



Investigation of Serum and Saliva CK, AST and LDH Levels in Livestock Guardings and Shelter Kangal Shepherd Dogs

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ABSTRACT

It is a fact that Kangal shepherd dogs, who serve as livestock guarding, have more daily physical activity than those living in the shelter. The relationship between physical activity and muscle metabolism is well known. However, no study has been found in the literature that reveals differences in muscle metabolism among Kangal shepherd dogs that protect their herd and live in shelters. The aim of this study is to investigate the muscle metabolism differences between the livestock guardings and those living in the shelter in Kangal shepherd dogs. In the study, a total of 32 young male and female Kangal dogs that guard the herd and live in the shelter were randomly divided into four equal groups (n=8/group). From serum and saliva samples, creatine kinase (CK), lactate dehydrogenase (LDH), and aspartate aminotransferase (AST) parameters related to muscle metabolism were analyzed with an autoanalyzer. Serum CK levels were lower in shelter dogs than in herd dogs, while the highest value was observed in females in the herd (P<0.05). The salivary CK levels of the females in the shelter were higher than the females in the herd (P<0.05). The highest serum AST value was seen in females living in the shelter, while the lowest was seen in sheltered males (P<0.05). However, salivary AST levels of the females in the shelter were higher than the females in the herd (P<0.05). Serum LDH level was higher in females in the shelter and herd than in males in the shelter (P<0.05). Salivary LDH levels were higher in males in the shelter than males and females in the herd. Females in the herd had lower salivary LDH values than males and females in the shelter (P<0.05). In this study, muscle metabolism-related variables (CK, AST, and LDH) were investigated for the first time in Kangal sheepdogs from serum and saliva samples.

Keywords: Aspartate Aminotransferase, Creatine Kinase, Kangal Shepherd Dogs, Lactate Dehydrogenase, Muscle Metabolism

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Introduction

Determination of species-specific values of serum biomarkers after exercise and training may contribute to distinguishing physiological responses from pathological conditions. Clinical profiles formed as a result of exercise and training, and muscle and heart biomarkers help determine the body fitness level of dogs. The increase in serum level of skeletal muscle enzymes may represent an index of cellular necrosis and tissue damage following acute and chronic muscle injuries (Szumilak et al., 1998). Changes in serum levels of muscle enzymes and isoenzymes have been detected in normal subjects and athletes after strenuous exercise (Brancaccio et al., 2006). The number of enzymes released from muscle tissue to the blood can be affected by physical exercise. Serum creatine kinase (CK), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) are indicators of the degree of metabolic adaptation of skeletal muscles

(depending on exercise level) (González Fernández et al., 2020). Serum concentrations of CK and LDH, which play a role in muscle metabolism, are normally very low as a result of physiological deterioration of the cell. CK and LDH serum concentrations increase significantly in humans after intense exercise and muscle pathology (Hood et al., 1991; Garry and McShane., 2000). There is little literature available on muscle biomarkers in dogs (Frank et al., 2015; Vlasakova et al., 2017). It has been reported that long-term endurance exercise and high-intensity races cause a significant increase in CK and AST levels of skeletal muscle biomarkers in dogs (Vlasakova et al., 2017; Cerqueira et al., 2018). In recent years, saliva has gained attention as an alternative diagnostic fluid to the blood. The increasing interest in the saliva-based analysis is due to its simplicity of sampling, noninvasiveness, inexpensiveness, and reliability. Saliva diagnosis is used in

tests for various hormones, stress markers and diabetes diagnosis (Jaedicke et al., 2012; Koçkaya and Şireli., 2015).

