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Is thermography the most effective tool to monitor arthritis in rats?

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Research Article

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ABSTRACT

In this study, it was aimed to compare the effect of clinical, radiological, and thermographic methods on the diagnosis and prognosis in experimentally rats with arthritis. A total of 24 rats were divided into 2 groups, each consisting of 12 rats. Arthritis was formed by administering 0.1 ml of FCA solution to the left-back extremities of the rats. The first group was called the control group. Cephalexin (60 mg/kg) and Diclofenac Sodium (1mg/kg) were applied to the second group to be the treatment group. From day 1 of the experiment, clinical evaluation and body weight measurements were performed every day. On the 7th, 14th, 21st, 28th days of the experiment, paw edema, radiographic, thermographic measurements were performed. While typical arthritis clinical findings obtain after FCA administration, no statistically significant difference was found when analyzing the changes of body weight measurements and paw edema measurements according to groups and time. The difference in the mean of the thermographic measurements of the arthritis-forming claws was statistically significant. Radiographically, degenerations in the joints and bones were found in the control group compared to the more in the treatment group. As a result, it was concluded that thermography can be a good alternative to clinical findings and radiography in the follow-up of arthritis.

Keywords: Inflammation of joint, monitoring, radiography, thermal camera.

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Introduction

Arthritis is the inflammation of one or more of the structures that make up the joint. In cases of arthritis, the joint cartilages lose their clarity while their color becomes darker or lighter. As long as joint movements continue, abrasions, which can vary from microscopic measurements to a few mm, are formed on the cartilage surface (Samsar and Akin, 2003). Clinical, radiological, and synovial fluid examinations are routinely used for diagnosis of arthritis (Gökhan and Öztürk, 2016). The most important criterion of joint radiography is the visualization of the joint space. The width and stenosis of the joint space may differ with the movement or position of the joint (Sarierler and

Alkan, 2002). While no finding can be determined by direct radiography in acute arthritis, the earliest finding in acute septic arthritis is joint effusion (Gökhan and Öztürk, 2016). In septic arthritis, the first radiographic findings are determined 24 hours after the disease occurs and begin to occur within 5-10 days at the earliest. There is an increase in joint volume during this period, but the radiographic data are not clear (Bumin et al., 2001). Radiographic findings are obtained after 4-5 weeks. At this stage, thickening of the joint capsule and increase in opacity, lytic changes in the subchondral bone, periarticular osteopenia, alignment disorders in the intercarpal bones, and bone erosions

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in the joint cartilage. Besides, osteomyelitis and soft tissue abscesses can occur. In the last stage, destruction is observed in the bone and joint (Bumin et al., 2001; Chew, 2010).

The thermographic examination is a non-invasive method used for diagnostic purposes in veterinary medicine (Hovinen et al., 2008). It is known that damaged tissues and organs are at different temperatures than normal. Diseased areas can be identified by detecting the heat spread that occurs. The infrared thermal camera determines the temperature and infrared radiation created by the blood circulation in the capillaries in the skin. Thanks to the thermographic devices, which are advanced technological products, the information about the temperature that occurs on the skin of the animals are transferred to the digital environment in the form of color images (Düzgün and Or, 2009). This technique is used to determine the physiological and functional disorders that occur in tissues, and to examine the changes before, during, and after the treatment of a diagnosed disease. It is very effective in early diagnosis as even very low-temperature differences can be detected (Laughmiller et al., 2001; Düzgün and Or, 2009).

In this study, it was aimed to compare clinical findings, radiography, and thermographic findings in arthritis. Besides, it was hypothesized that thermography could be as effective as clinical findings and radiography in the diagnosis and prognosis of arthritis.

Material and methods

This study was carried out within the framework of the experimental protocol approved by Atatürk University Animal Experiments Local Ethics Committee (HADYEK) (2016/136). The animal material of the study consisted of 24 male Sprague Dawley rats, 10 months of age, weighing 408-504 g. 24 rats were divided into 2 groups as 12 rats in each group. To induce arthritis in the hind left paws of all rats, 0.1 ml of FCA (Freund's Complete Adjuvant, Sigma, USA) solution was administered subcutaneously at four points circumferentially (Wang et al., 2018). Clinical signs of arthritis were formed in all rats after a three-day waiting period. At the next stage, while the treatment protocol was not applied to the control group, the rats in the treatment group were given cephalexin (60 mg/kg, SEF, 250 mg/5 ml Mustafa Nevzat, Turkey) and diclofenac sodium (1 mg/kg, Diclofenac Sodium, Sigma) by gavage 5 times a week.

Clinical evaluation: At the 7th, 14th, 21st, 28th days after arthritis has been experimentally established. Swelling in the left paws and all extremities, skin temperature and color changes, the presence and

severity of lameness during walking, the presence of reduced mobility, and the presence of open wounds, abscesses and fistulas in the area were noted. On the specified days, paw edema was measured by filling the test tube with water and calculating the amount of overflowing water by dipping the paw (Özbek and Öztürk, 2003) for all rats.

Thermographic measurements: Thermographic measurements were made on the 7th, 14th, 21st, and 28th days from the dorsal, medial, lateral, and plantar sides of the left paws. (IR Flexcam-S®, USA). Measurements were carried out at room temperature without anesthesia and made from the distance received the clearest image without the use of artificial light. During the measurement, care was taken that the paws were not wet.

Radiographic measurements: Radiographs of rats under general anesthesia (IM, 8 mg/kg xylazine hidroklorür, Rompun 2%, Bayer, Turkey, 30 mg/kg ketamine hidroklorür, Ketazol 10%, İnterhas, Austria) were taken on the 7th, 14th, 21st, and 28th days. Radiographic images (Meditronics 3L 103, Japan) were taken with the paws in the anteroposterior position.

Statistical analysis: Descriptive data (body weight and paw edema measurements) were analyzed by the mean procedure. Colmogorov-Smirnov method was used for the normality test. Two-way analysis of variance with repeated measurements method was used in the analysis of thermographic data. Dependent variables (thermographic data); group (control and treatment) and time (days) were modeled based on fixed variables. Main effect and interaction effect significance ($p < 0.05$) and group/subgroup means are presented with standard error values. SPSS version 16.0 package program (SPSS, Chicago, ILL) was used for data analysis.

Results

Clinical evaluation findings: On the 14th day of the experiment, 2 rats in the control group had a fistula on the dorsal side of the paw. On the 16th day, a fistula with pus flowing mixed with blood was detected on the plantar side of the paw in 1 rat in the treatment group. When the statistical analyzes of the body weight measurements were made until the experimental protocol was completed, it was determined that the body weights did not change significantly according to time and groups. The average paw edema measurements during the experiment were calculated as 2.37 ($\pm 0,149$) ml in the treatment group and 3.86 ($\pm 0,121$) ml in the control group. When the changes in the measurements taken were analyzed by groups and time, no statistically significant difference was found.

Thermographic findings: Thermographic measurements taken from the dorsal, medial, lateral, and plantar sides in the treatment and control groups on the 7th, 14th, 21st, and 28th days are given in Table 1. When the temperatures taken from the arthritic paws were evaluated according to the groups and time, no statistical difference was found. However, when the average temperature was evaluated until the end of the experiment, a significant difference was found. It was determined that the highest mean measurement was in the plantar side in the control group (Table 2). The biggest difference between the two groups was in the mean temperatures taken from the medial side (Table 2, Figure 1).

Table 1. Thermographic measurements taken from left paws.

Side	Days	Control (°)	Treatment (°)
Dorsal	7	36.77 ± 0.34	34.58 ± 1.30
	14	35.22 ± 0.30	34.52 ± 0.51
	21	32.32 ± 0.50	31.52 ± 0.92
	28	31.62 ± 1.70	29.57 ± 0.41
		NS	NS
Medial	7	36.94 ± 0.35	34.61 ± 1.29
	14	35.02 ± 0.33	34.16 ± 0.53
	21	32.60 ± 0.40	31.90 ± 0.67
	28	31.22 ± 1.26	29.60 ± 0.42
		NS	NS
Lateral	7	36.77 ± 0.25	34.51 ± 1.31
	14	34.75 ± 0.50	34.70 ± 0.52
	21	32.45 ± 0.31	31.22 ± 0.80
	28	30.80 ± 1.09	30.00 ± 0.32
		NS	NS
Plantar	7	37.05 ± 0.34	34.65 ± 1.30
	14	35.43 ± 0.44	34.88 ± 0.61
	21	32.40 ± 0.54	31.78 ± 0.65
	28	31.27 ± 1.57	29.83 ± 0.43
		NS	NS

NS = nonsignificant

Radiographic findings: In the radiographic images taken on the seventh day, an increase was observed in the metatarsophalangeal joint distances in the left extremities of the animals in both groups. On the 14th

Table 2. Thermographic measurements taken from different sides according to groups.

Side	Control (°)	Treatment (°)	P values
Dorsal	33.9 ± 0.40	32.5 ± 0.36	0.017
Medial	33.9 ± 0.32	32.5 ± 0.38	0.009
Lateral	33.6 ± 0.33	32.6 ± 0.39	0.043
Plantar	34.0 ± 0.33	32.7 ± 0.40	0.022

day of the experiment, it was determined that the metatarsophalangeal joint spaces were closed in the rats in the control group, but the joint space was normal in the rats in the treatment group. The phalanx and tarsal bones of the rats in the control group had degeneration on the 21st day of the experiment. Osteophytic formations in phalanges and tibiotarsal joint degenerations were observed in one rat. (Figure 2A). Metatarsophalangeal and tibiotarsal joint degenerations were seen in the treatment group. (Figure 2B). In the radiographs taken on the 28th day, it was seen that the rats in the treatment group had less bone and joint degeneration than the rats in the control group. In the radiographs of all rats in the control group, degenerations in all phalanges were determined. Fractures were seen in the 2nd, 3rd, and 4th phalanges of one rat. (Figure 2C). In all rats, it was determined that the joint spaces in the metatarsophalangeal joints were closed, joint integrity was impaired and tarsal joint alignment was changed. (Figure 2D).

Discussion

Arthritis is a disease that is very common in animals and takes time to treat. For this reason, many experimental arthritis studies have been conducted to date (Saricaoglu et al., 2008; Cenesiz et al., 2012; Kerimoğlu et al., 2017). In these studies, generally adult rats were used to create adjuvant arthritis (Gertel et al., 2015). In accordance with previous reports, experimental arthritis was created in adult rats and the disease was followed up for 28 days.

In the clinical examination of arthritis, lameness is inevitable due to swelling and pain. On palpation of the area, fistulization, and pus discharge are described in most cases with an increase in temperature (Rao et al., 2020). In our study, it was determined that temperature increase and bloating occurred on the 3rd day in accordance with previous reports. On the 14th day of the experiment, fistulas with a purulent discharge mixed with blood were detected on the dorsal side of the paw in two rats in the control group and on the plantar side of the paw in one rat in the treatment group. In the last week of the experimental protocol, it was observed that the fistulization in the

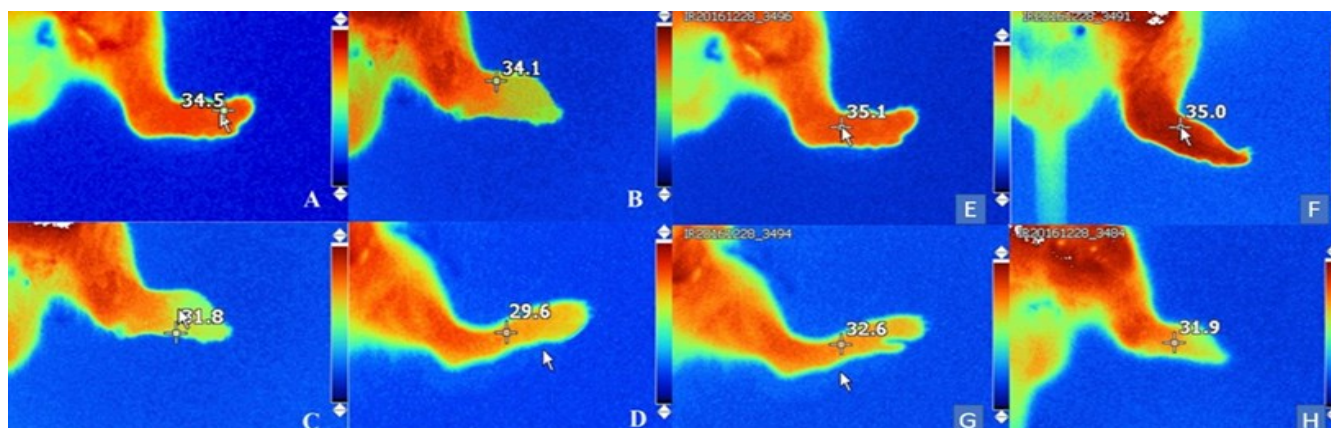


Figure 1: Thermographic images taken from the medial side of rats in the treatment and control groups. Treatment group (A; 7th day, B; 14th day, C; 21st day, D; 28th day), Control group (E; 7th day, F; 14th day, G; 21st day, H; 28th day).

rats in the treatment group decreased and the discharge disappeared. It was observed that the fistula formed in the treatment group closed more quickly due to the drugs used.

Disease symptoms have been studied for a long time. It has been mentioned that the temperature increase in the skin in ancient Egypt is an indicator of diseases. Although skin temperature can be affected by many factors, it is a known fact that it may be an indicator of inflammatory disease (Borojevi et al., 2011). Clinical findings in which skin temperature is also controlled, radiological findings, and laboratory tests are used for diagnosis in cases of arthritis (Naredo et al., 2005). The effectiveness of the use of thermography has begun to be investigated in both animal models and humans for diagnostic purposes (Jiang et al., 2005; Düzgün and Or, 2009).

Inflammatory lesions in the joints are characterized by an increase in temperature with increased vascularization in the inflamed tissue (Cuevas et al., 2015). Since joint damage may occur in the early stages of the disease, the use of a thermal camera becomes important (Düzgün and Or, 2009). To elaborate the thermographic findings, we made measurements from four different points: dorsal, medial, lateral, and plantar of the paw. It was determined that the temperature increases at four points in the rats in the treatment group were almost the same (dorsal 32.5 °C, medial 32.5 °C, lateral 32.6 °C and plantar 32.7 °C). In the control group, the temperature values on the dorsal, medial, and lateral sides were very close to each other (dorsal 33.9 °C, medial 33.9 °C and lateral 33.6 °C), while the temperature on the plantar surface was higher (34 °C). It was concluded that the increase in temperature taken from the plantar side resulted from the fact that the paws of the rats stepped on the ground with the plantar side and the blood circulation increased due to

the trauma during the press. This situation may be misinterpreted in the pathogenesis of the disease, especially in cases where treatment is delayed, if immobility is not achieved, as it will lead to an increase in local temperature. It was concluded that measurements taken from the dorsal, medial, and lateral sides would be more advantageous than the plantar side clinically. Although there was no significant change in body weight and paw edema measurements until the end of the experiment in our study, a significant difference was found when the thermographic measurements were evaluated. We thought that even in cases where clinical symptoms did not fully occur, thermography could yield results for the diagnosis of the clinician. Therefore, the determination of regional temperature should be considered as an important parameter in arthritis cases.

In the study performed on knee joints with osteoarthritis in humans, thermographic and radiological follow-up was performed and the thermographic temperatures obtained correlated with the severity of the radiological findings (Denoble et al., 2010). In arthritis cases when the direct radiographic examination is performed, an increase in joint spaces, osteomyelitis, porosity and subluxations on the surfaces of the bones forming the joint can be determined (Öztuna, 2010). In our study where we experimentally created arthritis, an increase in metatarsophalangeal joint distances in the left extremities of rats in both groups were detected on the seventh day, On the 14th day, it was found that the joint gaps were closed especially in the rats in the control group. In our radiographic findings on the seventh day, no findings emerging in chronic cases such as degeneration or bone growth on the bone surfaces were observed besides the increase in joint space. We thought that this was because the



Figure 2: Radiographs taken on the 21st and 28th day. Degeneration in the tibiotarsal joint on day 21 (A, Arrow 1) and osteophytic formations in the phalanges (A, Arrow 2), Degeneration in the tibiotarsal joint of a rat in the treatment group on the 21st day (B). It was determined that two rats in the control group had fractures in the phalanges on the 28th day (C) and another rat had tibiotarsal joint degenerations (D).

destructions in the joint tissue had not yet progressed. As stated in the study (Örgüç, 2014), the joint distance narrowing that occurs with the progression of the destruction process and the formation of fibrosis is among the findings of the 14th day of our study. Considering the 14 days of the experimental protocol, the initial period of arthritis is in line with the literature data (Moskowitz and Holderbaum, 2001). On the 7th day radiographically, the joint space was enlarged due to effusion, Then, due to cartilage destruction, erosions in the bone regions, and symmetrical narrowing in the joint space were detected radiographically, especially in the control group, in the second week of our study. Arthritis is considered a disease that can cause rapid destruction of joints and bones (Yanık et al., 2007). It is a chronic disease that is noticed with the emergence of edema at the earliest 48 hours after its formation (Nipate and Bhandarkar, 2020) and does not cause bone destruction until at least 1/3 of the bone matrix is affected (Öktem et al., 2016). It has been reported that the radiographic findings became clear a few weeks after the onset of the disease, and could not create data to determine the prognosis in the previous period (Yurdakul and Saritaş, 2013). In accordance with the literature, it is very important to use radiographic and other imaging methods together with the clinical examination of arthritis with bone destruction in terms of determining the prognosis. According to a published literature (Nipate and Bhandarkar, 2020), The narrowing of the joint spaces indicates the progression of the disease and there is little correlation with the clinical symptoms during these periods. On the 14th day of the experimental protocol, we determined that the

metatarsophalangeal joint gaps were closed in all rats in the control group. Our study findings are consistent with this study (Nipate and Bhandarkar, 2020). At the same time, it was concluded that imaging methods have a very important place in terms of diagnosis and prognosis in arthritis cases due to the low correlation with clinical symptoms.

