

## An Investigation on Serum Troponin Concentration in Healthy Ruminants <sup>[1]</sup>

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### Summary

The present study aimed to investigate the cardiac troponin (cTn I and cTn T) levels in healthy ruminants. The study involved 30 cattle (15 male, 15 female), 30 Akkaraman sheep (15 male, 15 female) and 30 goats (15 male, 15 female) and all animals were clinically healthy. The study animals were grouped as 0-6 months, 6-12 months and 12 months and over. Blood samples were obtained from jugular vein and cardiac troponin I and T levels, and biochemical parameters (CK, CK-MB, AST and LDH) were measured. Troponin T values were found as negative in all three species. Troponin I values were 0-0.23 ng/ml (mean 0.18 ng/ml) in cattle, 0-0.21 ng/ml (mean 0.15 ng/ml) in sheep and 0-0.24 ng/ml (mean 0.20 ng/ml) in goats. It was also found that the values were in declared limits for cattle and sheep. Although CK, CK-MB, AST and LDH values were in normal ranges AST levels were higher in cattle and sheep of 0-6 months of age and, LDH levels were higher in sheep and goat at 6-12 months of age. In conclusion, obtained troponin values in this study could be values for healthy ruminants.

**Keywords:** Cattle, Sheep, Goat, Troponin

## Sağlıklı Ruminantlarda Serum Troponin Düzeylerinin Araştırılması

### Özet

Bu çalışmada sağlıklı ruminantlarda kardiyak troponinlerin (cTn I ve cTn T) düzeylerinin belirlenmesi amaçlandı. Çalışmanın materyalini klinik olarak sağlıklı 30 sığır (15 erkek, 15 dişi), 30 Akkaraman koyun (15 erkek, 15 dişi) ve 30 keçi (15 erkek, 15 dişi) oluşturdu. Her üç türdeki hayvanlar 0-6 aylık, 6-12 aylık ve 12 ay ve üzeri olmak üzere gruplara ayrıldı. Vena jugularisten usulüne uygun olarak alınan ve elde edilen serum örneklerinden kardiyak troponin I ve T düzeyleri ve biyokimyasal parametreler (CK, CK-MB, AST ve LDH) ölçüldü. Troponin T değerleri her üç türde negatif (-) olarak belirlendi. Troponin I sığırlarda 0-0.23 ng/ml (ortalama 0.18 ng/ml), koyunlarda 0-0.21 ng/ml (ortalama 0.15 ng/ml) ve keçilerde de 0-0.24 ng/ml (ortalama 0.20 ng/ml) değerleri arasında olduğu, cinsiyet ve yaş açısından da üç türde de belirtilen sınırlarda olduğu tespit edildi. CK, CK-MB, AST ve LDH seviyelerinin normal sınırlar arasında olmasına rağmen AST sığır ve koyunlarda 0-6 aylıklarda, LDH koyun ve keçilerde 6-12 aylıklarda yüksek olduğu belirlendi. Sonuç olarak bu çalışmada sağlıklı ruminantlar için serum troponin değerlerini teşkil edebileceği kanısına varıldı.

**Anahtar sözcükler:** Sığır, Koyun, Keçi, Troponin

### INTRODUCTION

Muscle proteins are comprised of myoglobin, actin, myosin, titin, nebulin, tropomyosin and troponins. They exist in blood in certain amounts. The level of muscle proteins in blood is widely used in human medicine especially in cardiac originated diseases and also has been recently introduced into the use of veterinary medicine <sup>1-3</sup>.

Troponins and myoglobins among cardiac originated proteins and biochemical parameters covering the muscle originated enzymes are used in foreground to determine the existence and degree of myocardial injury. For this purpose, the inadequate sensitivity and specificity of creatine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST) and alanine amino-



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transferase (ALT) have led scientist to develop new methods<sup>1-4</sup>. CK enzyme has 3 isozymes classified according to their origin. Those are CK-MB (heart), CK-MM (skeletal muscle), and CK-BB (brain)<sup>5-7</sup>. Cardiac originated creatine kinase is among the diagnostic tests mostly used. However, the relatively short duration of increase in serum creatine kinase-MB level during cardiomyopathy and its existence in extracardiac muscle tissues limit its diagnostic value<sup>3,8,9</sup>.

