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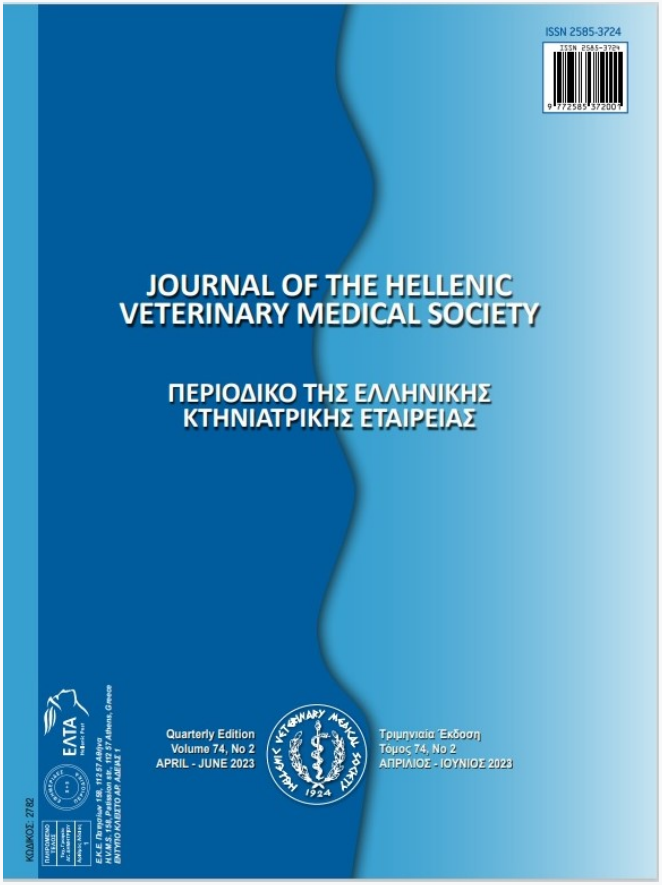

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Research article
Ερευνητικό άρθρο

Investigation of Acute Phase Proteins in Cattle Infected with *Mycobacterium bovis*

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ABSTRACT: Tuberculosis (TB) is a contagious and zoonotic disease that adversely affects human and animal health, caused by the formation of tubercules in a caseous character in the lungs, other tissues and organs. The causative of the disease is *Mycobacterium bovis*. An increase or decrease in acute phase protein (APP) levels is observed in various bacterial, viral and parasitic diseases in cattle. Therefore, in this study, it was aimed to determine the changes in the levels of APPs in cattle infected with *M. bovis*. In the study, 26 tuberculosis suspected and 10 healthy cattle blood serums collected from various enterprises in Samsun were used. Whole blood samples were subjected to gamma interferon ELISA test by drawing blood from the vena jugularis of cattle into tubes containing heparin. Haptoglobin (Hp), serum amyloid A (SAA), albumin and total protein (TP) levels, which are among the acute phase proteins, were determined in the blood serums taken from the groups. As a result of the analysis, it was found haptoglobin 1.166 g/L, SAA 40.353 µg/mL, total protein 73.2 mg/dL and albumin 3.703 mg/dL in the group infected with *M. Bovis*, while it was found haptoglobin 0.235 g/L, SAA 20.300 µg/mL, total protein 20.37 mg/dL and albumin 3.71 mg/dL in the healthy group. When the APP levels of the cattle infected with *M. bovis* and the cattle in the healthy group were compared, it was determined that haptoglobin ($p < 0.01$), serum amyloid A ($p < 0.01$) and total protein ($p < 0.01$) levels, which are APPs increased statistically significantly compared to the healthy group, while the albümin ($p > 0.05$) level decreased, but there was no statistically significant difference.

Keywords: acute phase protein (APP); cattle; *Mycobacterium bovis*; tuberculosis (TB)

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INTRODUCTION

Bovine tuberculosis is a zoonotic and economically important disease. It is subjected to tests and controls in slaughterhouse cutting in many countries (Schiller, 2010). The causative of the disease is *Mycobacterium bovis*, which aerobic, acid-resistant and sporeless bacterium (Gortazar et al., 2005). Although the main host of this disease is cattle, the disease can also be transmitted to various animals, especially domestic animals such as goats, sheep, horses, and wild animals such as foxes and jackals (Ramos et al., 2015; Pollock and Neill, 2002). Animals such as cattle, pigs and goats, which are especially economically important, are very susceptible to infection with *M. bovis*. In addition, *M. bovis* also threatens human health due to its zoonotic character (Cinoğlu, 2021). Therefore, the disease has important implications for veterinary medicine and public health (Amanfu, 2006). There are many ways that cattle can become infected with *M. bovis*. However, this can be affected by animal age, behavior, environment, climate and current farming practices (Pritchard, 1988). Tuberculosis, which is often transmitted through the respiratory tract, can also reach through the gastrointestinal tract. The lesion is usually found, in addition to being found in lymph nodules next to the lung, It is also detected in head, intestine, liver, spleen, and peritoneal nodules (Menzies and Neill, 2000). Lesions may occur with lymphatic spread, natural channels such as the bronchi, and hematogenous spread when massive miliary tuberculosis occurs. Unlike humans, lesions in cattle can be controlled by an immune response. The role of activated mononuclear macrophages in protection against its host, *M. bovis*, is considered important. These macrophages plays a role presenting to T-lymphocytes. Which the immune response to mycobacteria and a key recognition unit in processing mycobacterial antigens (Thoen and Himes, 1986). For this reason, in whole blood or monocular cell culture adapted with bovine antigens (ESAT-6, CFP-10) with the Gamma Interferon (IFN-γ) test used in the diagnosis of tuberculosis, the level of IFN-γ released from sensitive lymphocytes within 16-24 hours, Measured by sandwich ELISA using monoclonal antibovine IFN-γ antibodies (Rua-Domenech, 2006; Pollock and Andersen, 1997).

