et al., 2017; Pechová and Nečasová, 2018).

The digestive system of ruminants is an ideal environment for fermentation. More than 60% of digestive activities occur in reticulo rumen. In ruminants, nutrients that monogastric animals can not digest, thanks to the microorganism population in the reticulo can be digested (Özel and Sarıçiçek, 2009). In this anaerobic conditions environment under microorganism bacteria, protozoa and fıngus are present symbiotically (Bilal, 2003; Altuğ, 2014).

Indigestion may be caused by all nutritional factors that cause changes in the intra-rumenal environment (Poock, 2011). Sudden ration changes, unbalanced ration, excessive feeding of grain feeds, increased energy amount in the ration, unidirectional feeding, poor quality feed materials

(frozen or moldy feeds, poor quality or soil contaminated silage), feding of your time irregularity, insufficient water supply, long-term use of antibiotics, sulfonamides or disinfectants, inactivity or reduced feed intake as a result of being closed, some diseases (such as forestomach or abomasum dysfunctions and other organ diseases) occur as a result of impaired adaptation of rumen microorganisms (Gül, 2016). Subacute rumen acidosis is also one of the suggested causes (Foster, 2003).

One of the most commonly used examinations in the diagnosis of forestomach diseases of cattle is the analysis of rumen fluid. Many physical and biochemical analyzes of rumen fluid samples are made (Dabak, 2009). In 1 ml of rumen content there are approximately $1x10^{10}$ bacteria, $1x10^{6}$ ciliate protozoa and $1x10^{6}$ fungus. This microbial population allows the diet to ferment essential fatty acids (acetic acid, propionic acid and butyric acid) microbial proteins and minerals (Dehorrity, 1998).

Trace elements have great importance in increasing the resistance against diseases in living things (Sarıbay and Özsoy, 2019). It is emphasized that insufficiency or excess causes serious clinical symptoms, causes significant losses in the livestock economy, and the resulting losses are as important as those caused by infectious and parasitic diseases. In animals, diseases caused by macro and micro elements are of great importance (Durmuş and Eryavuz, 2012). Incomplete or excessive intake of one or a few elements disrupts normal functions, as well as deterioration of the ratios between elements can cause physiological changes in the organism (Yeşil and Sarıözkan, 2017; Sarıbay and Özsoy, 2019; Hervig et al., 2019).

Copper is one of the most important trace elements necessary for the body and essentially it must be taken from outside. (Paksoy et al., 2010). The amount of Cu in the blood of cattle is between 32.8-35.2 µg / dl (Küçükaslan, 2011). It has been reported that symptoms such as growth pause, weight loss and cachexia occur in copper deficiency due to the contraction of oxidation in tissues (İmren and Şahal 1991).

Zinc essentially enters the structure of many enzymes in different ways, and protein, carbohydrate, nucleic acid and lipid metabolisms also play a role through these enzymes (Tanyüksel, 1995). Total blood Zn amount in cow are 319±34 µg/dl (Küçükaslan, 2011). Zn deficiency; It causes many symptoms such as diarrhea, anorexia, weakening, growth retardation, mouth inflammation, decreased milk yield, parakeratosis, and allopesia in animals (Başoğlu, 2004; Gül, 2012).

Chlorine is an extracellular ion with a high concentration (Turgut, 2000; Şentürk, 2013). Serum normal value of Cl in cattle; It is 95-110 mEq/l (Şentürk, 2013).

Extracellular Cl concentration is determined by measuring the plasma Cl level. Plasma Cl concentration varies according to absorption from the intestines. In cases of diarrhea or food-related indigestion that delay gastric emptying and in abomasal displacements, a Cl level below 75 mmol/l is accepted as a negative plasma value (Bilal, 2012).

Calcium is the most important mineral that forms bone tissue (Guyton and Hall, 1996). The blood Ca level of adult cattle is approximately 8.5-10 mg/dl. It is possible that blood Ca can maintain its normal normal level, Ca absorption from the rumen and intestines and Ca mobilization from bone tissue are possible (Guyton and Hall, 1996). Two types of hypocalcemia are mentioned in dairy cattle. The most common of these is parturient hypocalcemia, which is formed due to birth-related Ca loss, and less commonly, a decrease in non-parturient Ca levels due to Ca loss due to gastrointestinal diseases such as indigestions rather than Ca loss due to the onset of lactation and secondary disorders such as stress (Başoğlu and Sevinç, 2004).

Magnesium is an essential macro element essential for numerous functions in the body of all mammals, including fattening and diurnal cattle (Schaff, 2014). The digestibility of Mg in the diet is around 40% in ruminants and 60% in horses, cats and dogs. Mg is absorbed in the forestomach in ruminants, the last part of the small intestines and colon in monogastric animals. Increasing serum Mg level causes its absorption to decrease. While absorption occurs from the small and large intestine up to 1 month in calves, absorption occurs from here after the forestomach develops (Bilal, 2012).

Cobalt, which is one of the essential elements for ruminant animals, is necessary for rumen microorganisms to synthesize vitamin B_{12} (Vit B_{12}) (Okatan et al., 2008; Küçükaslan, 2011; Gül, 2012). Rumen bacteria are used in Vit B_{12} synthesis to meet both the animal's and their own Vit B_{12} needs (McDowell, 1992; Uyanık, 2000). Vit B_{12} deficiency due to Co causes loss of appetite and weakening in ruminants (Uyanık, 2000).

Deficiency of B vitamins in indigestion occurs due to the activity of rumen microflora (George, 2006).

Because they provide the nutrients (B complex vitamins and all essential amino acids) that ruminant animals need (Ensminger et al., 1990). Because they provide the nutrients (B complex vitamins and all essential amino acids) that ruminant animals need (Ensminger et al., 1990).

In studies conducted on the use of organic minerals in ruminant nutrition, it has been reported that these minerals can be used in ruminant rations, increase yield performance, prevent low yield due to mastitis, have an effect on milk production and quality, using organic minerals reduces the number of somatic cells and has a positive effect on immunity (Boland, 2003).

In this study; It was aimed to compare serum and rumen content, trace element and serum vitamin levels, rumen content examination and hematological parameters in cattle with indigestion with healthy animals.

MATERIALS and METHODS

This study was approved by Yuzuncu Yil University Animal Experiments Local Ethics Committee (YUHADYEK) with the decision dated 25.06.2015 and numbered 08.

Animal selection criteria

Animal material included in the study was carried out on a total of 30 cattle with an average age of 3 (three) registered with the Ministry of Agriculture and Forestry in Van and surrounding districts. Twenty of these animals were indigestion and 10 of them constituted the control group. Routine clinical examination of all cattle was made (heart and respiratory frequency, body temperature, dehydration status, examination of mucous membranes) and the data obtained were recorded.

Among the cattle with suspicion of 20 indigestion with anorexia, decrease in milk yield and rumen movements according to anamnesis information; With the help of the content probe, the rumen content was taken into containers that were duly heated up to 36-38°C at room temperature. This content, which was brought to the laboratory, was first examined macroscopically and the pH value was measured. At the same time, the preparation was prepared and examined under a microscope in terms of infusoria. Samples detected as positive for indigestion were stored at -20°C until the number was completed. It was determined that the presence and mobility of protozoans in the indigestion group decreased compared to normal. Ten cattle without any symptoms in their anamnesis and showing compliance with physiological conditions as a result of rumen content controls constituted the control group.

In addition, blood samples were taken from the animals in both biochemistry and hematology tubes from the vena jugularis. The rumen contents and the serums separated by centrifuge were stored in a deep freezer (-20ºC) in the laboratory of the Van Yuzuncu Yil University, Faculty of Veterinary Medicine, Department of Internal Diseases.

Method

Hematological examinations

The blood collected was brought to Van Yüzüncü Yıl University Faculty of Veterinary Medicine, Department of Internal Diseases, and hematological parameters (Red blood cell (RBC), Haemoglobin (Hg) Haematocrit (HTC), MCV, White blood cell (WBC), Lymphocyte (LY), Monocyte (MO) Neutrophil (NE), Eozonophil (EO), Basophil (BA), Platelet (PLT), veterinary blood count device (Abacus - Junior Vet5).

Biochemical Examinations

Serum samples were seperated off by centrifugation for 3000/10 cycle /minute and stored in a deep freezer (-20°C) until biochemical analysis was performed.

Physical examinations of rumen contents

In the diagnosis of indigestion animals, pH, smell, color, consistency, number of protozoa and their movements (Başoğlu, 1998; Bilal, 2012; Şentürk, 2013; Altuğ, 2014) were evaluated.

Trace element measurements of rumen content

For the determination of trace elements and minerals in rumen content; The content samples were centrifuged before analysis (3000/5 rpm) and the supernatant part was removed. Diluted with 2 ml rumen contents and 2 ml 1/5 nitrochloric acid and centrifuged (3000/5 rpm) then filtered and clear filtrate. 3 ml was taken and placed in tubes. From trace elements and minerals from the supernatant taken; Zn, Cu, Ca, Mg, Co and Cl levels were measured by Atomic Absorption Spectrophotometer device.

Serum Vit B_1 liquid chromatography device (HPLC Agilend 1100), Vit B_{12} levels (Abbot C16200®) device and Zn, Cu, Ca, Mg, Co and Cl levels of trace elements were measured with Atomic Absorption Spectrophotometer device.

Statistical analysis

Descriptive statistics for control group and indigestion group; Average was expressed as Standard Deviation values. Independent-samples T test was performed to compare the control group and the indication group.

In addition, Spearman correlation analysis was performed to determine the relationships between blood serum and rumen content parameters.

In calculations, the statistical significance level was taken as 5% and the SPSS statistical package program was used for calculations (Suvak et al., 2014; Başbuğan et al., 2015).

RESULTS

Clinical findings

Anamnesis information was obtained in cattle with indigestion, no eating and drinking between 3-10 days, animals did not rumble, decreased milk yield was observed, fatigue and staggering, tympani in some animals and no defecation was obtained.

A rumen examination was performed along with the general examination of the animals whose anamnesis information was obtained. Hypomotility was detected. Rumenoreticular contractions were found to be lower than normal. It was also observed that the sounds heard from the rumen were weakened. In 10 of the animals, foreign bodies were found to be positive after ferroscopic examination. It was found that the indigestion was formed due to the foreign body.

Rumen content examination

In healthy animals (control group) included in the study, the pH of the rumen content was 6-7, the smell was normal, aromatic, its color changed from bright green to gray, its consistency was viscous and infsoria was dense and mobile, in the indigestion group the pH was 6-8, the odor was sour and moldy, dingy color changed from gray to dark green even black, its consistency was watery and foamy, the amount of infsoria was small and the presence of only small sized ones were found to be less mobile.

Table 1. Trace element levels with indigestion and control animals.

Parameters		Control (n=10)	Indigestion (n=20)	Reference value
Mg	Serum (mg/dl)	2.089±1.27	1.540±0.89*	1.8-2.3[1]
	Rumen content (mg/L)	27.21±0.18	27.00±0.75	
Zn	Serum (µg/dl)	1.17±0.04	0.96±0.05*	0.95-1.03[2]
	Rumen content (mg/L)	3.36±0.72	4.05±064	
Cu	Serum (mg/L)	0.6±0.04	0.48±0.04	0.5-2.2[3]
	Rumen content (mg/L)	0.45±0.13	0.24±0.03	
Ca	Serum (mg/dl)	13.87±3.84[a]	8.94±2.18*[b]	9.7-10.4[4]
	Rumen content (mg/L)	312.46±59.77[a]	374.07±81.63*[b]	
Cl	Serum (mEq/L)	98.05±6.04	91.62±4.30	97-111[4.5]
	Rumen content (mg/L)	22.36±0.8	26.04±1.03	
Co	Serum (µg/dl)	0.16±0.01	0.29±0.02**	0.26[2]
	Rumen content (mg/L)	0.49±0.02	0.56±0.09	

* Indicates the statistical significance on the same line. [a,b] Indicates the statistical significance in the different row. [1](Şentürk 2013), [2](Okatan ve ark., 2008), [3](Blood ve ark.,1987),[4](Bilal 2012), [5](Turgut, 2000)

Laboratory findings

Trace element levels obtained from indigestion and control group animals in the study are given in Table 1, and Vit B_1 and B_{12} levels are given in Table 2. Among the statistical correlation analyzes, no statistically significant correlation was found in the serum and rumen content values of Cu, Zn and Co, and the statistical correlation tables of the Mg and Ca parameters are presented in Table 3,4.

Biochemical findings

In the indigestion group, it was determined that Mg, Ca and Zn levels were statistically decreased compared to the control group. (P<0.05).

Table 2. Vitamin B_1 and B_{12} levels with indigestion and control animals.

Parameter	Control	Indigestion	Reference
Vit B_1 (µg/dl)	8.30±0.44	4.40±0.30**	7±1.1 [1]
Vit B_{12}(pg/ml)	193.00±7.69	186.62±15.84	155.13±19.74 [2]

*p<0.01

[1](Mert, 1996), [2](İssi ve ark., 2010)

It was observed that there was a statistically significant increase in serum Co levels in the indigestion group compared to the control group. (p<0.01).

It was determined that Ca level in rumen content in the indigestion group increased statistically compared to the control group (p<0.05)

It was determined that serum vit B_1 levels in the indigestion group were statistically significantly decreased compared to the control group (p<0.01). In the indigestion group compared to the control group, there was no statistical significance for the results obtained at vit B_{12} levels (p>0.05).

Table 3. Magnesium serum and rumen content levels with indigestion and control animals.

Parameter		Serum Mg		Rumen content Mg	
		Control (n=10)	Indigestion (n=20)	Control (n=10)	Indigestion (n=20)
Serum Mg (mg/dl)	Control	1			
	Indigestion	-0.217	1		
Rumen content Mg (mg/dl)	Control	0.870**	0.139	1	
	Indigestion	-0.212	0.283	-0.199	1

**: p<0.01

Table 4. Calcium serum and rumen content levels with indigestion and control animals.

Parameter		Serum Ca		Rumen content Ca	
		Control (n=10)	Indigestion (n=20)	Control (n=10)	Indigestion (n=20)
Serum Ca (mg/dl)	Control	1			
	Indigestion	-0.636	1		
Rumen content Ca (mg/dl)	Control	-0.040	-0.341	1	
	Indigestion	0.654	-0.897**	0.057	1

**: p<0.01

After Spearman correlation analysis performed to determine the relationships between properties in serum and index group, a 87.0% correlation was found between serum Mg value of control group and Mg value of rumen content of control group. Accordingly, while the serum Mg value in the control group increases, the rumen content of the control group also increases by 87.0%. In other words, while the Mg value of the rumen content of the control group increases, the serum Mg value of the control group also increases by 87.0%.

There was no statistically significant correlation among other features. After Spearman correlation analysis performed to determine the relationships between properties in serum and indigestion groups, a negative correlation of 89.7% was found between the serum Ca value of the indexed group and the rumen content Ca value of the indigested group. Accordingly, while serum Ca value in the indigestion group decreased, the rumen content Ca value in the control group increased by 89.7%. There was no statistically significant correlation between other features.

DISCUSSION

Forestomach diseases have an important place in the digestive system diseases of cattle (Dabak, 2009; Suvak et al., 2014). The yield level obtained from

ruminants is in parallel with the healthy work of the forestomach and abomasum (Başoğlu, 1998). One of the most common examinations used in the diagnosis of forestomach diseases of cattle is the examination of rumen fluid. Many physical and biochemical analyzes of rumen fluid samples (Dabak, 2009; Suvak et al., 2014). For this purpose, the analysis of rumen content, color, odor, pH, consistency and existing infsoria is an important criterion (Bilal, 2012; Şentürk, 2013).

According to researches, the color of the rumen content changes depending on the quality of the feed given to the animal. The color varying between gray and brown in those fed in pasture and in green barns. When healthy animals are fed with a diet rich in carbohydrates, rumen content is sour and an odor similar to ammonia is perceived when they are fed protein-rich diet. In cases of rumen acidosis, it turns into a sour dough odor. pH examination of rumen content is between 6.2-7.2 in healthy animals. In rumen acidosis, while the colour of rumen content turns from sesema paste-tahini- to brown, it smells like sourdough and the pH drops below 5. While the rumen content in rumen alkalosis has a sharp ammonia odor, the pH is between 7.5-8.0. In rumen smelling, while the color takes on a greenish color close to black, an odor resembling the smell of rotten and sour food is perceived (Başoğlu, 1998; Bilal, 2012; Şentürk, 2013).

In this study, it was observed that the rumen content of the indigestion group changed from dark green to gray brown, their pH was between 7-8 and had a musty and sour odor. In the control group, it was observed that the rumen content was green and brown in color, its odor was not aromatic and not alarming, but its pH was between 6-7. This situation is in line with what the researchers (Başoğlu, 1998; Batmaz, 2010; Clanie and Tesfaye, 2012; Şentürk, 2013) stated.

In the examination of infsoria, which has an important place in the examination of rumen content, it is stated that the density of animals fed with insufficient and poor quality ration decreases, when the pH is below 5, the infsoria completely disappear, and when the pH is 7 and above, their movements are reduced although they are dense and lively (İmren, 2003; Bilal, 2012; Şentürk, 2013).