Troponins are globular proteins responsible for contraction and relaxation of myofibrils<sup>9,10-13</sup>. They are placed on actin filament of striated muscle and regulate sliding of filaments over each other during contraction and relaxation<sup>12-13</sup>. It has been reported that troponins are also detected in blood and striated muscles of domestic animals, similar to that found in humans<sup>12-16</sup>.

Troponins consist of 3 subunits: cTn I is the largest subunit and bound to actin, cTn T is bound to tropomyosin and cTn C is placed between Tn I and Tn T and has a high affinity against Ca<sup>+2</sup> ion<sup>7-12,17,18</sup>. Troponins don't exist in the blood of healthy persons or are suggested to exist in very small amounts<sup>18</sup>.

It has been reported that cTn T levels lower than 0.05 ng/mL indicate no myocard injury, levels between 0.05-0.1 ng/mL are suspiciously positive, and levels between 0.1-2.0 ng/mL indicate myocardial damage in humans<sup>19</sup>.

In healthy dogs<sup>20,21</sup>, cats<sup>22,23</sup>, cattle<sup>4,13,17</sup> and sheep<sup>24</sup> cardiac troponin I levels have been reported to vary as reported earlier.

Recent reports suggested that serum concentration of cTn I in clinically healthy ruminants is around 0-0.04 ng/ml and this is increased to 0.89 ng/ml in ruminants with idiopathic pericarditis, and a reference value of 0-2 ng/ml for other large animal species is reported<sup>17</sup>.

Cardiac troponins (cTn-T and cTn-I) are highly sensitive markers for myocardial necrosis. In recent years, it has been suggested as an important marker of cardiac diseases in veterinary medicine<sup>4,13,17,25</sup>.

Lack of detailed study regarding to normal cardiac troponin levels in ruminants in Turkey led us to conduct this study. In this study, it was aimed to determine cardiac troponin (cTn-T and cTn-I) levels in healthy ruminants and to compare the obtained data with other cardiac parameters.

## MATERIAL and METHODS

The material in this study consisted of 90 ruminants

(cattle, sheep, goat) obtained from Van and its vicinity. All animals were found healthy on clinical examination by checking body temperature, respiratory and heart beat frequency and general clinical appearance. The animals were grouped according to gender (male and female) and age. Each group comprised of 15 male and 15 female and animals were also categorised as. 10 animals in 0-7 months old (n=10), 6-12 months old (n=10), 1 year old (n=10).

The blood samples from all animals were obtained from jugular vein into anticoagulant-free container. Sera were obtained by centrifugation at (3000 rpm for 10 min). Serum samples were preserved at -20°C until analyses.

Serum cardiac troponin I (cTn-I) and T (cTn-T) values were determined calorimetrically using commercial test kits (Troponin I kit - DRG Diagnostic and Troponin T Sfat Kit-Roche) on an ELISA readers (ELISA reader®-DAS for cTn-I and Elecsys® 2010-Roche for cTn-T).

Serum lactate dehydrogenase, aspartate aminotransferase, alanin aminotransferase, creatin kinase and myocard originated creatine kinase levels were measured spectrophotometrically (Photometer® 5010 Boehringer Mannheim) using commercial test kits (Randox®-UK) as instructed by producers.

The statistical evaluation of data was made by student's t test using SPSS statistical package<sup>26</sup>.

## RESULTS

### Clinical Findings

All animals included in the study were determined as healthy on clinical examination.

### Serum Troponin I Consantration

Cardiac Troponin T concentrations were below 0.010 ng/ml therefore classification according gender and species was not possible. cTn-I concentrations determined for the groups and species did not significantly differ (P>0.05) (Table 1).

### Biochemical Results

Serum Troponin I concentrations of healthy ruminants according to species, gender and age are shown in the Table 1 and biochemical findings (CK, CK-MB, AST and LDH) are given in the Table 2. CK, CK-MB, AST and LDH concentration in gender and age groups are given Table 3 and Table 4 respectively.