Conclusion

As a result, the significant increase in local temperature in arthritis cases, which can be seen in all animal species and may progress with bone destruction, has led to the use of thermography, which is one of the imaging methods. Especially in cases of acute arthritis, while bone and joint destruction cannot be determined exactly on the radiography, determining the temperature increase in the thermographic allows the diagnosis of the disease to be made very early. According to our study findings, we recommend performing a routine thermography screening and suspecting the presence of a lesion when a temperature increase of even one degree is detected. In arthritis cases where the treatment is more difficult with the late diagnosis, the advantage of thermography is that it can be diagnosed with thermographic temperature increase before the radiological symptoms appear. Another advantage of thermography is that the prognosis can be determined more easily by interpreting temperature changes in the tissue after the diagnosis of the disease and comparing it with healthy tissue.

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Adaptations of racing Thoroughbreds to a hypoxic chamber: A pilot study

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Research Article

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ABSTRACT

Hypoxic exposure results in physiological adaptations and enhanced human athletic performance. However, few reports exist regarding responses of horses to similar conditions. The goals of this preliminary work were to evaluate whether horses could be acclimated to a hypoxic chamber (HC) and to monitor their performance. Trial 1: Two 4-yr-old Thoroughbreds were used to establish protocols for adaptation to the HC. Horses were stalled in the HC for 8 to 10 h/d while O₂ concentrations were decreased over 2 wk until 13.5% O₂ was achieved. On d 37, horses were removed from the HC and shipped to a track for 7 d before reentering the HC for the remainder of the 2-month study. Resting hemoglobin (Hb) was measured on d 0, 37, and 61 and ranged from 14.8 to 15.2 g/dL. Trial 2: Two 2-yr-old Thoroughbreds were maintained in the HC at 13.5% O₂ for 8 to 8.5 h/d for 21 d, shipped to a track for 5 d, and then placed back in the HC 8.5 h daily for the remainder of the 31-d trial. Horses were conditioned on the treadmill or track 6 d/wk. Horses underwent a standardized exercise test (SET) prior to being initially placed in the HC. The SET was repeated on d 10 and 31. Peak heart rate (HR) reached during exercise, and HR at 3 and 5 min post-exercise were recorded. Hemoglobin was measured immediately upon cessation of exercise. There was no difference in HR at 3 min (P=0.18), and 5 min post-exercise (P=0.64). Hb was greater on d 31 compared to d 0 (P<0.01). Without controls for comparison, we cannot confirm that differences detected were caused by the effect of HC, due to potential training effects. Results demonstrated horses can be adapted to HC but improvements in race performance were not noted.

Keywords: oxygen, performance, high altitude, equine, horse

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Introduction

Since the 1968 Olympic Games in Mexico City (elevation 2,240 meters), when the benefits of altitude training on athletic performance became evident (Kasperowski, 2009), people started to use hypoxic conditions to improve aerobic performance. It has been recognized that boosting the oxygen transport capacity, either by training or through other means commonly referred to as "blood doping," is the powerful tool for improving athletic performance in aerobic sports (Segura and Lundby, 2014). However,

blood doping was banned by the International Olympic Committee (IOC) in 1985, though there was no specific test for it at the time (Milne, 2006). Moreover, Union Cycliste Internationale (UCI) decided to evict the male athlete with hematocrit (HCT) level >50% and female athlete HCT >47% from the competition (Vergouwen et al., 1999). Given the benefits of blood doping, many coaches and athletes still are trying to find ways to improve athletic performance in a similar fashion, but through legal means. Compared with blood doping, the

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function of “live high, train low” is to improve aerobic capacity legally and without harmful side effects (Mairböurl, 2013). In principle, human and animals exposed to high altitude conditions for an extended period of time, such as living on a mountain or sleeping in a hypoxic chamber that can simulate high altitude conditions, can allow humans or animals to obtain physiological acclimations, such as increases in red blood cell (RBC) volume and erythropoietin (EPO), and improvements in heart rate (HR) recovery (Naeije, 2010), thereby improving their aerobic capacity. Thus, athletes can perform the same intensity of exercise more efficiently when they return to lower elevations (Levine and Stray-Gundersen, 1997).

A number of studies have shown the benefits of high-altitude training to human athletes (Wolski et al., 1996; Stray-Gundersen et al., 2001), which raises interest in the horse industry. There is some anecdotal evidence to suggest some success of high-altitude training in horses. For instance, Canonero II, purchased for \$1,200 as a yearling, was shipped from Venezuela where he had been racing and training at altitude, shortly before racing in, and winning, the 1971 Kentucky Derby (Hunter, 2010). However, there is limited evidence in the scientific literature proving that horses can benefit from the physiological changes of chronic altitude exposure. Thus, there is interest in exploring the role of altitude training in horse racing, which could bring some scientific insights for horse owners and trainers for the future.

Given they are commonly used by elite human athletes, it is not surprising that “high-altitude” or hypoxic chambers have also been designed for horses (Stewart, 2013). However, testing of such chambers has been limited. This study was therefore designed using a high-altitude chamber with simulated low oxygen conditions to determine whether horses could be acclimated to a hypoxic chamber (HC) and to monitor their performance utilizing a Standardized Exercise Test (SET) and official races. We hypothesized that horses could adapt to a hypoxic chamber.

Materials and methods

The project was approved by the Michigan State University Institutional Animal Care and Use Committee via an exemption approved on June 15, 2018.

Design of hypoxic chamber (HC): Prior to the start of this work, the owner of the Thoroughbred facility retrofitted a room in his barn to make into a HC. The HC had to be air-tight, have an additional power supply, and be equipped to produce hypoxic conditions. Equipment was housed outside the HC and included a rotary screw air compressor with tank and

dryer (Aircenter SK15, Kaeser Compressors, Inc., Fredericksburg, VA) to make incoming gas compressed and flow into the Simulated Altitude Generator (Hypoxico Altitude Training Systems, Hypoxico, Inc., New York, NY). This generator provided oxygen-depleted air to a stall having ventilating openings for equivalent pressure inside. The system contained digital internal and external monitoring systems and could control oxygen concentration from 20.9% down to 9%.

The inside chamber contained two separated stalls (3.1×3.1 m) equipped with sealed door and walls, hay bags, water buckets, oscillating fans, air conditioning, and scrubber machines to remove CO₂, methane, and ammonia produced by horses. Moreover, there was an ammonia monitor and oxygen sensor to ensure horses’ safety with the HC equipped with an emergency ventilation door open if conditions inside the chambers were to exceed safety margins. The chamber temperature was approximately 18°C in the winter and 21°C in the summer. Stalls were equipped with a drainage system to allow for urine removal. Feces were manually removed every 12 h. Cartridges for CO₂ scrubber were replaced every 36 h.

Experimental design Trial 1: Two 4-yr-old Thoroughbreds, one gelding and one filly, with at least 6 prior racing starts were housed in the HC to test the adaptation of the horses and chamber equipment. All horses in study were owned by, and under the management of, a professional Thoroughbred breeding and racing facility. The horses were in healthy physical condition as needed for racing. At the beginning of the study, the two horses in Trial 1 were administered 200 mg/d iron orally for four wk in case iron deficiency caused by hypoxia might interfere with erythropoiesis.

Taking into consideration the horses’ safety and health, the oxygen concentration was gradually decreased daily to target setpoint (13.5%) that was reached after a two-week period to allow horses to adapt to low oxygen conditions. On a daily basis, within the hypoxic system, it took almost 1.5 h to decrease the oxygen concentration from 21% to 18% and then approximately an additional three hours to reach the setpoint 13.5% (simulated altitude at 3,200 m). Thereafter, horses stayed at the HC with 13.5% oxygen content for 8 to 10 h/d. All horses were provided with ad libitum access to water and grass hay while in the HC. When out of the HC, horses stayed in traditional box stalls and were turned out in a paddock if weather permitted. Additionally, horses were conditioned with 30-min training on a high-speed treadmill (Chadwick Engineering Ltd., Canada)

outside the HC, which included walking, trotting, and cantering 6 d/wk. On day 37, the horses were sent to a racetrack, at which point their daily exercise was on the dirt track with a rider. After 7 d, they returned to the farm and resumed daily HC occupation and both track and treadmill exercise through the completion of Trial 1 except when transported to the racetrack for racing. Horse 1 raced on day 62 (one day after the trial ended) and Horse 2 raced on day 41 and day 56. All training and racing regimens were under the control of the farm's resident trainer and were similar to what was prescribed for the other Thoroughbreds in race training at the farm. Trial 1 began in September 2018 and ended in November 2018.

Experimental design Trial 2: The second trial began in December 2018 and finished in January 2019, lasting for 31 days. Two 2-yr-old Thoroughbred fillies, with a minimum of 3 prior starts, were stalled in the HC, with the oxygen concentration gradually decreasing to 13.5% during the two-week adaptation. They stayed with a 13.5% oxygen concentration for 8 to 8.5 h/d. When out of the HC, horses were maintained in normal box stalls on the farm. Horses were conditioned on the treadmill or track 6 d/wk. When not provided controlled daily exercise, horses were allowed turnout in a paddock. The conditioning regimen consisted of 30-min of exercise including walk, trot, and canter on the high-speed treadmill or with a rider on a dirt track, consisting of a 15-min walk (warm-up), a 4-min trot, a 4-min canter, a 2-min gallop, and then a 15-min walk (recovery). The choice as to whether the exercise was performed on the treadmill or track was weather-dependent and, again, was made by the resident trainer and was consistent with the work being received by other Thoroughbreds in race training at the facility. The horses were shipped to a track on d 22 for 5 days and then reentered the HC for the remainder of Trial 2 except when transported to the racetrack for racing. Horse 3 raced two days after Trial 2 ended and Horse 4 raced 8 days after Trial 2 ended. Horses received race training on the dirt track with rider during the time between the end of the trial and racing. In this trial, a standardized exercise test (SET) was performed on the treadmill, consisting of walking 1.6 km at 1.8 m/sec with an 8% slope, trotting 1.6 km at 3.4 m/sec with 3% slope, and walking 0.8 km at 1.8 m/sec. Due to equipment limitations, the SET did not include canter exercise. The SET was performed at d 0 and repeated on d 10 and 31. No supplemental iron was provided in Trial 2.

Sample collection Trials 1 and 2: In Trial 1, blood samples were collected for resting hemoglobin (Hb) in

the morning on d 0, 37, and at the end of the trial (d 61). In Trial 2, blood samples were taken immediately after exercise ceased in each SET for Hb analysis. Peak HR, and the HR at 3 min and 5 min post-exercise was recorded after each of the SET on day 0, 10, and 31 by heart rate monitor (Polar, Bethpage, NY).

Sample analysis Trials 1 and 2: Blood samples were taken via jugular venipuncture with vacutainers (BD Vacutainer®, Becton Dickinson, Franklin Lakes). Samples were placed in refrigerated bags, transported immediately after collection to Michigan State University, where whole blood was used to analyze Hb levels following the procedure of Stan-bio (HemoPoint® H2). After 4-wk of iron supplementation, blood samples for iron analysis were taken and centrifuged at 3,500 x g for 20 min. The aliquots of serum were stored at -20° C until analysis. Serum iron was determined by a colorimetric method (Wako Pure Chemical Industries, USA) with an automated analyzer. Total iron binding capacity (TIBC) was calculated, following the manufacturer's instructions. The equation follows as:

$$\text{TIBC } (\mu\text{g/dl}) = (\text{Aunk} - \text{Ablk}) / (\text{Astd} - \text{Ablk}) \times 400$$

Where Aunk, Ablk and Astd are the absorbance of the plasma unknown, blank, and standard.

Race performance evaluations Trials 1 and 2: All horses' race results were obtained from Equibase (<https://www.equibase.com>) and average finishing position within a race and the average speed ratings from races before starting the trials were compared to the races after commencing the trials.

Statistics: Data from Trial 1 were not analyzed statistically but were simply used to look for anomalies outside of normal ranges. All data from the SET trial (Trial 2) are presented as mean \pm standard error. Hemoglobin concentration was assessed by ANOVA in the proc MIXED program of SAS version 9.0 (SAS Inc., Cary, NC, USA) with the fixed effect of day. Peak HR, and HR at 3 min, and 5 min post-exercise were compared using a paired Student's t-test to assess performance differences based on the physiological changes that occur with acclimatization. Significance was defined as $P \leq 0.05$.

Results

Blood Variables Trials 1 and 2: After four weeks of being on the study in Trial 1 and being supplemented with iron, serum iron concentrations for the two horses were 124 $\mu\text{g/dL}$ and 119 $\mu\text{g/dL}$, and TIBC were 404 $\mu\text{g/dL}$ and 416 $\mu\text{g/dL}$. The standard ranges of serum iron and TIBC are 53 to 209 $\mu\text{g/dL}$ and 244 to 480 $\mu\text{g/dL}$, respectively (Assenza et al., 2016). The concentrations of resting Hb (g/dL) of first two horses

in Trial 1 ranged from 14.8 to 15.2 g/dL. The standard range of resting Hb is 12 to 18 g/dL (Stewart et al., 1977).

In Trial 2, Hb measured immediately post-exercise was greater on d 31 (17.0 g/dL) compared to d 0 (16.0 g/dL, SEM=0.1; P<0.01) as seen in Table 1.

Table 1. Hemoglobin concentration in two Thoroughbreds taken immediately after a SET on day 0, 10, and 31 in Trial 2.

	Hb (g/dL)		
	day 0	day 10	day 31
Horse 3	15.8	15.9	17.1
Horse 4	15.9	16.1	16.9
Average	15.9 ^a	16.0 ^{ab}	17.0 ^b

SEM=0.1, P Value=0.005

^a^b Means not sharing similar superscripts differ (P < 0.05). Hb = hemoglobin. SEM = Standard error of the mean. SET = Standard exercise test

Heart rate Trial 2: The average peak HR measured on d 0, 10, and 31 during the SET was between 110 and 120 and did not differ between days. The HR at 3 min post-

Table 2. Average heart rate in two Thoroughbreds during and after a SET on day 0, 10, and 31.

	Average HR (bpm)		
	Peak	3-min post SET	5-min post SET
day 0	120	68	63
day 10	110	68	62
day 31	120	61	54

bpm = beats per minute. HR = Heart rate. SET = Standard exercise test

SET ranged from 61 to 68 (SEM=2, p=0.18) and HR at 5 min post-SET ranged from 54 to 63 (SEM=7, p=0.64). No differences in HR were seen between days (Table 2).

Race performance in Trials 1 and 2: The race results from all four horses studied in the two trials can be found in Table 3 – contrasting their performances after HC to their races prior to commencing this study. As can be seen, no improvement in the average finish position was noted and the average speed rating decreased in the races performed after HC adaptation compared to prior to HC adaptation.

Discussion

This preliminary study has many limitations. There is a small number of animal subjects, a lack of controls, and limited characterization of athletic performance – all of which complicate the interpretation of this study evaluating the use of a HC and prevent any firm conclusions from being made. The changes over time

Table 3. The average finish position and average speed rating score both prior to HC acclimation and after HC acclimation in four Thoroughbred racehorses along with the number of races from which the average was determined. Racetrack elevation ranged from 147 to 262 m.

Trial 1	Average finish position (# of races)	Average Speed Rating
Horse 1 Prior HC	6 (6 races)	52
Horse 1 After HC	5 (1 race)	41
Horse 2 Prior HC	6 (6 races)	23
Horse 2 After HC	9 (2 races)	17
Trial 2	Average finish position (# of races)	Average Speed Rating
Horse 3 Prior HC	4 (5 races)	36
Horse 3 After HC	5 (1 race)	14
Horse 4 Prior HC	5 (3 races)	31
Horse 4 After HC	5 (1 race)	2

HC = hypoxic chamber

seen in Trial 2 are confounded with potential training effects (Soroko et al., 2019). Despite these major limitations, the tremendous cost associated with establishing and maintaining HC would likely preclude this study to be performed in a normal research setting. Thus, by having the opportunity to study actual racehorses being placed into HC and then compare their performances before and after adaptation enabled some insights into the acclimation of horses in a HC that might not otherwise be afforded.

While not specifically measured, changes in behavior were noted throughout the study. In Trial 1, when the horses were first exposed to 13.5% oxygen, they remained sedentary and just stood in a corner with their heads down – suggesting they were possibly experiencing altitude sickness such as occurs with humans (Moore and Regensteiner, 1983). However, after two weeks of exposure to low oxygen, behavior appeared to return to normal. The return to normal behavior suggested that horses could adapt to a HC, but that adaptation was not immediate and horse welfare needs to be considered.

The interpretation of resting hematological parameters can be somewhat ambiguous. This is because the horse's spleen is capable of storing one-third to one-half of the RBC (Poole and Erickson, 2011). The spleen can make active contractions induced by chemicals called alpha agonists and mobilize stored RBC to the systemic circulation in response to varying stressful stimuli, such as hypoxia, excitement.

Releasing reserved RBC leads to raising systemic hematocrit from 35% at rest to 60 to 70% during maximal exercise (Poole and Erickson, 2011). Moreover, strenuous exercise and training could potentially boost blood values as well (Soroko et al., 2019). In Trial 2, Hb concentrations were increased after 31 days of being in the study and being stabled in the HC. Similar results were also reported among previous human and other animal studies (Heinicke et al., 2003). Wehrlin et al. (2006) showed Hbmass increased by 5.7% in human athletes after 24 days in a “live high, train low” protocol. Hb concentration is a favorable indicator that supports aerobic performance. However, in this pilot study, due to the lack of resting hemoglobin concentrations, the lack of control animals and possible confounding effects of training, post-SET Hb concentrations were not analyzed statistically. Thus, it is not possible to conclude that the changes in blood parameters were due to the HC.