It was observed that the infsoria in the stomach contents of the control group animals included in this study were dense, mobile, and equal numbers of large, small and medium sizes. In the indigestion group, the presence of fewer and less mobile infsorias was detected. It was understood that these findings were similar to what the researchers (Şentürk, 2013; Bilal, 2012) said about indigestion animals.

In this study, no statistical difference was found between hematological parameters examined in control and indigestion groups. Due to this situation, it was not included in the discussion. Although serum trace element and mineral levels have been determined in many studies, no study has been found on the mineral and trace element levels of rumen fluid. The aim of this study was to evaluate serum and rumen content, mineral and trace element levels together to aid in diagnosis and to be used as a reference value in animals with indigestion.

The Mg level in healthy cattle is reported as 1.8-2.3 mg/dl (Şentürk, 2013). In this study, serum Mg level was measured as 2.089±1.27 mg/dl in the control group and this was found to be parallel with the specified value. Serum Mg value was measured as 1.540±0.89 mg/dl'in the indigestion group. This value is lower than the control group and is statistically significant ($p<0.05$). While the Mg level of rumen content was 27.21±0.18 mg/L in the control group, it was 27.00±0.75 mg/L in the group with indigestion. It was observed that there was no difference compared to the control. Magnesium is an essential macro element essential for numerous functions in the body of all mammals (Schauff, 2014). Mg is absorbed in the forestomach of cattle. Increa

Serum Zn levels are reported to be 0,98±0,01(0,95-1,03) μg/dl in healthy cattle (Okatan et al., 2008). Serum Mg level causes decrease in absorption (Bilal, 2012). In this study, serum Zn level was measured as 1.17±0.04 μg/dl in the control group and 0.96±0.05 μg/dl in the indigestion group. As can be seen in Table 1, it was observed that this value decreased compared to the control group and was statistically significant ($p<0.05$). This level was measured as 4.05 ± 064 mg/L in animals with 3.36 ± 0.72 mg/L indigestion in the Zn control group with rumen content.

Blood et al. (1987) found serum Cu level 0.5-2.2 mg/L in healthy cattle. In our study given in Table 1, serum Cu level in the control group was 60±0.4 μg/dl, while it was found as 48±4 μg/dl in indigestion animals. This situation was not found statistically significant when compared with the indigestion group of the control group, which was parallel to the statements in Blood et al. In this

study, the rumen content was measured as 0.45±0.13 mg/L in the control group and 0.24±0.03 mg/L in the indigestion group, and these values are the first data feature detected in cattle.

Serum Ca level in healthy cattle 9.7-10.4 mg/dl in the control group (Bilal, 2012). In this study, serum Ca level was measured as 13.87±3.84 mg/dl in the control group. It was seen that this value was between the levels that the researchers gave for serum Ca levels of healthy cattle. Serum Ca level was measured as 8.94 ± 2.18 mg/dl in indigestion animals given in Table 1, and this level was found to be statistically significantly decreased ($p<0.05$) compared to the control group. Ca level of rumen content was found to be 312.46±59.77 mg / L in the control group and 374.07±81.63 in the group with indigestion.

Although it was determined that the rumen Ca level decreased in indigestion animals compared to the control group, this situation was not statistically significant ($p>0.05$). It is thought that there is a decrease due to indigestion in the group with Ca indigestion. The Cl level in healthy cattle is between 97-111 mEq/L (Turgut, 2000; Bilal, 2012).

In our study, the Cl level, which was parallel to the statements of the researchers (Turgut, 2000; Bilal, 2012), was found to be 98.05±6.04 mEq/L in the control group, while it was 91.62±4.30 mEq/L in the group with indigestion. It was concluded that the value, which was found to be lower than the control group, was statistically insignificant ($p>0.05$).

Rumen content Cl values are a frequently used marker in the differentiation of anter Rumen Cl level in healthy cattle has been reported as <25 mEq/L. prior and posterior functional stenosis. Posterior stenosis occurs in which serum levels below <85 and rumen content will be above >25 mEq/L (Turgut, 2000). The rumen content of the control group, which is similar to the statement of the investigators given in Table 1, was 22.36±0.8 mEq L, while it was found to be 26.04±1.03 mEq/L in the group with indigestion. In this context, it is an indication that there is no posterior functional stenosis in animals. Although in indigestion animals the rumen Cl control unit increased compared to the display, this is similarly insignificant ($p>0.005$).

One of the essential elements for ruminant animals, Co is necessary for rumen microorganisms to synthesize vitamin B_{12} (Okatan et al., 2008; Küçükaslan, 2011). The need for Co of ruminants results from the Co requirement of the bacteria in the rumen (Uyanık, 2000; Gül, 2012). Rumen bacteria are used in vit B_{12} synthesis to meet both the animal's and their own vit B_{12} needs (Uyanık, 2000). Serum Co levels in healthy cattle are reported to be between 0.26±0.15 μg/dl (Okatan et al., 2008).

In this study, the serum Co level was 0.16±0.01 μg/dl in the control group and it is parallel with the value in healthy cattle expressed by the researcher. Serum Co level was measured as 0.29±0.02 μg/dl in the indigestion group. It was determined that the increase in serum Co levels in animals with indigestion was statistically significant ($p<0.01$).

This situation is thought to be increased due to the fact that the rumen micro flora cannot be used in vit B_{12} synthesis, as stated by the researchers (Okatan et al., 2008; Küçükaslan, 2011; Gül, 2012). While the Co level of rumen content was measured as 0.49±0.02 mg/L in the control group, this value was measured as 0.56±0.09 mg/L in the indigestion group. Although the rumen Co level increased in the indigestion group compared to the control group, this increase was found to be statistically insignificant ($p>0.05$).

Vitamin B_1 is synthesized with the help of rumen microorganisms. (Evan, 1993; Karapınar et al., 2010). Therefore, this situation should be taken into consideration when adding thiamine to rations (Evan, 1993; Karapınar et al., 2010). Serum Vit B_1 level in healthy cattle is stated to be 7±1.1 μg/dl (Mert, 1996). In this study, vit B_1 level was measured as 0.83± 0.44 (μg/ml) in the control group and as 0.440±0.30 (μg/ml) in the indigestion group. Cyanocobalamin is synthesized in the digestive tract of ruminants (İmren, 1991). As there is sufficient cobalt and protein in the feed, ruminant animals do not need to take Vit B_{12} from outside (İmren and Şahal, 1991). It is synthesized in the digestive tract of ruminants.

As long as there is sufficient amount of cobalt and protein in feed, ruminant animals do not need to take Vit B_{12} from outside (Şahal and İmren, 1991). Serum Vit B12 level of healthy cattle is reported to be 155.13±19.74 pg/mL (İssi et al., 2010). In this study, vit B_{12} level in the control group was 193.00±7.69. 209.94 ± 27.65 in pg mL indigestion group. It is measured in pg/mL. Although it was observed that the Vit B_{12} level increased compared to the control group (Table 2), this increase was statistically insignificant ($p>0.05$).

In this study; There was no statistical difference in hematological findings between healthy animals and indigestive animals. Serum magnesium (Mg),

calcium (Ca), zinc (Zn) levels of trace elements decreased in the group with indigestion ($p<0.05$), while a significant increase in cobalt (Co) levels ($p<0.01$) was detected. Although there was a significant increase ($p<0.5$) in the group with rumen content Ca values indigestion compared to the healthy group, a decrease in serum levels ($p<0.05$) was detected. There was a positive correlation ($p<0.01$) between rumen content of indigestion cattle and blood serum values between control group serum Mg and control group rumen content. In addition, a negative correlation was found between the indigestion group serum Ca and the indigestion group rumen content Ca. Serum vitamin levels decreased in Vit B_1 statistically ($p<0.05$), while a non-statistical increase was determined in Vit B12 ($p>0.05$).

CONCLUSION

As a result, although serum trace element and mineral levels were determined in many studies, no study was found on the mineral and trace element levels in rumen fluid. With this study, it is thought that the values measured together with serum and rumen content, mineral and trace element levels will be helpful in diagnosis and can be used as a reference value in animals with indigestion. In addition, in this study, it was observed that the decreases in serum Vitamin B_1 as well as in Mg, Ca and Zn values were significant in animals with indigestion, and these decreases were due to indigestion. Thus, it was concluded that these vitamins and trace elements should be taken into consideration in the treatment.

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Investigation of the effect of aminoglycosides on angiotensin converting enzyme (ACE)

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ABSTRACT

Objective: The researcher's attention nephrotoxicity from antibiotics (as aminoglycosides), non-steroidal anti-inflammatory drugs, and antifungals, angiotensin-converting enzyme (ACE) inhibitors. Several drugs have resulted in produce an adverse effect on kidneys. Angiotensin-converting enzyme (ACE) is a component of the renin-angiotensin system (RAS), which leads to the conversion of Angiotensin-I to Angiotensin-II in vascular tissues. The aim of this work was to investigate the effect on serum angiotensin converting enzyme of the amikacin.

Material-Method: In this study, two different groups were formed as control (10 rats / Wistar-albino female) and experimental group (30 rats / Wistar-albino female). The experimental group was administered 15 mg/kg amikacin intraperitoneally (ip) for 14 days, and the control group was administered saline solution at the same rate.

Result: When the groups are compared according to the statistical results, it is seen that there is a significant increase in ACE activity of the experimental group compared to the control group ($p<0.001$).

Conclusion: As a result, it was determined that amikacin administered increased serum ACE activity and it was concluded that it may be useful to investigate the possibilities to evaluate it as a risk factor and indicator in the development of hypertension.

Keywords: *Amikacin, Angiotensin-Converting Enzyme, Antibiotics*

INTRODUCTION

The renin-angiotensin system (RAS) plays an important role in the pathogenesis of cardiovascular and renal diseases. The RAS system, maintains blood volume and regulates water, salt metabolism, vascular tone, kidney and heart function through the classical angiotensin-converting enzyme (ACE) angiotensin I-II receptor, types 1-2 pathway (Ma et al., 2014). The ACE is a monomeric and membrane-bound enzyme that activated with zinc and chloride. It is found in significant proportions of lungs, brain, testis, kidney tissues, in addition to physiological fluids such as plasma, semen, macrophages, vessels, endothelial cells (Burnier, 2001).

It has been determined that ACE is mostly found in the endothelium of large and small arteries and arterioles (Falkenhahn et al., 1995; Moncada et al., 1998). ACE plays a key role in cardiovascular and renal disease. The results of endothelial function impaired by response to risk factors such as hypertension, diabetes mellitus, smoking and hypercholesterolemia, is associated with the pathological activation of local ACE. At the same

time, this pathological activation has serious effects on the heart, blood vessels and kidneys (Dzau et al., 2001). ACE inhibitors are one of the main drug groups used in the treatment of hypertension (Mancia et al., 2009).

Although angiotensin receptor blockers (ARB) were initially considered as an alternative drug group to ACE inhibitors, they are now considered to be a completely different group. It has been observed in studies performed on isolated artery grafts that Angiotensin II receptor blockers suppress Angiotensin II response (Liu et al., 2000).

Aminoglycoside group antibiotics like amikacin are bactericidal antibiotics that are effective by inhibiting protein synthesis of Gram (-) bacteria. (Aygün, 2002; Yanagida et al., 2004). Aminoglycosides are potential of nephrotoxicity. Nephrotoxicity effects of aminoglycosides are either aggravating the previous kidney disease or creating new lesions in the kidney (Dilmener, 1986). Almost, 8% to 26% of the patient's aminoglycoside-inducing for several days improve slightly kidney impairment (Alimoradian et al., 2017). In study of Karahan et al. (2005), saw that aminoglycoside causes nephrotoxicity damage (Karahan et al., 2005).

The safe range of aminoglycosides is very narrow, and the most notable limitation of aminoglycosides is toxicity. The most common side effects are nephrotoxicity, ototoxicity, neuromuscular blockade. Nephrotoxicity can develop in all aminoglycosides and can be detected in 5-10% of aminoglycoside use (Mıstık, 2000; Aygün, 2002).

The aim of this work was to investigate the effect on serum angiotensin converting enzyme of the amikacin.

MATERIALS and METHODS

Animal Material

The experiments were conducted according to ethical guidelines and under the supervision of Yuzuncu Yil University Local Ethics Committee of Animal Experiments.

A total of 40 female Wistar-Albino rats weighing 200-250 g formed the study material. The rats were kept in cages at 22±2°C temperature with 12 hours dark-light period and continuous fresh food and water. The rats were fed standard pellet diet and distilled water ad libitum.

Study groups: The rats selected randomly were divided into two groups as the experimental (n=30) and the control (n=10) groups.

Control Group: The rats in this group were intraperitoneally administered 0.9% saline solution for 14 days.

Experimental Group: The rats in this group were administered 15 mg/kg amikacin from the aminoglycoside group (Sigma, St. Louis, MO, USA) every day for 14 days intraperitoneally.

Method

At the end of the experiment, blood samples were taken from the control and experimental group rats, under ether anesthesia, from the left ventricle of their hearts and put down in tubes with gel. Tubes were centrifuged. Serum samples obtained were transferred to eppendorf tubes and frozen at -18°C until the date of the experiment.

Assay of ACE enzyme activity

Measurement of ACE activity performed by using a commercial kit (Colorimetric ACE Angiotensin-Converting Enzyme, Assay-Enzymatic Bühlman Laboratories AG). The angiotensin-converting-enzyme catalyzes the conversion of angiotensin I to angiotensin II. This reaction stopped by the addition of hydrochloric acid (HCl) and complexes with cyanuric chloride after the release of hippuric acid. The absorption of this complex measured at 382 nm. One unit of ACE activity defined as the amount of enzyme required to release 1 µmol of hippuric acid per minute and liter in 37°C serum. Control/sample and control blank/sample blank readings calculated on the spectrophotometer (Boeco S–22 UV/Vis (Germany)).

Statistical analysis

The data from groups were analyzed with the Duncan test was applied for multiple comparisons. Differences were considered significant when the p-value was less than 0.05 (SPSS 22.0).

RESULTS

There is a significant increase in ACE activity between the amikacin-administered rat group and the control group (Table 1).

DISCUSSION

ACE is primarily found in the endothelial cell membrane of the lung, and it is mostly seen as small amounts in the kidney cells and plasma. The ACE in plasma is secreted by endothelial and renal cells.

Table 1. ACE activity of the control and experimental group.

	n	Control Group (Mean ± SD)	n	Experimental Group (Mean ± SD)	p
ACE activity (U/L)	10	82.19±8.87	30	92.82±9.52*	<0.001

*As a result of statistical analysis, the p-value is less than 0.001 and a significant difference observed as statistically.

ACE, a part of RAS, plays a vital role in regulating blood pressure and converts from Angiotensin-I to Angiotensin-II, a potent vasoconstrictor (Balcı-Ekmekçi et al., 2002). In the past, it was thought that almost all of the angiotensin 2 production was pulmonary because the ACE concentration in the lungs was higher than in other organs. However, today it has been shown that angiotensin 2 can be synthesized locally in many organs such as kidney, endothelium, adrenal and brain. One of the most common examples of local RAS activation occurs in the proximal tubule in the kidney. ACE and angiotensin 2 receptors of these cells are shown in proximal tubular cells. Besides, the concentration of angiotensin 2 in this region is approximately 1000 times higher than in the systemic circulation. This locally active system enables a number of physiological functions to be carried out independently of renin secretion (Koçak et al., 2017).

Nephrotoxicity due to aminoglycosides are characterized by direct tubular damage and increased glomerular filtrate rate (GFR) (Fauconneau et al., 1995; Mıstık, 2000). Ototoxicity, nephrotoxicity, and neurotoxicity are the three main side effects of aminoglycosides. Nephrotoxicity is characterized by the accumulation of aminoglycosides in the renal cortex and proximal tubules. Acute tubular necrosis, which is one of acute renal failure (ARF), causes vasoconstriction in afferent arterioles. Due to this response, renal blood flow decreases, and renin secretion increases in the juxtaglomerular apparatus, conversion from angiotensin-I to angiotensin-II. In addition to, studies suggest that there is a relationship between renal disease and activation of the intrarenal renin-angiotensin-aldosterone system (Siragy and Carey, 2010). Ziai et al. (2002) stated that serum ACE activity can be considered as an indicator in patients with bone fractures given 80 mg of gentamicin for 3 days. The significant increase in ACE activity of the experimental group treated with aminoglycosides seems to be consistent with the literature.

Diabetes is one of the most important causes of nephrotoxicity. The studies investigating ACE activity in diabetes-dependent nephrotoxicity have been conducted. Ozmutlu et al. (2012), determined that they found statistically significant increase in ACE activity between diabetes and control groups. They stated that nephropathy, a result of diabetical complications, might cause increase in the ACE activations. Ma et al. (2014), investigated relationship between renal injury in diabetic rats and the antagonism ACE/ACE-II. They were found significant pathological changes in the kidney of Streptozotosin (STZ)-treatmented rats. ACE/ACE-II mRNA levels were significantly higher STZ-treated groups than control groups. In the study presented, the same result was obtained in the nephrotoxicity induced by amikacin.

There are some studies investigating the change in ACE activity due to aminoglycoside and the reasons for this. It is claimed that the endothelial ACE in mice with diabetic nephropathy is a crucial player in the development of tubular ACE and is a central regulator of the glomerular filtration rate (GFR) (Eriguchi et al., 2018). Singhal and Prajapati (2011) reported that treatment with aminoglycoside (such as amikasin) causes an important free radical production, which causes oxidative stress and a significant damage to kidney and hepatic tissue.