The concentrations of AST, LDH, CK and CK-MB did

**Table 1.** Serum troponin I levels in healthy ruminants**Tablo 1.** Sağlıklı ruminantlarda serum troponin I düzeyleri

| Groups                 | N  | Cattle<br>Mean<br>(X <sub>min</sub> -X <sub>max</sub> ) | Sheep<br>Mean<br>(X <sub>min</sub> -X <sub>max</sub> ) | Goat<br>Mean<br>(X <sub>min</sub> -X <sub>max</sub> ) |
|------------------------|----|---|--|---|
| Overall<br>(ng/ml)     | 30 | 0.18<br>(0-0.23)  | 0.15<br>(0-0.21)                                       | 0.20<br>(0-0.24)                                      |
| Male<br>(ng/ml)        | 15 | 0.17<br>(0-0.19)  | 0.14<br>(0-0.18)                                       | 0<br>(0-0)  |
| Female<br>(ng/ml)      | 15 | 0.19<br>(0-0.23)  | 0.16<br>(0-0.21)                                       | 0.20<br>(0-0.24)                                      |
| 0-6 months<br>(ng/ml)  | 10 | 0.19<br>(0-0.21)  | 0.15<br>(0-0.21)                                       | 0<br>(0-0)  |
| 6-12 months<br>(ng/ml) | 10 | 0.15<br>(0-0.18)  | 0<br>(0-0)   | 0.20<br>(0-0.24)                                      |
| >12 months<br>(ng/ml)  | 10 | 0.20<br>(0-0.23)  | 0<br>(0-0)   | 0<br>(0-0)  |

**Table 2.** Muscle originated enzyme levels in healthy ruminants**Tablo 2.** Sağlıklı ruminantlarda kas kökenli enzim değerleri

| Parameters   | Cattle<br>(Mean±SEM)<br>(n=30) | Sheep<br>(Mean±SEM)<br>(n=30) | Goat<br>(Mean±SEM)<br>(n=30) |
|--------------|--------------------------------|-------------------------------|------------------------------|
| CK (IU/L)    | 56.71±7.31                     | 61.57±5.91                    | 45.96±2.64                   |
| CK-MB (IU/L) | 19.85±2.44                     | 30.07±2.24                    | 25.10±1.14                   |
| AST (IU/L)   | 59.16±6.54                     | 75.85±3.78                    | 58.0±1.56                    |
| LDH (IU/L)   | 993.4±49.21                    | 450.1±23.56                   | 327.8±14.0                   |

not significantly change in healthy ruminants according to species (*Table 2*) and gender (*Table 3*).

CK and CK-MB were higher in cattles aged 6-12 months old ( $P<0.001$ ) and aged 12 months old or over ( $P<0.001$ ) when compared to those aged 0-6 months old, but the value obtained for cattle aged 12 months or

**Table 3.** The distribution of muscle originated enzymes according to gender in healthy ruminants**Tablo 3.** Sağlıklı ruminantlarda kas kökenli enzim değerlerinin cinsiyete göre dağılımı

| Parameters      | Gender | N  | Cattle<br>(Mean±SEM) | N  | Sheep<br>(Mean±SEM) | N  | Goat<br>(Mean±SEM) |
|-----------------|--------|----|----------------------|----|---------------------|----|--------------------|
| CK<br>(IU/L)    | Male   | 13 | 63.3±12.3            | 14 | 69.9±10.0           | 15 | 46.2±4.44          |
|                 | Female | 15 | 49.4±7.13            | 15 | 53.2±5.6            | 15 | 45.7±3.13          |
| CK-MB<br>(IU/L) | Male   | 13 | 24.4±4.7             | 14 | 31.64±3.63          | 15 | 25.5±1.71          |
|                 | Female | 15 | 20.6±3.80            | 15 | 28.38±2.62          | 15 | 24.06±1.57         |
| AST<br>(IU/L)   | Male   | 13 | 65.90±12.63          | 14 | 69.42±3.65          | 15 | 60.06±2.42         |
|                 | Female | 15 | 53.2±5.16            | 15 | 82.7±6.41           | 15 | 55.93±1.92         |
| LDH<br>(IU/L)   | Male   | 13 | 951.8±82.4           | 14 | 419.5±29.6          | 15 | 342.3±20.5         |
|                 | Female | 15 | 1035.0±54.8          | 15 | 478.5±35.4          | 15 | 313.3±19.3         |