Acute phase proteins (APP) is a protein synthesized by the liver that occurs in response to stimuli such as tissue damage, infection and inflammation that cause the acute phase response (APR) (Eckersall and Bell, 2010; Murata et al., 2004; Petersen et al., 2004). Levels of APPs found insignificant in healthy animals, while increase rapidly in the presence of inflammation. Therefore, these proteins play a role as an indicator of inflammation (Petersen et al., 2004; Nikunen et al., 2007). The main causes of tissue destruction constituting the acute phase response can be of traumatic, neoplastic, immunological, parasitic infective origin (Gruys et al., 1994). The function of APR is to clean the molecules and residues that are harmful in the organism, to eliminate the infectious agents and to provide homeostasis by initiating the necessary repair process for the organism to return to its normal functions (Habif, 2005). Production of APPs is accelerated by some mediators and suppressed by others. While accelerating mediators form various mediators, especially interleukin 6 (IL-6), accelerating mediators, suppressing mediators are reported as insulin and okadaic acid (Onat et al., 2002). L-6 and IL-1 are inflammatory cytokines that activate endothelial and fibroblast cells in the local inflammatory region. In this way, by starting the secretion of cytokines again, a systemic inflammatory response is initiated thanks to the secondary cytokines that pass into the circulatory (Petersen et al., 2004). APPs can result in more than 200 protein exchange grouped as positive and negative. Albumin is defined as a major negative acute phase protein and its level in the blood decreases during the acute phase reaction. Serum amyloid A (SAA) and haptoglobin (Hp) are positive acute phase proteins and their blood levels increase during the acute phase reaction (Cray et al., 2009). Although the concentrations in the blood of these proteins the vary according to animal species, they are APPs of some diagnostic importance in sheep and cattle (Tothova et al., 2014). Especially, it is routinely used in the diagnosis and prognosis of the disease clinically (Eckersall et al., 2007). Low or high of protein levels are great importance in the diagnosis and prognosis of inflammation, tissue damage, infection and tumoral formations (Abdulkhaleq et al., 2018). However, there are limited studies on the biochemistry of tuberculosis and acute phase proteins in veterinary medicine (Javed et al., 2006; Lopez-Olvera et al., 2013; Miller et al., 1989; Moda et al., 1996). For this reason, this study was aimed, considering it may be useful the determination of APP levels in cattle infected with *M. bovis* in the diagnosis and prognosis monitoring and in the detection of the acute-chronic phase of the disease.

MATERIALS AND METHODS

In this study, 26 piece of tuberculosis suspected

and 10 healthy 2-5 year old female cattle blood serums collected from various business in Samsun were used. In the province of Samsun, where the study was conducted, tuberculosis infection in cattle is not very common. Researchers were able to reach 54 cattle showing clinical symptoms of tuberculosis infection at the time of the study. Among these cattle, those with brucella etc. diseases and those with negative test results despite showing tuberculosis symptoms were not included in the study. As a result of the power analysis, the number of animals with positive tuberculosis test to be used in the study was determined as 26. In addition, 10 cattle that did not show the clinical symptoms of tuberculosis infection, were evaluated as completely healthy when their general health status was examined, and gave a negative result in the tuberculosis infection skin test were identified. This number was considered sufficient as the control group. Whole blood samples taken from *vena jugularis* of cattle into heparinized tubes were subjected to gamma interferon ELISA test. Those who showed positive results in the whole blood gamma interferon ELISA test from suspected cattle showing clinical symptoms of tuberculosis infection were included in the study. Again for the control group, those who gave negative results in the gamma interferon ELISA test from whole blood taken from 10 cattle that were examined in terms of both tuberculosis infection and general health status and were evaluated as completely healthy were included in the control group. It was considered sufficient that the cattle included in the control group did not carry tuberculosis infection. Because the main target in the study is the change of acute phase proteins in the presence and absence of tuberculosis infection, and its evaluation in terms of other infections is not within the scope of the study. The study was carried out in accordance with the ethics committee decision numbered 2022/4 taken in accordance with the "Ethics Committee Directive" of the Animal Experiments Local Ethics Committee of the Samsun Veterinary Control Institute of the Ministry of Agriculture and Forestry.

Gamma Interferon ELISA testi: Approximately 5 mL of blood was taken from tuberculosis suspected and healthy cattle into heparinized blood collection tubes and quickly delivered to the laboratory. Preliminary preparation and ELISA test were performed according to the instructions of the kit manufacturer (Bovigam TB Kit). For this purpose, each blood sample was divided into three wells as 1 mL in 24-well sterile cell culture slides. The procedures were continued according to the procedure specified in the kit, and the results were evaluated by reading the optical densities (OD) in the ELISA plate reader (Mindray MR96A). The tests on the plates that gave a result of 0.7 and above for the positive control and 0.15 or less for the negative control were considered valid. Samples with a cattle PPD well OD-Avian PPD well OD $\geq$ 0.1 value were considered positive, and cattle PPD well OD-Avian PPD well OD < 0.1 values were considered negative.