Guard dogs have been bred for over a thousand years to protect sheep and other farm animals from wild animals such as wolf attacks, hunters, and thieves (Koçkaya and Şireli., 2015). In traditional farming practices, the herds that come out of the barn about three hours before sunset are taken to the pasture, accompanied by livestock guarding dogs and shepherds. The herd grazes on the pasture overnight and returns to the barn early the next morning to protect the sheep from the dangerous heat of the sun. Kangal Shepherd dogs are livestock guarding dogs unique to Turkey and they cover more than 16 km per day during herd protection, reaching an average speed of 30 km/h (Akyazi et al., 2017). However, it has been reported that dogs in shelters for commercial production and breeding cover approximately 2-5 meters per minute (Cafazzo et al., 2014). Based on this, it was hypothesized that there might be differences in muscle metabolism-related enzyme levels (CK, AST, and LDH) in serum and saliva samples of Kangal shepherd dogs that work as livestock guardings and live in shelters. In the literature review, no study was found in which serum and saliva CK, AST, and LDH enzyme levels were compared in Kangal shepherd dogs that protect the herd and live in a shelter. The aim of this study was to investigate serum and saliva CK, AST, and LDH enzyme levels in male and female Kangal shepherd dogs between livestock guardings and those living in the shelter.

Material and Methods

The study was carried out on a total of 32 herd-guarding and shelter-living male and female Kangal shepherd dogs (n=8/group), after obtaining ethical approval from the Sivas Cumhuriyet University Animal Experiments Local Ethics Committee (Date and Identity Number: 20.04.2021 and 65202830-050.04.04-525). Animals to be used in the study were selected according to appropriate prophylactic and health controls. It was emphasized that none of the dogs to be used in the study had a pre-existing or ongoing orthopedic and gingival disease, any history of muscle, heart, and liver problems, or any drug administration that could affect the metabolism of these organs. Blood samples from animals were taken from vena cephalica antebrachii to serum tubes between 19.00 and 20.00 at the same time interval during the study. The blood samples were centrifuged at 3000 rpm for 15 minutes and the serums were extracted. Serums were stored at -20°C for analysis. Saliva samples were obtained by placing a sponge in the dog's mouth for 1-2 minutes (Parra et al., 2005; Luke et al., 2015; Tvarijonavičiute et al., 2017). The sponge was then placed in a saliva tube (Salivette®, Sarstedt AG & Co., Nümbrecht, Germany) and the samples were stored at +4°C until analysis. Saliva samples were centrifuged at 3500 rpm x 15 minutes as previously reported (Tvarijonavičiute et al., 2017). Saliva supernatants were stored at -20°C until analysis. CK, AST, and LDH enzyme

levels were determined from serum and saliva with an autoanalyzer (Mindray BS 200).

Statistical Analysis

The obtained data were analyzed in terms of sex and lifestyle effects with two-way ANOVA test and post hoc Tukey test in GraphPad Prism 8.0.1 (Graph Pad Software Inc., San Diego, CA, USA) software. Results are given as mean ± standard deviation. P<0.05 was considered significant.

Results

Serum AST, CK, and LDH levels results were represented in Figure 1. While the main effect of sex (P=0.004, F=11.94) and sex-life interaction (P=0.002, F=15.01) were significant on serum AST levels, the main effect of lifestyle was not observed (P=0.722, F=0.132). As a result of post hoc analysis, the highest serum AST value was observed in females living in the shelter (P<0.05), while the lowest was observed in males in the shelter (P<0.05). However, there was no difference between livestock guarding male and female dogs (P>0.05).

Lifestyle (P=0.000, F=82.773) and sex-life interaction (P=0.013, F=8.014) were found to be significant on serum CK levels, but sex had no effect (P=0.120, F=2.740). Serum CK levels were found to be lower in those living in the shelter than in the herd (P<0.05). The highest value was observed in females in the herd (P<0.05).

While sex influenced serum LDH levels (P=0.017, F=7.368), lifestyle effects (P=0.149, F=2.332) and interaction were not significant (P=0.367, F=0.868). It was higher in females in the shelter and herd than in males in the shelter (P<0.05).