**Table 4.** The distribution of muscle originated enzymes according to age in healthy ruminants**Tablo 4.** Sağlıklı ruminantlarda kas kökenli enzim değerlerinin yaşa göre dağılımı

| Parameters      | Age         | N | Cattle<br>(Mean±SEM)       | N  | Sheep<br>(Mean±SEM)        | N  | Goat<br>(Mean±SEM)          |
|-----------------|-------------|---|----------------------------|----|----------------------------|----|-----------------------------|
| CK<br>(IU/L)    | 0-6 months  | 9 | 26.55±4.72                 | 10 | 94.6±11.3                  | 10 | 35.80±3.34                  |
|                 | 6-12 months | 9 | 96.0±8.9 ***               | 9  | 54.7±3.98 **               | 10 | 45.40±2.42 *                |
|                 | >12 months  | 8 | 62.6±5.23 *** <sup>b</sup> | 10 | 39.0±2.75 ** <sup>b</sup>  | 10 | 62.70±6.58 ** <sup>a</sup>  |
| CK-MB<br>(IU/L) | 0-6 months  | 9 | 9.88±1.67                  | 10 | 41.00±4.72                 | 10 | 20.80±1.58                  |
|                 | 6-12 months | 9 | 33.3±2.98 ***              | 9  | 29.7±2.48 *                | 10 | 26.00±1.15 *                |
|                 | >12 months  | 8 | 21.3±1.25 *** <sup>b</sup> | 10 | 21.60±1.40 ** <sup>a</sup> | 10 | 27.8±2.55 *                 |
| AST<br>(IU/L)   | 0-6 months  | 9 | 35.0±4.32                  | 10 | 65.37±3.89                 | 10 | 55.20±2.92                  |
|                 | 6-12 months | 9 | 89.5±12.8 **               | 9  | 94.33±7.46 **              | 10 | 60.80±2.42                  |
|                 | >12 months  | 8 | 56.00±6.53 * <sup>a</sup>  | 10 | 67.60±2.46 <sup>b</sup>    | 10 | 58.00±2.74                  |
| LDH<br>(IU/L)   | 0-6 months  | 9 | 892.3±91.3                 | 10 | 416.0±42.3                 | 10 | 395.0±18.5                  |
|                 | 6-12 months | 9 | 1125.6±82.3                | 9  | 550.6±35.4 *               | 10 | 321.4±17.1 **               |
|                 | >12 months  | 8 | 958.5±63.5                 | 10 | 386.9±25.4 <sup>b</sup>    | 10 | 267.1±19.2 *** <sup>a</sup> |

The statistical significance in the same column among the animals aged 0-6 months, 6-12 months and more than 12 months

\*  $P<0.05$ , \*\*  $P<0.01$  and \*\*\*  $P<0.001$

The statistical significance in the same column among the animals aged 6-12 months and more than 12 months

<sup>a</sup> $P<0.05$  and <sup>b</sup> $P<0.01$

over was higher than that of cattle aged 6-12 months ( $P < 0.01$ ). A gradual decrease in the concentrations of CK and CK-MB in sheep was observed as animals aged the concentrations significantly decreased (*Table 4*). An increase in CK concentrations was noted in goats over age 12 months or over however a fluctuating decrease was evident in CK-MB values in the same animals. AST values obtained in all ruminant aged 6-12 months were significantly higher than the other age groups except for goats. A fluctuation of LDH was determined in cattle and sheep in all age groups but a gradual decrease was determined in goats.

## DISCUSSION

Animals included in the study were healthy based on examination and cardiac troponin I and T values and other biochemical parameters were determined.