Iron plays a role in enhancing Hb concentration and the activity of RBC. Exercise can induce changes in iron metabolism to mimic iron deficiency, thereby decreasing Hb and ferritin concentrations in humans (Zoller and Vogel, 2004). Iron supplements are often used by elite athletes during extreme exercise (Beutler, 2002; Zoller and Vogel, 2004). In humans, Govus et al. (2015) suggested that sufficient iron stores are required to support increased erythropoiesis during prolonged altitude exposure. Moreover, a three- to five-fold increase in erythropoiesis occurs and was associated with 100% erythroid iron uptake during the first few days of adaptation. Recognizing this, the racehorse facility owner supplemented the two initial horses from Trial 1 with iron to ensure their iron status was sufficient to facilitate erythropoiesis. However, TIBC and serum iron values were similar to previous results with a standard training program (Assenza et al., 2016). Some studies have reported increased serum iron concentrations in horses following exercise, probably resulting from the release of iron from reticuloendothelial cells (Assenza et al., 2016; Assenza et al., 2017). Iron metabolism can be influenced by the duration and intensity of exercise (Assenza et al., 2016).

The 2007 Horse NRC suggested iron 40 ppm in the diet for adult horses. Typically, there is no need to provide additional iron supplements for horses due to an abundance of iron in the diet of horses (Richards and Nielsen, 2018) and it is unusual for horses in training to have an iron deficiency. Chronic excessive iron intake may result in toxicosis or potential disease in equids (Theelen et al., 2019). At this time, there is no indication that iron supplementation is needed or

beneficial to racehorses and the owner, after seeing the initial results from the horses in Trial 1, decided to not continue with iron supplementation in Trial 2.

In terms of indoor air control, when opening the door of the HC to lead a horse through, air within the HC is allowed to mix with air from outside the HC resulting in small changes in oxygen concentration (2 to 3%) that were noted for 15 to 20 min before returning to the setpoint. However, with the oxygen sensor near the door, it is unlikely that the oxygen concentration of the whole room was altered much during those brief periods. Regardless, these brief alterations were noted as a reminder that any time the door is opened, there is a period in which the gas concentrations are altered before homeostasis is once regained.

Conditions of air quality and sanitation in the HC can have a direct effect on the health and performance of horses. Humans sleeping in a hypobaric chamber or high-altitude tent experience different environmental conditions than would a horse residing in one. Air pollutants originate mainly from their feces, urine, feed, and bedding. Moreover, horses undergo substantial hindgut fermentation – resulting in carbon dioxide, methane, and ammonia being released from horses – gases which should be removed from the chamber. Horses can potentially produce 20.7 kg of methane gas (Elghandour et al., 2019) and 12.2 kg of ammonia (EPA, 2004) per year. From this standpoint, filter systems are a critical part of the insulated chamber to remove those biochemical wastes and keep the airflow in the chamber clean and fresh. At the time of this study, to purchase cartridges to remove CO₂ for the chamber, the cost would have been around US\$350, and the cartridges would need to be replaced every 36 hours - resulting in high operating costs.

Beyond gas production, horses can produce large amounts of sweat and also release substantial moisture through their respiration. After racing poorly, one of the horses in Trial 2 had an endoscopic evaluation done. An accumulation of mucus within the airways was found and it is believed the high humidity in the chambers likely contributed to mucus production and secretion (Gerber, 1973). This likely had a negative effect on racing performance.

As a pilot field study, we confronted various unexpected challenges with using the HC. While this study was not able to demonstrate improved performances with the use of HC, the findings are useful in providing guidance to others that may be interested in exploring work in this area. For example, lowering the oxygen concentration in the chambers

lowering the oxygen concentration in the chambers needs to be done over two to three weeks to allow adaptation without compromising animal welfare. When the oxygen concentration was decreased too quickly after initially placing the horses into the HC, a lack of voluntary movement in the chambers suggested adaptation had yet to occur. As horses seemingly adapted to the HC, their behavior returned to normal. While this study was originally designed to evaluate physiological acclimation, the behavioral adaptation should also be taken into consideration.

In terms of oxygen level, a 13.5% concentration, similar to that reported by Wickler and Anderson (2000), was targeted. The initial assumption was that an ideal concentration could be determined and then used for a larger study with more horses. Towards the end of the two-wk adaptation period in Trial 1, the oxygen concentration was temporarily dropped to 13% but the behavior of the horses was concerning (they appeared to be abnormally depressed and lethargic) and the concentration was returned to 13.5% at which point the horses resumed eating and normal behavior within the HC. Again, this study serves as a reminder that, even if improvements in athletic performance were gained, the well-being of the horses needs to always be considered. Adaptation needs to be gradual and an ideal oxygen concentration for an equine HC still needs to be elucidated.

By monitoring horses performance after exposure to HC, we found their performance actually appeared to decrease, it also revealed potential concerns with using such a system. Beyond needing to remove large quantities of gases such as carbon dioxide, methane, and ammonia, high humidity within the chambers may have been the cause of the excess mucus found in the airway of one of the study horses that had an endoscopic evaluation after a poor race performance. These are not issues that would be present to a great degree with human HC but are challenging to deal with in an equine HC. In addition to the gas removal, a dehumidifier should be used to keep moisture within the chambers to an acceptable level.

While a HC allows oxygen concentrations to be controlled allowing horses to “live high, train low” without actually living at a high altitude, there are many limitations. Using a HC is a time-consuming and installing and operating the system is expensive. While there is vast experience using such systems with humans, many modifications need to be made to a HC system to allow it to be used for horses given that horses excrete waste while in the chambers and this waste must be removed promptly. Besides the high cost and large investments of time needed to manage

horses in the chambers, horse welfare must be continually emphasized – particularly during the initial adaptation stage as horses are becoming acclimated to decreasing oxygen concentrations.

Conclusions

Although limitations existed in this pilot study, the findings of this study demonstrate that horses could adapt to a HC, but this process takes time. Also, by not having control horses housed outside the HC, any increases in Hb that may be associated with HC use were confounded with a potential effect of training. While having control horses should be the standard for any research project, this rare opportunity to study horses in a HC and undergoing actual racing provided some knowledge that could be used in further controlled studies. Further, this preliminary work showed no improvements in finish position and speed rating after HC. HC is a costly and time-consuming investment and any desire to invest in such should be taken only after great consideration. Whether HC could enhance horse race performance is still in question.

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Macroanatomical comparison and examination of external carotid artery and last branches in the Abaza (*Capra aegagrus*) and Gurcu (*Capra falconeri*) goats

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Research Article

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ABSTRACT

The aim of this research is to macroanatomically compare and examine the arteria carotis externa and its last branches that provide arterial vascularization of the head region of Abaza and Gurcu goats. For this purpose, a total of 10 Abaza and 10 Gurcu goat heads were used. In both goat breeds, a. carotis communis continued as a. carotis externa. In both goat breeds, according to the vessel order not taking place in Nomina Veterinaria Anatomica, a common root belonging to a. temporalis superficialis and a. transversa faciei was observed. In addition to this common root, the presence of a common stem belonging to a. malaris and a. infraorbitalis, not mentioned in the nomina, in both goat breeds was detected. In Gurcu goats, a. lingualis dextra disappeared by getting thinner after being separated from a. carotis externa in 70% of the materials. As a result, although the distribution of a. carotis externa in the head region of Abaza and Gurcu goats is generally similar to other small ruminants, some differences were determined in the origin points, courses and the number of sub-branches of the branches originating from the a. carotis externa.

Keywords: Abaza goat, A. carotis externa, Gurcu goat

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Introduction

Abaza goat (*Capra aegagrus*): Abaza goats, which are mainly bred in the province of Artvin in Turkey, are generally bronze, white, black and brown in color (figure 1). The mouth, around the eyes, horns and legs may be black. It is in thin form in terms of body structure and therefore, it is likened to gazelle. The females can be horned or hornless, while the males have xiphoid, flat and broadly shaped horns. The horn structure of the females with horns is small and similar to that of a male goat (Batu 1951, Sezgin et al 2010).

Gurcu goat (*Capra falconeri*): 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, which is mostly in black, gray



Figure 1. Abaza goat

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and white colours, originates from the auger horned goat *Capra falconeri* (Batu 1951, Sezgin et al 2010, Yalçın et al 1990). The horn structure of the male goats differs from the hair goats. The long and upright horns of male Gurcu goats touch each other at the tip (Figure 2). Gurcu goats' horns are sometimes seen to reach 50 cm (Batu 1951).



Figure 2. Gurcu goat

Considering the importance of the anatomy and circulatory system, the circulatory system is a system in which many variations of the artery and vascular system can be seen (Dursun, 2000). There are several methods that solidify into the vessel and allow us to examine the course of the vessels and their sub-branches in order to successfully examine and understand this highly complex system. There studies examining the circulatory system in ruminantia using these methods in the literature, (Akbulut and Aslan 2013, Beki 2017).

The aim of this research is to macroanatomically compare and examine the a. carotis externa and its last branches that provide arterial vascularization of the head region of Abaza and Gurcu goats.

Materials and Methods

Abaza and Gurcu skulls were used in this study. First of all, permission was obtained from the Kafkas University Animal Experiments Local Ethics Committee (KAÜ-HADYEK/2018-003). After receiving the approval a total of 10 Abaza and Gurcu goat heads were provided from the education, research and application farm, regardless of gender. The arteries of the materials were washed with a 0.9% saltwater solution (Erençin et al 1967). Then, the arteries were filled with latex (300-400 cc. latex and 25 cc. artdeco fabric dye) colored with red dye (Ayçan and Bilge 1984, Beşoluk et al 2006, Bugge 1963). After the latex solidified, the skin on the skulls was removed. The arteria carotis externa and its branches running ventral to the glandula parotid were dissected along their course

and findings were obtained.

Results

At the level of processus transversus of the atlas, the arteria carotis externa (a.) is seen to be separated from common carotid artery (Figure 3-4/1). In both goat breeds, it was observed that the common root belonging to a. auricularis caudalis, a. temporalis superficialis and a. transversa faciei and a. lingualis originated from the a. carotis externa.

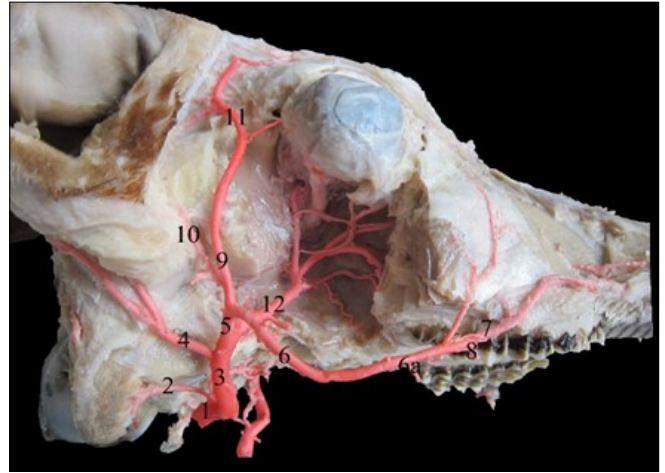


Figure 3. Arteria carotis communis and its branches in the Abaza goat. 1. a. carotis communis, 2. a. occipitalis, 3. a. carotis externa, 4. a. auricularis caudalis, 5. a common stem belonging to a. temporalis superficialis and a. transversa faciei, 6. a. transversa faciei, 6a. r. massetericus, 7. a. labialis superior, 8. a. labialis inferior, 9. a. temporalis superficialis, 10. a. auricularis rostralis, 11. a. cornualis, 12. a. maxillaris.

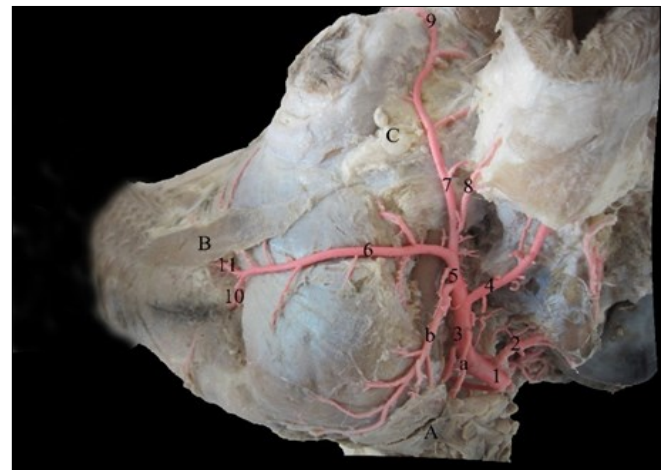


Figure 4. Arteria carotis communis and its branches in the Gurcu goat. 1. a. carotis communis, 2. a. occipitalis, 3. a. carotis externa, 4. a. auricularis caudalis, 5. a common stem belonging to a. temporalis superficialis and a. transversa faciei, 6. a. transversa faciei, 7. a. temporalis superficialis, 8. a. auricularis rostralis, 9. a. cornualis, 10. a. labialis inferior, 11. a. labialis superior, a. ramus glandularis, b. branch from the artery carotis externa to the ventral edge of the mandible, A: Gl. mandibularis, B: M. zygomaticus, C: Corpus adiposum.

In both goat breeds, it was determined that the rr. glandularis, which goes to gl. parotis, is originated from the stem belonging to a. temporalis superficialis and a. transversa faciei that separates from a. carotis externa as a common stem (Figure 3-4). Furthermore, a strong branch originating from the common stem in Gurcu goats was found to move towards the ventral edge of the mandible (Figure 4/5). In both goat breeds, it was seen that the a. auricularis caudalis provided the arterial vascularization of the ear by dividing into last two branches after giving branches primarily going to gl. Parotis (Figure 3-4/4).

In the Abaza and Gurcu goats, it was seen that a. temporalis superficialis gave a. auricularis rostralis after the origin, then gave the common stem belonging to a. palpebralis superior lateralis and a. palpebralis inferior lateralis towards the orbita, ramus lacrimalis and a. cornualis going towards gl. lacrimalis and ended by spreading in the frontal region. In 30% of the Abaza goats, it was seen that the a. auricularis rostralis was separated from the common stem of a. temporalis superficialis and a. transversa faciei.

In both breeds, it was observed that the a. transversa faciei provided m. masseter's arterial vascularization, and it divided into two branches as a. labialis superior and a. labialis inferior at the front edge of the m. masseter (Figure 4/10-11).

In the Abaza and Gurcu goats, it was observed that the a. lingualis was separated from the lateral face of a. carotis externa. In 70% of the materials in Gurcu goats, a. lingualis dextra was observed to be disappeared after being separated from a. carotis externa and the right side of the tongue was fed by a strong branch coming from a. lingualis sinistra (Figure 5/1). In both goat breeds, it was determined that the a. profunda lingua, which is the continuation of a. lingualis, anastomosed with a similar vessel in the opposite side of the apex of the tongue (Figure5-6).



Figure 5. A. lingualis and branches in Gurcu goat. 1. a. lingualis, 2. rr. perihyoidei, 3. a. profunda linguae, a. rami dorsales linguae, A: Apex linguae, B: Os hyoideum.

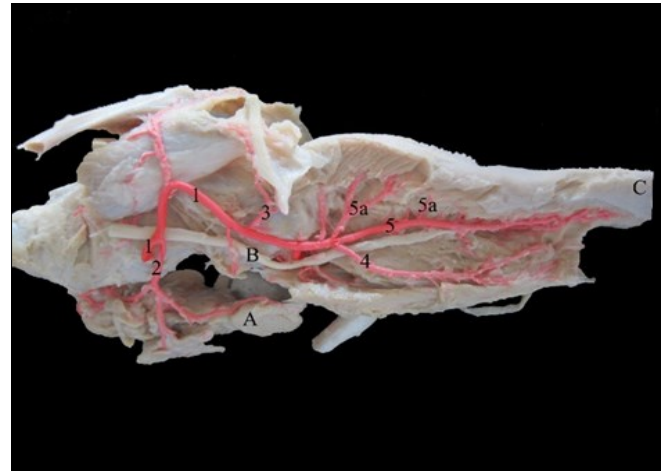


Figure 6. A. lingualis and branches in Abaza goat. 1. a. lingualis, 2. Ramus glandularis, 3. rr. perihyoidei, 4. a. sublingualis, 5. a. profunda linguae, 5a. Rami dorsales linguae, A: Glandula mandibularis, B: Nervus lingualis, C: Apex linguae.

In both goat breeds, it was also observed that the a. maxillaris, which is the continuation of a. carotis externa, ended by giving a common stem for a. alveolaris inferior, a. buccalis, a. temporalis profunda, a. ophthalmica externa, a. malaris and a. infraorbitalis, respectively, and finally for a. sphenopalatina and a. palatina descendens (Figure 7-8).

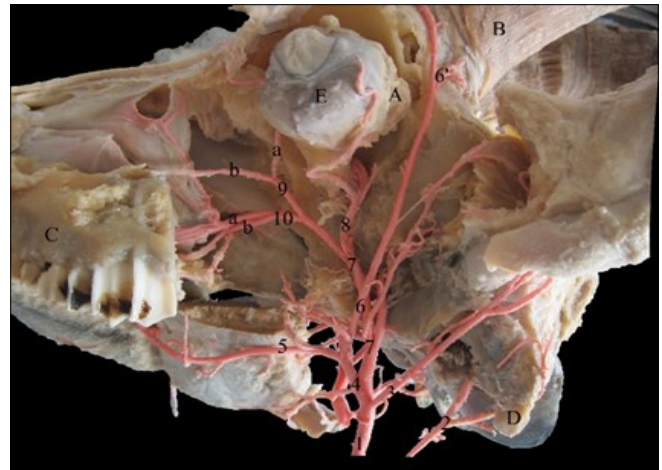


Figure 7. Arteria maxillaris and terminal branches in Gurcu goats. 1. a. carotis communis, 2. a. occipitalis, 3. a. auricularis caudalis, 4. common root of arteria temporalis superficialis and arteria transversa faciei, 5. a. transversa faciei, 6. a. temporalis superficialis, 6. a. cornualis, 7. a. maxillaris, 8. a. ophthalmica externa, 9. common root of arteria malaris with arteria infraorbitalis, a. a. malaris, b. a. infraorbitalis, 10. common root of arteria sphenopalatina with arteria palatina descendens, a. a. palatina descendens, b. a. palatina minor, A: Gl. lacrimalis, B: Proc. cornualis, C: Os maxilla, D: Condylus occipitalis, E: Bulbus oculi.