Saliva AST, CK, and LDH levels results were represented in Figure 2. The effects of sex (P=0.380, F=0.784) and interaction (P=0.146, F=2.23) on salivary AST levels were not significant. However, the effect of lifestyle was significant (P=0.007, F=9.49). The salivary AST levels of the females in the shelter were higher than the females in the herd (P<0.05). However, it was not statistically different from the other groups (P>0.05).

There was no effect of sex (P=0.085, F=3.485) and interaction (P=0.177, F=2.035) on salivary CK levels. However, the effect of lifestyle was significant (P=0.042, F=5.073). The salivary CK levels of the females in the shelter were higher than the females in the herd (P<0.05). However, it was not statistically different from the other groups (P>0.05).

Sex (P=0.233, F=1.539) and interaction (P=0.862, F=0.031) had no effect on salivary LDH levels. However, the effect of lifestyle was significant (P=0.000, F=23.19). Salivary LDH levels were higher in males in the shelter than males and females in the herd. Females in the herd had lower salivary LDH values than males and females in the shelter (P<0.05).

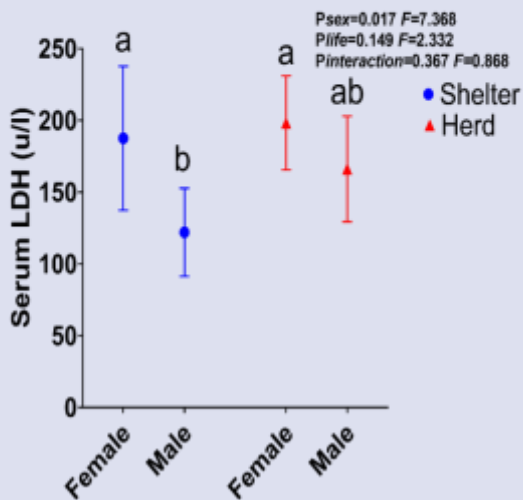
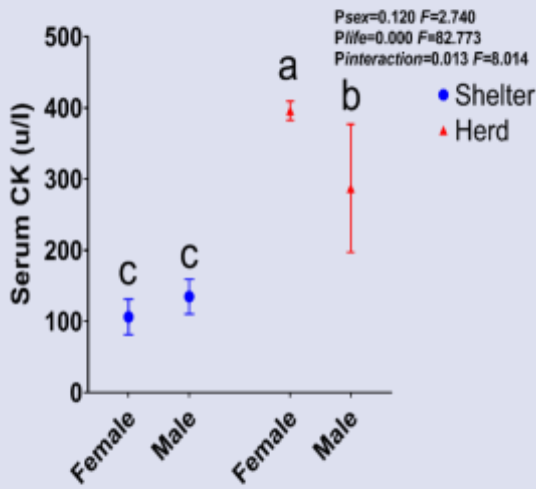
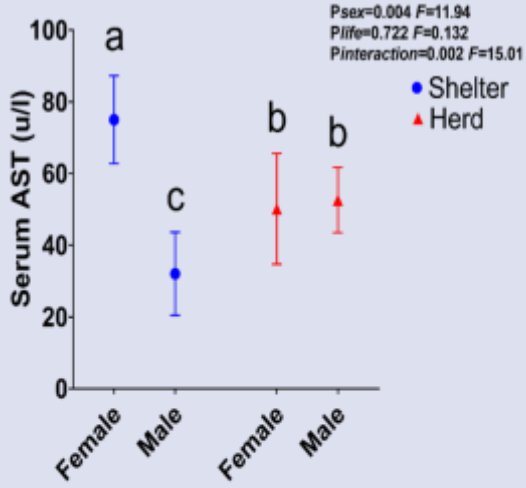


Figure 1. Results of serum AST, CK, and LDH levels in female and male Kangal shepherd dogs that work as livestock guardings and live in shelters a,b,c: different letters represent the differences between the groups according to the post hoc Tukey test performed after the two-way ANOVA test

