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Haptoglobin and Amyloid A Levels in Milk of Clinical and Subclinical Mastitic Cows in Turkey

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NU and OM designed the study and wrote the article. NU, OM and SS performed the experiments and analyzed the data. SS, URF and AA critically reviewed and revised the manuscript.

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ABSTRACT

Mastitis is one of the most common diseases in dairy cows and causes significant economic losses globally. We aimed to investigate whether haptoglobin (Hp) and amyloid A levels in milk may be an alternative method for diagnosing subclinical mastitis (SM) in dairy cows. Ten subclinical-, 16 clinical mastitis and 39 healthy Holstein cows were allocated to the study following California Mastitis Test (CMT). Thus, a total of 65 Holstein cows participated in the study. In the study, *Staphylococcus aureus* (n = 10) was isolated as the most dominant bacterial species seen in clinical and subclinical mastitis samples. Somatic cell counts (SCC) in cows with clinical mastitis were significantly higher than the milk of cows with subclinical mastitis and healthy cows. Milk serum haptoglobin (Hp) and amyloid A levels were not determined statistically different between groups ($p > 0.05$). No correlation was found between CMT scores, SCC values, Hp and milk amyloid A levels in milk serum. There were no significant differences in Hp, MAA levels and SCC in the milk of clinically healthy cows and cows with clinical and subclinical mastitis ($p > 0.05$). As a result, Hp and amyloid A levels in milk showed no proper parameters for diagnosing subclinical mastitis in dairy cattle and monitoring treatment efficacy. Acute-phase proteins have not yet been adequately studied and clinically used in dairy animals, routinely diagnostic and prognostic, but they are used in human medicine for these purposes.

INTRODUCTION

Mastitis is a multifactorial disease characterized by decreased milk yield and a change in its composition in dairy animals. It is an inflammatory response of the mammary gland to physiological and metabolic alterations, injuries, allergies and, more often, damage caused by microorganisms. The loss of yield and economic losses caused by the disease still is a devastating problem in the worlds' dairy cow breeding (Pir Yağcı, 2008; Deb *et al*., 2013; Ismail *et al*., 2018).

Accurate and precise diagnosis of mastitis determines the fate of the udder health, specifically at early stages. Although several methods are routinely used in the indirect diagnosis of mastitis, the most practical application is the California Mastitis Test (CMT) (Schalm and Noorlander, 1957; Ashraf and Imran, 2018). Despite the use of many chemical and microbiological tests in diagnosing subclinical mastitis, tests based on determining the somatic cell count (SCC) per ml of milk have gained more importance in recent years (Rişvanlı and Kalkan, 2002; Duarte *et al*., 2015). However, studies for new diagnostic approaches continue.

Determination of the levels of positive acute-phase proteins (APP), serum amyloid-A (SAA) and haptoglobin (Hp) in differentiating acute and chronic inflammations in cattle are more valuable than using haematological tests, and the SAA levels are higher in acute inflammation than in chronic inflammation (Alsemgeest *et al*., 1994; Thomas *et al*., 2015). The APP levels in body fluids are related to the extent of tissue damage and the severity of the problem in affected animals and can provide clinical diagnostic and prognostic information (Coşkun and Şen, 2011; Dhama *et al*., 2019).

It has been suggested that the SAA levels are an indicator in the diagnosis of chronic subclinical mastitis (Grönlund *et al*., 2005). Serum Hp and SAA levels below the detection limit were evaluated as healthy nipple markers (Grönlund *et al*., 2003). Haptoglobin is also locally synthesized in the mammary gland (Hiss *et*

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al., 2004; Thielen *et al*., 2007). However, Hp in milk has been determined to have originated from the circulation (Pedersen *et al*., 2003; Jain *et al*., 2011). Since the APP levels in milk increase significantly with increasing SCC, it has been stated that milk APPs can indicate the severity of infection (Dalanezi *et al*., 2020). Due to the direct synthesis of milk amyloid-A (MAA), a specific isoform of SAA, from breast epithelial cells, it is stated that MAA is a more sensitive indicator in mastitis cases and can be used in the diagnosis of clinical and subclinical mastitis (Hussein *et al*., 2018). Studies conducted on milk Hp (mHp) and the MAA levels with acute clinical mastitis have revealed that APPs in milk can be determined more accurately than in serum and thus can be used as a valuable tool in the diagnosis of mastitis and can be an indicator of milk quality (Nazifi *et al*., 2008; Gerardi *et al*., 2009; Kováč *et al*., 2011; Alkan *et al*., 2014).