It has been determined that free radicals and their derivatives cause tissue damage due to amikacin, which is an antibiotic drug of aminoglycosides category and significantly, reduce antioxidant enzyme activities (SOD, Catalase and Glutathione reductase) along with increased free radical mediated damage (as evidenced by enhanced MDA levels) as well as some extracellular antioxidants (Creatinine, Uric acid and Total bilirubin) in treated mice (Singhal and Prajapati, 2011; Caner and Değer, 2018).

In rats with renal toxicity caused by gentamicin, ACE activity was measured in serum, lung and kidney. The damage to the proximal kidney tubule was evident by the histological analysis and increase in the urinary excretion of N-acetyl-β-d-glucosaminidase (NAG). Kidney ACE activity decreased while lung and serum ACE activity didn't change until day 7 (administration 1, 3, 5, and 7 consecutive days). Ziai et al. (2003), evidenced that occur renal toxicity by presence of proteinuria, polyuria, and declined creatinine clearance at rats with gentamicin-induced. ACE activity was measured in serum, lung and kidney.

ACE activity decreased in kidney, and didn't change in serum, lung. In this study, ACE activity increased in serum. The reason for this increase may be related to the amount of antibiotics administered.

Physiologically, ACE is a crucial enzyme in the renin-angiotensin system, converting angiotensin I into the potent vasopressor angiotensin II and also inactivating the vasodilator bradykinin. Increased serum ACE activity (SACE) has been reported in pathologies involving stimulation of the monocytic cell line, primarily granulomatous diseases. Angiotensin II stimulates the production of various profibrotic and proinflammatory cytokines in tissues independent of their hemodynamic effects. (Bénéteau-Burnat and Baudin, 1991; Ecder, 2009). In parallel with this study, we saw that the serum ACE activity increased in our study as well.

CONCLUSION

ACE activity is increased in rats aminoglycoside administered and the increased ACE activity has many side effects as mentioned in the sources given above. As a result, it was concluded that the monitoring of the renin-angiotensin-aldosterone system may be important in preventing the progression of renal failure in aminoglycoside treatment and alternative studies should be conducted on this subject.

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Detection of extended-spectrum β-lactamase (ESBL) producing *Escherichia coli* in chickens

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ABSTRACT

Objective: Indiscriminate use of antibiotics in poultry farms increases the chance of antibiotic resistant and extended-spectrum β-lactamase (ESBL) producing bacteria in Bangladesh. Therefore, the study was under taken to detect ESBL producing *Escherichia coli* (*E. coli*) in chickens.

Materials and methods: A total of 60 cloacal swab samples (20 from commercial layer, 20 from commercial broiler and 20 from commercial sonali chickens) were collected from Rajshahi district of Bangladesh. The *E. coli* was isolated from these samples and identified based on cultural, staining, and biochemical characteristics. The disk diffusion method was used to assay the antibiotic resistant/sensitivity patterns of the isolated *E. coli*. Phenotypc detection of ESBL producing *E. coli* was also done.

Results: The prevalence of *E. coli* in chickens was 61.67% in Rajshahi district of Bangladesh, where the prevalence was 60%, 60%, and 65% in commercial layer, commercial broiler, and commercial sonali chickens, respectively. The antibiotic sensitivity assay of *E. coli* isolated from commercial layer chickens showed 100%, 80%, 50%, 40%, and 40% resistant to amoxicillin, tetracycline, cefotaxime, ciprofloxacin, and ceftazidime, respectively. *E. coli* isolated from commercial broiler chickens showed 100%, 100%, 60%, 50%, and 40% resistant to amoxicillin, tetracycline, cefotaxime, ceftazidime, and ciprofloxacin, respectively. *E. coli* isolated from commercial sonali chickens showed 90%, 70%, 50%, 50%, and 40% resistant to amoxicillin, tetracycline, cefotaxime, ciprofloxacin, and ceftazidime, respectively. In phenotypic detection, the overall prevalence of ESBL producing *E. coli* was 43.33%, where 40%, 50%, and 40% in the commercial layer, commercial broiler, and commercial sonali chickens, respectively in Rajshahi district of Bangladesh.

Conclusion: These results indicated that chickens are a potential reservoir for ESBL producing *E. coli* and their antibiotic resistances are obviously significant. These findings will help us to make proper guideline for the treatment, prevention and control of *E. coli* prevalent in chickens in Bangladesh.

Keywords: *Antibiotic resistance, Chickens, E. coli, ESBL, Prevalence*

INTRODUCTION

The β-lactamases are bacterial enzymes that hydrolyze β-lactam ring of antibiotics which results in ineffective compounds. Extended-spectrum β-lactamases (ESBLs), have the capability of hydrolyzing and causing resistance to various types of β-lactam antibiotics, including the penicillins, 1st, 2nd and 3rd generation cephalosporins and aztreonam. They are not active against the cephamycins (cefoxitin and cefotetan), but are susceptible to β- lactamase inhibitors (clavulanic acid) (Mohanty et al., 2010). The main ESBL types are TEM (trimethylamine), SHV (sulfhydryl variable), and CTX-M (cefotaximase). These

enzymes confer resistance to β-lactam antibacterial drugs, particularly cephalosporin, and may be accompanied by co-resistance to drugs of other classes (Paterson and Bonomo, 2005; Canton and Coque, 2006). Specifically, the ESBL enzymes are increasingly expressed by pathogenic bacteria like *Escherichia coli* (*E. coli*) with potential for dissemination. These enzymes have also been identified in several other members of the family *Enterobacteriaceae* and in certain non-fomenters (Jacoby et al., 2005). The *E. coli*, ESBLs has increased the resistance traits and the evolution of different genes worldwide (Paterson and Bonomo, 2005). Indiscriminate uses of antibiotics for the treatment of poultry diseases increase the chance of antimicrobial exposure to microorganisms resulting in resistant and ESBL producing bacteria in poultry (Hasan et al., 2012). With the misuse and overuse of antibiotics to treat diseases, resistance to the drugs has begun to appear and has become more serious because of selective pressure. In Bangladesh, there is limited data on this perspective. Therefore, the aim of this study was to detect ESBL producing *E. coli* in chickens as well as antibiogram assay of these isolates.

MATERIALS and METHODS

The study area and period

The study was conducted in the commercial layer, commercial broiler, and sonali chicken's farms in Rajshahi district of Bangladesh. Cloacal samples were collected with the sterile swab and brought to the Microbiology Lab., Department of Veterinary and Animal Sciences, University of Rajshahi for bacteriological analysis. The study was conducted during the period from July to December, 2020, with the ethical number 144/320/IBSc.

Sample collection

A total of 60 cloacal swab samples were collected from randomly selected chicken farms. Out of 60 swab samples, 20 were collected from commercial layer farms, 20 were collected from commercial broiler farms, and 20 were collected from commercial sonali farms. These samples were collected from four upazila (Charghat, Durgapur, Godagari and Paba) and metropolitan area of Rajshahi district of Bangladesh. From each study area (4 upazila and 1 metropolitan area) 4 swab samples from commercial layer chickens, 4 swab samples from commercial broiler chickens, and 4 swab samples from commercial sonali chickens were collected.

Isolation and identification of E. coli

The collected samples were separately inoculated into freshly prepared nutrient broth and incubated at 37°C for 24 hours in the bacteriological incubator for enrichment. The incubated tubes were examined for the growth of bacteria. Then the broth culture of bacteria was inoculated on nutrient agar by streak plate techniques and inoculated 37°C for 24 hours for the development of colonies. The colonies on primary culture was repeatedly sub-cultured on different selective culture media (EMB agar, MacConkey agar and SS agar) by the streak plate method until the pure culture with homogenous colonies was obtained.

Colony morphology: The colony morphology of the isolated *E. coli* was studied as mentioned by Merchant and Packer (1967). Colony morphology such as shape, size, surface texture, edge and elevation, color and opacity developed after 24 hours of incubation were carefully studied and recorded.

Gram's staining: Gram's staining was performed according to the method described by Cheesbrough (1985).

Biochemical identification of isolated bacteria: Pure culture of bacteria was subjected to different biochemical tests like sugar fermentation test (with five basic sugars for the production of acid with or without H_2S gas), catalase test, indole test, MR test, VP test and TSI agar slant reaction. Standard methods were followed to conduct these tests and interpretation (Cowan, 1985).

Antibiogram assay of the isolated E. coli

The disk diffusion method (Bauer et al., 1966; Jorgensen and Turnidge, 2015) was used to test the susceptibility of the *E. coli* isolates. In brief, pure colonies of the *E. coli* isolates were inoculated in nutrient broth and incubated at 37°C for overnight. Then 100 µl of broth culture (OD 0.5) was taken and placed onto Mueller Hinton agar plate and spread evenly with a sterile glass rod spreader. The antibiotic discs were dispensed onto the surface of the inoculated agar plates keeping about 1 cm apart. After 18 to 20 hours of incubation at 37°C, each plate was examined. The susceptibility test of the *E. coli* was done against nine antibiotic disks including; penicillin (10 IU), amoxicillin (10 µg), tetracycline (30 µg), ciprofloxacin (5 µg), cefotaxime (10 µg), ceftazidime (30 µg), gentamicin (10 µg), meropenem (10 µg), and imipenem (10 µg). Using a ruler, the susceptibility zones were measured and interpreted according to criteria (Table 1) set by the

Clinical and Laboratory Standards Institute document M100-S17 (CLSI, 2016).

Table 1. Used antibiotics with their disc concentration and standard zone of inhibition.

Antimicrobial agent	Disc concentration (µg/disc))	Interpretation of results (zone diameter in mm)		
		R	I	S
Penicillin	50 µg	≤11	12-21	≥22
Gentamicin	10 µg	≤12	13-14	≥15
Tetracycline	30 µg	≤14	15-18	≥19
Amoxycillin	30 µg	≤13	14-17	≥18
Ciprofloxacin	5 µg	≤15	16-20	≥21
Cefotaxime	30 µg	≤13	14-17	≥18
Ceftrazidine	30 µg	≤16	18-20	≥19
Imipenem	10 µg	≤13	14-15	≥16
Meropenem	10 µg	≤15	16-22	≥23

µg: Microgram, SL: Serial, mm: millimetre. S: Sensitive, I:Intermediately sensitive, R: Resistant, ≥: Greater than or equal to, ≤: Less than or equal to

ESBL screening and confirmatory tests

To identify potential ESBL producer's disk diffusion breakpoints were used for cefotaxime (10 µg) and ceftazidime (30 µg) according to (CLSI, 2016) guidelines. An ESBL-producing strain might hydrolyze one or more of these agents. Results were interpreted based on the CLSI guidelines as follows: zones of inhibition of ≤22 mm for ceftazidime and ≤27 mm cefotaxime or combination of two. The less susceptible or resistant isolates were subjected to confirmatory test using double discs diffusion method according to (CLSI, 2016). The intermediate and resistant *E. coli* isolates were tested with both cefotaxime and ceftazidime alone and in combination with clavulanic acid (10µg). The zone diameter increased ≥5 mm compared to when tested without clavulanic acid, confirms ESBL production (CLSI, 2016).

RESULTS

Cultural characteristics

The growth of *E. coli* on nutrient agar was indicated by the development of smooth, circular, white to grayish white colony and on EMB by the development of smooth, circular, black color colonies with metallic sheen (Figure 1). The growth of *E. coli* on MacConkey agar was indicated by the development of bright pink colored colony and on Salmonella-Shigela (SS) agar by the development of pink to rose red colonies.

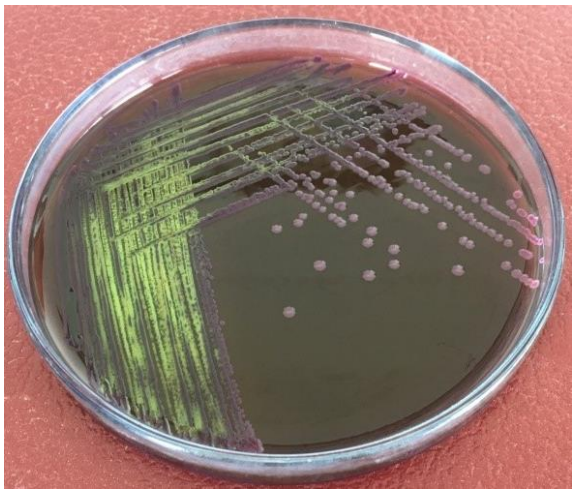

Figure 1. Growth of *E. coli* on EMB agar (produced greenish-black colonies with metallic sheen)

Biochemical properties

Isolated *E. coli* fermented dextrose, lactose, sucrose, maltose and mannitol with the production of acid and gas (Figure 2). Isolated *E. coli* showed positive results in catalase test, indole test and MR test but showed negative result in VP test. Isolated *E. coli* produced acidic slant and acidic butt (yellow slant, yellow butt) with gas production in TSI agar slant reaction (Table 2).

The overall prevalence of *E. coli* in the present study was 61.67% in chickens in Rajshahi district of Bangladesh, where the prevalence was 60%, 60%, and 65% in the commercial layer, commercial broiler, and commercial sonali chickens, respectively (Table 3).

Figure 2. Fermentation activity of isolated *E. coli* with five basic sugars (fermented dextrose, lactose, sucrose, maltose and mannitol with the production of acid and gas).

The overall prevalence of *E. coli* in the present study was 61.67% in chickens in Rajshahi district of

Bangladesh, where the prevalence was 60%, 60%, and 65% in the commercial layer, commercial broiler, and commercial sonali chickens, respectively (Table 4).

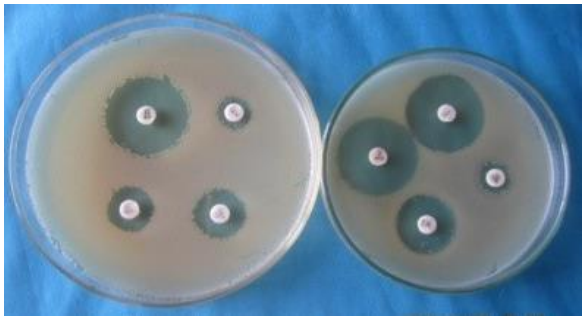

Figure 3. Antibiotic sensitivity patterns of isolated *E. coli* on Muller Hinton agar media (showed sensitive to meropenem, imipenem and gentamicin but resistant to penicillin).

The antibiotic sensitivity assay of isolated *E. coli* from commercial layer chickens showed 100%, 90%, 90%, 50%, 40%, and 40% sensitive to meropenem, imipenem, gentamicin, ciprofloxacin, ceftazidime, and cefotaxime, respectively. *E. coli* isolated from commercial broiler chickens showed 100%, 90%, 80%, 30%, 30%, and 20% sensitive to meropenem, imipenem, gentamicin, cefotaxime, ceftazidime, and ciprofloxacin, respectively. *E. coli* isolated from commercial sonali chickens showed 100%, 90%, 60%, 30%, 30%, and 20% sensitive to meropenem, imipenem, gentamicin, cefotaxime, ceftazidime, ciprofloxacin, respectively. Whereas, *E. coli* isolated from commercial layer chickens showed 100%, 100%, 80%, 50%, 40%, and 40% resistant to penicillin, amoxicillin, tetracycline, cefotaxime, ciprofloxacin, and ceftazidime, respectively. *E. coli* isolated from commercial broiler chickens showed 100%, 100%, 100%, 60%, 50%, and 40% resistant to penicillin, amoxicillin, tetracycline, cefotaxime, ceftazidime, and ciprofloxacin, respectively. *E. coli* isolated from commercial sonali chickens showed 100%, 90%, 70%, 50%, 50%, and 40% resistant to penicillin, amoxicillin, tetracycline, cefotaxime, ciprofloxacin, and ceftazidime, respectively (Figure 3, Table 4).

Table 2. Biochemical properties of isolated *E. coli.*

Tests	Used sugars	Acid production	Gas production	Results
Fermentation reaction with five basic sugars	Dextrose	+	+	+
	Maltose	+	+	+
	Lactose	+	+	+
	Sucrose	+	+	+
	Mannitol	+	+	+
Indole test				+
Catalase test				+
MR test				+
VP test				-
TSI agar slant reaction	Acidic slant and acidic butt (Yellow slant, yellow butt) with gas production			+

Table 3. Prevalence of *E. coli* in chickens.

Types of chickens	No. of the samples tested	Prevalence of *E. coli* (%)	Overall prevalence of *E. coli* (%)
Commercial layer chickens	20	12 (60)	61.67
Commercial broiler chickens	20	12 (60)	
Commercial sonali chickens	20	13 (65)	
Total	60	37	

Table 4. Antibiotic sensitivity and resistant pattern of isolated *E. coli*.

Name of antibiotics used	Sensitivity patterns (%)								
	E. coli from commercial layer chickens			*E. coli* from commercial broiler chickens			*E. coli* from commercial sonali chickens		
	S	I	R	S	I	R	S	I	R
Penicillin	0	0	100	0	0	100	0	0	100
Amoxicillin	0	0	100	0	0	100	0	10	90
Cefotaxime	40	10	50	30	10	60	30	20	50
Tetracycline	0	20	80	0	0	100	0	30	70
Ciprofloxacin	50	10	40	20	40	40	20	30	50
Ceftazidime	40	20	40	30	20	50	30	30	40
Gentamicin	90	10	0	80	10	10	60	30	10
Meropenem	100	0	0	100	0	0	100	0	0
Imipenem	90	10	0	90	10	0	90	10	0

S: sensitive, I: intermediate sensitive, R: resistant.