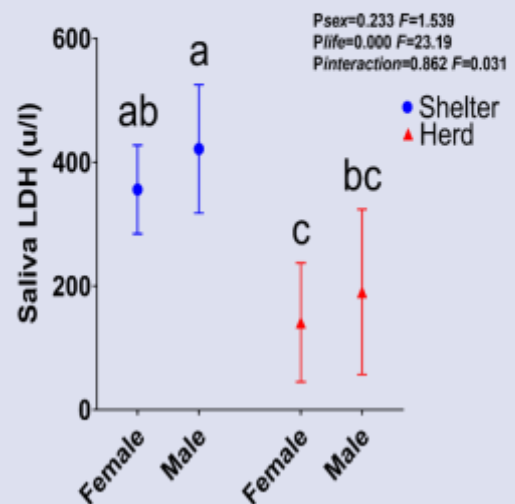
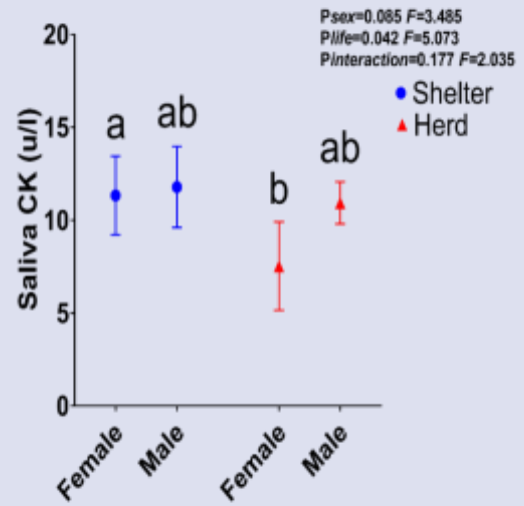
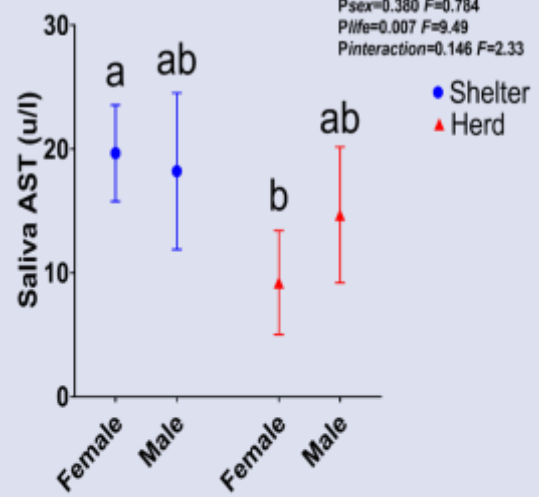


Figure 2. Results of saliva AST, CK, and LDH levels in female and male Kangal shepherd dogs that work as livestock guardings and live in shelters a,b,c: different letters represent the differences between the groups according to the post hoc Tukey test performed after the two-way ANOVA test.

Discussion

In this study, muscle enzyme changes were investigated for the first time in serum and saliva samples of Kangal shepherd dogs that are herd-guarding and sheltered. In our study, muscle metabolism variables were examined through saliva, which is non-invasive, non-time-consuming, less stressful, and easy to collect, and serum samples. As a result of our study, variations were observed in serum and saliva AST, CK, and LDH levels.

Kangal shepherd dogs cover walking distances exceeding 16 km at an average speed of 3-4 km/h during their herd protection duties. It also undertakes the task of protecting and attacking foreigners by running at a speed of over 30 km/h (Akyazi et al., 2017). However, it has been reported that dogs in shelters for commercial production and breeding cover approximately 2-5 meters per minute (Cafazzo et al., 2014). In the light of this information, it is highly likely to expect changes in muscle metabolism since livestock guarding dogs are more active during the day.