Since mastitis is a global health issue for dairy cows, this study aimed (i) to measure and assess mHp, MAA, SCC together with microbiological results in detecting various degrees of subclinical and clinical mastitis, and (ii) to compare the biochemical and microbiologic result with the SCC.

MATERIALS AND METHODS

Animals

In this study, 65 Holstein cows in the postpartum period (lactation period) between 2-8 years of age, were selected from family-type farms in Çankırı Center and Eldivan District and were used as healthy animals and animals with mastitis. Cows with the same diets were included in the study. Milk samples of 25 healthy cows (those with no signs of mastitis and no microbiological growth as a result of microbiological examinations), 14 healthy controls (those with no signs of mastitis, but bacterial growth detected in microbiological cultivation), 16 cows with clinical mastitis and 10 cows with subclinical mastitis, were allocated into four groups.

California mastitis test (CMT)

According to the previously described method, the CMT was conducted (Schalm and Noorlander, 1957). Milk samples obtained from each 4-udder group were collected into a test container with four separate compartments. Equal amounts of milk were mixed with the test solution containing “bromocresol purple” an anionic detergent. Changes such as color changes or gel formation were evaluated by rotating the test plate slowly. The CMT scores were graded as follows: 0 for no reaction, 1 for weak positive, 2 for a significant positive and 3 for a strong positive reaction.

Bacteriological examinations

Milk samples were incubated at +37°C for 1-3 days in an aerobic environment after inoculation on MacConkey’s agar and blood agar medium. For isolation, bacteria were also cultivated from each milk sample in broth medium with serum and incubated at +37°C for 48 h under microaerophilic conditions. After the media on which the growth was observed were examined microscopically, pure cultures were formed by passaging from single falling colonies in mixed growing cultures. Based on the obtained analysis results, routine microbiological examinations were used to see other biochemical properties for identification.

Measurement of somatic cell count (SCC)

The SCC was calculated with the Bentley Bactocount IBC-M (Bentley Instruments, MERKİM®, USA) device.

Determination of haptoglobin and amyloid-A levels

The milk samples were brought to the laboratory and centrifuged at 26.000 x g for 30 minutes. The fat layer accumulated over the tubes was removed, milk serum was collected, placed into centrifuge tubes and kept at -80°C until the analysis. Quantification of MAA and mHp was performed using commercially available ELISA kits (respectively, Serum Amyloid A PhaseTM Analysis and Bovine Haptoglobin PhaseTM Enyme Immunassay, Tridelta Ltd. Company, Ireland).

Statistical analysis

The data obtained from our study were statistically analyzed using SPSS 11 program. The control of the difference between the groups, analysis of variance and multiple comparison tests were performed. The results were given as X ± Sx: mean ± standard error. $P<0.05$ was considered statistically different.

RESULTS

The mean number of SCC, mHp and MAA values and standard error rates in healthy Holstein cows with clinical and subclinical mastitis are presented in Table I. As a result of the variance analysis, the SCC difference between the groups was detected significant ($p<0.001$), while the difference between the groups was not significant for mHp and MAA ($p>0.05$). The analysis of variance for SCC showed similarity in the healthy and the healthy control groups, while SCC was significantly lower than in the subclinical and clinical mastitis groups. The mean SCC was highest in clinical mastitis, followed by the mean SCC in subclinical mastitis.

Table I. Somatic cell count (SCC), haptoglobin and amyloid A mean values and statistical significance of groups in clinical mastitis, subclinical mastitis and healthy cows.

		Healthy	Healthy control	Subclinical mastitis	Clinical mastitis
SCC	n	23	14	10	9
	Mean ± SD	61,60 ± 59,39[a]	36,14 ± 35,21[a]	517,30 ± 284,87[b]	2519 ± 934,11[c]
	SEM	12,38	9,41	90,08	311,37
Haptoglobin (mg/ml)	n	25	14	10	16
	Mean ± SD	0,3409 ± 0,0314	0,3429 ± 0,0386	0,3370 ± 0,0213	0,3396 ± ,0215
	SEM	0,0062	0,0103	0,0067	0,0053
Amyloid A (ng/ml)	n	15	8	6	12
	Mean ± SD	136,40 ± 126,89	207,50 ± 159,64	126,66 ± 137,88	228,75 ± 115,57
	SEM	32,76	56,44	56,29	33,36

a, b, c, The difference between the averages of the groups with these different media in the same row is significant. As a result of the correlation analysis, there was no statistically significant correlation between SCC, haptogloblin and amyloid A variables. (p>0,05).

Table II. Somatic cell count, CMT and microbiological analysis results in clinical mastitis, subclinical mastitis and healthy cow milk.