Biochemical Analysis

The blood samples were centrifuged at 3000 rpm for 10 minutes and their serums were separated. Biochemical analysis was performed with the obtained serums. Haptoglobin and serum amyloid A analyzes (Tridelta Development Limited, Ireland) were measured in the ELISA plate reader (Tecan Infinite F50) with Elisa test kits.

The serum amyloid A kit is a solid-phase sandwich enzyme-linked immunosorbent assay. A monoclonal antibody specific to SAA is covered on the wells of the microtiter strips. When standards and serum samples with known SAA content are added to these wells, SAAs bind to specific monoclonal antibodies. Then, it incubated at 37°C added HRP-labeled anti-SAA antibody into the wells. At the end of the incubation, the wells are washed to remove unbound material. A blue product is obtained direct proportion to the amount of SAA present in the original sample or calibrator with the addition of TMB. Stop reagent is added to stop the reaction. As a result of these procedures performed in accordance with the procedure, the OD of the measurement were read and evaluated.

In the Haptoglobin kit, on the other hand, deoxyhemoglobin exhibits inhibited peroxidase activity at a low pH, and haptoglobin in the sample combines with hemoglobin. Thus, at a low pH, it preserves the peroxidase activity of deoxyhemoglobin. The maintenance of peroxidase activity of hemoglobin is directly proportional to the amount of haptoglobin present in the sample. Based on this principle, analyzes were performed according to the procedure in the commercial test kit and the results were calculated according to the OD read.

Total protein and albumin levels were measured in an autoanalyzer (Biosistem A25, Spain) device using Biosistem kits.

Statistical Analysis

SPSS (version 17.0) program was used for statistical analysis. In order to determine the normality of the distribution, it was determined that it was a normal distribution by looking at the skewness, kurtosis values and Kolmogorov-Smirnov test. T test was used to compare the groups for the groups showed normal distribution. P values less than 0.05 were considered significant. Pearson test was used to determine the correlation between the groups since the data obtained showed normal distribution.

RESULTS

In the study, some acute phase protein values were determined in blood samples taken from *M. bovis* positive cattle and *M. bovis* negative cattle. Serum acute phase protein values of healthy and *M. bovis* infected cattle are given in Table 1.

When the acute phase proteins of *M. bovis* negative and *M. bovis* positive cattle were compared, haptoglobin, serum amyloid A and total protein levels of *M. bovis* infected cattle increased statistically significantly ($P < 0.01$) compared to *M. bovis* negative cattle, It was determined that the levels of the albumin decreased but there was no statistically significant difference ($P > 0.05$).

Haptoglobin levels were determined to be higher in *M. bovis* infected cattle (1.166 ± 0.715 g/L) compared to healthy cattle (0.235 ± 0.141 g/L) ($P < 0.01$) and are given in Figure 1.

Serum Amyloid A levels were determined to be higher in *M. bovis* infected cattle (40.353 ± 30.228 µg/mL) compared to healthy cattle (20.300 ± 7.203 µg/mL) ($P < 0.01$) and are given in Figure 2.

Total protein levels were determined to be higher in *M. bovis* infected cattle (73.2 ± 8.361 mg/dL) compared to healthy cattle (27.37 ± 3.687 mg/dL) ($P < 0.01$) and are given in Figure 3.

Albumin levels were determined to be higher in *M. bovis* infected cattle (3.703 ± 0.302 mg/dL) compared to healthy cattle (3.71 ± 0.251 mg/dL) ($P > 0.05$) and are given in Figure 4.

As a result of the data we obtained, no significant correlation was found between the groups as a result of the Pearson test performed to determine the correlation between the groups.

Table 1. Haptoglobin, serum amyloid A, albumin and total protein levels in clinically healthy and *M. bovis* infected cattle (mean ± SE)

Parameters	Healthy group	Group infected with *M. bovis*	P value
Haptoglobin (g/L)	0.235 ± 0.141	1.166 ± 0.715	P < 0.01
SAA (µg/mL)	20.300 ± 7.203	40.353 ± 30.228	P < 0.01
Total protein (mg/dL)	27.37 ± 3.687	73.2 ± 8.361	P < 0.01
Albumin (mg/dL)	3.71 ± 0.251	3.703 ± 0.302	P > 0.05

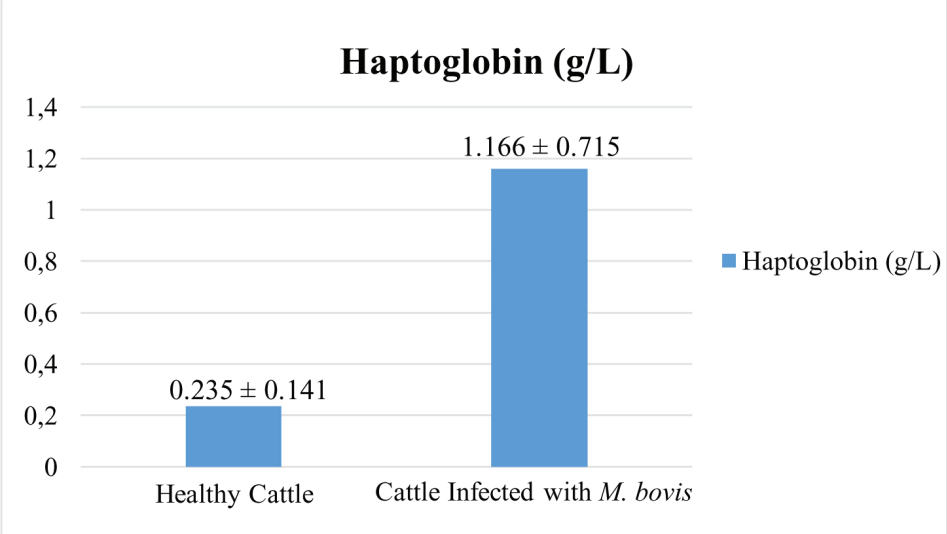

Figure 1. Haptoglobin levels in the serum of healthy and *M. bovis* infected cattle