In the Abaza and Gurcu goats, it was observed that the a. alveolaris inferior was originated from a. maxillaris, and ended as a. mentalis after coming out from for. mentale by following a straight course within the canalis mandibularis.

In both goat breeds, it was seen that the a. ophthalmica externa was separated from a. maxillaris as a single stem (Figure 7/8, Figure 8/10). It was observed that the vessel formed a. lacrimalis first and then rete mirabile ophthalmicum after giving various branches to the eye muscles. Then, it gave a. ethmoidalis externa and a. supraorbitalis with two branches. In both goat breeds, it was observed that a common stem belonging to a. malaris and a. infraorbitalis is separated from a. maxillaris, a. malaris moves towards the medial angle of the eye and feeds this area, and the a. infraorbitalis is ended on the sides of the nose by passing through the canalis infraorbitalis (Figure 9). In the Abaza and Gurcu goats, it was determined that the a. palatina descendens and a. sphenopalatina were separated from a. maxillaris with a common stem, the palatina descendens feeds the nasal cavity, and the a. sphenopalatina was divided into two branches as a. palatina major and a. palatina minor (Figure 7-8).

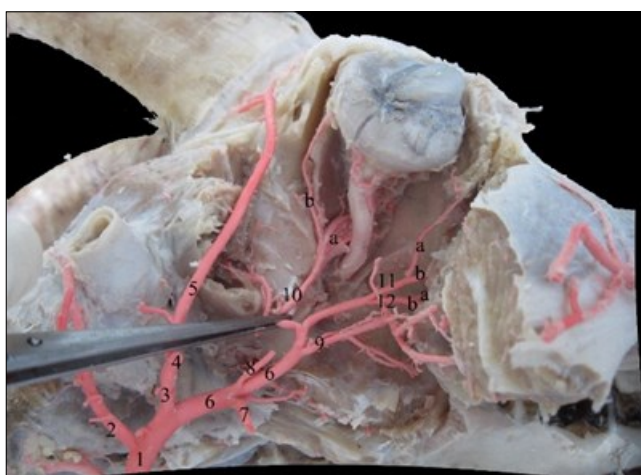


Figure 8. Arteria maxillaris and terminal branches in Abaza goats. 1. a. carotis externa, 2. a. auricularis caudalis, 3. common root of arteria temporalis superficialis and arteria transversa faciei, 4. a. transversa faciei, 5. a. temporalis superficialis, 6. a. maxillaris, 7. a. alveolaris inferior, 8. r. caudalis ad rete mirabile epidurale rostrale, 9. a. buccalis, 10. a. ophthalmica externa, a. rete mirabile ophthalmicum, b. ramus lacrimalis, 11. common root of arteria malaris with arteria infraorbitalis, a. a. malaris, b. a. infraorbitalis, 12. common root of arteria sphenopalatina with arteria palatina descendens, a. a. sphenopalatina, b. a. palatina descendens

Discussion

Researches stated that (Dursun 2000, Doğuer and Erençin 1966, Najafi et al 2008, Ghoshal 1975, Dalga and Aslan 2016), a. carotis externa is a continuation of a. carotis communis in terms of its course and thickness. In our study, in both Abaza and Gurcu goats, the a. carotis externa was determined to be the continuation of a. carotis communis.

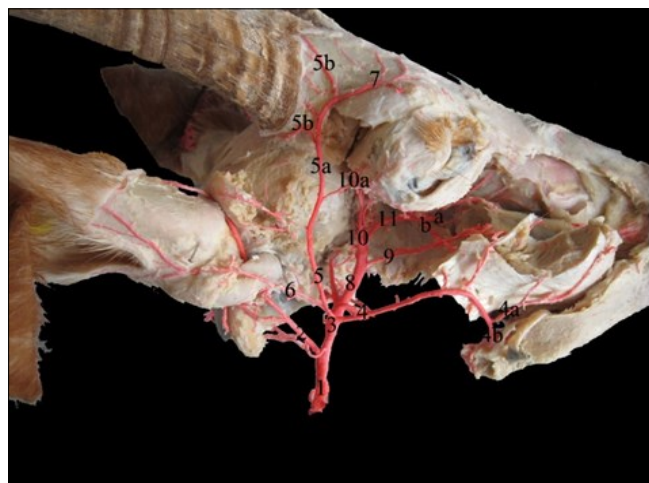


Figure 9. Arteria maxillaris and terminal branches in Abaza goats. 1. a. carotis externa, 2. a. auricularis caudalis, 3. common root of arteria temporalis superficialis and arteria transversa faciei, 4. a. transversa faciei, 4a. a. labialis superior, 4b. a. labialis inferior, 5. a. temporalis superficialis, 5a. branch of the arteria temporalis superficialis scattering to the regio temporalis, 5b. a. cornualis, 6. a. auricularis rostralis, 7. A. dorsalis nasi, 8. a. maxillaris, 9. a. buccalis, 10. a. ophthalmicus externa, 10a. rr. musculares, 11. common root of arteria malaris with arteria infraorbitalis, a. a. malaris, b. a. infraorbitalis.

Dalga and Aslan (2016) reported that the a. lingualis originates from the lateral face of a. carotis externa in the Hemşin sheep, while Nur and Dursun (1992a) reported that the a. lingualis originates from the front face of the a. carotis externa in the Akkaraman sheep. Beki (2017) examined the Tuj sheep and reported that a. lingualis originates from the front face of a. carotis externa. Furthermore, in the Abaza and Gurcu goats, it was determined that the a. lingualis originates from the front face of the a. carotis externa, and participated in arterial nutrition of gl. mandibularis, m. masseter, m. hyoglossus and m. genioglossus. Then, it was divided into the last end branches called a. sublingualis and a. profunda linguae. Nur and Dursun (1992a, 1992b), it was reported that the rr. dorsales lingules ranging from 6 to 8 in number originates from a. profunda linguae for the dorsum linguae in Akkaraman sheep and Angora goat. Dalga and Aslan (2016), reported that the a. profunda linguae follows a spiral course. Baki (2017), examined the Tuj sheep and reported that the a. profunda linguae, which is the continuation of a. lingualis, advances in a straight line and gives the rr. dorsales linguales, whose number varies between 10-12, for the dorsum linguae. In the Abaza and Gurcu goats, it was determined that a. lingualis and its continuation a. profunda linguae follow a straight course and gives a smaller number of rami dorsales linguales for the dorsum linguae. As stated in the findings, a finding that is not included in the literature

review was obtained for Gurcu goats. In 70% of the materials in Gurcu goats, a. lingualis dextra was observed to be disappeared after being originated from a. carotis externa and the right side of the tongue was fed by a strong branch coming from a. lingualis sinistra.

According to the studies conducted on the Akkaraman sheep, Angora goat, (Nur and Dursun 1992a, 1992b), Hemşin sheep (Dalga and Aslan 2016) and Tuj sheep (Beki 2017), a. temporalis superficialis and a. transversa faciei originate from the a. carotis externa as a common root. It has been reported that the a. temporalis superficialis and a. transversa faciei in buffalo (Özdemir and Tıprıdamaz 2002) and Zavot breed cattle (Akbulut ve Aslan 2013) originates at different levels from the a. carotis externa. In this study conducted on the Abaza and Gurcu goats, the results were found to be consistent with the results of the studies conducted on the Hemşin and Akkaraman sheep, and it was determined that a. temporalis superficialis and a. transversa faciei are separated from a. carotis externa as a common root. However, this common root was not seen in the terms included in Nomina Anatomica Veterinaria (2017). In addition, in our study, it was determined that the common root length was longer in Gurcu goats compared to Abaza goats. Dalga and Aslan (2016), it was stated that the a. auricularis rostralis originates from the common root of a. temporalis superficialis and a. transversa faciei in the Hemşin sheep. It was also determined that the a. auricularis rostralis originates from a. temporalis superficialis and a. transversa faciei at a rate of 80% in the Akkaraman sheep (Nur and Dursun 1992b) and originates from a. temporalis superficialis in the Angora goat (Nur and Dursun 1992a). On the other hand, it was reported that the a. auricularis rostralis originates from the caudal face of a. temporalis superficialis in the Tuj sheep (Beki 2017). In our study, it was determined that the a. auricularis rostralis was separated from the common root of a. temporalis superficialis and a. transversa faciei in %30 of the Abaza goats, which is similar to the arterial structure in the Hemşin and Akkaraman sheep. This situation in Gurcu goats was determined to be consistent with the Tuj sheep and Angora goat. Nur and Dursun (1992a, 1992b), reported that the a. palpebralis superior lateralis and a. palpebralis inferior lateralis are separated from a common root originated from a. temporalis superficialis in 45% of the the Angora goats and 30% of the Akkaraman sheep. In our study, it was observed that the a. palpebralis superior lateralis and a. palpebralis inferior lateralis are separated from a common root originated from a. temporalis superficialis in both goat breeds. In our study, the finding stating that the a. temporalis superficialis is weaker than a. transversa faciei is consistent with the

literature (Beki 2017, Doğuer and Erençin 1966, Bilgiç 1987).

Ghoshal (1975) and Dursun (2000) reported that the a. maxillaris, which is one of the end branches of a. carotis externa, is the continuation of a. carotis externa with its thickness. In addition, the a. maxillaris was observed to be the continuation of a. carotis externa in the Abaza and Gurcu goats. The fact that the a. maxillaris gave the a. ophthalmica externa after giving the a. alveolaris inferior and a. buccalis, formed the rete mirabile ophthalmicum between m. rectus dorsalis and m. retractor bulbi at the level of for. orbitorotundum showed that it is consistent with the information presented in the literature (Dursun 2000).

Akbulut and Aslan (2013) reported that the a. alveolaris inferior originates from a. maxillaris, and follows a spiral course within the canalis mandibularis after entering the mandible foramen, and continued to the mental foramen in Zavot breed cattle. As a matter of fact, Dalga and Aslan (2016) reported that the a. alveolaris inferior follows a straight course in the Hemşin sheep. Furthermore, Beki (2017) reported that the a. alveolaris inferior follows a straight course in the Tuj sheep. It was observed that the a. alveolaris inferior follows a straight course within the canalis mandibularis in both Abaza and Gurcu goats similar to Hemşin and Tuj sheep. Özdemir and Tıprıdamaz (2002) reported that the a. ophthalmica externa originates from a. maxillaris as two roots, while Diesem (1975), Ghoshal (1975) and Schummer et al (1981) reported that the a. ophthalmica externa is separated from a. maxillaris as a single root in the Tuj sheep Beki and Akbulut (2017). It was observed that the a. ophthalmica externa is separated from a. maxillaris as a single root in both Abaza and Gurcu goats. In our study, as stated in the literature Dursun (2000), Ghoshal (1975), the a. lacrimalis, which is originated from rete mirabile ophthalmicum, follows a course towards gl. lacrimalis and ends in gl. lacrimalis.

Dursun (2000) reported that the a. malaris originates from a. maxillaris, Dalga and Aslan (2016) reported that the mentioned vessel originates from a. infraorbitalis. Beki (2017) stated that the a. malaris originates from a. maxillaris with a. infraorbitalis as a single root in the Tuj sheep. In the Abaza and Gurcu goats, this situation is consistent with the Tuj sheep. In both goat breeds, it was observed that a. malaris is separated from a. maxillaris with a. infraorbitalis as a single root. In the terms included in Nomina Anatomica Veterinaria (2017), it was stated that the a. malaris and a. infraorbitalis are separately originated from a. maxillaris. Despite the literature information reporting that there is an anastomosis between the a. malaris and a. maxillaris in sheep, no such finding was encountered in our study.

As a result, in the Abaza and Gurcu goats, although the distribution of a. carotis externa in the head region of Abaza and Gurcu goats is generally similar to other small ruminants, some differences were determined in the origin points, courses and the number of sub-branches of the branches originating from a. carotis externa. Accordingly;

In 70% of the materials in Gurcu goats, a. lingualis dextra was observed to be disappeared after being originated from a. carotis externa and the right side of the tongue was fed by a strong branch coming from a. lingualis sinistra. In Nomina Anatomica Veterinaria (2017), it was stated that the a. transversa faciei and a. temporalis superficialis are separately branched from a. carotis externa. However, in our study, these vessels in both goat breeds were determined to be branched from a common root separated from dorsal face of the a. carotis extern. Despite the fact that Nomina Anatomica Veterinaria (2017) stated that the r. sternocleido mastoideus are separated from a. auricularis profunda, it was not found in either goat breed. In the Abaza goats, it was seen that 30% of the materials of a. auricularis rostralis was seperated from the common root of a. temporalis superficialis and a. transversa faciei. Unlike the vessel names specified for the goats in Nomina Anatomica Veterinaria (2017), it was determined that the a. malaris and a. infraorbitalis were seperated from a. maxillaris as a common root in both goat breeds. It is thought that our study will compensate the deficiency on this subject relatively and will be a reference in determining the head region vascular differences between species in future studies.

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Evaluation of kallistatin and some biochemical parameters in rats with experimental liver injury

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Research Article

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ABSTRACT

This study aims to determine the possible relationship between the levels of Kallistatin and the severity of liver injury in rats that were exposed to carbon tetrachloride (CCl₄). According to the results, serum ALT, AST, LDH, GGT and Collagen-I and Collagen-III levels in rats in the severe group were higher than control. Histopathological examination of rats in injury groups showed severe morphological changes that were resulted in cell dissociation and disruption of the liver lobe architecture in the liver parenchyma of rats that received CCl₄. Kallistatin serum level decreasing respectively in mild group (M1), moderate group (M2), and severe group (S) groups compared to the control group, and the lowest amount was belonging to the severe group. As a result, there was a reverse connection between Kallistatin serum level and the liver injury intensity. Serum kallistatin levels are an essential parameter in determining liver tissue damage levels, and measuring it may help provide a treatment prognosis.

Keywords: kallistatin, liver injury, CCl₄,

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Introduction

Liver is the largest organ in the body; this organ has more biochemical functions than other body organs, mainly consisted of hepatocytes, sinusoidal cells, and biliary epithelium (Vdoviaková, Petrovová, Maloveská, 2016; Kruepunga et al., 2019). The liver has specific functions, such as removing exogenous and endogenous toxins and synthesizing vital substances like blood clotting agents, albumin, and enzymes. It is also played a fundamental role in the metabolism of proteins, fats, carbohydrates, storage of vitamins and minerals, the process of conjugation and excretion of bilirubin in the bile, activation of glycogen and triglyceride, various hormones balance, and generating bile salts (Vdoviaková, Petrovová, Maloveská, 2016).

The complexity of liver dysfunction and the influence of many environmental factors on the

appearance of the disease and response to treatment indicate the need to study serum-based function to find markers that can be sensitive, accurate, diagnostic, and prognostic (Cheng et al., 2015). Elevated liver enzymes, a conjugated or separated changing of biochemical markers in patients with liver abnormalities, are a massive reason for debate among clinicians. These serum biochemical enzymes are considered as one of the most common laboratory challenges which can make a mistake during the identification of liver diseases (primary belonging to hepatic problems or related to some other parts of the body, secondary due to extrahepatic issues). For this reason, it is important to find a new diagnostic method to help to solve this problem; therefore, this study aims to explore changes in the level of blood Kallistatin

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(as a possible specific diagnostic biomarker which is mostly produced in the liver) and some biochemical liver indicators like blood serum of ALT, AST, the ratio of aminotransferases, alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH), collagen I (COL-I) and Collagen III (COL-III) in rat with experimental liver injury and to determine if serum kallistatin levels could be considered as a diagnostic indicator of healthy hepatic status or not (Cheng et al., 2015; Moreira et al., 2017). In some studies, it has been declared that the administration of kallistatin can decrease the progression of liver-related diseases (Cheng et al., 2015). Initially, based on other researchers, it was hypothesized that serum kallistatin levels could be a potential biomarker, and measuring its serum amount along with other liver parameters could be a useful approach for more accurate diagnosis of liver-related problems (Cheng et al., 2015; Li et al., 2015).

Results from the current study, both in human medicine and veterinary medicine, could be useful in gathering more information about introducing new biomarkers for liver-related problems. Besides, it may also help studies based on introducing new biomarkers as a clue for the diagnosis and treatment of clinical liver diseases and other investigations focused on kallistatin effects.

Materials and Methods

Animal material: As the animal material of the study, 32 Wister Albino male rats with live weights ranging from 200-400 gr were used in the Experimental Animal Breeding and Research Center of Van Yuzuncu Yil University. The Animal Ethics committee approved animal experiments. The rats cared for according to protocols approved by the Animal Care and Use Committee. 7 days before the investigation; all rats were housed and acclimated to the environment at 75±2% relative humidity in a temperature-controlled environment (20-22 °C) and with a standard light / dark cycle.