Cardiac Troponin I and T parameters are usually investigated for cardiac muscle injuries<sup>8,25,27,28</sup> and reported to attain sufficient sensitivity for acute cardiac damage<sup>8,29</sup>. Although cTn T was claimed to be specific for cardiac muscle<sup>29</sup>, cTn I was reported to have high sensitivity in the diagnosis of cardiac diseases<sup>30</sup>. In recent years their use as important markers of diseases causing cardiac damage in veterinary medicine was also reported<sup>17</sup>.

cTn I was reported to be 0-0.25 ng/ml in healthy cattle and 0.1-1.3 ng/ml in cattles with pericarditis<sup>31</sup>, Jesty et al.<sup>32</sup> reported a value of 0-0.04 ng/ml for healthy cattles, and Gunes et al.<sup>17</sup> reported cTn I levels as 0.052 ng/ml for healthy cattles and 0.39-7.74 ng/ml for cattles with pericarditis. Tunca et al.<sup>24</sup> reported cTn I to be 0.32 ng/ml in healthy lambs and 10.49 ng/ml in lambs with white muscle disease.

In this study, cTn I in each of three species was found to be 0-0.23 ng/ml (0.18 ng/ml) in cattles, 0-0.21 ng/ml (0.15 ng/ml) in sheeps and 0-0.24 ng/ml (0.20 ng/ml) in goats. The results obtained for sex and age groups (0-6 months, 6-12 months, and 12 months or over) were within the reference range. The results obtained for cattle and sheep are in congruity with the data reported by many other researches<sup>17,24,31-33</sup>. Shober and Kirbach<sup>20</sup> reported cTn T levels in healthy dogs ranging between minimum 0 ng/ml and maksimum 0 ng/ml. In humans, detected Tn T levels below 0.05 ng/mL indicate no myocardial damage, levels between 0.05-1.0 ng/mL suspicious positive and levels between 0.1-2.0 ng/mL show myocardial damage<sup>19</sup>.

In this study, Tn T levels in all ruminants were detected to be 0.010 ng/ml and these findings are in parallel with

the results of other researchers<sup>19,20</sup>. This finding also confirms that the ruminants used in our study were clinically healthy.

No data is available with regard to cTn I and cTn T levels in goats. The made discussion of the data obtained here difficult, however the values were similar to the figures reported for other ruminant species. Consequently, serum cTn I and cTn T levels obtained for goats in this study may be of help for future studies

Among biochemical parameters for the diagnosis of cardiac muscular diseases, CK-MB, LDH ve AST have commonly been used. However many disadvantageous of these parameters have been reported and thus these parameters were replaced with newly developed cardiac markers such as cTn I and cTn T. CK enzyme exists in high amounts in striated muscles. Increased serum activity indicates skeletal or cardiac muscle injury<sup>5</sup>. The normal level of CK in cats has been reported as 0-195 U/L. The reference values for CK was reported to be 44-228 IU/L in cattles<sup>34</sup>, 39-97.5 IU/L in sheeps<sup>35</sup> and less than 200 IU/L in goats<sup>36</sup>. AST is mostly localized in muscle cells. They are found in cardiac muscle and liver in lower amounts. AST is not an organ specific enzyme. The reference levels for AST are reported to be 48-132 IU/L in cattles, 60-280 IU/L in sheeps<sup>9</sup> and 68-94 IU/L in goats<sup>37</sup>. LDH levels are increased during skeletal muscle diseases, cardiac muscle diseases and cellular liver diseases<sup>3</sup>. In cardiac diseases including trauma, necrosis, neoplasia and tissue degeneration, LDH activity is low<sup>3</sup>. The reference values of LDH is reported to be 692-1445 IU/L in cattles<sup>9</sup>, 60-440 IU/L in sheeps<sup>3,7</sup> and 123-393 IU/L in goats<sup>9</sup>. In this study, CK, CK-MB, AST and LDH levels were found to be within the limits for each three species and sex suggested by other researchers<sup>3,7,34-37</sup> (*Table 2* and *Table 3*). Nevertheless, statistically higher levels in some age groups (*Table 4*) may be related to some other factors leading to changes in physiological conditions such as increased metabolism depending on growth and development and different care and nutrition status of the animals used in the study.

In conclusion the results regarding to cardiac troponins may serve as complementary data to already known information as values of healthy subject especially those obtained for goats.

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