Table 2. T test results of haptoglobin levels in healthy and *M. bovis* infected cattle

	Levene's Test for Equality of Variances		t-test for Equality of Means						
Haptoglobin								95% Confidence Interval of the Difference	
	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Equal variances assumed	16.192	0.000	-3.626	42	0.001	-0.889768	0.245368	-1.384941	-0.394596
Equal variances not assumed			-6.746	41.138	0.000	-0.889768	0.131896	-1.156110	-0.623427

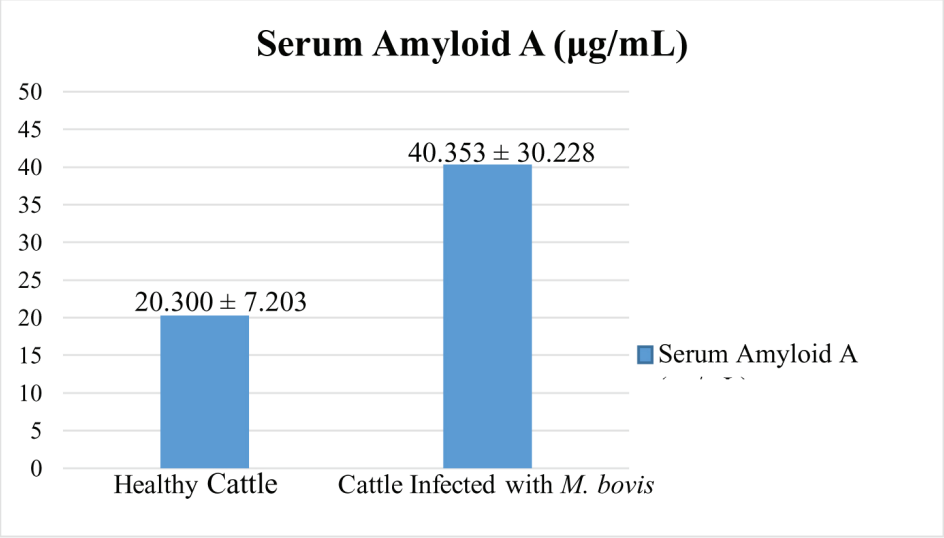

Figure 2. Serum amyloid A levels in the serum of healthy and *M. bovis* infected cattle

Table 3. T test results of serum amyloid A levels in healthy and *M. bovis* infected cattle

	Levene's Test for Equality of Variances		t-test for Equality of Means						
Serum amyloid A								95% Confidence Interval of the Difference	
	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Equal variances assumed	12.339	0.001	-2.056	32	0.048	-20.05267	9.752260	-39.91737	-0.187963
Equal variances not assumed			-3.049	28.350	0.005	-20.05267	6.577286	-33.51813	-6.58720

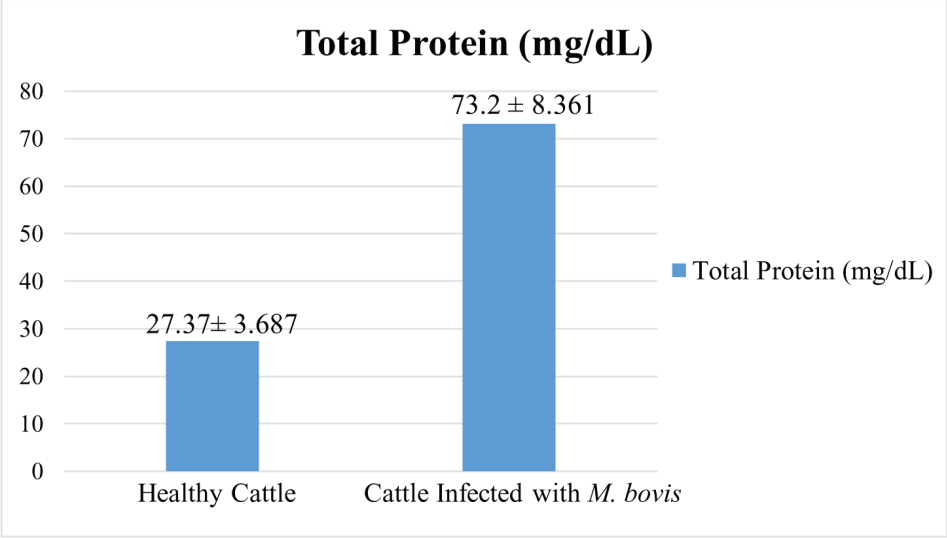

Figure 3. Total Protein levels in the serum of healthy and *M. bovis* infected cattle

Table 4. T test results of total protein levels in healthy and *M. bovis* infected cattle

	Levene's Test for Equality of Variances		t-test for Equality of Means						
Total protein								95% Confidence Interval of the Difference	
	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Equal variances assumed	6.246	0.017	-16.606	34	0.000	-45.8300	2.7599	-51.4387	-40.2213
Equal variances not assumed			-22.777	33.139	0.000	-45.8300	2.0122	-49.9231	-41.7369