No	Somatic cell count (SCC) x 1000 cells/ml)	Group	California mastitis test (CMT)	Microbiological analysis
1	48	Healthy	negative (-)	*Corynebacterium* spp.
2	29	Healthy control	negative (-)	No microbiological growth
3	1302	Clinical mastitis	positive (+++) 3	*Staphylococcus aureus*
4	480	Subclinical mastitis	suspicious	*Staphylococcus aureus* /*Micrococcus* spp.
5	24	Healthy	negative (-)	*Bacillus* spp. / *Pseudomonas* spp.
6	21	Healthy control	negative (-)	No microbiological growth
7	2948	Clinical mastitis	positive (+++) 3	*Bacillus* spp. / *Streptococcus* spp.
8	10	Healthy	negative (-)	*Bacillus* spp. / *Staphylococcus* spp.
9	12	Healthy	negative (-)	*Staphylococcus* spp.
10	22	Healthy	negative (-)	*Bacillus* spp. / *Staphylococcus intermedius*
11	12	Healthy	negative (-)	*Escherichia coli* /*Staphylococcus aureus*
12	31	Healthy control	negative (-)	No microbiological growth
13	51	Healthy	negative (-)	*Staphylococcus aureus* / *Micrococcus* spp.
14	68	Healthy	negative (-)	*Alcaligenes* spp. /*Streptococcus dysgalactiae*
15	8	Healthy control	negative (-)	No microbiological growth
16	581	Subclinical mastitis	positive (+) 1	*Staphylococcus aureus*
17	18	Healthy	negative (-)	*Staphylococcus* spp.
18	9	Healthy control	negative (-)	No microbiological growth
19	333	Subclinical mastitis	suspicious	*Staphylococcus aureus* / *Escherichia coli*
20	8	Healthy control	negative (-)	No microbiological growth
21	10	Healthy control	negative (-)	No microbiological growth
22	193	Healthy	negative (-)	*Staphylococcus aureus* / *Micrococcus* spp.
23	674	Subclinical mastitis	positive (+) 1	*Pasteurella haemolytica*
24	183	Healthy	negative (-)	No microbiological growth

No	Somatic cell count (SCC) x 1000 cells/ml)	Group	California mastitis test (CMT)	Microbiological analysis
25	55	Healthy	negative (-)	*Escherichia coli*
26	36	Healthy control	negative (-)	No microbiological growth
27	39	Healthy control	negative (-)	No microbiological growth
28	201	Subclinical mastitis	suspicious	*Staphylococcus aureus* / *Staphylococcus* spp.
29	68	Healthy control	negative (-)	No microbiological growth
30	6	Healthy	negative (-)	*Staphylococcus* spp.
31	271	Subclinical mastitis	suspicious	*Staphylococcus* spp.
32	443	Subclinical mastitis	suspicious	*Staphylococcus* spp.
33	76	Healthy	negative (-)	*Staphylococcus intermedius*
34	194	Healthy	negative (-)	*Staphylococcus intermedius* / *Staphylococcus* spp.
35	16	Healthy control	negative (-)	No microbiological growth
36	3254	Clinical mastitis	positive (+++) 3	*Streptococcus* spp.
37	314	Subclinical mastitis	suspicious	*Micrococcus* spp.
38	142	Healthy control	negative (-)	No microbiological growth
39	3460	Clinical mastitis	positive (+++) 3	*Staphylococcus aureus*
40	107	Healthy	negative (-)	*Corynebacterium* spp.
41	40	Healthy control	negative (-)	No microbiological growth
42	129	Healthy	negative (-)	Streptecoccus dysagalactia
43	45	Healthy	negative (-)	*Corynebacterium* spp.
44	58	Healthy	negative (-)	*Staphylococcus* spp.
45	32	Healthy	negative (-)	*Corynebacterium* spp. / *Bacillus* spp.
46	2270	Clinical mastitis	positive (+++) 3	*Staphylococcus* spp.
47	1167	Subclinical mastitis	positive (+) 1	*Alcaligenes* spp. / *Streptococcus dysgalactiae*
48	28	Healthy	negative (-)	*Bacillus* spp. / *Enterococcus* spp.
49	1455	Clinical mastitis	positive (+) 1	*Corynebacterium* spp.
50	28	Healthy	negative (-)	*Bacillus* spp.
51	18	Healthy	negative (-)	Staphylococcus intermedius
52	49	Healthy control	negative (-)	No microbiological growth
53	1336	Clinical mastitis	positive (+++) 3	Staphylococcus intermedius
54	709	Subclinical mastitis	positive (+) 1	*Streptococcus* spp.
55	3364	Clinical mastitis	positive (+++) 3	*Staphylococcus aureus*
56	3282	Clinical mastitis	positive (+++) 3	*Staphylococcus intermedius* / *Staphylococcus* spp.
57	Could not be measured	Clinical mastitis	positive (+++) 3	*Streptococcus dysagalactia* / *Corynebacterium* spp.
58	Could not be measured	Healthy	suspicious	*Staphylococcus* spp.
59	Could not be measured	Healthy	suspicious	Staphylococcus aureus/Micrococcus spp.
60	Could not be measured	Clinical Mastitis	positive (++) 2	Microbiological analysis could not be done
61	Could not be measured	Clinical Mastitis	positive (++) 2	Microbiological analysis could not be done
62	Could not be measured	Clinical Mastitis	positive (++) 2	Microbiological analysis could not be done
63	Could not be measured	Clinical Mastitis	positive (+) 1	Microbiological analysis could not be done
64	Could not be measured	Clinical Mastitis	positive (++) 2	Microbiological analysis could not be done
65	Could not be measured	Clinical Mastitis	positive (++) 2	Microbiological analysis could not be done