The rats were randomly divided into four groups, and each group consisted of eight male rats. The groups were named below: Control group (group C), which was healthy, and it was given a regular standard pellet diet. The mild group (group M1) was injected subcutaneously with 2 ml/kg of 25% CCl₄ in paraffin oil (Sigma Co., Milan, Italy) twice a week for four weeks. Moderate group (group M2), the same procedure as the mild group was applied to this group (injected subcutaneously with 2 ml/kg of 25% CCl₄ in paraffin oil twice a week for four weeks), and to make a moderate group, the procedure was followed by subcutaneous injection of 2 ml/kg of 50% CCl₄ in

paraffin oil for the two weeks (6 weeks in all). Severe group (group S), which CCl₄ was administered to this group in three stages with three different doses for a total of 8 weeks. Firstly, rats were subcutaneously applied twice a week for four weeks with a 25% CCl₄ solution dissolved in paraffin oil at a dose of 2 ml/kg (as to mild group). Secondly, 50% CCl₄ solution dissolved in paraffin oil was applied subcutaneously twice a week for two weeks at a dose of 2 ml/kg (as to moderate group), and finally, 62.5% CCl₄ solution dissolved in paraffin oil was applied subcutaneously at a dose of 2 ml/kg twice a week for two weeks (Li et al., 2016).

At the end of the study, all rats were anesthetized with the administration of a combination of xylazine and ketamine (60 mg / kg+7.5 mg/kg), and then were sacrificed on the same day. Blood samples were directly gathered into tubes and then centrifuged at 3000 g for 15 min at 4°C for serum preparation. Liver samples were prepared and washed with an ice-cold PBS to eliminate blood, and they were fixed in a 10% formalin solution for histopathological surveys.

Hematological analysis: Blood samples were taken from both posterior vena cava and direct heart puncture to analyze hematological and biochemical parameters by 25 gauge needle. Anticoagulant tubes with 3 ml EDTA were used for hematological examinations and jelly biochemical serum tubes for biochemical tests. Measurement of hematological parameters: Lymphocyte (Lym) and monocyte (Mon), neutrophil (Neu), hematocrit value (Hct), hemoglobin concentration (Hb), white blood cell (WBC) count, red blood cell (RBC) count, mean corpuscular volume (MCV), mean cell hemoglobin (MCH), mean platelet volume (MPV), plateletcrit (Pct), and mean corpuscular hemoglobin concentration (MCHC) were measured by veterinary hemogram device (Veterinary MS4-s-Melet Schloesing Laboratories in France).

Biochemical analysis: As mentioned, according to the method to be used in biochemical examinations, blood samples taken in anticoagulant-free tubes were centrifuged at 3000 rpm for 15 minutes. Then their serum was collected and stored at -20 °C for further analysis. ELISA (enzyme-linked immunosorbent assay) was done using a polyclonal antibody to rat kallistatin. ELISA techniques were implemented for analyzing rat serum kallistatin levels and measured by commercial ELISA kits (YL biont, Shanghai YL biotech company, China) (catalog No: YLA1624RA) due to the producer's instructions. As well Rat COL-I (YL biont) (catalog No: YLA0195RA) and Rat COL-III (YL biont) (catalog No: YLA0605RA) was measured due to the producer's instructions (Li et al., 2015). In short, the kallistatin sample and standards were added, in duplicate, into a

96-well microtiter plate previously coated with none labeled anti kallistatin IgG. All plates had incubated at 37°C for 90 min; then, the washing process had performed and then was followed by adding biotin labeled anti kallistatin IgG; the plate was incubated at 37°C for 60 min. Before substrate addition, Peroxidase avidin was added and incubated at 37°C for 60 min. The plate was read at 414 nm using an ELISA reader. After a 30 min color reaction. The same technique was applied for both COL-I and COL-III. All levels were measured using commercial ELISA test kits according to their instructions. Measurement of aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), Lactate dehydrogenase (LDH), and gamma-glutamyl transferase (GGT) levels, the blood serum was measured by commercial kits using auto-analyzer (BS-120 Vet-Mindray, Mindray Animal Care Co., Ltd., China)

Histopathological analysis: At the end of the experiment, the rats were necropsied following their euthanasia by anesthesia. Following the macroscopic examination, samples taken from the liver were detected in 10 % neutral formalin for histopathological examination. At the end of routine tissue follow-up, 4-5 microns thick sections taken from paraffin blocks were stained with hematoxylin-eosin and Masson trichrome. Semi-quantitatively; fatty, necrosis and fibrosis in hepatocytes in sections stained with hematoxylin-eosin were scored by two pathologists by taking the average value of each damage parameter by counting 10 and 20 lenses in 10 different areas in each slide.

Statistical analysis: Descriptive statistics were used for the properties of biochemical parameters of liver rats and healthy rats; Mean were expressed as Standard Deviation. The Biochemical parameters were compared between groups by Kruskal-Wallis test. Duncan multiple comparison test were used to Identifying different groups. To determine the relationship between these variables, Spearman correlation coefficients were calculated separately. Statistical significance levels were taken as 5% in the calculations and SPSS statistical package program were used for the calculations.

Results

Hematologic findings: In statistical analysis, WBC and Neu values of rats with mild, moderate, and severe liver damage were significantly higher ($P < 0.05$) compared to the control group. The Lym and Mon values of the rats with moderate liver damage were significantly higher ($P < 0.05$) than the Lym and Mon values of rats with control, mild and severe liver damage groups. While Hct values of rats with mild liver damage were significantly higher ($P < 0.05$) than

the Hct values of the control, moderate and severe liver damage groups, Hct and Hb values of the group with severe liver damage were significantly lower ($P < 0.01$) than the Hct and Hb values of the control, mild and moderate liver damage groups. MCV, MCH, and MCHC values of rats with severe liver damage were significantly lower ($P < 0.05$) compared to control rats with mild and moderate liver damage. Similarly, the MCH values of the rats with mild and moderate liver damage were significantly lower ($P < 0.05$) compared to the control group's MCH values. The Pct values of rats with moderate and severe liver damage were significantly higher ($P < 0.05$) compared to the Pct values of rats with mild and moderate liver damage (Table 1).

Table 1. Hematological parameters in control and liver damage of rat

Parameter	Control (Group1)	Mild liver disorder (Group 2)	Moderate liver disorder (Group 3)	Severe liver disorder (Group 4)
WBC ($10^3/mm^3$)	5.2 6 ± 1.8 ^a	13.4 ± 4.01 ^b	22.1 ± 11.07 ^b	7.2 0 ± 2.71 ^c
RBC ($10^6/mm^3$)	6.78 ± 0.47 ^a	7.87 ± 0.74 ^a	7.84 ± 1.15 ^a	6.35 ± 1.62 ^a
Neu ($10^3/mm^3$)	2.57 ± 0.91 ^a	9.12 ± 4.96 ^b	15.8 ± 6.52 ^b	4.44 ± 2.02 ^c
Lym ($10^3/mm^3$)	2.35 ± 0.85 ^a	3.59 ± 1.84 ^a	5.78 ± 6.64 ^b	2.25 ± 0.73 ^a
Mon ($10^3/mm^3$)	0.26 ± 0.10 ^a	0.47 ± 0.14 ^b	0.74 ± 0.23 ^b	0.28 ± 0.14 ^a
Hct (%)	39.0 ± 2.54 ^a	43.8 ± 3.81 ^b	40.9 ± 3.49 ^a	35.8 ± 9.35 ^b
Hb (g/dl)	16.7 ± 0.53 ^a	17.5 ± 1.75 ^a	15.2 ± 1.38 ^a	12.9 ± 3.70 ^b
MCV (fl)	57.6 ± 1.41 ^a	55.6 ± 1.18 ^a	55.5 ± 3.22 ^a	52.8 ± 6.09 ^c
MCH (fl)	24.7 ± 1.48 ^a	22.2 ± 0.94 ^b	20.7 ± 1.06 ^b	18.6 ± 2.30 ^c
MCHC (g/dl)	43.0 ± 2.19 ^a	40.03 ± 2.00 ^a	37.1 ± 1.66 ^c	35.4 ± 2.43 ^c
Pct (%)	0.31 ± 0.05 ^a	0.30 ± 0.14 ^a	0.52 ± 0.30 ^b	0.50 ± 0.20 ^b

Data are expressed as mean ± standard deviation. N number is 8 in each group. a, b, c: Different lower cases in the same column represent statistically significant differences ab, ac, bc: $p < 0.01$ or $p < 0.05$, and same lower cases in the same column represent statistically not substantial differences. aa, bb, and cc: $P > 0.05$. WBC = White blood cell, RBC = Red blood cell, Neu = Neutrophil, Lym = Lymphocyte, Mon = Monocyte, HCT = Hematocrit, Hb = Hemoglobin, MCV = Mean cell volume, MCH = Mean corpuscular hemoglobin, MCHC = Mean corpuscular hemoglobin concentration, Pct = Plateletcrit

Biochemical findings: In the statistical analysis of biochemical parameters, serum ALT, AST, LDH, and GGT levels in rats with severe liver injury were significantly higher than those of the control group and rats with mild and moderate liver injury. ALT and GGT levels did not show any significant difference between rats belonging to control, mild, and moderate liver injury groups. LDH levels in rats with mild and moderate liver injury groups were higher than the control group.

Table 2. Biochemical parameters in control and rat with liver injury

Parameter	Control (Group1)	Mild liver disorder (Group 2)	Moderate liver disorder (Group 3)	Severe liver disorder (Group 4)
ALT (IU/L)	30.8 ± 4.2 ^a	104.9 ± 45.6 ^a	261.6 ± 72.1 ^a	1076.46 ± 864.4 ^b
AST (IU/L)	96.07 ± 33.2 ^a	213.6 ± 75.1 ^a	448.8 ± 84.04 ^b	968.86 ± 218.1 ^c
LDH (IU/L)	414.1 ± 181.3 ^a	804.5 ± 82.1 ^b	1077.2 ± 191.9 ^c	2090.8 ± 679.7 ^d
GGT (IU/L)	1.37 ± 0.51 ^a	2.00 ± 0.7 ^a	2.12 ± 0.99 ^a	4.00 ± 2.07 ^b
Col-I (ng/l)	218.1 ± 31.4 ^a	339.3 ± 18.5 ^b	380.8 ± 17.9 ^c	488.8 ± 30.4 ^d
Col-III (ng/l)	7.96 ± 1.7 ^a	6.48 ± 0.18 ^b	5.91 ± 0.24 ^b	5.12 ± 0.01 ^c

Data are expressed as mean ± standard deviation. N number is 8 in each group. a, b, c, d: Different lower cases in the same column represent statistically significant differences ab, ac, ad, bc, bd, and cd: p<0.01 or p<0.001 and same lower cases in the same column represent statistically not significant differences. aa, bb, and cc: p>0.05. ALT: alanine aminotransferase, AST: Aspartate aminotransferase, LDH: Lactase dehydrogenase, GGT: Gamma-glutamyl transferase, Col-I: Type 1 collagen, Col-III: Type III collagen,

rats belonging to liver injury groups was significantly higher than the control group. Serum kallistatin levels were lower in all groups with liver damage compared to the control group. Comparison between groups with liver injury showed that although there was a statistically significant difference between kallistatin levels in the mild and severe groups, there was no significant difference between the mild and moderate groups and the moderate and severe groups (Table 2).

Histopathological findings: In histopathological examination of tissue sections prepared from the livers of rats; in the control group, normal histological appearances of the portal regions and remark cords were recorded (Figure 1A). In CCL₄ applied groups, macro and microvesicular oil vacuoles (arrows and *) of different sizes in hepatocytes (oil degeneration), especially around the vena centralis, were noted in large areas in the parenchyma (Figures 1 B, C, D). In some of these hepatocytes, the core was pushed aside and was pycnotic (necrosis). Also, necrotic hepatocytes with basophilic pycnotic core and dark eosinophilic cytoplasm (arrowheads) were seen in the parenchyma (coagulation necrosis), they were particularly more common around the vena centralis. Mononuclear cell infiltrates in the parenchyma and in the portal spaces and an increase in the number of perisinusoidal cells were noted. Fibrosis was particularly observed in the portal regions and around the vena centralis. In some regions, fibrous bridgings was detected between the portal regions, and extending from the portal regions to the vena centralis. As a result of all these lesions in the liver parenchyma, the remark cord structure of hepatocytes was distorted and the sinusoids were narrowed (dissociation) in proportion to the extent and severity of the lesions. As a result of severe vacuolar degeneration, bile pigment accumulation was observed in some hepatocytes (intrahepatic cholestasis) due to obstruction of bile flow. Similar morphological changes were observed in "Mild (M1),

Moderate (M2) and Severe (S)" application groups, although different in the prevalence and severity of lesions. Scoring of the basic morphological changes (degeneration, necrosis and fibrosis) observed in the livers of the groups are shown in Table 3.

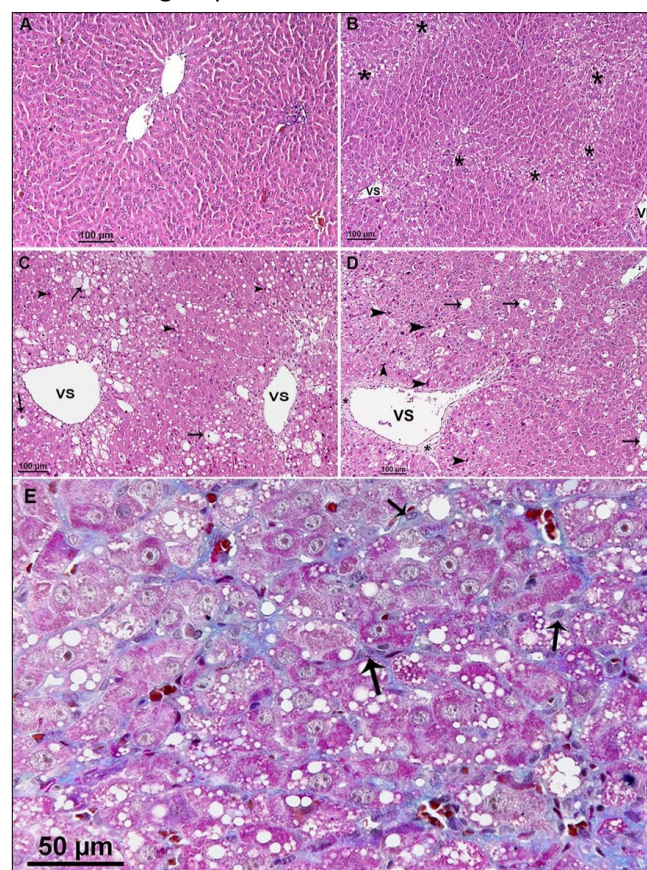


Figure 1. (A) Control group: Normal histological appearance of the liver. (B) M1 group: Mild vacuolar fat degeneration (*) more prominent around the vena centralis (VS). (C) M2 group: showing widespread macro and microvesicular fat vacuoles (arrows) of different sizes, and sporadic necrotic hepatocytes (arrowheads) in hepatocytes around the vena centralis (VS) and midzonal regions. (D) S group: Fibrosis (*) around the vena centralis (VS), necrotic changes in hepatocytes (arrowheads), diffuse macro and microvesicular oil vacuoles (arrows). H.E., Bar; 100. (E) Increase in perisinusoidal cells (arrows) and bluish colored collagen accumulation (fibrosis) in Disse intervals. Masson trichrome staining, Bar; 50.

Table 3. Effects of CCl₄ on the liver tissue of rats

Changes/ Lesions In liver	Groups							
	Control	Mild		Moderate		Severe		
Degeneration		8/6	+	8/2	++++	8/5	++++	
	8/8	0	8/2	++	8/4	++	8/2	++
				8/2	+	8/1	++	
Necrosis		8/6	+	8/2	+++	8/5	+++	
	8/8	0	8/2	++	8/4	++	8/2	++++
				8/2	+	8/1	++	
Fibrosis		8/6	0	8/2	+++	8/5	++++	
	8/8	0	8/2	+	8/4	++	8/2	+++
				8/2	+++	8/1	++	

Control group, which was healthy, and it was given a regular standard pellet diet. The Mild liver injury (group 2) was injected subcutaneously with 2 ml/kg of 25% CCl₄ in paraffin oil twice a week for four weeks. Moderate liver injury (group 3), the same procedure as the mild group was applied to this group (injected subcutaneously with 2 ml/kg of 25% CCl₄ in paraffin oil twice a week for four weeks). And to make a moderate group, the procedure was followed by subcutaneous injection of 2 ml/kg of 50% CCl₄ in paraffin oil for the two weeks (6 weeks in all). Severe liver injury (group 4), which CCl₄ was administered to this group in three stages with three different doses for a total of 8 weeks.

Discussion

Today, liver diseases are one of the most common diseases globally. Hence, to assess the success and achieves early diagnosis and prognosis of the treatment, alternative diagnostic methods and additional parameters are necessary. According to this, in addition to the methods and parameters used in the diagnosis and prognosis of hepatic problems, this study was objective to disclose the kallistatin serum levels alterations in rats with different degrees of liver injury. Carbon tetrachloride is one of the most common hepatotoxin drugs used in experimental hepatopathy studies (Eidi et al., 2015). This drug has been widely used in rodents as a model for studying and monitoring hepatic injury mechanisms (Raafat et al., 2015). In the present study, when hematological, biochemical, and histopathological findings of rats-induced by different doses of CCl₄ and different liver damage were evaluated, it was found that CCl₄ is a good hepatotoxic agent for liver injury as reported by other researchers (Eidi et al., 2015; Raafat et al., 2015). It has been reported (Nwidu et al., 2018) that

in rats induced with CCl₄, RBC and Hb concentrations were decreased, and microcytic hypochromic anemia was observed. Another study stated that using CCl₄ induces leukocytosis (Asmaa, Al-Diwan, AL-Jadaan, 2018; Nwidu et al., 2018). In one research based on rats where CCl₄ was used orally, RBC, Hb, and PCV values were reported to be decreased, and all hematological parameters were affected (Mandal et al., 1998). The connection between liver-related problems and serum WBC counts has been identified in some studies. Studies have been indicated that WBC counts are related to the risk of hepatic diseases occurring. Therefore, counting of serum WBC could be useful in the process of detecting liver-related diseases (Chung et al., 2016; Jie et al., 2018). Decrease the ratio of Platelet to White Blood Cells may be an indicator of the progression of the liver disease to a worse condition, which is due to the body's comprehensive response to infection (Jie et al., 2018). Results obtained from the present study, according to Table 1, revealed that results came in line with other studies related to this topic (Chung et al., 2016; Jie et al., 2018).