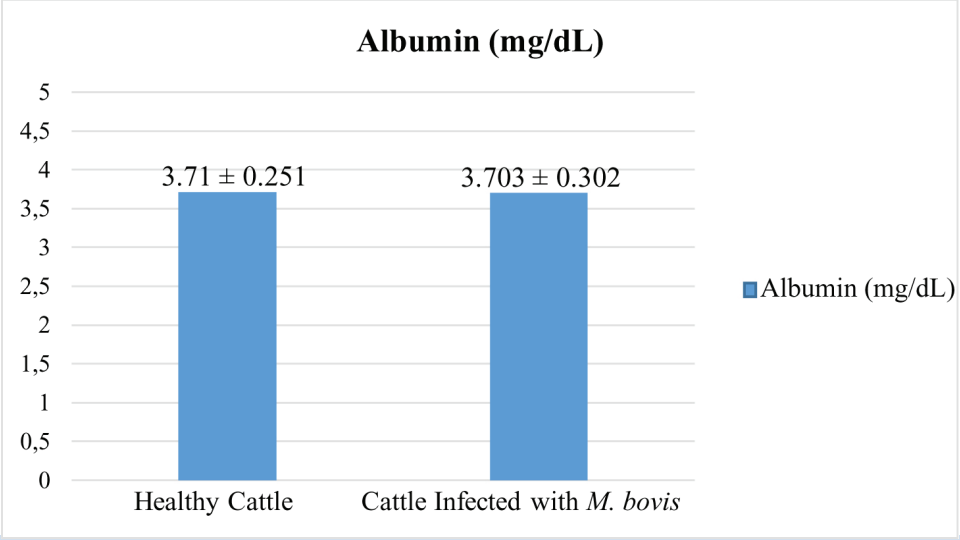

Figure 4. Albumin levels in the serum of healthy and *M. bovis* infected cattle

Table 5. T test results of albumin levels in healthy and *M. bovis* infected cattle

	Levene's Test for Equality of Variances		t-test for Equality of Means						
Albumin								95% Confidence Interval of the Difference	
	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Equal variances assumed	0.600	0.444	0.057	34	0.955	0.0062	0.1079	-0.2131	0.2254
Equal variances not assumed			0.062	19.629	0.951	0.0062	0.0992	-0.2011	0.2134

DISCUSSION

Bovine tuberculosis is a chronic infection characterized by the formation of white-yellow, calcified granuloma, which occurs due to the relationship between bacilli and the host's inflammatory cells (Sayın and Erganiş, 2010). In this disease, acute phase proteins can be a guide in addition to radiological follow-up, when the response to treatment is monitored bacteriologically (Yanagisawa, 1996). APPs produced during this response, which occurs in cases of infection and inflammation, can generally directly destroy the agents that cause inflammation and help the healing and regeneration of the tissue (Gruys et al., 1994; El-Deeb and El- Bahr, 2017). It has been reported that APP blood concentrations increase and decrease according to animal species and diseases (Gruys et al., 2005; Petersen et al., 2004). Among these proteins, SAA and Hp are the most important acute phase proteins known in cattle (Erkılıç et al., 2019). In our study, it was aimed to determine the APP levels in cattle infected with *M. bovis*, and it is thought that it can be included among the parameters that can be routinely checked in cases of suspected tuberculosis, in line with the data obtained. In addition, it is thought that it may be useful in understanding whether acute or chronic, in addition to the clinical findings of tuberculosis depending on the status of acute phase proteins.

Although Hp and SAA levels with important positive APPs in cattle, are quite low in healthy cattle, their levels increase in the presence of inflammation (Gruys et al., 2004; Petersen et al., 2004). Although Hp is either absent or at very low levels (<0.1g/L) in healthy cattle serum (Eckersall and Conner, 1988), its level can increase 50-100 times in the acute phase response. This makes Hp the most important APP in cattle (Alsemgeest et al., 1994). One of the functions of Hp is to is to render harmless by hydrolyzing the peroxides in the inflammation area and released from neutrophils. It also acts as an immunomodulator in lipid metabolism regulation and lymphocyte functions (Ametaj et al., 2011). For this reason, it is stated that Hp concentration can be used in monitoring the immune functions of cattle (Murata and Miyamoto, 1993). In natural and experimental studies, it has been determined that the levels of Hp increase in bacterial, viral and parasitic diseases such as brucella, tuberculosis, alum, *Toxocara vitulorum*, hypodermosis (Bozukluhan et al., 2016; Merhan et al., 2017). Another important APP in cattle is SAA (Murata et al., 2004). Although the concentration of this protein is quite low in healthy cattle, its concentration increases in cases of inflammation. Therefore, it is considered to be a highly sensitive APP against inflammation (Hayes, 1994). In the studies carried out, it was stated that SAA increases after bacterial (Horadagoda et al., 1994; Horadagoda et al., 1993) and viral infections (Heegard et al., 2000; Ganheim et al., 2003), ketosis (Karreman et al., 2000) and endotoxin applications (Boosman et al., 1989). It has been reported that it can be used in the early diagnosis of acute cases for SAA levels increase within 2-5 hours and reach their peak within 24 hours SAA stimulates inflammatory cells in tissues, prevents from losing their structure due to oxidation of leukocytes and manages the immune response (Ceron et al., 2005). When these two APPs are evaluated together, it has been reported to be important in the differential diagnosis of acute and chronic cases (Alsemgeest et al., 1994; Horadagoda et al., 1999). Merhan et al. (2017) in a study, stated that the levels of Hp and SAA, which are positive acute phase proteins in *M. Bovis* infected cattle, significant increase in *M. bovis* infected cattle compared to healthy cattle. Horadogada et al. (1999), in their study of 81 acute and chronic sick cattle, found that Hp