In this study, 65 cows in their lactation period were examined with CMT. As a result of the microbiological evaluation of milk samples obtained from CMT positive cows, inoculation was not possible in 6 of 65 samples, and in the remaining 59 samples, isolation and identification were carried out. No growth was observed in 20 of 59 samples, and no aerobic microorganism was isolated. While aerobic microorganisms were isolated from 39 specimens with bacterial growth, *Staphylococcus aureus* was identified in 15 of them. Besides, mixed infections were determined in 24 of 39 milk samples (Table II).

DISCUSSION

Bovine milk is an essential nutrient for human health. Besides, it is a critical production element in terms of the country's economy. Therefore, the health of the mammary gland and protection against possible diseases, diagnosis and treatment regimes are significant efforts of veterinary medicine (Wiley, 2008; Mahmood *et al*., 2017).

During inflammation of the mammary gland, the SCC in milk increases, which is a good indicator of the degree of inflammation in mastitis (Souza *et al*., 2016; Mahmood *et al*., 2017; Darbaz *et al*., 2019). The presented study revealed a significant difference in milk SCC between the groups ($p<0.001$), while the highest mean values were found in the clinical mastitis group, followed by the subclinical mastitis group. The mean values of the healthy and the control groups were low and close to each other. The significant increases in SCC values in milk with clinical and subclinical mastitis were remarkable and consistent with the literature.

Detection of *Staphylococcus aureus* in 15 of 39 specimens with positive CMT and bacterial growth and mixed infection was determined in 24. This result may be due to that all milk samples were obtained from the family-type farm, and the farmers in the region did not know or did not apply milking hygiene and milking rules well due to lack of knowledge of methods of protection from mastitis such as disinfecting the udders before milking, and lack of the dry period treatment habit.

Alkan *et al*. (2014) collected 109 milk samples from 112 udders of 28 Holstein cows and detected a difference between the mean SCC values of the groups with and without bacterial growth was not statistically significant ($p>0.05$). The SCC results of the present study were also compatible with the literature. Besides, Alkan *et al*. (2014) suggested that CMT alone was insufficient in determining subclinical intramammary infections in dry cows and that the CMT results should be evaluated together with bacteriological examination results. In our study, evaluation was made based on CMT and the bacteriological examination results. It was determined that although there was bacterial growth in samples obtained from some udders, the CMT test was negative, and although the CMT test was positive in some samples, no bacteria were isolated. It is thought that the main reasons for this situation may be factors such as weak sensitivity and specificity of the CMT test, which may not be determinative for intra-mammary infections in all cases, as well as the difference in SCC values in mastitis cases due to microorganisms, the number of bacterial colonies and the inoculation technique.

Rişvanlı and Kalkan (2002) has been determined that the mean SCC in milk samples obtained from CMT (+) lobes with microbiological growth was 313.001; the mean SCC in milk samples obtained from CMT (++) lobes with microbiological growth was 559.007, and the mean SCC in milk samples obtained from CMT (+++) lobes with microbiological growth was 1.563.618. Results of our study were that the mean value of milk SCC of the CMT (+) (subclinical mastitis) group with microbiological growth was 517.000 ± 90.000 / ml; the mean value of milk SCC in the CMT (++) and (+++) (clinical mastitis) group with microbiological growth was 2.519.000 ± 311.000 / ml (Table I). Thus, our results were compatible with the literature, and the SCCs in milk with clinical and subclinical mastitis were significantly higher than those in healthy milk ($p <0.001$).