Serum enzymes like ALT, AST, LDH, and ALP are frequently used parameters to examine liver dysfunction (Rahmouni et al., 2017). ALT and AST are now widely used as reliable biomarkers for the diagnosis of CCl₄-induced liver cell damage, in addition to other biochemical enzymes known as intracellular biomarkers, which are found in the bloodstream after cell membrane injury (McGill, 2016). Serum LDH was another biomarker that is not reputable to detect liver diseases; however, it could be remarkably increased due to ischemic problems, hepatic disorders related to hemolysis, and some tumors (Gitlin, Serio, 1992). The current study found that blood serum enzyme concentrations of ALT, AST, LDH, and GGT of rats with mild, moderate, and severe liver injury were higher than the control group. Also, there was a positive correlation between the increase in enzyme activity concentrations and liver damage. The highest increase in enzyme activities was detected in rats belonging to the severe group. Findings of serum enzyme activities in the current study are in line with other researchers' results and support their data (Recknagel et al., 1989; Gitlin, Serio, 1992; Althnaian, Albokhadaim, El-Bahr, 2013; McGill, 2016).

Histological survey of liver tissues has shown that CCl₄ induces fibrosis, cirrhosis, and hepatocarcinoma (Althnaian, Albokhadaim, El-Bahr, 2013). Studies show that CCl₄ brings about an alteration in lipid together with the raise of the inflammation complex, occurring fiber parts and collagen accumulation, and also

increase the loss of liver cells (Dong et al., 2015). Chronic hepatic injury induced by CCl₄ in rats develops hepatic fibrosis and histological and biochemical be similar to hepatic cirrhosis in both human and animals. Therefore, liver cirrhosis development in the rat model has been useful for conducting research based on liver function in human and veterinary medicine (Yanguas et al., 2016).

In the liver of CCl₄ applied rats, it was determined that degenerations in the group M1 were limited in centrilobular regions, no large macrovesicles were formed, coagulation necrosis in hepatocytes was very little, and fibrosis did not occur in most of the rats due to the low parenchymal damage. It was noted that degenerations in the group M2 also included the midzonal regions, severe and widespread macro and micro vesicular fat vacuoles were formed in hepatocytes, and hepatocellular necrosis and fibrosis were observed in most rats. It was noted that the morphological changes in the group S were similar to those in the group M2, but inflammatory, necrotic and fibrotic changes were more prominent. Differences in morphological changes between groups in rats; It is understood to be related to the density of CCl₄ applied. These results were found to be similar to those of many researchers (Abdelghany et al., 2016). Extracellular matrix consisting of connective tissue and interstitial collagen in normal liver; it is located in the portal spaces, around the central veins, and in the capsular regions. In addition, there is a thin roof consisting of extracellular matrix in which there are myofibroblastic stellate cells in the Disse gap between hepatocytes and sinusoidal endothelial cells. In cases of fibrosis and cirrhosis, primarily, excessive amounts of collagen are produced in perisinusoidal stellate cells in the Disse range. These cells, where vitamin A is normally stored, are activated during the development of fibrosis and turn into myofibroblasts. It is believed that cytokines such as TNF, IL-1 and lymphotoxins and reactive oxygen derivatives, which are thought to be produced by injured hepatocytes or by stimulated Kupffer cells and sinusoidal endothelial cells, play an important role in this transformation (Kocabayoglu, Friedman, 2013).

There are many studies on the effect of CCl₄ on the making and progress of liver damage (Cheng et al., 2015; Idu, Ovuakporie-Uvo, Okojie, 2017). In this study, it was found that CCl₄-applied led to liver damage and progressed it to fibrosis (Figure1; Table 3), hence finding of this study comes in line with previous studies which was mentioned (Cheng et al., 2015; Idu, Ovuakporie-Uvo, Okojie, 2017). Liver fibrosis is an expansion changing that initially includes

Collagen's raised deposition in the liver (Rojkind, Perez-Tamayo, 1983; Ala-Kokko et al., 1987). It has been known that Collagen forms approximately 1 mg/g of fresh rat liver tissue (80% belonging to I and III collagens, and the rest 20% belonging to types IV and V altogether) (Ala-Kokko et al., 1987). In this study, it was found that the amount of Collagen I increased in mild, moderate, and severe groups (9.36 ± 0.35 ng/L, 10.75 ± 0.68 ng/L, and 13.3 ± 0.78 ng/L), respectively compared to the control group ($P < 0.001$) (Table 2). While these findings indicate significant differences between all groups, it states that the highest serum level belongs to the severe group. There was a considerable difference between the control and liver injury groups and within the liver injury groups themselves. Also, it was shown that the concentration serum level of Collagen III increased in mild, moderate, and severe groups (respectively 339.3 ± 18.5 ng/L, 380.8 ± 17.9 ng/L, and 488.8 ± 30.4 ng/L) compared to the control group ($P < 0.001$). The highest amount was found to belong to the severe group (Table 2). There was a significant difference between the control group and the liver injury groups and between the liver injury groups. The current study results are consistent with previous studies mentioned (Rojkind, Perez-Tamayo, 1983; Ala-Kokko et al., 1987).

According to some articles based on research in humans and rats, it has been proven that there is a decrease in the serum level of kallistatin in liver fibrosis and liver cirrhosis (Cheng et al., 2015). In this study, serum kallistatin level in the control group was (7.96 ± 1.76 ng/ml). Compared to the control group, it was observed to decrease in the mild, moderate and severe groups (6.48 ± 0.18 ng / ml, 5.91 ± 0.024 ng/ml, and 5.12 ± 0.01 ng/ml), respectively ($P < 0.001$). Besides, the lowest amount was found to belong to the severe group. According to data, there was a significant difference between the control group and groups exposed to liver injury, and also, there was a significant difference between mild and severe groups. However, there was no difference between the mild and moderate groups, as well as between the moderate and severe groups. This data is in agreement with previous studies which was mentioned (Cheng et al., 2015; li et al., 2015; Chao, Bledsoe, Chao, 2016). In this study, it was determined that there was a negative relationship between kallistatin and other biochemical parameters; in other words, these biochemical serum parameters increased with the decrease in kallistatin serum level (Table 2). Histopathology examination of liver tissue showed no histological changes in rats belonging to the control

group. The results of this study showed that the bile duct, parenchymal cells, and Kupffer cells were normal. Hepatocyte necrosis, inflammatory cell infiltration, and bleeding foci were detected in the CCl₄-induced liver injury groups. Histological findings, changes in serum biochemical, and hematological parameters of rats with the moderate and severe liver injury caused by CCl₄ were evidence of severe cell damage in liver tissue. The histopathological results of this study support the data of other investigators (Thanh et al., 2015).

To the best of our knowledge, this is the first study to show that the measurement of serum kallistatin concentration is a potentially useful marker in rats with liver injury induced by different CCl₄ doses. This study demonstrates that serum kallistatin amounts are markedly lower in rats belonging to study groups (various degrees of liver injury caused by CCl₄) than healthy (control) group. It also indicates that as the intensity of hepatic injury develops, the serum kallistatin levels decrease. This study's importance is to evaluate the level changes of serum kallistatin and other biochemical parameters in rats with varying degrees of liver injury. This study aimed to evaluate the level of serum kallistatin and other biochemical parameters by inducing varying degrees of liver damage in rats by CCl₄. Despite the liver, damage to other organs such as the kidneys and lungs can also cause changes in kallistatin serum level. The protective role of kallistatin has been described in many studies (Chao, Bledsoe, Chao, 2016; Chao, Li, Chao, 2017; Wang et al., 2020). Administration of kallistatin has been shown to reduce liver disease progression (Cheng et al., 2015). According to the present study results, it is thought that measuring serum kallistatin level and other parameters could be useful in evaluating and interpreting the response to treatment in liver-related disorders. It can also be considered as a potential biomarker to detect the progression of liver injuries. Moreover, kallistatin may be promising as a candidate drug for the prevention and treatment of liver disease and may also be a treatment of other inflammatory organs discussed previously, and may add clues for kallistatin therapy/prevention approaches.

Conclusions

As a result, in this study, it was found that decreased serum kallistatin levels were associated with liver injury. This finding can lead us to introduce kallistatin as a potential biomarker that can help for more accurate detection besides other diagnostic methods of liver disorders. As discussed earlier, kallistatin may aid early diagnosis and be considered a promising

biomarker for liver disease, but more research is needed on this topic.

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Serous multicystic papillary adenoma of the ovaries and glandular cystic hyperplasia of uterus in a Guinea Pig (*Cavia porcellus*)

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Case report

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ABSTRACT

Cystic changes of ovarian tissue are crucial as they cause infertility in laboratory animals. A 4 years old, weighing approximately 500 g adult, intact guinea pig had pruritic progressive alopecia over the flanks at the dorsal area and abdomen symmetrically, bilaterally for a month. Beside, an abdominal enlargement was observed outside on the guinea pig. Large polycystic ovaries were detected behind the kidneys during ultrasonography. Also abdominal radiography revealed the cystic structures at the same region. Ovariohysterectomy was performed on the animal, multiple thin walled, fluctuant fluid-filled, large cysts measured as 3.9 x 2.5 and 4.5 x 2.8 cm diameter were detected at the left and right ovary, respectively. The uterine body was observed normal macroscopically. The guinea pig was died after the operation during reanimation because of the hypothermia. The organ pieces were submitted to pathology for histopathological examination after the operation. Histologic examination demonstrated a diagnosis of serous multicystic papillary adenoma of the ovaries and glandular cystic hyperplasia of uterus. In conclusion, this case report describes the necessity of not to ignore the ovarian pathologies with guinea pigs demonstrate symmetric, bilateral alopecia on the skin and indicates the importance of postoperative care in laboratory animals despite a successful anesthetic choice and operation.

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Introduction

Cystic changes of ovarian tissue are crucial as they cause infertility in laboratory animals (Keller et al., 1987; Eden and Warren, 1999). Ovarian and uterine diseases are very frequent in intact guinea pig females with an incidence of >75% (Capello, 2006). Polycystic ovarian syndrome (PCOS) is a disorder related with the hormonal variations in LH: FSH ratios, intraovarian concentrations of androgen to estrogen or follicular atresia (Barnes and Rosenfield, 1989). Ovarian cysts

are usually organized from ovarian follicles, corpora lutea, ovarian surface epithelium, remnants of the mesonephric and paramesonephric ducts, or rete ovarii (Young et al., 1938; Nalbandov, 1952; Sauramo, 1954). There are 3 types of ovarian cysts seen in guinea pigs differentiated only by histopathology; 1) Serous cysts (cystic rete ovarii), 2) Follicular cysts, 3) Parovarian cysts. The response to medical therapy is important in these type of cysts (Pilny, 2014). Keller et

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al. (1987) reported various cystic structures on the ovaries between 0.5-7 cm diameter in guinea pigs in their study.

The most common detected cysts are serous cysts (cystic rete ovarii), which remain present throughout the estrus cycle of guinea pigs. Serous cysts are composed of a simple cuboidal-to-columnar epithelium composed of cells with solitary cilia or tufts of cilia. These cysts are incapable of steroidogenesis and do not respond to surges of luteinizing hormones (LHs) similar to follicular cysts (Pilny, 2014). Keller et al. (1987) reported cystic endometrial hyperplasia, mucometra, or endometritis in appropriate placental tissue, or leiomyofibroma in 21/54 guinea pigs with cystic ovaries, but in only 1/17 guinea pigs without cystic ovaries, these cysts were determined as cystic rete ovarii histopathologically. Follicular cysts are the second most observed cysts and are derived from preovulatory follicles that fail to ovulate. These structures reach ovulatory size, fails to ovulate, and alters normal ovarian cyclicity. The wall of these cysts is lined by granulosa cells in histopathological examinations (Pilny, 2014). Parovarian cysts are the rarest type of the cysts seen in the guinea pigs and are cysts of the parovarium. They are vestigial structures that is associated with the ovary and consists of

mesonephric tubules and a portion of the mesonephric duct (Pilny, 2014). The aim of the present case report is to suggest the importance of the ovarian pathologies in guinea pigs demonstrate symmetric, bilateral alopecia on the skin and the postoperative care in laboratory animals despite a successful anesthetic choice and operation.

Case History

A 4 years old, weighing approximately 500 g adult, intact Guinea pig was presented to Department of Obstetrics and Gynecology clinic. The animal was housed in a stainless steel cage and was fed with pellet feed and grass, ad libitum water. Some nutritious and beneficial dietary sources for vitamin C such as; tomatoes, broccoli, kiwi, spinach, etc. were supplemented to the cavy's diet by the owner daily. The guinea pig had pruritic progressive alopecia over the flanks at the dorsal area and abdomen symmetrically and bilaterally for a month (Figure 1A). The owner complained about the anorexia. Because the patient demonstrated abdominal enlargement bilaterally at the caudal region of the kidneys, ultrasonography was performed. Large polycystic ovaries (Figure 1B) were detected behind the kidneys during ultrasonography (Easote MyLab Five Vet,

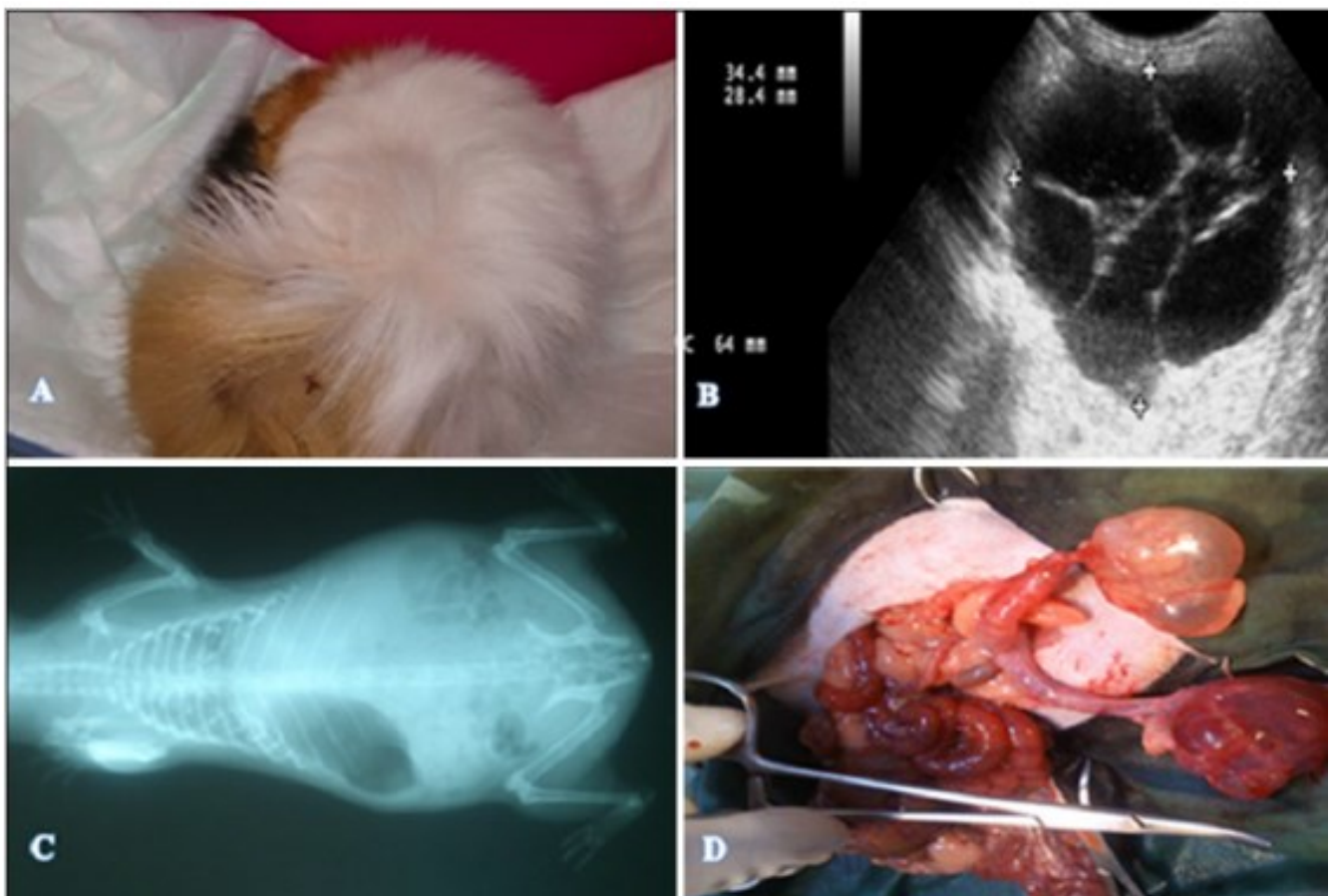


Figure 1. Symmetric, bilateral and pruritic alopecia on the skin (A), Polycystic right ovary of a guinea pig, ultrasonographically (B), Radiographic findings of the cysts (C), Cystic structures after the operation of the ovaries (D).