levels increased 68% in the acute phase, 24% in the chronic phase, and SAA levels increase 100% in the acute phase and 54% in the chronic phase. Espinosa et al. (2020) in another study, stated that Hp and SAA values increased significantly in cattle with paratuberculosis compared to healthy cattle. In a study on camels, Hp and SAA levels were found to be higher in camels with tuberculosis than in healthy camels (El-Deeb et al., 2014). Vicente et al. (2019), in a study, it was determined that the serum Hp concentration in 88 red deer (*Cervus elaphus*) naturally infected with tuberculosis was higher than in red deer (*C. elaphus*) negative with tuberculosis. Duran and Cenesiz (2019), in a study, while the serum haptoglobin level in cattle with paratuberculosis was found to be higher in infective cattle (0.820 ± 0.615 mg/mL) compared to healthy cattle (0.354 ± 0.177 mg/mL), the serum SAA level was also found to be higher in cattle with paratuberculosis (369. 9 ± 17.0 ng/mL) compared to healthy cattle (253.1 ± 82.7 ng/mL). In another study conducted in 35 naturally infected Saanen goats with paratuberculosis and 10 negative Saanen goats with paratuberculosis in the control group, SAA and Hp levels were found to be high in saanen goats with paratuberculosis (Sevgisunar ve Şahinduran, 2021). In our study, Hp and SAA levels were found to be statistically significantly higher in *M. bovis* infected cattle compared to healthy cattle. It was seen that the results of our study were similar to the results of the studies of other researchers. The purpose of acute phase proteins is to prevent further damage to a tissue or organ, to limit the proliferation of the infected organism, and to initiate the repair process necessary for the tissue or organ to be revert (Habif, 2005). It is thought that the reason for the increase in SAA and Hp concentrations may be related to the tissue damage and the severity of the infection in cattle. In this study, it is thought that the levels of SAA and Hp increased due to the infection in cattle infected with *M. bovis*.

It has been reported that the level of albumin, which is a negative APP, decreases during AFR (Coşkun and Şen 2011; Gökce and Bozukluhan 2009). In this study, it was determined that the level of albumin in *M. bovis* infected cattle was lower than in healthy cattle, but there was no statistically significant difference. In a study conducted in camels, it was determined that the serum albumin level was lower in camels with tuberculosis compared to healthy camels (El-Deeb et al., 2014). In another study conducted in cattle with paratuberculosis, the level of albumin was found to be lower in the infective group (2.977 ± 0.699 mg/dL) compared to the control group (3.713 ± 0.407 mg/dL) (Duran ve Cenesiz, 2019). Opolot et al. (2015), in another study, albumin levels of 71 cattle with diagnosed tuberculosis and 89 control patients were found to be lower in tuberculosis-positive cattle compared to the control group. It is thought that reason for this may be due to a decrease caused by hypoproteinemia or the infiltration of albumin into the damaged tissue. It is also thought that it may be due to the AFR that occurs or due to the increased consumption of albumin in animals. The decrease in albumin level in the current study is similar to the results of other studies. In this study, the level of total protein was found to be statistically significantly higher in *M. bovis* infected cattle compared to healthy cattle. It is thought that the reason for this may be that the level of total protein increased as a result of the increase in the level of globulin due to increased APPs.

CONCLUSIONS

As a result of the data we obtained in our study, it was determined that acute phase proteins occur in cattle infected with *M. bovis*. It was determined that while serum amyloid A and haptoglobin levels, which are positive acute phase proteins, increased statistically in cattle infected with *M. bovis*, the decrease in albumin level, which is a negative acute phase protein, was not statistically significant, but decreased compared to cattle infected with *M. bovis*. In addition, total protein levels were found to be statistically significantly increased in cattle infected with *M. bovis*. When these results are evaluated, acute phase proteins can be a guide in monitoring the prognosis n this disease. Therefore, it is thought that these proteins can be used to identify cattle infected with *M. bovis*, which have pathological forms associated with potent cell-mediated immune responses. In addition, it is thought that acute phase proteins can be useful in evaluating the prognosis of cattle tuberculosis as well as understanding whether it is acute or chronic. It is thought that determining acute phase proteins in acute and chronic tuberculosis and carrying out studies to determine the levels of these parameters, it can be used routinely to diagnose the disease with these parameters, as well as to determine the status of the disease phase.

CONFLICT OF INTEREST

There is no declared conflict of interest by the authors.

REFERENCES

Abdulkhaleq LA, Assi MA, Abdullah R, ZamriSaad M, Taufiq-Yap YH, Hezmee MNM (2018) The crucial roles of inflammatory mediators in inflammation. Veterinary World 11(5): 627-635.

Alsemgeest SPM, Lalsbeek HC, Wensıng T, Koeman JP, Van Ederen AM, Gruys E (1994) Concentrations of serum amyloid-A (SAA) and haptoglobin (Hp) as parameters of inflammatory diseases in cattle. Veterinary Quarterly 16(1): 21-23.

Ametaj BN, Hosseini A, Odhiambo JF, Iqbal S, Sharma S, Deng Q, Lam TH, Farooq U, Zebeli Q, Dunn SM (2011) Application of acute phase proteins for monitoring inflammatory states in cattle. Acute Phase Proteins as Early Non-Specific Biomarkers of Human and Veterinary Diseases; DOI: 10.5772/19492 · Source: InTech

Boosman R, Niewold TA, Mutsaers CW, Gruys E (1989) Serum amyloid A concentrations in cows given endotoxin as acute phase stimulant. American Journal of Veterinary Research 50(10): 1690-94.