Table 5. Prevalence of extended-spectrum β-lactamase producing *E. coli* in chickens.

Types of chickens	Antibiotics used in combination with CVA	I	R	Increased Zone diameter*	ESBL producing *E. coli* (%)	Overall ESBL producing *E. coli* (%)
Commercial layer chickens	CTX with CVA	1	5	4	40	13 (43.33)
	CAZ with CVA	3	3			
Commercial broiler chickens	CTX with CVA	1	5	5	50	
	CAZ with CVA	2	5			
Commercial sonali chickens	CTX with CVA	2	5	4	40	
	CAZ with CVA	3	4			

*Increased zone diameter (≥5 mm than the previous zone) in a combination with CVA, CTX: cefotaxime; CAZ:ceftazidime; CVA: clavulanic acid.

The overall prevalence of ESBL producing *E. coli* was 43.33% in chickens in Rajshahi district of Bangladesh, where 40%, 50%, and 40% were in commercial layer, commercial broiler, and commercial sonali chickens, respectively (Table 5).

DISCUSSION

In the current study, the results of cultural, staining and biochemical tests of isolated *E. coli*, was successfully done. Our results are similar to the findings of Freeman (1985), Buxton and Fraser (1977) and Merchant and Packer (1967). In the present study the overall prevalence of *E. coli* in commercial chickens was 61.67%; where 60%, 60%, and 65% were in commercial layer, commercial broiler and commercial sonali chickens, respectively in Rajshahi district of Bangladesh. Previously the prevalence of *E. coli* was reported in commercial chickens as 64%, 71%, and 65% at Bogura, Gazipur and Joypurhat districts, respectively in Bangladesh (Hadiujjaman et al., 2016). They also reported that the prevalence of *E. coli* was 56%, 80%, and 68% in the commercial broiler, commercial layer and commercial sonali chickens, respectively. The present study also showed that *E. coli* isolated from commercial layer, commercial broiler and commercial sonali chickens were 90%, 80%, and 60% sensitive, respectively to gentamicin. Almost similarly result was reported previously (Hadiujjaman et al., 2016). They

reported that all *E. coli* isolates (100%) from commercial layer chickens were sensitive to gentamycin. The current study revealed that 20% *E. coli* isolates from commercial sonali chickens were sensitive to ciprofloxacin. It was previously reported that 12.5% *E. coli* from commercial sonali chickens were sensitive to ciprofloxacin (Hadiujjaman et al., 2016). The sensitivity patterns of our study showed that *E. coli* isolated from commercial chickens were sensitive to meropenem and imipenem in 100% and 90%, respectively. This finding is more likely because meropenem and imipenem are not commonly practiced in chickens in Bangladesh. More or less similarly result was reported previously (Ahoyo et al., 2007; Muvunyi et al., 2011). They reported that 96.4% (Ahoyo et al., 2007) and 93% (Muvunyi et al., 2011) *E. coli* isolates from commercial chickens were sensitive to imipenem. The results of our study showed that *E. coli* isolates from commercial layer chickens were resistant to penicillin, amoxicillin, tetracycline, ciprofloxacin, and ceftazidime in 100%, 100%, 80%, 40%, and 40%, respectively. Similarly, the high resistance rates of *E. coli* isolates were reported to commonly used antibiotics such as ampicillin (97.6%) and amoxicillin (95.2%) in Benin (Anago et al., 2015). The overall ESBL producing *E. coli* in the present study was 43.33%. This finding is agreed with other studies (Costa et al., 2009; Moyo et al., 2010; Kashyap et al., 2013). In this study, the prevalence of ESBL producing *E. coli* was 40%, 50%, and 40% in the commercial layer, commercial broiler, and commercial sonali chickens, respectively. Similarly, result was reported previously (Charles et al., 2017). They reported that ESBL-positive *E. coli* were 87% in the commercial broiler, 42% in commercial layer and 49% in commercial layer chickens.

CONCLUSION

The overall prevalence and the prevalence of ESBL producing *E. coli* was 61.67% and 43.33%, respectively in chickens in Rajshahi district of Bangladesh. Out of 60%, 60% and 65% prevalence the ESBL producing *E. coli* was 40%, 50%, 40%, respectively in commercial layer, in commercial broiler, and commercial sonali chickensin this study area. The prevalence of ESBL producing *E. coli* in chickens and their antibiotic resistance is obviously significant. These resistance genes are transmitting to the human body through the food chain. Therefore, the poultry sector should be provided with immediate attention by the government to control the indiscriminate use of antibiotics.

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Determination of *Malassezia* spp. infection and flea allergy incidences in pet dogs found in Kırıkkale and Ankara provinces

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ABSTRACT

Objective: The aim of this study is to determine the incidences of *Malassezia* infection and flea allergy in dogs with dermatitis complaints which were brought to veterinarians for examination in Ankara and Kırıkkale provinces.

Materials and Methods: The material for this study consists of 40 pet dogs of different breeds genders and ages found in Ankara and Kırıkkale provinces of Turkey. Malassezia examination was conducted by staining smear samples taken with the acetate band method with Modified Wright's Stain method. Flea existency examination was performed by using a flea comb.

Results: Out of the 40 dogs with dermatitis which were included in this study, 6 dogs (15%) were found to have *Malassezia* infection, 12 dogs (30%) were found to have flea infestation and 4 dogs (10%) were found to have together.

Conclusion: In light of the results that were found in this study, *Malassezia* and flea infestation hold an important place for dogs which were brought with complaints of dermatitis to veterinary clinics for examination in Ankara and Kırıkkale provinces. Clinical veterinarians must keep in mind that, there is high incidence rates of these 2 dermatitis causing agents and also remember to especially consider them in the list of differential diagnosis.

Keywords: *Ankara, Kırıkkale, pet dog, dermatitis, Malassezia, flea*

INTRODUCTION

Malassezia species are lipophilic yeasts which are found in the natural microbiota of the skin and mucous membranes of the animals and human beings (Silva et al., 1981). Malassezia yeasts are opportunistic pathogens. In the presence of predisposing factors, malassezia plays a role in creation of various diseases (Faergemann, 2002; Çomak and Ceylan, 2018).

Yeasts from the *Malassezia* genus have a grooved inner surface, a multi layered membrane and a thick (0.12 µm approx.) cell wall and the cell wall forms 26-37% of the total cell volume. The protoplasmic membrane is tightly adherent to the cell wall (Kantarcıoğlu et al., 2005).

Malassezia dermatitis is a secondary problem that can develop after allergic diseases like flea allergy, atopic dermatitis and also after recurrent pyoderma, hyperadrenocorticism, hyperthyroidism, diabetes mellitus, cutaneous, internal neoplasia or other metabolic diseases (Bajwa, 2017).

The most common clinical symptom is average or extreme itchiness that may partially recover as a response to the corticosteroids and antibiotics. As a result of the itchiness the patient rubs their faces,

shake their heads, lick their feet or chew them (Aytuğ, 2012). In some dog's behavioral problems due to the intensity of the itching can be misinterpreted as a neurologic or a behavioral problem which results in an incorrect diagnosis (Patterson et al., 2002).

Lesions usually seen on the ventral parts of body such as neck, abdomen, inguinal and axillary area and in ears, lips, mouth and medials of the extremities are the other important clinical signs (Şentürk et al., 2001; Conkova et al., 2011). The skin and fur can become oily and alopecia may be observed as a result of this. In chronic cases, lichenification may be observed prominently. Hyperpigmentation can be variable among different breeds (Bond et al., 2020). In chronic cases secondary lesions are observed due to itching and licking (Kamaljyoti et al., 2017).

The most practical diagnosis method for *Malassezia* dermatitis is cytological examination. Samples collected through various methods such as swap smear, superficial skin scrapes, acetate band smear, impression smear are examined under the microscope (Bajwa, 2017).

Focal lesions can be treated with antifungal creams or ear drops. Substances containing chlorhexidine, climbazole, miconazole, boric acid and shampoos are recommended for topical treatment of diffuse infections. Oral antifungals are used when topical treatment cannot be used or provide effective treatment. Itraconazole, ketoconazole and terbinafine, flucanazole are the most commonly used drugs (Carlotti, 2005; Paterson, 2008).

Fleas are obligatory hematophagous ectoparasites of mammals. They are arthropods with small, flattened from the sides and wingless bodies. They have an important role as pathogen carriers in the world (Bitam et al., 2010). Fleas, which are important parasites in both veterinary and human medicine, act as vectors of allergy causing agents in both humans and animals such as *Bartonella*, *Yersinia* and *Rickettsia*. Furthermore, they cause damage to *Dipylidium caninum*, *Hymenolepis dimunata* ve *Hymenolepis nana* by acting as an intermediate host. Fleas, which are more concentrated in the mild temperate zone, multiply fast, parallel to the increasing temperature rates (Acıöz and Aydın, 2020).

Fleas, unlike lice, do not spend all their life's on the host. Egg, larva, pupa and adult stages are seen in fleas (Kandemir et al., 2019). Fleas that find a host such as a dog or a cat do not leave their host unless they are exposed to insecticides. Fleas that live on cats have a minimum movement (Dryden, 2014).

Fleas, may cause skin diseases like dermatitis and cause anemia in the pets and their owners (Lam and Yu, 2009). Clinical symptoms related to flea allergy vary in accordance with the frequency of exposure to the fleas, secondary and simultaneous skin diseases, degree of hypersensitivity and effects of previous treatments. Itchiness related to the flea allergy dermatitis in dogs is intense and spreads throughout the body. Caudal and medial parts of the thigh, ventral abdomen, neck and ears develop a sensitivity in the affected dogs. Due to the itching and licking of these regions, color changes in the fur and fur loss is observed. The first lesion observed in flea allergy dermatitis is the papule formation at the flea bite spot. Alopecia may also be observed simultaneously with a red-brown crusted papule. Injuries which occur in the hair roots may secondarily lead to pyoderma. Pyotraumatic dermatitis may form as a result of intense itching by the affected dogs. Potential chronic diseases may present themselves with symptoms like diffuse alopecia, severe seborrhea, hyperkeratosis and hyperpigmentation. In cats however, the first lesion is a typical milier lesion which is seen on the face, the back and the neck (Dryden and Blakemore, 1989). Erythemia, alopecia, excoriation, papules, crusts and itchiness is seen in dogs with flea allergy dermatitis while alopecia, milier dermatitis and itchiness is observed in cats with the same disease (Traversa, 2013).

Anamnesis, physical examination and clinical findings, flea or flea excreta, intradermal tests and elimination of other possible dermatologic diseases are the most important methods for the diagnosis of flea allergy dermatitis (Dryden, 2014). Diagnosis is usually done by detection of flea on a pet's body. Fleas and their excreta may be observed by using a flea comb. Flea excreta is reddish black. When excreta put on a moist towel forms a varying range of reddish brown colors when crushed. Flea excreta is generally cylindrical or comma shaped (Dryden, 2014).

In the first step of flea allergy dermatitis treatment, prevention of flea bites in the pets is important. Flea collars are commonly used for this purpose, although they are not effective enough as the only prevention method. Hence, orally and topically applicable products are preferred (Paterson, 2008; Bruner, 2011; Aytuğ, 2012).

The aim of this study is to determine the incidences of *Malassezia* infection and flea allergy in dogs with dermatitis complaints which were brought to veterinarians for examination in Ankara and Kırıkkale provinces.

MATERIALS and METHODS

Animal material

The experiments were conducted according to ethical guidelines and under the supervision of Kırıkkale University Local Ethics Committee of Animal Experiments.

The animal material for this study includes 40 dogs of different ages, breeds and genders that were brought to the Kırıkkale University Veterinary Faculty Animal Hospital and Başkent Animal Hospital with a complaint of dermatological problems.

Clinical examination findings and sample collection

Upon general examination of dogs that were brought to the above mentioned places with complaints of skin rash, itching, alopecia, hyperkeratosis etc., no signs of a different disease were found. A systematic dermatological examination was done afterwards and the findings were noted down on a dermatological evaluation form.

The patients were combed with a fine toothed comb. The red-black colored flea excreta and adult fleas that were got collected on the comb were put aside for further tests. Variations in the reddish-brown colors that formed in the collected excreta when in contact with a moist towel were noted and evaluated.

Samples for dermatological tests were taken from the periorbital, perioral, external ear canal, interdigital and ventral abdominal regions of the dogs using acetate band smear and skin scraping methods. Dermatological samples that were taken using the acetate band smear were stained with Modified Wright's Stain. Samples which had 5 or more than 5 *Malassezia* agents were accepted as positive for *Malassezia* dermatitis.

Laboratory analysis

Samples taken using the acetate band smear method were stained with Modified Wright's Stain and put on the microscopic slide. The samples taken with the acetate band smear method were and examined with oil immersion under 100x magnification. From the samples included in the analysis, the ones which had 5 or more than 5 *Malassezia* agents were accepted as positive for *Malassezia* dermatitis.

Statistical analysis

Chi-Square test was used for the comparison of proportional data and analysis results were interpreted in accordance with the Pearson Chi-Square or Fisher's Exact Test. 0.05 was taken into account as the significance level. SPSS (version 23) program was used for data analysis.

RESULTS

The data of dogs which submitted to the study were shown in Table 1.

Table 1. Distribution of 40 dogs included in the study by age, gender and breed.

Case Number	Age	Gender	Breed
1	3	Female	Pug
2	6	Female	Golden Retriever
3	1	Male	Husky
4	4	Female	German Shepherd
5	7	Male	Bull Terrier
6	2	Male	Labrador Retriever
7	4	Female	Pekingese
8	8	Male	Mixed
9	3	Male	Spitz
10	4	Female	Golden Retriever
11	6	Female	Mixed
12	1	Male	Pug
13	2	Female	Pug
14	9	Female	Golden Retriever
15	3	Female	Terrier
16	4	Male	Cavalier King Charles
17	1	Female	Mixed
18	4	Female	Maltese Terrier
19	6	Male	Pekingese
20	7	Male	Mixed
21	1	Male	Cocker Spaniel
22	2	Female	Golden Retriever
23	3	Male	Jack Russel
24	3	Female	Cavalier King Charles
25	5	Female	Pitbull
26	4	Male	Mixed
27	7	Female	Mixed
28	10	Male	Golden Retriever
29	3	Male	Kangal
30	2	Female	Chow Chow
31	6	Male	Maltese Terrier
32	4	Female	German Shepherd
33	7	Female	Labrador Retriever
34	3	Male	Cocker Spaniel
35	1	Female	Poodle
36	5	Female	Setter
37	4	Female	Mixed
38	2	Male	Bernese
39	3	Female	Mixed
40	6	Male	Golden Retriever

Table 2. Skin lessions of cases.

Skin lessions	Case Number																																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Itching without lession								+										+						+																
Primary Skin Lessions																																							
Erythema	+		+						+	+	+	+		+		+			+	+			+			+	+	+	+	+					+					
Papul		+					+				+	+			+				+			+					+		+	+	+			+		+	+	+	+	
Pustula		+		+											+							+			+					+	+					+		+			
Secondary Skin Lessions																																								
Epidermal collarete					+										+							+				+												+		+
Crusting				+	+	+	+							+	+						+						+		+		+	+		+	+		+	+	+	+
Staining with saliva	+								+														+																	
Alopecia	+				+	+			+	+	+	+	+		+		+			+			+		+	+			+			+			+			+	+
Lichenification							+				+				+		+			+					+	+					+	+			+					
Hiperpigmentation					+	+			+		+	+		+			+			+						+	+					+			+	+				

No any other pathology was seen except dermatologic problems after the clinical examinations performed. The examination findings were recorded and classified for each patient after the dermatological examination. While in 3 of the patient no existence of dermatologic lesions determined, only itching was observed as a symptom and in the reminder of the 37 patient were seen different dermatological lesions. The determined dermatologic lesions were shown in Table 2.

On 6 of the patients, 5 or more *Malassezia* spp were detected and accepted as *Malasssezia* positive. On the 10 of patient adult fleas were determined either on inspectional examination and on testing with flea comb manipulation. On 2 of the patients no flea feces was determined by the flea comb grooming. On 4 of the patients (case no 1-7-11-12) both *Malassezia* spp and flea existence were determined together (Table 3).

Table 3. *Malassezia* spp. and flea detected case numbers and percentages.

	Malassezia spp.	Flea	*Malassezia spp.* and Flea
Case Numbers	1-7-9-11-12-25	1-7-11-12-14-20-27-29-31-34-37-39	1-7-11-12
Percentages	15%	30%	10%

DISCUSSION

Malassezia species are lypophilic yeast which exists in the natural microbiata of the skin and mucous membranes of the animals and human beings (Silva *at al*., 1981). *Malassezia* yeasts are opportunist pathogens. They play a role in various diseases via various predisposing factors (Faergemann, 2002). These predisposing factors are excess production of and the decreasing quality of sebum, destruction of epidermis, increasing of humidity, persistence of skin folds, changing of the cutaneous pH levels, application of antibiotic treatments and long term corticosteroid medications (Bajwa, 2017). *Malassezia pachydermatidis* could be found in ear channel, anal glands, lips, chin, vagina, rectum and even skin and causes alopecia, both localized or generalized ertyhtrema, papulas and maculas, and crustaceous and squamous appearance in face, body perianal and interdigital areas and also in skin folds (Patteron et al., 2002).