Milano, Italy). Also abdominal radiography revealed the cystic structures at the same region (Figure 1C). Ovariohysterectomy was recommended immediately. During the surgery procedure, the guinea pig was warmed with heating thermophores. The anesthesia was conducted with 50 mg/kg ketamine hydrochloride (Ketalar, Eczacıbaşı, Turkey) and 7.5 mg/kg xylazine hydrochloride (Rompun, Bayer, Germany) intraperitoneally on the animal. Median line through linea alba was preferred for the surgery. There were so many adhesions and fluid accumulation in the abdominal cavity due to the mass. The surgery was carried out very carefully, both of the ovaries contained multiple thin walled, fluctuant fluid filled, large cysts which were measured as 3.9 x 2.5 cm and 4.5 x 2.8 cm diameter at the left and right ovary (Figure 1D), after the surgery respectively, the uterine body was observed normal macroscopically. They were removed according to the three clamp procedure successfully, the abdominal cavity was closed with interrupted 3/0 absorbable suture material and the subcutaneous layer was closed with 4/0 absorbable suture material, the skin was closed with 3/0 non-absorbable suture material. The

operation was completed in 30 minutes totally. Although the guinea pig was in the intensive care stall, she died after the operation during reanimation because of the hypothermia. The organ pieces were submitted to pathology for histopathological examination after the operation. The tissue samples were fixed in 10% buffered formalin, embedded in paraffin wax and sectioned at 2-3µm, stained with hematoxylin and eosin (HE) for histopathological evaluations.

Macroscopical and histological findings: On macroscopical examination, there were numerous cysts in varying sizes on the ovaries. Microscopic examination showed multiple serous cysts of varying sizes lined by a simple cuboidal-to-columnar epithelium with prominent papillary projections in some areas (Figure 2A and B). Ovarian tissue was compressed by the cyst formations (Figure 2C). In uterus, marked multifocal cystic degeneration of the mucosal glands and hyperplastic changes were seen in the endometrium (Figure 2D). Histologic examination demonstrated a diagnosis of serous multicystic papillary adenoma of the ovaries and glandular cystic hyperplasia of uterus.

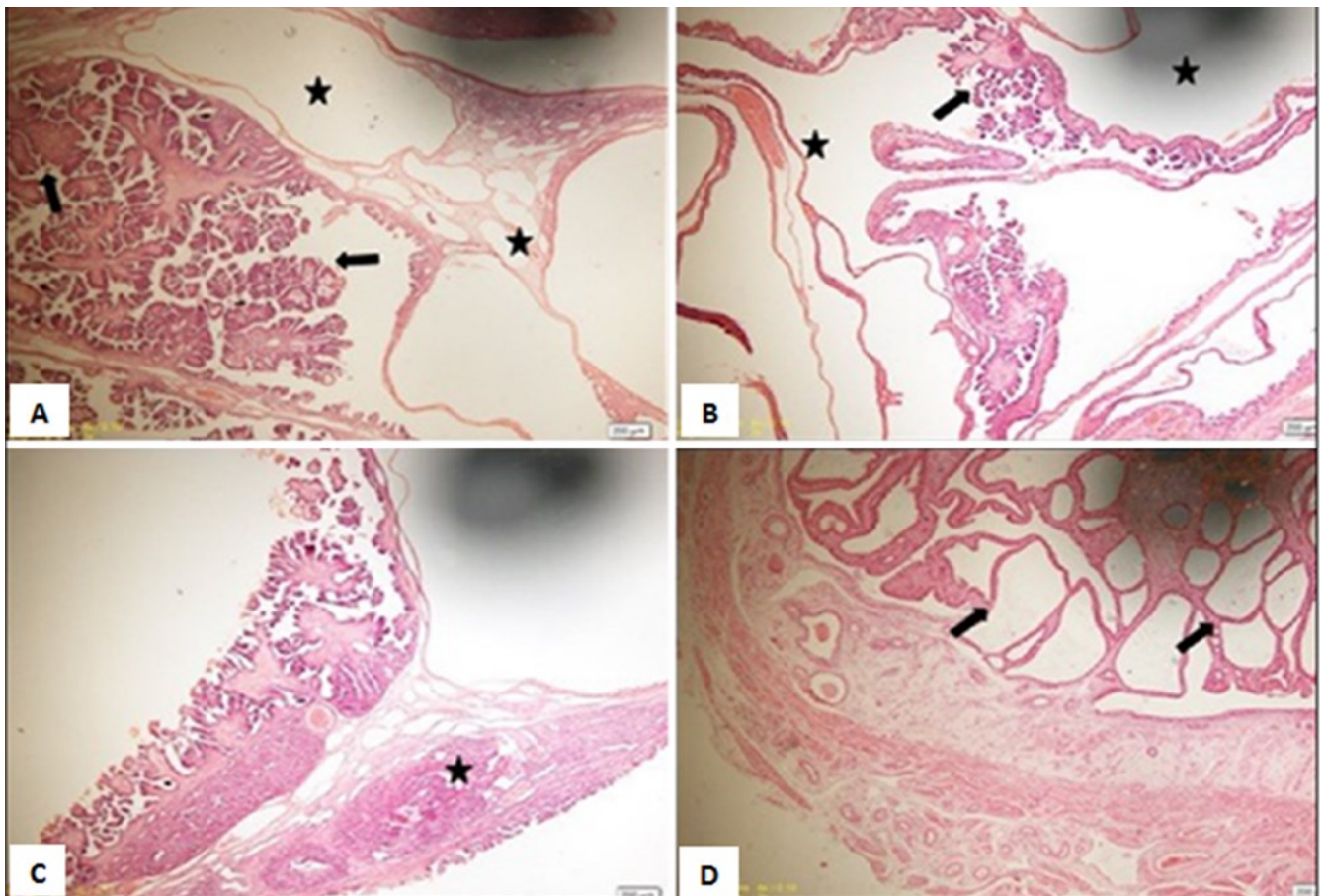


Figure 2. A and B: Cysts in varying sizes (stars), papillary projections (arrows), ovary (H&E), C: Compressed ovarian tissue (star), ovary (H&E), D: Cystic glands in endometrium (arrows), uterus (H&E).

Discussion and conclusion

Although cystic ovaries are common in guinea pigs, there are some reports about their treatment approaches. Quandt and Hutz (1993) treated the guinea pigs with cystic ovaries by estradiol 17- β (E2) for a duration of 48 hour and detected the induction of the cysts in all animals treated with this regimen in their study. They also has detected that exogenous E2 initiates an atresia-like process in the dominant follicle (Dierschke et al., 1985), and they reported that this increased incidence of atresia could reduce the adequate and healthy preovulatory follicles and this might lead to cystic degeneration again. In this case we did not use any hormonal compounds to treat the cystic ovaries because of the recurrence risk of the lesions. Keller et al. (1987) reported cystic ovaries in 76% (71/54) cases diagnosed during necropsy in guinea pigs. They reported that small sized cysts were noticed incidentally but larger cysts could be distinguished as an abdominal mass by palpation, like in this case report which the diagnose was supported by ultrasonography and radiography. Paterson (2006) reported the high incidence risk of cysts between 2-4 years in the guinea pigs, the animal in this case was 4 years old. Also the same researcher, Paterson (2006), implicated the reason for the cystic ovarian disease could be attributed to the estrogenic substances in the hay. We are not sure about the existence reason of these cystic structures on the ovaries might be related with the estrogenic effect caused by the possible food intake, further examinations are required to support this hypothesis. Ketamine/xylazine combinations are the most commonly used injectable combinations for anesthesia of mice, rat, hamsters, guinea pigs (Van Pelt, 1977; Green et al., 1981; Branson, 2001). Dang et al. (2008), have compared the anesthesia protocols in their study such as; 30 mg/kg ketamine and 2.5 mg/kg xylazine subcutaneously, intramuscularly or intraperitoneally, 37 mg/kg sodium pentobarbital intraperitoneally and 0.5 mg/kg medetomidine intramuscularly. They recommended intraperitoneal 30 mg/kg ketamine and 2.5 mg/kg xylazine regimen as the best choice for time to recovery after the operation but they used the anesthesia for blood collection from anterior vena cava and they did not require long time for this procedure. Green et al. (1981) reported ketamine-xylazine combination in a single dose im injection at 60 mg/kg and 8 mg/kg, respectively in guinea pigs, and they developed maximum muscular relaxation. But they reported the existence of pain and corneal reflexes still. Gaertner et al. (1997) recommended

ketamine/xylazine mixture given by intraperitoneal (IP) route to avoid muscle necrosis that may occur given intramuscularly. Muscle necrosis is most likely due to the pH of 3 of the ketamine component. Brodbelt et al. (2008) reported the risk of animals dying because of anesthetics as 1/26 (3.8%) in guinea pigs. In present case we performed 50 mg/kg ketamine and 7.5 mg/kg xylazine intraperitoneally and there was no problem observed associated with the anesthesia and analgesia during the operation period. Olson and Bruce (1986) reported that rodents and rabbits could become hypothermic easily after the operations so heating pads, waterbottles or isothermic pads were all recommended by them for to avoid hypothermia but although the guinea pig was warmed with heating thermophores during the operation and was transferred to the intensive care stall in order to increase the body temperature after the operation in this case, we could not able to recover her and she died because of the hypothermia.

In conclusion, this case report describes the necessity of not to ignore the ovarian pathologies with guinea pigs demonstrate symmetric, bilateral alopecia on the skin and indicates the importance of postoperative care in laboratory animals despite a successful anesthetic choice and operation.

Conflict of interest

The authors have no conflicts of interest to declare.

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University students' perception of animal welfare and opinions on consumption of milk and dairy products

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Research Article

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ABSTRACT

The aim of this study is to determine the opinions of university students on the consumption of milk and dairy products within the scope of animal welfare. The research was carried out with 361 randomly selected students from Samsun Ondokuz Mayıs University, Turkey, using a face-to-face questionnaire. In the study, the rate of students who think that, they do not have information about the presence and amount of cholesterol in milk and dairy products was 77.35%, milk and dairy products contain all the nutritional elements was 35.62%, UHT milk is not spoiled for a long time due to heat treatment was 28.90%, milk, cheese, yoghurt prices are normal were 63.34%, 40.41%, 68.31%, respectively, there was a welfare problem in farm animals was 56.00%. Also, the rate of students who want label information about milk and dairy products produced from animals raised under welfare conditions was 75.43%. The rates of students who can pay up to 10%, 11-25%, and more than 25% for milk and dairy products because they are suitable for animal welfare were determined as 64.74%, 26.14% and 9.12%, respectively. As a result, it was determined that university students' perceptions of animal welfare and their views on milk and dairy products consumption were generally at an acceptable level. However, more information and necessary training support should be provided to bring them to the desired level.

Keywords: animal welfare, dairy product, milk consumption, student perception.

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Introduction

Milk and dairy products have been widely consumed around the world for thousands of years and serve as an important food source. The proteins, fats, carbohydrates, vitamins and minerals in its content serve as a healthy complete food by containing all important nutrients (Vanga and Raghavan, 2018). Because of the potential of probiotic bacteria to colonize the intestinal tract and affect health, consuming fermented dairy products is beneficial for health (Kok and Hutkins, 2018). However, it is also

extremely important that they are obtained from husbandry practices where basic animal welfare conditions are met. It is a particularly important aspect, because animal welfare is an important element of sustainable development, including food consumption and human diet and can positively contribute to food quality (Sajdakowska et al., 2020).

During more recent years, the society's awareness of animal welfare and farming issues has grown. Improvements in farm animal welfare are ultimately

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grounded in society, and as a consequence the literature on animal welfare has paid substantial attention to the views of citizens on animal welfare (Ingenbleek et al., 2013). In a study it was determined that Brazilian citizens had higher perception of animal welfare and sentience than French citizens. Concerning the Brazilian respondents, ordinary citizens and biologists seemed to have similar perceptions of animal welfare and emotions. In addition, the results show a relationship between the perception of animal welfare and sentience with gender and age, as women and older respondents tended to show higher concerns about animal welfare issues (Tamioso et al., 2018).

In a study conducted by Köseman and Şeker (2019), it was reported that despite the positive developments in recent years, farms in Turkey cannot be said to have optimal fattening conditions that take into account animal welfare in general. In their study, Harvey and Hubbard (2013) reported that consumers should purchase animal-friendly products in order to increase production suitable for animal welfare. Frey and Pirscher (2018) reported that there is a significant positive relationship between the willingness to pay extra for food to increase the welfare of farm animals and consumers' environmental awareness, dedication and interest in animal welfare.

It is known that education and income level are effective in food preferences (Erdoğan and Çiçek, 2015). In a study conducted in China, it was determined that insufficient milk intake is a worrying nutritional problem and income level is effective in this (Zhao et al., 2017). In another study, it was reported that having health awareness has an important effect on regular milk consumption, and education has an important place in food preferences (Hoque et al., 2018). Among animal foods, those produced in accordance with welfare conditions are more preferred (Sajdakowska et al., 2020). In another study which was conducted in 11 universities, the relationship between animal welfare and gender was revealed. Accordingly, in countries where females were more empowered, principally Sweden, Norway and Great Britain, females had much greater concern than males for animal welfare, whereas in other countries the responses of males and females were more similar (Phillips and McCulloch, 2005; Phillips et al., 2011).

By improving public knowledge, awareness and understanding of animal welfare in food production, we can elevate knowledge to align with current societal concerns, thus redefining socially acceptable methods of food production and improving the lives of billions of animals that are farmed for food annually (Cornish et al., 2016). The aim of this study was to determine the animal welfare perception on milk and dairy products consumption in the context of

university students.

The present study was conducted to the Ondokuz Mayıs University (OMU) in Turkey. The OMU is a large state university established in 1975 in Samsun province in Turkey. OMU is in the 1001+ group of The Times Higher Education (THE) 2020 World Universities ranking. In the 2019-2020 academic years, OMU had about 30000 students (Anonymous, 2020).

Materials and Method

The material of this research was composed of the data obtained from the questionnaire conducted by face-to-face interviews with 379 students at OMU between 01 March 2019 and 31 May 2019. However, some incorrect questionnaires were excluded from the study and data from a total of 361 answers were included in the study. The questionnaire was applied to students who were chosen by random sampling method and volunteered for participation. The questionnaire was prepared based on previous work by Şeker et al. 2012.

The sample size formula (Üstün 2016):

$$n = \frac{N t^2 p q}{d^2 (N - 1) + t^2 p q}$$

In the formula; N = Number of individuals in the population, n = The number of individuals to be sampled, p = Frequency (probability) of the event to be examined, q = frequency of absence of the event to be examined (1-p), t = The theoretical value found in the t table at certain degrees of freedom and detected error level. d = It is represented as the \pm deviation required to be made according to the frequency of occurrence of the event. t: = 1.96. α = 0.05 is the t value at ∞ degrees of freedom.

Statistical analyses: Frequencies (number, %) were calculated for each question. The chi-square test was used for the comparisons between the gender groups regarding milk consumption habits and preferences (Akgül, 2005). Considering the results of this test, the Pearson chi-square test was used if the percentage of those less than 5 among the theoretical frequencies calculated for each eye were less than 20%, and the significance levels of the exact method were used if greater than 20%. The SPSS version 22.0 was used for the analyses and calculations (SPSS, 2015).

Results and discussion

Data obtain students' opinions on milk and dairy products consumption have been presented in Table 1. According to the research, the rate of male students who think that the society is not encouraged enough to consume drinking milk and dairy products was 75.32%, and the rate of female students was 82.18% ($p=0.112$) (Table 1).

Table 1. Students' opinions on milk and dairy products consumption

Questionnaire		Gender		Total (n: Total, %: Average)	p	
		Male	Female			
Is the community encouraged enough to drink milk and dairy products consumption?						
Yes	n	39	36	75	0.112	
	%	24.68	17.82	20.83		
No	n	119	166	285		
	%	75.32	82.18	79.17		
What do you think is the most effective way to encourage the community to consume drinking milk and dairy products?						
Written and visual media-communication tools	n	104	147	251		0.142
	%	67.10	74.24	71.10		
Seminars, meetings	n	51	51	102		
	%	32.90	25.76	28.90		
What do you think of the school milk program?						
Necessary	n	120	171	291	0.016	
	%	75.95	85.93	81.51		
Unnecessary	n	38	28	66		
	%	4.05	14.07	18.49		
Do you know about the presence and amount of cholesterol in milk and dairy products?						
Yes	n	36	40	76		0.457
	%	25.00	20.74	22.65		
No	n	108	152	260		
	%	75.00	78.76	77.35		
At what age do you think milk should be consumed the most?						
Infancy-childhood	n	47	39	86	0.110	
	%	30.32	19.50	24.22		
Youth	n	5	5	10		
	%	3.00	2.50	2.82		
In old age	n	1	2	3		
	%	0.87	1.00	0.85		
At all ages	n	102	154	256		
	%	65.81	77.00	72.11		
Your knowledge about the primary nutritional values of drinking milk and dairy products?						
Protein source	n	64	99	163		0.363
	%	42.38	49.50	46.44		
Gives energy	n	12	11	23		
	%	7.95	5.50	6.55		
Vitamin and mineral source	n	9	16	25		
	%	5.96	8.00	7.12		
Contains all the nutrients needed	n	57	68	125		
	%	37.75	34.00	35.62		
None	n	9	6	15		
	%	5.96	3.00	4.27		
Your opinions about the reason why sterilized UHT milk can not spoil for a long time?						
Heat treatment	n	50	52	102	0.002	
	%	32.47	26.13	28.90		
Containing preservatives	n	56	109	165		
	%	36.36	54.77	46.74		
I do not know	n	48	38	86		
	%	31.17	19.10	24.36		

In the study conducted by Şeker et al. (2012), it was reported that 86.3% of the participants thought the society was not encouraged enough about milk consumption. Improving the level of knowledge about nutrition related to milk can improve the intake of milk and dairy products (Huang et al., 2019).

According to the study, the rate of male students who thinks that the most effective way to encourage the consumption of milk and dairy products is through print and visual media-communication tools was 67.10%, while the rate of female students was 74.24%. Also, the rate of male students who thinks that the most effective way to encourage is seminars and meetings was 32.90%, while the rate of female students was 25.76% ($p=0.142$) (Table 1).