Bozukluhan K, Merhan O, Büyük F, Çelebi Ö, Gökçe G (2016) Determination of some acute phase proteins level in cattle with brucellosis. Ankara University Veterinary Faculty journal 63(1): 13-16.

Ceron JJ, Eckersall PD, Martinez-Subiela S (2005) Acute phase proteins in dogs and cats: current knowledge and future perspectives. Veterinary Clinical Pathology 34(2): 85-99.

Cinoğlu H (2021). Adıyaman ilinde tüberküloz şüphesi olan sığırlarda *Mycobacterium bovis* varlığının araştırılması ve epidemiyolojik tiplendirilmesi. Yüksek Lisans Tezi, Tıbbi mikrobiyoloji, Adıyaman.

Coşkun A, Şen İ (2011) Sığırlarda akut faz proteinleri ve klinik kullanım alanları. Sağlık Bilimleri Dergisi 20(3): 240-246.

Cray C, Zaias J, Altman NH (2009) Acute phase response in animals. Comparative Medicine 59(6): 517-526.

Duran U, Cenesiz S (2019) Determination of acute phase protein levels in cattle with paratuberclosis. International Journal of Advances in Science Engineering and Technology 7(1-2).

Eckersall PD, Bell R (2010) Acute phase proteins: biomarkers of infection and inflammation in veterinary medicine. Veterinary Journal 185(1): 23-7.

Eckersall PD, Conner JG (1988) Bovine and canine acute phase proteins. Veterinary Research Communications 12: 169-178.

Eckersall PD, Lawson FP, Bence L, Waterston MM, Lang TL, Donachie W, Fontaine MC (2007) Acute phase protein response in an experimental model of ovine caseous lymphadenitis BMC Veterinary Research 3(35): 1-6.

El-Deeb WB, El-Bahr SM (2017) Biomarkers of ketosis in dairy cows at postparturient period: acute phase proteins and pro-inflammatory cytokines. Veterinarski Arhiv 87(4): 431-440.

El-Deeb WM, Fouda TA, El Bahr SM (2014) Clinico-biochemical investigation of paratuberculosis of dromedary camels in Saudi Arabia: Proinflammatory cytokines, Acute phase proteins and oxidative stress biomarkers. Pakistan Veterinary Journal 34(4): 484-488.

Erkılıç EE, Merhan O, Kırmızıgül AH, Öğün M, Akyüz E, Çitil M (2018) Salivary and serum levels of serum amyloid a, haptoglobin, ceruloplasmin and albumin in neonatal calves with diarrhoea. Kafkas University Veterinary Faculty Journal 25(4): 583-586.

Espinosa J, Morena R, Benavides J, García-Pariente C, Fernández M, Tesouro M, Arteche N, Vallejo R, Ferreras MC Pérez V (2020) Assessment of Acute-Phase Protein Response Associated with the Different Pathological Forms of Bovine Paratuberculosis. Animals 10(10): 1925.

Ganheim C, Hulten C, Carlsson U (2003). The acute phase response in calves experimentally infected with bovine viral diarrhea virus and / or Mannheimia haemolytica. Journal of Veterinary Medicine. B, Infectious Diseases and Veterinary Public Health 50(4): 183-190.

Gortazar C, Vicente J, Samper S, Garrido JM, Fernandez-De-Mera IG, Gavin P, Juste RA, Martin C, Acevedo P, De La Puente M, Höfle U (2005) Molecular characterization of *Mycobacterium* tuberculosis complex isolates from wild ungulates in south-central Spain. Veterinary Research 36(14): 3-52.

Gökce Hİ, Bozukluhan K (2009) Çiftlik hayvanlarında önemli akut faz proteinleri ve bunların veteriner hekimlik alanındaki kullanımı. Dicle Üniversitesi Veteriner Fakültesi Dergisi 1(1): 1-14.

Gruys E, Obwolo MJ, Toussaint MJM (1994) Diagnostic significance of the major acute phase proteins in veterinary clinical chemistry. Veterinary Bulletin 64(11): 1009-18.

Gruys E, Toussaınt MJM, Nıewold TA, Koopmans SJ (2005) Acute phase reaction and acute phase proteins. Zhejiang University Science 6B(11): 1045-1056.

Habif S (2005). İnflamatuar yanıtta akut faz proteinler. İzmir Atatürk Eğitim Hastanesi Dergisi 43 (2): 55-65.

Hayes MA (1994). Functions of cytokines and acute phase proteins in inflamation. In: Lumsden J.H. (ed) VIth Congress of the ISACB Proceedings. Guelp Canada, 1-7.

Heegard PMH, Godson DL, Toussaint MJM (2000) The acute phase response of haptoglobin and Serum Amyloid A in cattle undergoing experimental infection with bovine respiratory syncytial virus. Veterinary Immunology and Immunopathology 77(1): 151-159.

Horadagoda A, Eckersall PD, Hodgson JC, Gibbs HA, Moon GM (1993) Purification and quantitative measurement of bovine SAA. Research Veterinary Science 55: 317-25.

Horadagoda A, Eckersall PD, Hodgson JC, Gibbs HA, Moon GM (1994) Immediate responses in serum TNF-α and acute phase protein concentrations to infection with *P. haemolytica* A1 in calves. Research Veterinary Science 57: 129-132.