There is no age and gender predisposition on *Malassezia* dermatitis. The predisposing breeds are; Westhighland White Terrier, Basset Hound, English Setter, Poodle, American Cocker Spaniel, Jack Russel Terrier, Shih Tzu, Silky Terrier, Boxer, German Shepherd, Maltese, Australian Terrier, Chihuahua, Shar Pei, Shetland Sheepdog, Lhaso Apso, Springer Spaniel and Daschund (Çamak and İçen, 2010). In our study, 6 dogs suffered from dermatitis which got diagnosed *Malassezia* spp., were not belong to any of predisposed breeds. The mentioned 6 dog's breeds were as Pug, Pekingese, Spitz, Pitbull and mix. The average ages of 4 female and 2 male dogs who got *Malassezia* diagnosed were 3.66 years.

Because of the itching, patient rubs his face to certain surfaces, shakes his head, licks or bites his legs (Aytuğ, 2012). Lesions usually seen on the ventral parts of the body such as neck, abdomen, inguinal and axillary area and in ears, lips, mouth and medials of the extremities. Usually there is an unpleasant and soury smell exists (Jasmin, 2011). The other important findings are erythema, plaque, papule and nodule formation, hyperpigmentation, seborrhoea, otitis externa which are relevant on the areas rich from the sebum glands (Şentürk et al., 2001; Conkova et al., 2011). The red-brown discharge is very specific in cases of *Malassezia* related otitis externa (Şentürk et al., 2001). Alopeci were seen in all 6 dogs included to the study and got diagnosed *Malassezia*. The other skin lesions determined frequently were as erythema (5 into 6), hyperpigmentation, liquenification and papule formation (3 into 6), getting stain with saliva (2 into 6) and pustula formation (1 into 6).

Demir and Sancak (2013), diagnosed *Malassezia* dermatitis on the 16 dogs of 566 dermatologic patient (2.82%). Canpolat et al. (2018), diagnosed *Malassezia* dermatitis in only two of the 653 dermatologic patients (0.3%). In the presented study in 6 of the 40 dermatology patients *Malassezia* was diagnosed and the incidence of *Malassezia* was found as 15%. The results obtained from this study about *Malassezia* coincidence were determined severely high from the other studies.

The fleas which are the obligatory hematophofagous ectoparasites of mammals and birds. They are small insects that flattened from lateral sides and wingless. They have big importance because of they are pathogenic carriers all over the world (Bitam et al., 2010). As an important parasite for both human and veterinary medicine, they cause allergic reactions and serve as vectors for *Bartonella*, *Yersinia* and *Ricketsi*a kind of agents. Besides they cause harm as they are intermediate hosts for *Dphylidium caninum*,

Hymenolepis dimunata, and *Hymenolepis nana* (Acıöz and Aydın, 2020).

Fleas can cause allergic dermatitis kind of dermatologic diseases and anemia both on the animals and their owners (Lam and Yu, 2009). The flea bite dermatitis caused allergic itching could be intense and generalized all over the body in the dogs. The sensitivity develops on the caudal and medial femoral areas, ventral abdomen, neck and ears in the effected dogs. The color changes and hairloss can be seen on these areas due to licking and itching. The first lesion seen in allergic flea bite dermatitis is the hyperemic areas occurred on the bite site. These lesions can become as a papula formation. Besides the red brown crustous covered papulas and alopecia can be seen. The damage occurred on the hair follicules can cause pyoderma secondarily. Also due to the self-itching trauma, wet dermatitis can develop. As the disease getting chronical, generalized alopecia, severe seborhhoea, hyperkeratosis and hyperpigmentation become evident. In cats, the primary lesion is the typical miliary dermatitis where seen on the face, back and neck (Dryden et al., 1989; Traversa, 2013). The lesions of the dogs which flea agent were frequently seen determined as papula (9 into 12), erythrema and incrustation (8 of 12), hyperpigmentation (6 of 12), alopecia (5 of 12), liquenification (4 of 12), pustula and staining with saliva (1 of 12). The average age of dogs was found as 4.66 year overall 12 dogs (7 female and 5 male) which no existence of predispositon neither gender nor breed.

It has been stated that, the 10-20% of the allergic dog disease are flea bite allergies (Bourdeau et al., 2004). Acıöz and Aydın (2020), performed a flea scanning on the 142 dogs at the localization Datça, Muğla, and submitted the flea infestation on 27 dogs (19%). Canpolat et al. (2018), informed that, on the study conducted with 1000 dogs which suffered from dermatological conditions, the rate of the "flea hypersensitivity" is 38% among the icthy dermatoses and 10% of overall dermatologic diseases. Demir and Sancak (2013), reported 29 allergic flea bite dermatitis (5.12%) between on total of 566 dogs with dermatologic problems. In Yılmaz et al's study (2002), including 7831 dogs which registered for the distribution of several diseases, the incidence of flea allergies was reported as 5%. In our study 40 dogs with dermatologic problems were subjected, and on 12 of these dogs (30%) even flea or feces of flea were determined. Based on the evaluation of the results obtained from our study, it can be state that the incidence of flea existency on the icthy dermatoses is higher than the results of Bourdeau (2004), which is 10-20%, and is lower than the results of Canpolat et al. (2008), which is 38%. An average rates reached according to these two studies. Despite the other research results given, were about to survey of the flea infestation on overall population, the results obtained from our study were evaluated between only the dogs with dermatologic problems and that why a severely high incidence was determined.

CONCLUSION

In conclusion, the incidence of *Malassezia* infection and flea infestation in dogs brought to clinics in the Ankara and Kırıkkale provinces with a dermatitis complaint was found to be 15% and 30% respectively. The results obtained have shown that the incidences of these two agents are very high and that they need to be placed in the list of differential diagnosis. Especially the 30% occurrence rate of flea infestation in pet dogs, found in this study, raises a question about whether ectoparasite treatments are done on an adequate level or not.

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Investigation of the nutritional and quality properties of meatballs added with bee pollen and apigenin

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ABSTRACT

Objective: The present study was carried out to investigate the possibility of using different levels of bee pollen and apigenin extract in beef meatballs to evaluate shelf-life, nutritional and quality properties of beef meatballs under different storage conditions.

Materials and Methods: It was determined, using the HPLC method, that bee pollen contains a high level of apigenin under optimal conditions. Bee pollen and apigenin were added to meatballs at different concentrations in five groups. Meatballs were made with control, 1-2% bee pollens/apigenin. Quality and safety evaluation of meatballs were determined by sensory, physicochemical, biochemical and microbiological tests. The analyses were conducted at 1st, 3rd, 7th and 14th days of interval.

Results: A statistically significant decrease was found in FFA, POV and TBARS levels of meatballs on different days of storage ($p<0.05$). When compared to the control group, the bioactive compounds preserved the microbiological and chemical properties of meatballs during frozen storage (14 days).

Conclusion: It was concluded that the extracts with 2% bee pollen concentrations can be used as bio-preservative agents for meat and meat products.

Keywords: *Bee pollen, Apigenin, Meatballs, Food quality*

INTRODUCTION

Natural compounds such as omega-3 fatty acids, vitamin E, conjugated linolenic acid (CLA), selenium (Se), herbs, spices, pre- and probiotics, phytochemicals and bioactive peptides are among the technologies used in developing qualified meat products (Zhang et al., 2010; Manessis et al., 2020). Meat and meat products are effective substrates for the development and growth of common food-borne pathogens. Thus, these foods are highly risky and easily spoiled, causing possible public health issues (Chen et al., 2012; Doulgeraki et al., 2012). Food spoilage is a process that causes foods to be undesirable or unacceptable for consumers for changes in sensory characteristics (Burkepile et al., 2006). Oxidative changes (lipid and protein oxidation) occur faster in meat. It was reported that it might be because the meat surface area in contact with air significantly increases after mincing the meat (Turgut et al., 2016).

Oxidative reactions are among the major interactions of meat spoilage after the butchering process. The high levels of myoglobin and ferrous content of beef meat, which is in the class "red meat", are one of the reasons for strong peroxide formation (Jiao et al., 2020). Increasing the functional and nutritional value of foods and prolonging the storage period by adding phenolic compounds into meats and meat products are among the focus points of studies carried out on this subject (Hintz et al., 2015). Natural additives containing phenolic compounds are added directly

into meats and meat products in order to improve the antimicrobial and antioxidant properties of the product, as well as the quality characteristics during the storage period (Ahn et al., 2004).

Bee pollen is a fine powder-like material produced by flowering plants pollen, mixed with nectar and bee secretions and collected by honey-bees (Graikou et al., 2011). Bee pollen is an apicultural product and it has been recommended in nutrition, for its nutritional contents and health benefits (Freire et al., 2012). It is known that bee pollen contains lipids, sugars, proteins, amino acids, vitamins, mineral substances, trace elements, carotenoids, polyphenolics such as flavonoids, and carbohydrates with these features, bee pollen is a source of biologically active substances (Human and Nicolson, 2006).

Bioactive phytochemicals compounds are contemplated beneficial for immune system support since they decrease the risk of inflammation by reducing oxidative stress and inhibiting free radicals. (Demir and Ağaoğlu, 2020; Demir, 2021). They have been shown to possess free-scavenging and metal chelating activity in addition to their reported antimicrobial and antioxidant properties (Morais et al., 2011). The high antioxidant potential of bee pollen is associated with the presence of flavonoids and polyphenols in its content (Rzepecka-Stojko et al., 2012). The present study was carried out to investigate the possibility of using different levels of bee pollen and apigenin extract in beef meatballs to evaluate shelf-life, nutritional and quality properties of beef meatballs under different storage conditions.

MATERIALS and METHODS

Preparation of bee pollen extracts, standard phytochemical (Apigenin) and meatball groups

Bee pollen were obtained from producer (Turkey). Extraction solution was prepared before grouping. The extract was concentrated to dryness under low pressure and controlled temperature. For the extraction of phenolic compounds in bee pollen, samples were mixed with methanol at 1:10 (g:mL) ratio and vortexed for 10s. The methanolic phase was separated by centrifugation at 6000 rpm x 3 g at room temperature for 10 min. High Performance Liquid Chromatography (HPLC) grade apigenin was used compared against the natural extracts. In the preparation of standard solutions, the purchased firm was made by examining the SDS package insert to Sigma-Aldrich (St. Louis, MO, ABD). The control (C) and four treatment groups were determined for meatballs. These groups were 1% bee pollen (P1), 2% bee pollen (P2), 1% Apigenin (A1), 2% Apigenin (A2), respectively.

Preparation of meatballs

Ground beef was purchased from a local butcher in Sivas, Turkey. Beef minced meat and salt were used in preparing the samples. The meat is mixed and 20 g of salt per kilo is added, and it is minced once in a medium thickness in the meat grinder. Bee pollen or Apigenin were added to the formulation of meatballs. The remaining group without any addition was used as a control. Kneading was performed after each component was added to the meatball mortar at the rate of 1% and 2%, respectively. In order for the meatballs to be of standard size and shape, samples were prepared as one meatball weighing 25 g, with the help of a shaping machine. All the experiments were performed three times (three different groups).

Biochemical analysis and microbial assessment

Free fatty acid values (FFA): Samples (5 g) were dissolved with 30 mL of chloroform using a homogenizer (IKA Ultra-Turrax T18 Basic, Staufen, Germany) (10,000 rpm; 1 min). Whatman No. 1 was used to remove the filtrate. The resulting filtrate (added phenolphthalein) was titrated (0.01 N KOH). The FFA value was calculated with the formula (Rukunudin et al., 1998).

Peroxide value (POV): The POVs of meatball samples were determined according to the method by Volpe et al. (2015) and Shahbazi et al. (2018). The samples (3 g) were weighed and heated in a water bath at 60 °C for 3 min (to melt the oil). The flasks were then shaken for 3 min with acetic acid-chloroform solution (3:2 by volume). Whatman No. 1 was used to re-move the filter. Saturated potassium iodide solution (0.5 mL) was added to the final filtrate (indicator starch). The titration process was continued against the standard sodium thiosulfate solution. The POV was determined in the total lipid extracts and calculated with the formula. Results are given as POV (meq/kg).

Thiobarbituric acid reactive substances (TBARS): Lipid oxidation was measured with 2-thiobarbituric acid reagent, as modified by Sharma et al. (2012). Values (1,1,3,3-tetraethoxypropane) were calculated by drawing the standard curve (y = ax + b) and expressed as milligram of malondialdehyde per kilo-gram of meatball sample (mg MA/kg).

Total Viable Count (TVC), Total Coliform Count (TCC) and Total Yeast Mold Count (TYMC) were determined according to TS EN ISO 4833-1, TS ISO 4832, TS ISO 21527-1 (TSE, 2010; TSE, 2012; TSE, 2014;). All analyses were done in triplicate and mean value was reported.

Analysis of phenolics

Phenolic compounds were separated using HPLC (Agilent 1200), analysis (Mohdaly et al., 2015) were performed by modifying the HPLC method. The column used ZORBAX SB-C18 (4.6mm x 250mm x 5µm), (Agilent Technoliges, USA). Different phenolic components were separated using a gradient of water containing 30% methanol (Solvent A) and methanol 100% (Solvent B), at a flow rate of 40 µL/min in following gradient elution: 25-100% Solvent B over 40 min, 50% Solvent B for 10 min, followed by 10 min for coloum calibration. The column temperature was maintained at 25°C and the phenolic compound was carried recorded at 280 nm. Solutions of pure eleven phenolic compounds were chromatographed as external standards. All standards were dissolved in methanol before injection in the analytical HPLC system.

Sensory evaluation

Different sensory attributes were examined. The meatball samples were assessed by a trained 8-member panel. The sensory questionnaires were calculated intensity on a 5-point balanced semantic scale for the attributes of color, smell, tenderness, juiciness, and overall acceptability. The evaluation method was based on the method used by Rubito et al. (2007). Sensory evaluation was had accomplished at 0 day and repeated at 1, 3, 7 and 14 days.

RESULTS

Results of the microbiological analyses of the meatballs formulated with different levels of bee pollen/apigenin during the 14 days storage (period) time are showed in Table 1. The results of all microbiological analyses were influenced by both bee pollen added (except 0 days of storage) and storage period ($p<0.05$). The range of TVC value, TCC value and TYMC values at different days of interval was 4.60 to 4.61, 0.98 to 1.13 and 1.22 to 1.71 logs cfu/g, respectively. In all storage periods the highest TVC was observed in control samples ($p<0.05$), and in general, TVC decreased as the pollen content/apigenin content increased (P2<P1, A2<A1) (Table 1). Among these five treatments, the TCC in control sample (1.11 logs cfu/g) was significantly higher than in the samples treated with P1, P2, A1, and A2 of bee pollen/apigenin extracts. Similar to TVC, in all storage periods (except 0 days of storage), the highest TCC count was control samples and at the end of the storage period, TCC count decreased to minimum levels of 1.11 (C), 1.10 (P1), 1.04 (P2), 1.03 (A1) and 1.01 logs cfu/g (A2) for control and meatballs added with 1%, 2% bee pollen and 1%, 2% apigenin, respectively (Table 1).

Sensory scores for parameters such as color, flavor, tenderness, juiciness and overall acceptability were significantly affected ($p<0.05$) by the addition of bee pollen and apigenin (Table 2). The analysis of phenolic compounds is very challenging due to the great variety and reactivity of these compounds. For the purpose of separation and quantification of individual phenolic compounds, HPLC is most frequently used because of its high separation capacity and relative simplicity. To know that are responsible active ingredients in bee pollen extract, methanol extracts were used for further investigations toward identification by HPLC. The biochemical effects of bee pollen/apigenin on meatball samples are shown in Figures 1-3. The overall FFA, POV and TBARS value at different treatment was 0.37-0.46%, 4.23-4.54 meq/kg and 0.52-0.66 mgMA/kg, respectively.

DISCUSSION

Considering the data obtained from this study, it was observed that there were significant differences in TYMC values between the five experimental groups. Among five treatments, TYMC in the control sample (1.68 logs cfu/g) were significantly higher than in the samples treated with bee pollen (P1, P2) and apigenin (A1, A2) extracts. Many bacteria might be available in the products, however these microorganisms growing may be controlled under storage conditions (Fernandes-lopez et al., 2005).

These results show that bee pollen and apigenin inhibited the bacterial growth (Graikou et al., 2011). The antimicrobial activity of bee pollen could be property chiefly to the effective contents of flavonoids and phenolic compounds such as apigenin, gallic acid, quercetin and kaempferol whichever are identified to possess antimicrobial and antifungal activity (Mohdaly et al., 2015). In present study was explored that sensory quality after added bee pollen/apigenin extracts was changed with the storage process.

Table 1. Effect of bee pollen and apigenin extracts on microbial population in beef meatballs (log cfu/g).