The most significant indicator of damaged striated muscles, particularly in skeletal muscle, is creatine kinase (CK). The greater release from the injured cell membrane is what causes the rise in plasma CK activity in dogs, which is frequently accompanied by muscle inflammation, necrosis, or degeneration (Spinella et al., 2021). Schneider et al., reported the most significant change in CK activity measured after physical stress. Similarly, in a study conducted in humans, an increase in CK values was observed in serum and saliva after futsal match (Barranco et al., 2018). In a previous pilot dog study, it was reported that salivary CK values in dogs with muscle damage were higher than in healthy dogs, and as a result, it was positively correlated with serum CK value (Tvarijonavičiute et al., 2017). In our study, serum CK levels were lower in shelter dogs than in herd dogs, while the highest value was observed in females in the herd. The salivary CK levels of the females in the shelter were higher than the females in the herd. However, it was not statistically different from the other groups. In the interpretation based on serum samples, the low CK level in shelter dogs can be interpreted as less developed muscle mass than livestock guardings, skeletal muscle not being as strained as livestock guarding dogs, and consequently less muscle damage. The high CK value in females in the herd may be due to the greater response of the muscle mass of these dogs to physical stress. On the other hand, saliva CK values do not support this interpretation. There could be several reasons for this. Salivary enzymes may have been affected at different levels, independent of serum enzymes, in Kangal shepherd dogs for CK. There may be a delay in the transition of this enzyme from the blood to the saliva.

Increases in blood concentrations of AST and LDH in addition to CK have been used to assess muscle effort at the end of physical stress (Rovira et al., 2008). Because these enzymes are located in the cytoplasmic matrix of muscle cells, their presence in serum or plasma serves as a sign that there are cell lesions (Brancaccio et al., 2008). AST is more common in the liver and is less organ-specific than CK. Evaluation of AST together with CK may give more accurate

results during muscle damage or recover (Lucas et al., 2015). The skeletal muscle biomarkers CK and AST were shown to be moderately but significantly elevated after long-term endurance exercise and high-intensity racing in previous research on sports dogs (Lucas et al., 2015; Vlasakova et al., 2017). Similarly, CK, serum and saliva AST levels were increased in muscle damage dogs (Tvarijonavičiute et al., 2017). The highest serum AST value was seen in females living in the shelter, while the lowest was seen in sheltered males. However, salivary AST levels of the females in the shelter were higher than the females in the herd. However, it was not statistically different from the other groups. This confusing result is quite contradictory and shows that the AST level is affected by many metabolic variables, especially the liver. The rise in serum concentrations of CK and AST, on the other hand, is dependent on the kind of activity, its intensity, duration, and the time of blood collection (McKenzie et al., 2007). Because CK and AST have half-lives ranging from 2 to 6 h (Vlasakova et al., 2017), the dynamics of these biomarkers following exercise, training or clinical examination may correctly represent the physiological or damage responses of the muscle.

Pyruvate and lactate are transformed by LDH into one another. An indicator of peripheral muscle fatigue, high LDH shows a switch from aerobic to anaerobic ATP synthesis (Finsterer, 2016). The levels of AST, CK, and LDH in blood and saliva samples were found to vary following strenuous activity in a variety of sports in previous human research (Brancaccio et al., 2008; Barranco et al., 2018). In a previous study, it was reported that the activity of muscle enzymes (CK, LDH and AST) was significantly higher for Spanish Greyhounds after exercise compared to the activity of these enzymes for dogs of other breeds at rest (Lucas et al., 2015). In our study, serum LDH level was higher in females in the shelter and herd than in males in the shelter. Moreover, salivary LDH levels were higher in males in the shelter than males and females in the herd. Females in the herd had lower salivary LDH values than males and females in the shelter. Although it appears that following intense exercise, serum LDH activity is elevated in humans and horses, contradictory findings have been found in dogs (Lucas et al., 2015).

Conclusion

In this study, muscle metabolism-related variables (CK, AST, and LDH) were investigated for the first time in Kangal sheepdogs from serum and saliva samples. Although the results were variable, it was predicted that changes in serum levels of muscle metabolism-related variables could yield more accurate results. We believe that these results can give an idea to similar studies with large participation.

Conflict of interest

All authors have read and approved the article. There is no conflict of interest.

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