Horadagoda NU, Knox KMG, Gibbs HA, Reid SWJ, Horadagoda A, Edwards SER, Eckersall PD (1999) Acute phase proteins in cattle discrimination between acute and chronic inflammation. Veterinary Research 144: 437-41

Javed MT, Usman M, Irfan M, Cagiola MA (2006) Study on tuberculosis in buffaloes: some epidemiological aspects, along with haematological and serum protein changes. Veterinarski Arhiv 76(3): 193-206.

Karreman HJ, Wentink GH, Wensing T (2000) Using serum amyloid A to screen dairy cows for subclinical inflammation. Veterinary Quarterly 22(3): 175-178.

Lopez-Olvera JR, Fernandez-de-Mera IG, Serrano E, Vidal D, Vicente J, Fierro Y, Gortazar C (2013) Sex-related differences in body condition and serum biochemical parameters in red deer (*Cervus elaphus*) naturally infected with *Mycobacterium bovis*. Veterinary Journal 198(3): 702-6.

Menzies FD, Neill SD (2000) Cattle-to-cattle transmission of bovine tuberculosis. Veterinary Journal 160(2): 92-106.

Merhan O, Bozukluhan K, Gokce HI (2017) Acute phase proteins and biochemical and oxidative stress parameters in Hypoderma spp. infested cattle. Journal Hellenic Veterinary Medical Society 68: 35-540.

Miller LD, Thoen CO, Throlson KJ, Himes EM, Morgan RL (1989) Serum biochemical and hematologic values of normal and *Mycobacterium bovis* infected American bison. Journal of Veterinary Diagnostic Investigation 1(3): 219-22.

Moda G, Daborn CJ, Grange JM, Cosivi O (1996) The zoonotic importance of *Mycobacterium bovis*. Tubercle and Lung Disease 77(2): 103-8.

Murata H, Miyamoto T (1993) Bovine haptoglobin as a possible immunomodulator in the sera of transported calves. The British Veterinary Journal 149(3): 277.

Murata H, Shimada N, Yoshioka M (2004) Current research on acute phase proteins in veterinary diagnosis: an overview. Veterinary Journal 168(1): 28-40.

Nikunen S, Hartel H, Orro T (2007) Association of bovine respiratory disease with clinical status and acute phase proteins in calves. Comparative Immunology, Microbiology and Infectious Diseases 30(3): 143-51.

Onat T, Emerk K, Sözmen EY (2002) İnsan Biyokimyası, Palme Yayıncılık Ankara.

Opolot JO, Theron AJ, Anderson R, Feldman C (2015) Acute Phase Proteins and Stress Hormone Responses in Patients with Newly-Diagnosed Active Pulmonary Tuberculosis. Lung 193(19): 13-8.

Petersen HH, Nielsen JP, Heegaard PM (2004) Application of acute phase protein measurements in veterinary clinical chemistry. Veterinary Research 35(2): 163-87.

Pollock JM, Andersen P (1997) Predominant recognation of the ESAT-6 protein in the first phase of infection with *M. bovis* in cattle. Infection and Immunity 65(7): 2587-2592.

Pollock JM, Neill SD (2002) *Mycobacterium bovis* infection and tuberculosis in cattle. Veterinary Journal 163(2): 115-27.

Pritchard DG (1988) A century of bovine tuberculosis 1888-1988: Conquest and controversy. Journal of Comparative Pathology 99(4): 357-399; doi.org/10.1016/0021-9975(88)90058-8

Ramos. DF, Silva, PEA, dellagostin OA (2015) Diagnosis of bovine tuberculosis. Brazilian Journal of Biology 75: 830-837.

Rua-Domenech R, Goodchild AT, Vordermeier HM, Hewinson RG, Christiansen KH, Sahin F, Yildiz P (2013) Distinctive biochemical changes in pulmonary tuberculosis and pneumonia. Archives of Medical Science 9(4): 656- 61.

Sayın Z, Erganiş O (2010) Tüberkülozunun Teşhisinde Kullanılan Metotlar. Kocatepe Veteriner Dergisi 3(2): 77-82.

Schiller I. Vordermeier HM, WR, OA Whelan, Coad M, Gormley E, Buddle BM, Palmer M, Thacker T, McNair J, Welsh M, Hewinson RG, Oesch B (2010) Bovine tuberculosis: Effect of the tuberculin skin test on in vitro interferon gamma responses. Veterinary Immunology and Immunopathology 136(1-2): 1-11.

Sevgisunar NS, Şahinduran Ş (2021) Evaluation of Some Ecute Phase Proteins, Cytokines and Hepcidin Levels in Naturally Infected Saanen Goats with Paratuberculosis. Health Science Instution 9(3): 29-37.

Thoen CO, Himes EM (1986) Pathogenesis of *Mycobacterium bovis* infection. Progress in Veterinary Microbiology and Immunology 2: 198-214.

Tothova C, Nagy O, Kovac G (2014) Acute phase proteins and their use in the diagnosis of diseases in ruminant: a review. Veterinarni Medicina 59(4): 163-180.

Vicentea J, Martinez-Guijosaa J, Tvarijonaviciutec A, Fernandez-de Meraa IG, Gortazara C, Ceronb JJ, Martinez-Subielab S (2019) Serum haptoglobin response in red deer naturally infected with tuberculosis Comparative Immunology. Microbiology and Infectious Diseases 64: 25-30.

W Amanfu (2006) The situation of tuberculosis and tuberculosis control in animals of economic interest. Tuberculosis 86(3-4): 330-335.

Yanagisawa N, Takahashi M, Namiki M, Okajima S, Lchinose Y, Toyama K (1996) Study of erythrocyte sedimentation rate in patients with pulmonary. Zasshi 70(9): 955-962.