Microbiological analysis	Storage Period	C	SD	P1	SD	P2	SD	A1	SD	A2	SD	Mean*
TCC (Total Coliform Count)	1	1.18	0.03	1.13	0.02	1.11	0.02	1.12	0.03	1.10	0.01	**1.13**
	3	1.13	0.02	1.11	0.01	1.07	0.02	1.06	0.03	1.06	0.04	**1.09**
	7	1.10	0.02	1.10	0.02	1.03	0.03	1.01	0.03	0.96	0.04	**1.04**
	14	1.03	0.02	1.06	0.02	0.96	0.03	0.94	0.07	0.91	0.07	**0.98**
	Mean*	**1.11**	**0.01**	**1.10**	**0.01**	**1.04**	**0.01**	**1.03**	**0.03**	**1.01**	**0.03**	
TVC (Total Viable Count)	1	4.86	0.02	4.63	0.02	4.53	0.03	4.61	0.03	4.42	0.02	**4.61**
	3	4.88	0.04	4.65	0.04	4.60	0.02	4.59	0.04	4.53	0.02	**4.65**
	7	4.99	0.07	4.67	0.01	4.62	0.01	4.60	0.03	4.46	0.04	**4.67**
	14	5.09	0.02	4.52	0.02	4.53	0.02	4.50	0.01	4.34	0.04	**4.60**
	Mean*	**4.95**	**0.03**	**4.62**	**0.01**	**4.57**	**0.00**	**4.57**	**0.02**	**4.44**	**0.02**	
TYMC (Total Yeast Mould Count)	1	1.82	0.02	1.68	0.04	1.69	0.05	1.62	0.05	1.71	0.03	**1.71**
	3	1.77	0.02	1.50	0.03	1.46	0.02	1.42	0.02	1.36	0.04	**1.50**
	7	1.62	0.05	1.34	0.03	1.27	0.04	1.28	0.03	1.18	0.02	**1.34**
	14	1.51	0.09	1.25	0.02	1.16	0.03	1.12	0.02	1.06	0.03	**1.22**
	Mean*	**1.68**	**0.01**	**1.44**	**0.02**	**1.40**	**0.02**	**1.36**	**0.02**	**1.33**	**0.02**	

Means with different superscripts in each column and row are significantly different (*p<0.05). C, Control; P1, 1% Pollen; P2, 2% Pollen; A1, 1% Apigenin; A2, 2% Apigenin

Table 2. Effect of bee pollen and apigenin extracts on sensory parameters in beef meatballs.

Sensory attributes	Storage Period	C	SD	P1	SD	P2	SD	A1	SD	A2	SD	Mean*
Color	1	5.00	0.00	5.00	0.00	5.00	0.00	5.00	0.00	5.00	0.00	5.00
	3	4.83	0.41	4.83	0.41	4.50	0.55	4.83	0.41	4.50	0.55	4.70
	7	4.67	0.52	4.67	0.52	4.33	0.52	4.67	0.52	4.17	0.41	4.50
	14	4.50	0.55	4.33	0.52	4.17	0.41	4.33	0.52	4.00	0.00	4.27
	Mean	**4.75**	**0.32**	**4.71**	**0.29**	**4.50**	**0.32**	**4.71**	**0.29**	**4.42**	**0.20**	
Flavor	1	5.00	0.00	5.00	0.00	5.00	0.00	4.83	0.41	4.83	0.41	4.93
	3	4.83	0.41	5.00	0.00	4.83	0.41	4.67	0.52	4.50	0.55	4.77
	7	4.50	0.55	4.67	0.52	4.83	0.41	4.33	0.52	4.17	0.41	4.50
	14	4.00	0.63	4.17	0.41	4.33	0.52	3.83	0.75	3.67	0.52	4.00
	Mean	**4.58**	**0.20**	**4.71**	**0.10**	**4.75**	**0.00**	**4.42**	**0.26**	**4.29**	**0.25**	
Tenderness	1	5.00	0.00	5.00	0.00	5.00	0.00	5.00	0.00	5.00	0.00	5.00
	3	4.67	0.52	4.67	0.52	4.67	0.52	4.50	0.55	4.50	0.55	4.60
	7	4.33	0.52	4.50	0.55	4.67	0.52	4.33	0.52	4.17	0.41	4.40
	14	4.00	0.00	4.00	0.00	4.50	0.55	3.83	0.41	3.83	0.41	4.03
	Mean	**4.50**	**0.00**	**4.54**	**0.10**	**4.71**	**0.25**	**4.42**	**0.26**	**4.38**	**0.21**	
Juiciness	1	5.00	0.00	5.00	0.00	5.00	0.00	5.00	0.00	5.00	0.00	5.00
	3	4.83	0.41	4.83	0.41	5.00	0.00	4.67	0.52	5.00	0.00	4.87
	7	4.33	0.52	4.67	0.52	4.83	0.41	4.50	0.55	4.83	0.41	4.63
	14	3.67	0.52	3.83	0.41	4.00	0.00	3.67	0.52	4.00	0.00	3.83
	Mean	**4.46**	**0.25**	**4.58**	**0.20**	**4.71**	**0.10**	**4.46**	**0.29**	**4.71**	**0.10**	
Overall acceptability	1	5.00	0.00	5.00	0.00	5.00	0.00	5.00	0.00	5.00	0.00	5.00
	3	5.00	0.00	5.00	0.00	5.00	0.00	4.83	0.41	4.83	0.41	4.93
	7	4.50	0.55	4.50	0.55	4.50	0.55	4.50	0.55	4.50	0.55	4.50
	14	4.33	0.52	4.33	0.52	4.33	0.52	4.33	0.52	4.00	0.63	4.27
	Mean	**4.71**	**0.19**	**4.71**	**0.19**	**4.71**	**0.19**	**4.67**	**0.20**	**4.58**	**0.30**	

Sensory scores were 5 for excellent, 4 for very good, 3 for good, 2 for fair, and 1 for poor. Mean in each row having different superscript varies significantly at values *$p < 0.05$.

Table 3. Phenolics composition of bee pollen extracts.

Compounds	Bee pollen (mg/mL extract)
Gallic acid	5.17±0.04
Vanilic acid	0.81±0.01
p- Coumaric acid	1.9±0.11
Ferulic acid	4.73±0.08
Rutin	2.83±0.13
Myricetin	1.88±0.15
Quercetin	5.42±0.10
Kaempferol	4.94±0.03
Apigenin	8.16±0.04
Naringenin	3.58±0.08
Luteolin	2.24±0.07

Mean values ± standard deviation of triplicate determinations are reported.

The range of overall recorded color-score was 4.75 to 4.29, tenderness score was 4.71 to 4.38, juiciness score was 4.71 to 4.46 and overall acceptability score was 4.71 to 4.58 (Table 2). The range of different day's intervals of overall observation of overall acceptability score 4.27 to 5.00. Among five treatments most preferable color and juiciness was observed from 1% (P1) tenderness and overall acceptability were observed from 2% (P2) and the flavor was observed from 2% (P2) bee pollen extract. With increasing of sensory scores were also reported some researches (Huang et al., 2005; Turhan et al., 2014).

Apigenin, quercetin, gallic acid, ferulic acid, kaempferol, naringenin, rutin and luteolin were recognized as the main phenolic components in bee pollen extract as shown in Table 3. Some components such as myricetin, p-coumaric acid and vanilic acid were also found in traces. All these components are known as phenolic composition of bee pollen samples (Mohdaly et al., 2015). Some other studies were developed concerning the phenolic composition of bee pollen extract (Almeida et al., 2017), apigenin (8.16 mg/mL) was the flavonoid most abundant in the bee pollen extract, followed by quercetin (5.12 mg/mL). Almeida et al. (2017) identified seven phenolic compounds in the bee pollen from lyophilized extracts. Among the identified constituents, several compounds with known antioxidant, antimicrobial, anticancer and anti-inflammatory such as flavonoids and phenolic acids, were present (Demir et al., 2020).

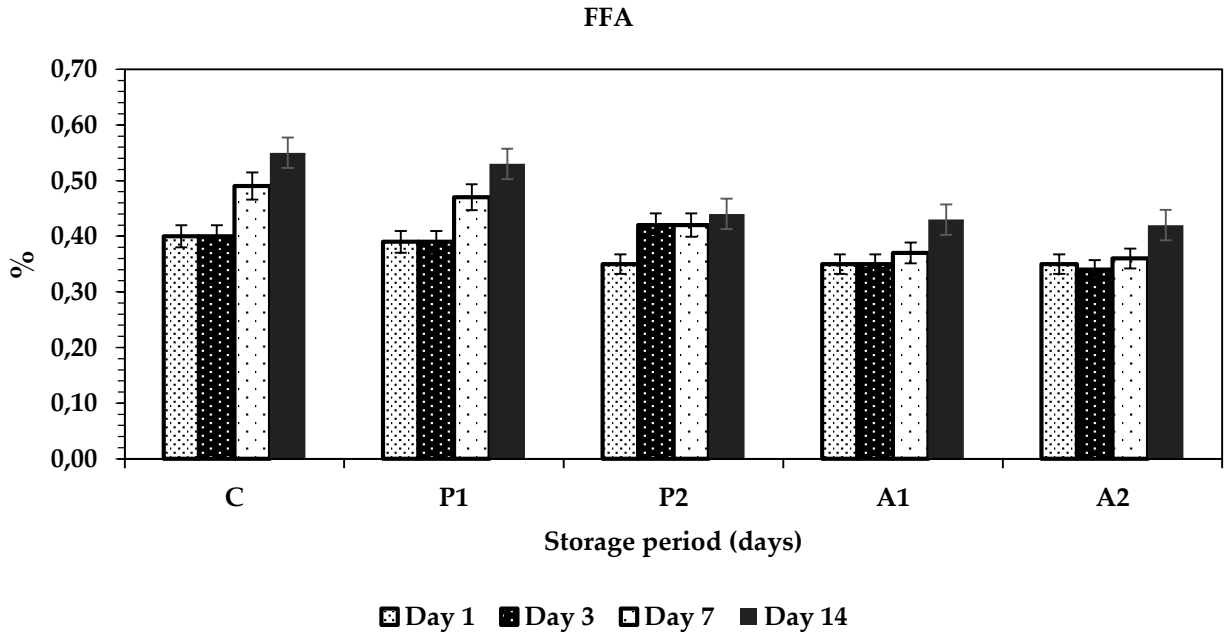

Figure 1. Effects of bee pollen and apigenin on free fatty acids value of meatballs at different storage durations.

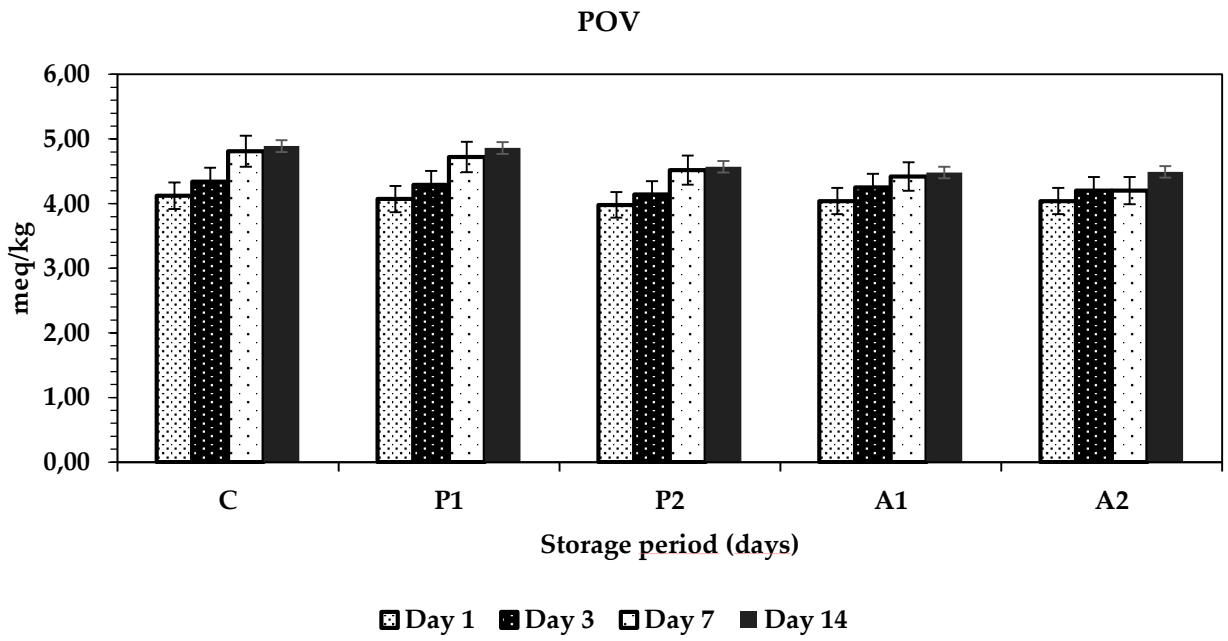

Figure 2. Changes in POV and meatballs during storage.

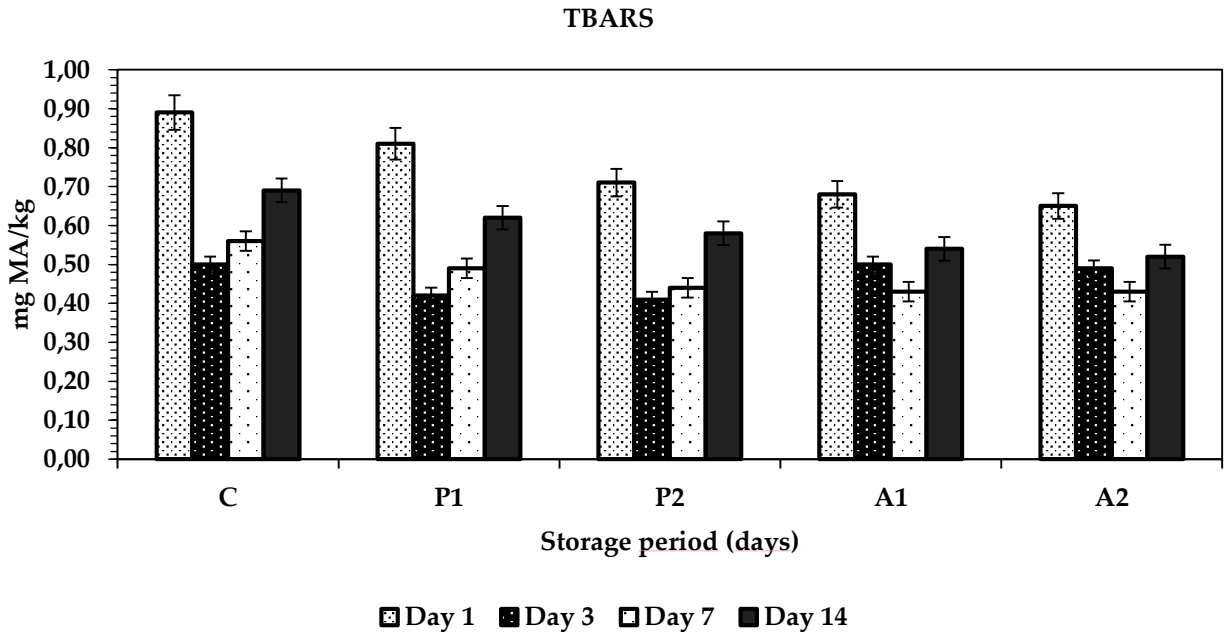

Figure 3. Changes in TBARS of meatballs during storage.

The most preferable FFA value was observed on the 1st day and less preferable FFA value was observed on the 14th day. The most preferable value was observed from 2% apigenin (A2) extract and then observed 1% A2, 2% P2 and 1% P1 (Figure 1). The FFA value (0.46) in the control group was remarkably ($p<0.05$) higher than the values of the samples added with bee pollen extracts (P1>P2). In a study found that sausages showed an increasing FFA content over time (Siddiqua et al., 2018).

Throughout the storage period, POV was generally higher in the control group compared to treatment groups (Figure 2). The most preferable POV was observed at 2% bee pollen (P2)/apigenin (A2) extracts. The lowest amount of POV indicates that this product is most preferable for consumer's

health. Siddiqua et al. (2018) obtained similar results in beef meatball with tulsi (*Ocimum sanctum*) leaves extract.

TBARS value is one of the most completely used experiments for measuring the lipid oxidation of meat and meat products. TBARS values of meatball samples were affected by bee pollen and apigenin added (except 0 days of storage) and storage process ($p<0.05$). While on the 7th day of the storage process, the maximum TBARS values were defined in control and meatballs prepared with 1% and 2% bee pollen, the minimum values were defined in meatballs prepared with 1% and 2% apigenin extracts ($p<0.05$). A similar situation was also observed on the 14th day of the storage. TBARS values of the samples achieved max grades of 0.89, 0.81, 0.71, 0.68 and 0.65 mgMA/kg for control and meatballs formulated with 1%, 2% bee pollen/apigenin, respectively (Figure 3). Mincing, mixing ferric hematin pigments and sodium chloride were accountable for this rise. Mincing and mixture break muscle structure and enhancement the surface picked out to oxygen and another oxidation catalysis. Ferric hematin pigments simplify the transference of electrons significant to raised ratios of free radical creation and thus supports the lipid oxidation (Turhan et al., 2017; Rubio et al., 2018). Natural antioxidants, particularly polyphenols, are the major plant compounds which have the capability to decreased the oxidative damage of a tissue implicitly by increasing natural defenses of cell and/or straight by depurating the free radical species struggle pathological irregularities created by physicochemical reactive oxygen species (Du et al., 2010). Jung et al. (2010) recorded that natural antioxidant agents have considerable administration prospective for consumer's acceptability, stability and during storage of meat products.