In a study conducted at Gümüşhane University, 71.7% of the students stated that they found the advertising and propaganda activities to encourage the consumption of milk and dairy products insufficient (Şahinöz and Özdemir, 2017). Another study conducted at Celal Bayar University, 76.8% of the students stated that television, newspaper and radio and advertising / propaganda activities are the most important tools that encourage milk consumption (Karagözlü et al., 2005). Also, in the study conducted at Yüzüncü Yıl University; it has been reported that 12.1% of the students found the studies of the press and media organs sufficient in encouraging the consumption of milk and dairy products, while 60.8% found it insufficient (Durmaz et al., 2002). It is understood that print and visual media-communication tools play a very important role in promoting consumption of milk and dairy products.

According to the research, the rate of male students who think that the school milk program is necessary is 75.95%, and the rate of female students is 85.93% ($p=0.016$) (Table 1). Şeker et al. (2012) reported that 84.6% of the participants found the school milk program very necessary. The findings obtained from the studies show that the school milk program is believed to be appropriate and necessary both to raise conscious generations about healthy nutrition and to popularize milk and dairy products from childhood. In a study conducted in South Korea, it was reported that the school milk program contributed to the increase in milk consumption and was beneficial for general nutrition (Lee et al., 2019).

According to the research, the rate of male students who think that they do not have information about the presence and amount of cholesterol in milk and dairy products was 75.00%, and the rate of female students was 78.76% ($p=0.457$) (Table 1). According to the research conducted at Yüzüncü Yıl University, it was reported that 29.78% of the students thought that cholesterol was harmful,

19.89% of them thought that cholesterol was not harmful, and 50.83% of them did not have any information about cholesterol (Selçuk et al., 2003). Approximately 64% of patients perceived high cholesterol as 'very serious'. The factors most significantly associated with desire to improve cholesterol control were perceiving hyperlipidemia as 'very serious and self-efficacy for cholesterol control (Zullig et al., 2016). Access to accurate information should be ensured by implementing educational programs for the society, including university students.

According to the study, the rate of male students who think that milk and dairy products should be consumed mostly in infancy and childhood was 30.32% and the rate of female students was 19.50%, the rate of male students who thinks that should be consumed at all ages was 65.81%, and the rate of female students was 77.00%, the rate of male students who thinks that should be consumed in youth ages was 3.00%, and the rate of female students was 2.50%, and also the rate of male students who thinks that should be consumed in old age was 0.87%, and the rate of female students was 1.00% ($p=0.110$) (Table 1). In a study conducted at Süleyman Demirel University, it was reported that 81.0% of the students thought that milk should be consumed at all ages (Şimşek and Açıkgöz, 2011). In both universities, the vast majority of university students participating in the survey indicated that milk and dairy products should be consumed at all ages and showed an appropriate perspective to scientific facts.

The intake of milk and dairy products in the elderly in China is still seriously insufficient. Liquid milk is the main type of milk intake. The awareness rate of "drinking milk and eating dairy products are beneficial to health" was 79.30% (Huang et al., 2019). The milk consumption of people in ≥ 70 years old was significantly higher than the people in 60- 69 years old (Liu et al., 2016). The most recent evidence suggested that intake of milk and dairy products was associated with reduced risk of childhood obesity. In adults, intake of dairy products was shown to improve body composition and facilitate weight loss during energy restriction (Kongerslev Thorning et al., 2016). For this reason, consumption of milk and dairy products is recommended for healthier eating habits in children and adolescents (Campmans-Kuijpers et al., 2016). Milk and dairy consumption should be encouraged at all ages.

According to the study, the rate of male students who thinks that the primary nutritional value of milk and dairy products is a source of protein was 42.38% and the rate of female students was 49.50%, the rate

of male students who thinks that source of vitamins and minerals was 5.96%, and the rate of female students was 7.12%, the rate of male students who thinks that source of energy was 7.95%, and the rate of female students was 5.50%, the rate of male students who thinks that milk and dairy products contain all the nutritional elements needed was 37.75%, and the rate of female students was 34.00%, and also the rate of male students who thinks that none of them was 5.96%, and the rate of female students was 3.00% ($p=0.363$) (Table 1). Milk and dairy products provided 9.1% of the total energy supply. A high share (above 20%) in the supply of nutrients was noted in the case of calcium (54.7%), riboflavin (28.1%), vitamin B12 (26.1%), and phosphorus (24.6%). Supply at the level of 10–20% was observed for protein, saturated fatty acids, zinc, total fat, cholesterol, potassium, magnesium, and vitamin A. Of the amino acids, the share above 20% from dairy category was recorded in the case of 6 amino acids (proline, tyrosine, serine, lysine, valine, and leucine) and at the level of 10–20% for 10 amino acids (isoleucine, histidine, threonine, tryptophan, phenylalanine, methionine, glutamic acid, aspartic acid, alanine, and arginine) (Górska-Warsewicz et al., 2019).

In a study conducted at Yüzüncü Yıl University, the rate of students who thinks that milk is a source of protein was 19.11%, thinks that milk is a source of vitamins was 16.02%, thinks that milk is a source of energy was 8.30%, the rate of thinks that it contains all of them was 35.33% (Tarakçı et al., 2003). The importance of milk and dairy products in nutrition should be conveyed to all segments of society through education.

According to the study, the rate of male students who thinks that UHT milk is not spoiled for a long time due to heat treatment was 42.38% and the rate of female students was 49.50%, the rate of male students who think that UHT milk does not spoil due to its preservative content was 36.36%, and the rate of female students was 54.77%, and also the rate of male students who do not know why UHT milk does not spoil for a long time was 31.17%, and the rate of female students was 19.10% ($p=0.002$) (Table 1). In a study conducted at Süleyman Demirel University, 41.6% of the students stated that long-life milks contain preservatives as the reason for their long-term durability, 36.8% as the reason for heat treatment, while 21.6% of them stated that they did not have any information about this subject (Şimşek and Açıkgöz, 2011). There is a lack of information for both university students on what UHT milk is and how it is obtained. Education programs that include

students should be applied to segments of society.

In the present study, students' opinions on milk and dairy products prices are given in Table 2.

According to the study, the rates of male students who thinks that milk, cheese, yoghurt, kefir, ayran and ice cream prices are cheap were 30.00%, 57.31%, 28.67%, 42.00%, 41.89%, and 21.48%, respectively, while the rates of female students were 16.24%, 53.61%, 14.43%, 43.01%, 40.41%, and 9.27%, respectively. Also, the rates of male students who thinks that prices are normal were 51.33%, 33.33%, 58.00%, 44.76%, 39.19%, and 50.33%, respectively, while the rates of female students were 72.77%, 45.88%, 76.29%, 51.81%, 50.78%, and 57.22%, respectively (Table 2). In a study conducted at Süleyman Demirel University, it was reported that 68.3% of the students determined the price of milk normal, and 23.2% determined it expensive (Şimşek and Açıkgöz, 2011). Addition, in a study conducted at Yüzüncü Yıl University, exactly half of the students evaluated the price of milk as expensive (Tarakçı et al., 2003), and in another study conducted at same University, the ratio of students who found the prices of cheese, yogurt, butter and ice cream expensive was 67.27%, 45.51%, 79.33% and 61.81%, respectively (Selçuk et al., 2003).

The fact that the input prices in animal husbandry are expensive causes the product price to be expensive. It also carries great risks. Therefore, to think that milk and dairy products are expensive is due to not knowing these facts. However, other products, which are much easier to produce and less costly, are sold more expensive than milk and dairy products. These facts need to be explained to the whole society, including students.

In the present study, students' opinions on animal welfare in the milk and dairy products consumption context are given in Table 3.

According to the research, the rate of male students who visited a place where animals is raised (farm, barn, fence, etc.) was 83.87%, and the rate of female students was 75.13% ($p=0.046$) (Table 3). Compared to those who have never visited a place related to animal husbandry, the rate of visitors is quite high. However, another research has revealed that; allowing citizens to tour a dairy farm improved their performance in a knowledge-based quiz of dairy husbandry practices, but did not improve perceptions of dairy cattle welfare for most participants. The implication is that the livestock industries cannot expect one-way education efforts to resolve changes in animal welfare perceptions after touring a dairy farm societal concerns about animal welfare. Rather, engagement between the livestock industries and the

Table 2. Students' opinions on milk and dairy products prices

Questionnaire		Gender		Total (n: Total,%: Average)	p
		Male	Famale		
Students' opinions on milk prices					
Cheap	n	45	31	76	0.000
	%	30.00	16.24	22.29	
Expensive	n	28	21	49	
	%	18.67	10.99	14.37	
Normal	n	77	139	216	
	%	51.33	72.77	63.34	
Students' opinions on cheese prices					
Cheap	n	86	104	190	0.000
	%	57.34	53.61	55.23	
Expensive	n	14	1	15	
	%	9.33	0.51	4.36	
Normal	n	50	89	139	
	%	33.33	45.88	40.41	
Students' opinions on yoghurt prices					
Cheap	n	43	28	71	0.001
	%	28.67	14.43	20.64	
Expensive	n	20	18	38	
	%	13.33	9.28	11.05	
Normal	n	87	148	235	
	%	58.00	76.29	68.31	
Students' opinions on kefir prices					
Cheap	n	63	83	146	0.026
	%	42.00	43.01	42.56	
Expensive	n	20	10	30	
	%	13.33	5.18	8.75	
Normal	n	67	100	167	
	%	44.67	51.81	48.69	
Students' opinions on buttermilk prices					
Cheap	n	62	78	140	0.011
	%	41.89	40.41	41.06	
Expensive	n	28	17	45	
	%	18.92	8.81	13.19	
Normal	n	58	98	156	
	%	39.19	50.78	45.75	
Students' opinions on ice cream prices					
Cheap	n	32	18	50	0.006
	%	21.48	9.27	14.58	
Expensive	n	42	65	107	
	%	28.19	33.51	31.19	
Normal	n	75	111	186	
	%	50.33	57.22	54.23	

livestock industries and the public should be two-way such that industry stakeholders strive to hear, and respond to, concerns that result from increased transparency. This type of communication might allow industry stakeholders to better identify welfare concerns in society and to highlight shared values, providing a foundation to resolve more contentious issues (Ventura et al., 2016).

According to the study, the rate of male students who know what the concept of animal welfare means was 85.16% and the rate of female students was 91.28% (p=0.091) (Table 3). In a study in China, approximately two thirds of the respondents had never heard of animal welfare (You et al., 2014). In another study, a total of 15.2 % ordinary citizens Brazil responded that they have never heard of

animal welfare. As 43.5% ordinary citizens Brazil and 60.3% ordinary citizens France have heard of the subject superficially, and 42.3% ordinary citizens Brazil and 35.1% ordinary citizens France have heard of the subject more deeply (Tamioso et al., 2018). The findings reveal that college students have higher rates have heard about animal welfare in Turkey. However, although countries and populations included in the study are different, it appears that there is a general lack of information on animal welfare. Informative training activities should be carried out on this subject.

According to the study, the rate of male students who thinks that there was a welfare problem in farm animals was 56.21% and the rate of female students was 55.84%. Also, the ratio of male students who have no idea about this issue was 27.45% and that of female students was 39.59% ($p=0.000$) (Table 3). It is estimated that the news about this issue is effective in students' thinking that there is an animal welfare problem. Although not at the desired rate, it is appreciated that at least some of the students have an opinion on this subject.

According to the this study, the rate of male students who thinks that the welfare problems that farm animals are most exposed to are the negativities in physical conditions was 22.44% and the rate of female students was 17.53%, the rate of male students who think that the negativity in the conditions of care and feeding was 13.46%, and the rate of female students was 7.73%, and also the rate of male students who think that diseases and negativities in treatment processes was 0.64%, and the rate of female students was 0.00%. In addition, according to the study, the rate of male students who thinks that the welfare problems that farm animals are most exposed to are the negativities in physical and care and feeding conditions was 24.36% and the rate of female students was 28.35%, the rate of male students who think that negativities in physical conditions and, diseases and negativities in treatment processes was 9.62%, and the rate of female students was 2.58%, the rate of male students who think that negativity in the conditions of care and feeding, and diseases and negativities in treatment processes was 1.92%, and the rate of female students was 1.03%, and also the rate of male students who think that all of them was 27.56%, and the rate of female students was 42.78% ($p=0.004$) (Table 3). The findings reveal that university students think that physical conditions are mostly insufficient in terms of animal welfare. The findings show that

the insufficiency of care and nutritional conditions is less. The rate of those who think that lack of animal welfare is related to disease and treatment processes is extremely low. Findings show similarities in single and multiple preference order.

In a study, in farming context, most stakeholders (Vietnam, Malaysia Thailand China India and Bangladesh) placed the lack of food and water as the most serious animal welfare issues (Sinclair and Phillips, 2019). In another study also, revealed that on-farm prevention of thermal stress and air quality were not sufficiently included. All groups also agreed that animal behavior and animal health are the most effective animal-based indicators (Averós et al., 2013).

According to the research, the rate of male students who want label information about milk and dairy products produced from animals raised under welfare conditions was 71.24% and the rate of female students was 78.68%. Also, the ratio of male students who have no idea about this issue was 12.42% and that of female students was 14.72% ($p=0.014$) (Table 3). There is a synergy between food safety and animal welfare. The importance of animal welfare in terms of food security is relevant increasing of animal products consumption (Demirel and Çak, 2016). A food label with the information that it is manufactured in accordance with animal welfare responds to the concerns of the community and consumer's request for ethical food purchase (Bozkurt and Koçak, 2017).

According to the study, the rate of male students who agreed to pay extra for milk and dairy products because suitable for animal welfare was 75.71% and the rate of female students was 81.77% ($p=0.253$). The rates of male students who could pay up to 10%, 11-25%, and more than 25% were 60.14%, 30.07%, and 9.79%, respectively, while the rates of female students were 62.28%, 23.12%, and 8.60%, respectively ($p=0.294$) (Table 3).

In a study in China, more than half of the respondents were willing, to pay more for high-welfare animal products, whereas 45.5% of the respondents were not willing to pay more (You et al., 2014). Another study show that the market is the most viable direction to improve farm animal welfare and willingness to pay for animal welfare the differences between European countries are considerable (Ingenbleek et al., 2013). It is important and valuable to have groups willing to pay more for animal products with high welfare.

Table 3. Students' opinions on animal welfare in the milk and dairy products consumption context

Questionnaire		Gender		Total (n: Total,%: Average)	p	
		Male	Female			
Have you ever visited a place where animals are breeding (fence, pen, barn, etc.)?						
Yes	n	130	148	278	0.046	
	%	83.87	75.13	77.87		
No	n	25	49	74		
	%	16.13	27.87	22.13		
Do you know what the concept of animal welfare means?						
Yes	n	132	178	310		0.091
	%	85.16	91.28	88.57		
No	n	23	17	40		
	%	14.84	8.72	11.43		
Do you think is there farm animals welfare problems in Turkey?						
Yes	n	86	110	196	0.000	
	%	56.21	55.84	56.00		
No	n	25	9	34		
	%	16.34	4.57	9.71		
No idea	n	42	78	120		
	%	27.45	39.59	34.29		
Which of the welfare problems do you think farm animals suffer from?						
Physical conditions	n	35	34	69	0.004	
	%	22.44	17.53	19.71		
Care and feeding conditions	n	21	15	36		
	%	13.46	7.73	10.29		
Diseases and treatment processes	n	1	0	1		
	%	0.64	0.00	0.29		
Physical conditions and care and feeding conditions	n	38	55	93		
	%	24.36	28.35	26.57		
Physical conditions and diseases and treatment processes	n	15	5	20		
	%	9.62	2.58	5.71		
Care and feeding conditions and diseases and treatment processes	n	3	2	5		
	%	1.92	1.03	1.43		
All	n	43	83	126		
	%	27.56	42.78	36.00		
When purchasing milk and dairy products, would you like to have product labels that read "made from animals raised in animal welfare conditions"?						
Yes	n	109	155	264	0.014	
	%	71.24	78.68	75.43		
No	n	25	13	38		
	%	16.34	6.60	10.86		
No idea	n	19	29	48		
	%	12.42	14.72	13.71		
Would you consider paying extra for products made from animals raised in animal welfare conditions?						
Yes	n	112	157	269	0.253	
	%	76.71	81.77	79.59		
No	n	34	35	69		
	%	23.29	18.23	20.41		
How much will you agree to pay extra for the animal products you buy because they have been raised in animal welfare conditions?						
Up to 10%	n	86	127	213	0.294	
	%	60.14	68.28	64.74		
11-25%	n	43	43	86		
	%	30.07	23.12	26.14		
More than 25%	n	14	16	30		
	%	9.79	8.60	9.12		

Conclusion

In this study, it was determined that university students' perception of animal welfare and opinions on consumption of milk and dairy products were generally at an acceptable level. However, in the light of the findings more information and the necessary training support should be provided on the following issues. a) The consumption of milk and dairy products should be encouraged more. b) Accurate and sufficient information about the presence, amount and functions of cholesterol in milk and dairy products should be conveyed more. c) Milk and dairy products are not only used by children, young people or old people; it should be explained that these should be consumed at all ages. d) Milk and dairy products are not just sources of energy, protein or vitamins; it should be explained more that it is a healthy food source that includes all of them. e) What the pasteurized and UHT milk is and how it is obtained should be explained more. f) It should be explained that milk and different dairy products are not expensive at all in the context of the difficulties and high costs of the production process from farm to table. g) Correct and sufficient information about animal welfare should be explained more. h) The importance of finding information on the labels of milk and dairy products showing that they are produced from animals suitable for welfare conditions should be explained more. i) It should be explained more about the need to pay more for milk and dairy products produced from animals in welfare conditions. Mass media are also recommended to be used in order to raise awareness of consumption of milk and dairy products to a better point.

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