CONCLUSION

From the study it may be concluded that 2% of bee pollen extract as natural antioxidant may be used beef meatball preparation. On the basis of sensory evaluation, physicochemical properties, biochemical analysis and microbial assessment indicated that 2% bee pollen extract showed better results in the preparation of beef meatball compare to control and other treatments. The results of this study indicated that bee pollen addition had a significant effect on the color changes, lipid oxidation and microbial quality of meatballs during frozen storage. The addition of pollen retarded lipid oxidation and inhibited microbial growth. Thus, bee pollen can be successfully used as a natural antioxidant and antimicrobial in meatballs.

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The effect Chia seeds (Salvia hispanica L.) on gastrointestinal echogenicity in cats

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ABSTRACT

Objective: It was aimed to investigate whether chia seeds provide an advantage during ultrasonographic imaging in cats when added to food by means of its high water-holding capacity.

Materials and Methods: Ten healthy cats were ultrasonographically examined following 12- hour fasting period and after feeding with 3 different food types at 1-week intervals. Thus, 4 study group were formed as follows; FST; fasting 12- hour period, WF; feeding with wet cat food following 12- hour fasting period DWF; feeding with ½ of same wet food diluted with the same volume drinking water, CWF; feeding with ½ of same wet food mixed with 36 ml swollen chia seeds obtained by holding 2 hours 3ml chia seeds in 33 ml drinking water. Cats were evaluated ultrasonographically immediately after feeding, and at the 30th minutes and 60th minutes.

Results: Especially in terms of thickness, gastric wall evaluation was easier after food intake. In the CWF, the stomach was fully imaged on the frame at all times. The gastrointestinal tract content moved faster when cats were fed DWF or CWF; In the examination immediately after feeding the contents were in duodenum and seen in the jejunum and ileum in the 30- and 60-minutes' examinations. CWF and DWF revealed more heterogeneous images then WF and also CWF provided better visualization for the intestinal canal wall layer.

Conclusion: Ultrasonographic examination after feeding with chia seed added formula can be used as a complementary method following the fasting examination.

Keywords: *Cat, Chia seed, Echogenicity, Food, Gastrointestinal, Ultrasonography*

INTRODUCTION

Ultrasonography is an imaging method based on the principle of sending high-frequency sound waves to the area of the body to be examined and the reflection of these waves in different tissues (Burk and Feeney, 2003). Gastrointestinal tract ultrasonography is often performed for cases of acute abdominal pain, vomiting, diarrhea, melena, intestinal wall thickening, and also it is used for determination of dilatation and obstruction of the intestines, presence of palpable masses, investigation of mesenteryomentum and peritoneal masses (Agthe, 2009).

Most sources suggest 12- hour fasting before the ultrasonographic examination, and also state that giving water to the patient before the examination provides a better assessment of the stomach wall (Penninck, 2008; Mattoon, 2009). It is even recommended to remove the intraluminal gas and fill the stomach with water by applying gastric catheter in humans and animals (Machi et al., 1986; Penninck et al., 1989), because the intraluminal gas causes frequently artefacts such as reverberation, comet-tail and acoustic shadow (Burk and Feeney, 2003; Peiretti and Meineri, 2008).

There is no much information about how the composition of the content remaining in the digestive tract affects the echogenicity during ultrasonographic imaging (Gaschen et al., 2016). Normal intestinal mucosa can be considered almost as anechoic, and some recent studies in dogs suggest that food intake and food quality will increase digestive tract echogenicity (Pollard et al., 2013; Gaschen et al., 2016).

Chia seeds (*Salvia hispanica L.*) is a unique food substance is known as hydrophilic, absorbing up to 12 times their weight in liquid when soaked and forms a gel layer around it (Muñoz et al., 2012). Also, it is used in many studies for human and animal nutrition due to its nutritional and therapeutic properties (Ayerza et al., 2002; Peiretti and Meineri, 2008; Ullah et al., 2016). In addition, since it does not have a noticeable taste and smell, it is easily consumed by the animals when mixed in to the food (Ullah et al., 2016). It was hypothesized that food intake containing swollen Chia seeds before ultrasonographic examination will positively affect the echogenicity in ultrasonographic examination due to the fact that sound waves propagation is much better in liquid and gel environments.

MATERIALS and METHODS

Animals

Study material was 10 healthy cats from different breed, gender, age and weight brought to our clinics (Table 1).

Table 1. Signalmen of the cats that constitutes study material

No	Breed	Age (year)	Gender
1	Mix	6	Male
2	Chinchilla	11	Female
3	British Short Hair	3	Male
4	Persian-Himalayan Mix	1	Female
5	Persian-Himalayan Mix	1	Male
6	Mixed	1.5	Female
7	Mixed	5	Female
8	Exotic Short Hair	6.5	Female
9	British Short Hair	5	Female
10	Mixed	1.5	Male

This study was approved by Aydin Adnan Menderes University Animal Experiments Local Ethics Committee (on 30/01/2018 and numbered 2018/022). All the procedures were conducted in accordance with good practices for animal experimentation. The owners of all animals were informed about the study and their approval was obtained. Neither sedation or anesthetic agent was administered to any cat before or during the study, nor was force-fed.

Experimental groups

The same 10 cats were fed with 3 different methods at a week interval, following 12-hour fasting period. Firstly, ultrasonographic examination were performed after fasting period and then repeated immediately after feeding and at the 30th and 60th minutes. Thus, 4 different assessment groups were generated.

FST: Fasting 12-hour period; **WF:** feeding with wet cat food (Proplan Gourmet Gold Ground Tuna 72 ml) following 12-hour fasting period; **DWF:** feeding with ½ of same wet food diluted with same volume drinking water; **CWF:** feeding with ½ of same wet food mixed with 36 ml swollen chia seeds obtained by holding 2 hours 3ml chia seeds in 33 ml drinking water.

Ultrasonographic Examinations

The abdominal area to be examined was shaved and local ultrasound gel was applied, and all examinations were performed in dorsal recumbency B-Mode imaging method by using 8.0 MHz probe (Mylab 30-Esaote, Genova, Italy). A rubric was generated (Table 2) and data was scored according to this table. After evaluation of each group within itself, a single score was revealed for each group in the light of the proportionally weighted data.

RESULTS

The findings are summarized and presented in Table 2. The rugal folds of the stomach could be imaged totally in all FST cats. The stomach enlarged after food intake and rugal folds became invisible in all cats. However, total images of the stomach were taken at all times in the CWF, and at the 30th and 60th minutes in most cats in the DWF. Because of hyperechogenicity and artifacts caused by the stomach content the total image could not be taken at any time point in WF. The stomach lumen was anechoic in the FST and echogenicity was significantly increased in the all postprandial evaluations. This hyperechogenicity became homogeneous hypoechoic at 60th minute in CWF, but did not change in the other feeding protocols.

Table 2. Pre- and postprandial ultrasound findings at 0, 30 and 60 minutes

Stomach	Min.	FST	WF	DWF	CWF
Mucosal Echogenity	0	1	1	1	1
	30		2	2	2
	60		2	2	1
Luminal Echogenity	0	AE	HE	HE	HT
	30		HE	HE	HT
	60		HE	HE	HE
Viewing the entire stomach	0	+	-	-	+
	30		-	+	+
	60		-	+	+
Rugal Folds	0	+	-	-	-
	30		-	-	-
	60		-	-	-
Pathologies	0	-	-	-	-
	30		-	-	-
	60	0	-	-	-
Duedonum					
Mucosal Echogenity	0	1	1	1	1
	30		2	2	1
	60		2	2	1
Luminal Echogenity	0	HO	HO	HT	HT
	30		HE	HE	HT
	60		HE	HE	HT
Jejenum					
Mucosal Echogenity	0	1	1	1	1
	30		2	2	2
	60		2	1	1
Luminal Echogenity	0	HO	HO	HO	HO
	30		HE	HT	HT
	60		HE	HT	HT
Ileum					
Mucosal Echogenity	0	1	1	1	1
	30		1	1	2
	60		1	1	1
Luminal Echogenity	0	HO	HT	HT	HT
	30		HT	HT	HE
	60		HT	HT	HT

Mucosal Echogenity (Gaschen et al. 2016) = (0): Anechoic mucosa; (1): Small number of mucosal speckles present; (2): Large concentration of mucosal speckles present
Lumen= (A): Anechoic; (HO): Hypoechoic; (HT): Heterogeneous hyperechoic reflections in the hypoechoic field; (HE): Hyperechoic
Rugal Fold & Pathology = (+): Present; (-): Absent

There was little mucosal hyperechogenicity in the all postprandial examinations at the first stage but then increased in all the feeding protocols. Hyperechogenicity was decreased only in the CWF at the 60th minutes (Figure 1).

In the postprandial examination at the first, lumen of the duodenum was generally hypoechoic in the groups of FST and WF, and heterogeneous hypoechoic with hyperechoic areas in the DWF and CWF. Duodenum lumen became hyperechoic at the 30th and 60th minutes in WF and DWF, while it remained heterogeneous in CWF (Figure 2).

Hypoechogenicity of the jejunum lumen in fasted animals turned into hyperechogenicity at the 30th and 60th minutes postprandially in WF. In the other feeding protocols, the hyperechogenicity increased at the 30th minute and decreased in the 60th minute (Figure 3).

Hypoechoic ileum lumen in fasting became heterogeneous postprandially at the 0, 30th and 60th minutes. Hyperechogenicity was dominant in most cats' ileum at the 30th minute only in the CWF (Figure 4).

DISCUSSION

In our study, considering the better propagation of sound waves in liquid and gel environments, the effect of food intake containing swollen Chia seeds (*Salvia hispanica L.*) on the ultrasonographic examination was investigated. Other advantage of Chia seeds in terms of its' usage in veterinary medicine is that it is easily consumed by animals when mixed into food since it has no noticeable taste and smell (Ullah et al., 2016). For the study, small particled food as possible was preferred to prevent artefacts that may arise from food particles, in the study.

Apart from this, it was also important that it is a product that cats love to eat to avoid force-feeding the cats. All cats included in the study ate voluntarily the food containing Chia seeds following a 12-hour fasting period.

Approaches and techniques may vary in ultrasonographic examination of the gastrointestinal system (Larson and Biller, 2009). The examination can be performed in standing, dorsal, right or left lateral recumbency for obtaining a more suitable acoustics in imaging (Penninck, 2008). However, imaging in the dorsal recumbency allows almost every part of the gastrointestinal system to be imaged (Larson and Biller, 2009). In our study, there was no problem obtaining the images of the targeted tissues in the dorsal recumbency.

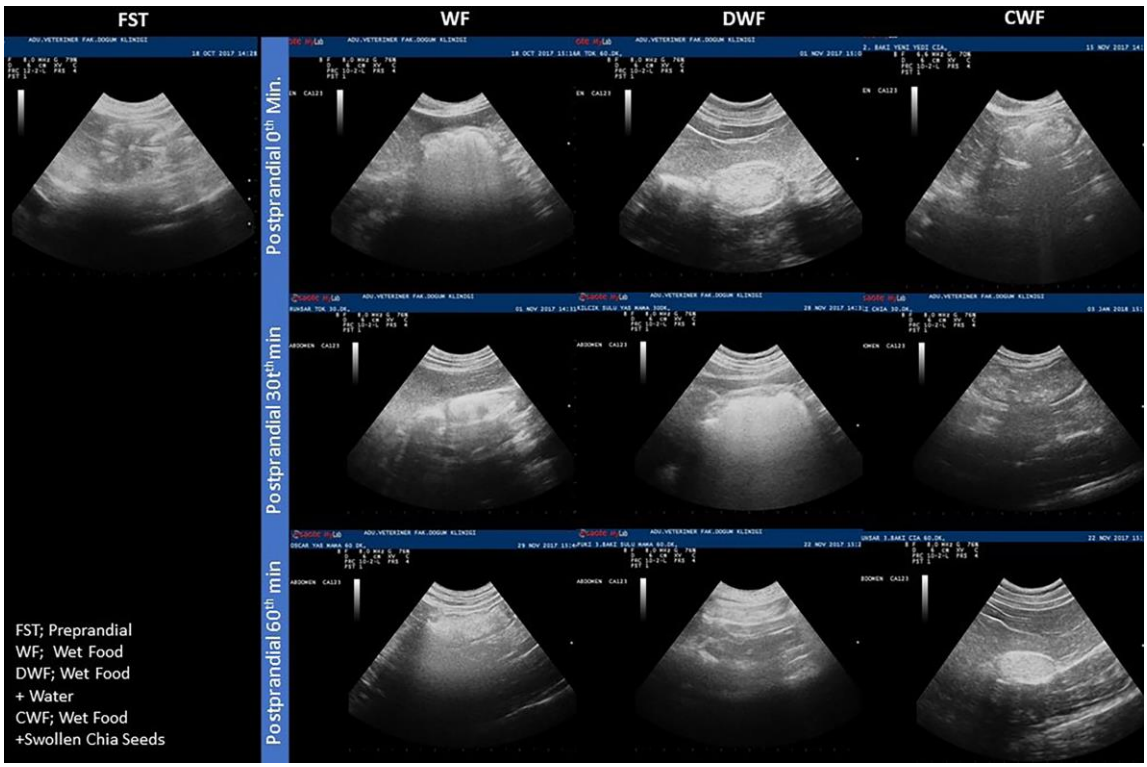

Figure 1. Ultrasonographic images of the stomach at pre- and postprandial 0, 30, 60 minutes

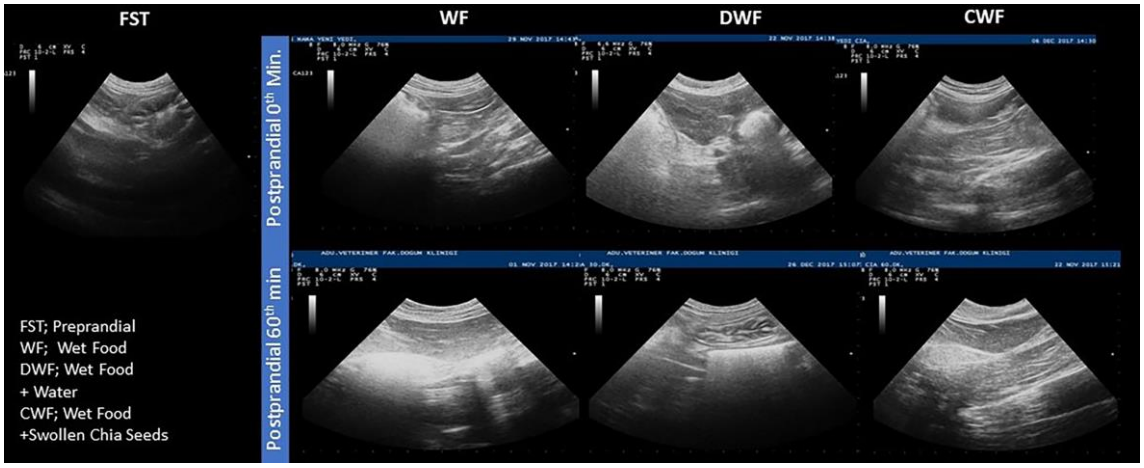

Figure 2. Ultrasonographic images of the duedonum at pre- and postprandial 0, 30, 60 minutes

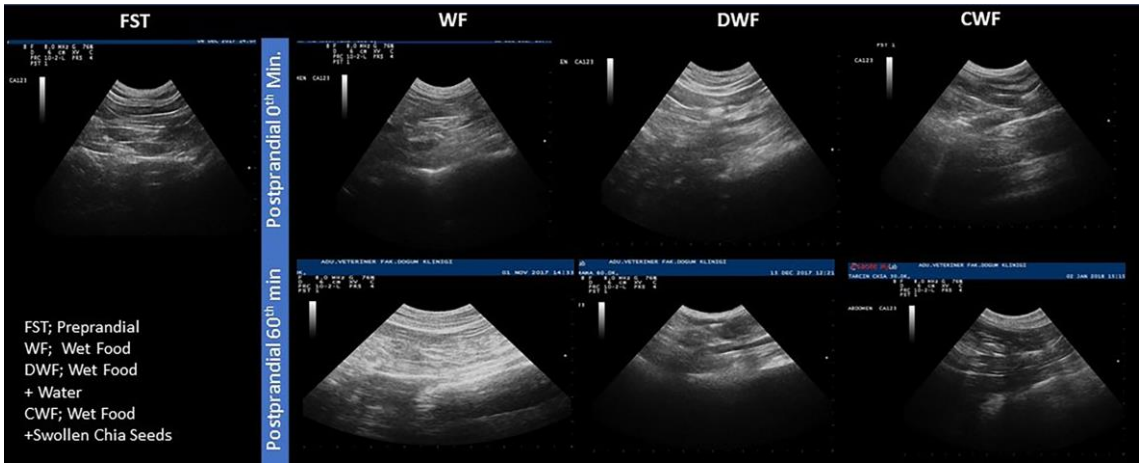

Figure 3. Ultrasonographic images of the jejenum at pre- and postprandial 0, 30, 60 minutes

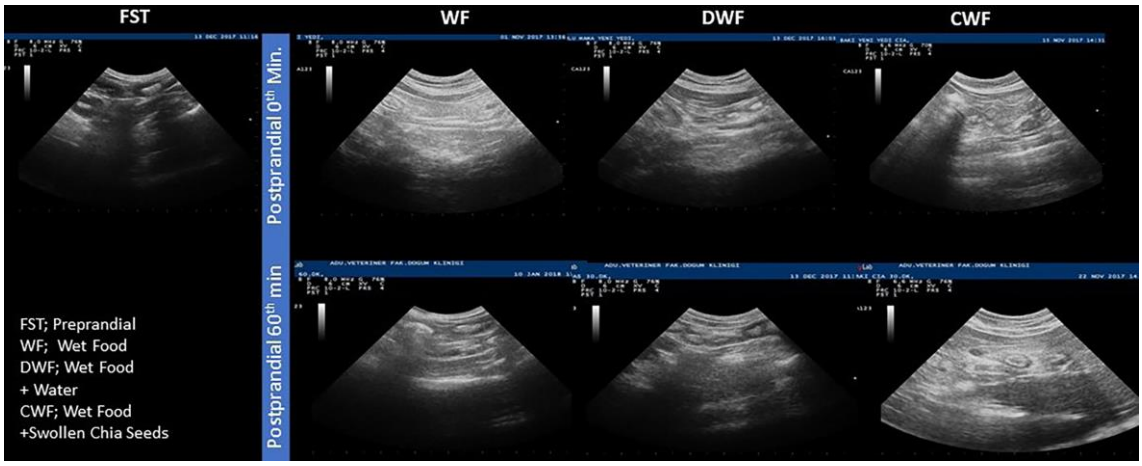

Figure 4. Ultrasonographic images of the ileum at pre- and postprandial 0, 30, 60 minutes

During ultrasonographic imaging of the gastrointestinal tract artifacts such as reverberation, comet-tail and acoustic shadow are frequently seen because of the intraluminal gas (Burk and Feeney, 2003; Penninck, 2008; Agthe, 2009). In our study, although no artifact was observed in the fasted animals' stomach but acoustic shadow artifact was observed due to gas in the intestines. After feeding, reverberation artifacts in the stomach, and reverberation and acoustic shadow artifacts in the intestines were seen mostly.

In the presented study, the stomach when it is empty totally entered to the field of the view together with its rugal folds described as in the literature (Agthe, 2009). Also, the stomach in most animals, could be viewed totally at the all times in CWF and at the 30th and 60th minutes in DWF. There are studies arguing that both the stomach can appear much thicker than normal when the stomach is completely empty (Agthe, 2009), and that gastric enlargement does not cause a significant change in the thickness of the rugalfolds or inter rugal region (Newell et al., 1999). Gastric wall thickness was not measured in this study, but observed that the appearing of the completely empty stomach wall was thicker. In addition, while the stomach walls initially gave a small number of echogenic areas in the all groups, the amount of the echogenicity increased as time passed after the food intake, however, it decreased again in the 60th minute in the CWF.

In cats and dogs, the duodenum descendens and distal ileum can usually be observed in the same area, but other small intestine segments are distributed within the abdomen (Agthe, 2009). The duodenum in cats is close to the midline and has a straight course (Agthe, 2009; Larson and Biller, 2009). In our study, duodenum could be observed in the longitudinal view distal to the stomach in the dorsal recumbency in FST and the nutritional status did not affect the position of the duodenum. Apart from this, according to the type of food taken, the wall echogenicity of the duodenum was affected at different levels at different times. The echogenicity increase that occurs 60 minutes after feeding in dogs is caused by physiological lacteal dilatation (Gaschen et al., 2016). In this study, it was also thought that the echogenicity change over time was caused by mucosal absorption and lacteal dilatation. When evaluated in this way, it can be suggested that Chia seeds addition to the food may shorten both the absorption and transition time.

Carefully scanning the entire middle abdomen is necessary to examine the whole jejunum (Larson and Biller, 2009). The hypoechogenicity observed in the jejunum lumen when fasted turned into hyperechogenicity at the 30th and 60th minutes in WF, however it decreased in the 60th minute in DWF and CWF.

The terminal ileum can be identified in the right dorsal quadrant, close to the ileocecocolic junction, slightly caudal to the costal arch in the right lateral recumbency (Agthe, 2009). In cats, the ileocolic junction is usually seen adjacent to the colic lymph node and just medial to the right kidney and is displayed in a cross section similar to a wagon wheel (Larson and Biller, 2009). In our study, it was generally imaged exactly as described, and the nutritional status did not affect the position of the ileum. Also, it is very likely that the echogenicity changes occurring in the 60-minute examination period are independent of the food type.

Diffuse or multifocal wall thickening and the indistinct wall layers and mesenteric

lymphadenopathy are seen in cases of gastrointestinal inflammation (Baez et al., 1999; Penninck, 2002; Gaschen et al., 2008; Penninck, 2008). In pathological conditions where intestinal wall infiltration is evaluated, residual ingesta affects mucosal echogenicity (Gaschen et al., 2016). Lines and/or speckles on ultrasonographic images of the intestinal wall that form in pathological conditions such as inflammatory bowel disease can also cause from residual ingesta as artifacts (Sutherland-Smith et al., 2007). Some substance such as oil added to food are reported to reduce the formation of these artifacts (Gaschen et al., 2016, Pollard et al., 2013). Based on this information, food intake containing swollen Chia seeds before ultrasonographic examination will positively affect the echogenicity in ultrasonographic examination was hypothesized in this study.

CONCLUSION

Swollen Chia seeds and wet cat food mixture can be consumed by cats without any force because it is odorless, tasteless and colorless. The stomach wall can be evaluated easier after the cat consumed it, especially in terms of thickness. In the small intestines, digestive tract content affected the echogenicity and the artifact densitydecreases by Chia seeds addition. It was concluded that ultrasonographic examination after feeding with chia seed added formula can be used as a complementary method following the fasting examination.

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Treatment of humerus Salter-Harris type II fracture with double pin combination

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ABSTRACT

In this case report, the clinical and radiographic results of the treatment of Salter Harris Type II fracture in the left humerus of a 10-month-old female and crossbred dog with parallel pin technique was evaluated. The dog with complaining of lameness was brought to Siirt University, Faculty of Veterinary Medicine, Clinic of Surgery Department and local fracture findings were found in the distal 1/3 of the left humerus. On radiological examination, it was found that the distal physeal line of the left humerus was detached. Also, it was seen that the integrity of the bone cortex was disrupted through in a line that included the metaphysis at the medial angle. In the operation, following the reduction of the fracture fragments, 2 krischner pins with 2 mm in diameter parallel to each other were applied from the medial cortex of the humerus to the lateral side of the distal condule and fixation was achieved. After the operation, the limb was taken to a backed bandage. In the radiological examination of the case on post-op 3rd week, it was found that the formation of the collus began. On the post-op 21st day, the bandage was removed and physical therapy applications were started to apply. On the post-op 4th week, it was seen that the dog used the extremity functionally and it was discharged. As a result, it was concluded that parallel double pin applications can be used successfully in the treatment of Salter Harris Type II fractures that are formed in the distal of dog's humerus.

Keywords: *Humerus, Dog, Salter-Harris fracture*

INTRODUCTION

Limb fractures in dogs can occur due to various reasons such as traffic accidents, hitting and falling from height (Aslanbey, 2002; Çağatay and Sağlam, 2013). Among these, traffic accidents take the first place with a rate of 70-80% (Aslanbey, 2002; Ermutlu et al., 2016). Shoulder fractures due to traffic accidents are less common than other extremity fractures (Deny and Butterworth 2000; Yanık, 2004). Fractures of the long extremity bones in the proximal or distal epiphyseal regions of dogs that are 3-11 months are called "Salter-Harris" fractures (Lefevbre et al., 2008; Ermutlu, 2016). It has been reported that the ratio of epiphyseal fractures to total fractures in dogs is 30%. Also, it has been reported that distal epiphyseal fractures of the femur take the first place among epiphyseal fractures with the ratio of 37% (Sağlam et al., 1999; Yanık, 2004). Articular and epiphyseal fractures of the humerus are rarely encountered, and it has been reported that these fractures occupy 8% of all fractures (Aslanbey, 2002; Seaman and Simpson, 2004). It has been observed that Salter-Harris Type I fractures are more prevalent in cats and dogs younger than 6 months while Salter-Harris Type II fractures in those older than 6 months (Sukhiani and Holmberg, 1997; Sağlam et al., 1999; Çağatay and Sağlam, 2013). These fractures do not affect the growth mechanism significantly in the growing age, but it is known that some epiphyseal fractures

can cause serious complications such as shortness of the extremity, angular deformities and joint disharmony (Ermutlu et al., 2016).

In the treatment of fractures, it is aimed to preserve the biological potential in bone healing and to restore its anatomical function (Palmer, 1999; Altunatmaz, 2004). In order to be successful in osteosynthesis, the least traumatic operative approach, a fixation system that can minimize vascular lesion, and the least traumatic methods of fixation should be preferred. In this respect, closed reduction and immobilization are considered as the first treatment option. However, in these fractures, it has been reported that the treatment failed with closed reduction and immobilization due to the inclusion of soft tissues between the fractured hematoma and fragments (Yanık, 2004; Ermutlu et al., 2016). It is known that cross-pin, single-crossing double-pin and single-pin and tension wire applications can be used for treatment of the fractures (Aslanbey, 2002; Yanık, 2004; Hayes et al., 2011; Ermutlu et al., 2016).

In this study, it was aimed to evaluate the results of the treatment of distal humeral Salter-Harris Type II fracture using the double parallel pin technique in a large dog.

CASE HISTORY

A 10-month-old, female, crossbred dog was brought to Siirt University, Faculty of Veterinary Medicine, Clinic of Surgery Department with complaints of lameness in the front extremity as a result of a traffic accident. No pathology was found in the routine clinical examinations of the patient. Medical treatment was started in terms of trauma and its general condition was stabilized. On inspection, it was observed that the dog could stand on three legs, but could not use its left forearm and the area was shaking with a pendulum during walking. In orthopedic examination, local fracture findings were detected in the distal 1/3 of the left humerus near the joint. In radiological examination, craniocaudal and mediolateral radiographs of the left humerus, laterolateral radiographs of the thorax and abdomen were evaluated. No pathology was found in the thorax and abdomen. However, separation in the distal physeal line of the left humerus and disruption of bone integrity in a line involving the metaphysis at the medial angle were seen (Figure 1A, B). The case was evaluated as a Salter Harris Type II fracture of the left humerus and the operation was decided. Following routine asepsis and antisepsis procedures, the patient was sedated with 2 mg/kg, intramuscular xylazine HCL (Xylazine 2%, Intermed, Ankara), and intramuscular 8 mg/kg Ketamine HCL (Ketasol 10%, Interhas, Ankara) were administered. Maintenance of the anesthesia of the intubated patient was carried out with 2% sevoflurone (Sevorane Liquid Abbvie, Istanbul). Intravenous fluid therapy (0.9% isotonic saline) was provided to patient during operation.

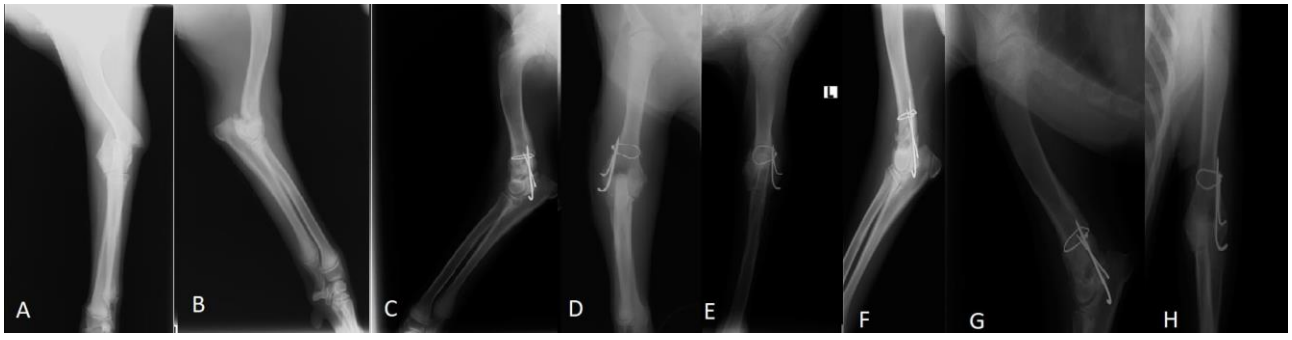

Figure 1. A: Pre-Op A/P**, B:** Pre-Op M/L**, C:** Post-Op 1st week M/L**, D:** Post-Op 1st week A/P**, E:** Post-Op 2nd week A/P**, F:** Post-Op 2nd week M/L, **G:** Post-Op 3rd week M/L, **H:** Post-Op 3rd week A/P

The distal condyle of the humerus was reached with a lateral approach. Fracture fragments were reduced. Two krischner pins 2mm in diameter parallel to each other were sent out from the lateral side of the distal condule to the medial cortex of the humerus and fixation was achieved. The operation site was closed in accordance with the technique (Aslanbey, 2002). After the operation, the relevant extremity was taken to a bandage. The bandage was extending from the distal to the other scapula on the lateral aspect of the extremity. The bandage was in the form of a cane and made of synthetic plaster. The bandage was changed every week for post-op external stabilization of the extremity. Post-op

medical treatment with ceftriaxone disodium (Unacefin® 1000 mg, Yavuz Drug, Istanbul) was applied for 5 days. Meloxicam (0.2 mg/kg, Maxicam, Sanovel, Istanbul) was administered to reduce postoperative inflammation and pain for 3 days

Radiological examinations of the relevant extremity were evaluated after the operation weekly. It was observed that complications such as loss of reduction and pin migration were not encountered in the 1st and 2nd week of radiological examinations and that the fracture line preserved its stabilization (Figure 1C, D, E, F). On the post-op 3rd week, it was found that the callus formation had begun to take shape and the fracture line could not be followed exactly (Figure 1G, H). On the post-op 21st day, the bandage was removed and physical therapy applications were started to apply. Mild lameness was observed in the extremity. It was seen that the dog could use its extremity functionally on the post-op 4th week. In addition, light walks and cage rest were recommended to continue for 1 month.

DISCUSSION

Epiphyseal fractures are frequently encountered in dogs. These are usually Salter Harris Type I and Type II fractures. (Sukhiani and Holmberg, 1997; Çağatay and Sağlam, 2013). According to the classification made by Salter-Harris for the first time in 1963, epiphyseal region fractures were categorized in 5 five (Type I, II, III, IV and V) separately. When evaluated in this context, the type of fracture accompanied by a metaphyseal fragment that occurs along the epiphyseal line is called Salter-Harris Type II (Aslanbey, 2002; Guille et al., 2004; Yanık, 2004; Simpson, 2004). Radiographs of this case revealed that the fracture was in the form of a Salter-Harris Type II fracture in the distal humerus.

It has been reported that traffic accidents and blunt traumas affecting the region can play a role in the formation of such fractures (Sukhiani and Holmberg, 1997; Guille et al., 2004; Ermutlu et al., 2016). In this case, it was observed that the fracture was formed as a result of a motor vehicle crash.

Distal humeral fractures are divided into group as Salter-Harris Type I, Salter-Harris Type II, Type III, Type IV and Type V (Yanık, 2004; Guille et al., 2004; Lefevbre et al., 2008). In cases in which a rigid fixation is provided there is a common belief that epiphyseal splitting fractures heal is faster than other long bone fractures. In the clinical and radiological examination performed on the post-op 4th week, it was determined that our patient could use the relevant extremity without any problem and treatment was performed in the fracture line successfully.

Anatomic reduction and rigid internal fixation are necessary for a painless, functional and ideal repair and proper recovery of physique. It has been reported that open reduction is required for anatomical reduction (Lefevbre et al., 2008; Ermutlu et al., 2016). It has been reported that in distal epiphyseal fractures of the humerus, a tension band applied with double pins and cerclage wire are sufficient for fixation. In some cases, a screw support is recommended in addition to this practice (Ermutlu et al., 2016). The case was over 1 year old and weighed 30 kg. It was decided to treat the fracture with a minimally invasive method. In this context, reduction and stabilization was achieved by applying a parallel double pin / cerclage wire to the lateral condule and then the limb was taken to backed bandage. The fact that there is no adverse event in the postoperative period indicates a successful fixation.

Common complications in the treatment of Salter Harris fractures have been reported as loosening of the implant, seroma formation and limitation of joint movements. (Lefevbre et al., 2008). In the radiography and clinical examinations taken at the end of the 4th week, it was found that the pins preserved their integrity, and there was no migration or any reaction to the material used.

It is generally recommended to take implants after fracture healing is completed (4-8 weeks) (Deny and Butterworth, 2000). The applied pins were not removed in this case. Because the growth age of the dog in our case was about to complete and no complications were encountered until the last control.

As a result, it was concluded that the parallel double pin fixation method preferred for the treatment of Salter Harris Type II fractures in the distal of dog humerus is sufficient in terms of providing stabilization in the desired period, clinically and radiologically. Based on the results of this case, it was determined that this technique could be an alternative to other methods and it was thought that it would contribute to the literature by increasing the number of similar cases.

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Author's Contributions: AG, MBA and NŞ designed the study. AG and MBA applied the operation technique. NŞ evaluated the results. AG and MBA wrote the manuscript. NŞ provided technical and supervisory support (AG Ali Gülaydın, MBA: M. Barış Akgül, NŞ: Nihat Şındak).

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