SCC was found to be 379,000 in Staphylococcal-infected lobes and 63,000 in non-infected lobes (Hillerton and Walton, 1991). In milk samples obtained from lobes infected with *S. aureus*, SCC was determined as 364,866 in CMT (+) cows, 504,306 in CMT (++) cows and 1,675,008 in CMT (+++) cows (Rişvanlı and Kalkan, 2002). In the presented study, High SCC values of *S. aureus* or *Staphylococcus* spp. infected lobes were determined to be remarkable and compatible with the literature.

The acute phase response is an essential process against inflammation and infection. Hepatic adaptation responds by synthesizing acute-phase proteins, such as Hp and amyloid A, which restores homeostasis (Eckersall *et al*., 2006; Suojala *et al*., 2008). Many studies have shown that *S. aureus* is in the first place among microorganisms isolated from clinical and subclinical mastitis cases (Alaçam *et al*., 1989; Shitandi and Kihumbu, 2004; Schröder *et al*., 2005; Pumipuntu *et al*., 2019).

Increasing levels of MAA in the mammary gland with mastitis were compared with healthy breasts, and it was determined that some udders be affected by infection processes and result in a negative CMT (Nazifi *et al*., 2008). It has been established that milk and serum Hp and SAA increases rapidly in the acute phase response in cows with mastitis caused by *Staphylococcus* spp. (Grönlund *et al*., 2003). Besides, mHp and MAA levels were reported to

increase significantly with the increase of SCC, which may indicate clinical mastitis severity (Nielsen *et al*., 2004). In the presented study, there was no significant difference between the groups for mHp and MAA ($p>0.05$), and no statistically significant relationship was found between the variables SCC, mHp and MAA ($p>0.05$) (Table I). Contrary to our findings, Eckersall (2004) reported a significant difference in udder Hp and SAA concentrations in cows with chronic subclinical mastitis. We believe that the difference between the results of the two studies may be related to the difference in the severity of mastitis and whether it is acute or chronic. It has been reported that the difference between the APP levels determined in serum and milk may be caused by the duration and severity of breast infection (Nielsen *et al*., 2004) and that the increases in Hp, MAA and SCC may address the severity of clinical mastitis.

MAA and mHp levels below the detection limit were evaluated as healthy nipple markers, and significant increases were observed in Hp and SAA levels in the milk of cows with chronic subclinical mastitis (Grönlund *et al*., 2005). The disease stages are suggested to be evaluated by monitoring more than one APP, and in this way, chronic and acute conditions can be evaluated and characterized with an APP profile (Eckersall, 2004; Thomas *et al*., 2015). Our results were consistent with to study of Gultiken *et al*. (2012) that reported no relationship between the CMT scores, SCC values, plasma Hp and milk Hp levels presence in subclinical mastitis.

Due to the ability to synthesis of Hp from udder tissue (Eckersall *et al*., 2006), Hp has been evaluated as a mastitis marker in many studies (Grönlund *et al*., 2005; Akerstedt *et al*., 2007, 2009). Our study result on milk Hp was not found to be consistent with these literature data. We think that the fact that the milk Hp values in mastitis cases were not as high as expected could be associated with the mild severity of the mastitis cases because the Hp level in cattle has been reported to be higher in moderate and severe cases compared to mild cases of clinical mastitis (Nielsen *et al*., 2004; Wenz *et al*., 2010). According to the presented study results, the milk MAA levels were not a significant indicator in the diagnosis of mastitis. However, Grönlund *et al*. (2005) found significant differences in the mHp and MAA levels in cows with chronic subclinical mastitis. It is thought that the chronic and acute nature of the mastitis cases examined in our study may have affected the results.

Increased mHp and MAA levels were found in cows with mastitis, and the levels were significantly higher in cows with moderate mastitis than those with mild mastitis. Besides, it was stated that MAA had a higher potential in detecting mastitis than mHp, because of high sensitivity, specificity and efficiency in differentiating healthy cows and cows with mastitis (Eckersall *et al*., 2001; Taghdiri *et al*., 2018). In our study, no significant increase was found in the mHp and MAA levels of cows with clinical and subclinical mastitis, and the mild course of mastitis can explain this.

In chronic subclinical mastitis cases, only the milk MAA levels were significantly higher than the pre-infection status and significantly higher than in healthy controls (Grönlund *et al*., 2003; Tomazi *et al*., 2015). Subsequent studies have shown that increased APP levels in milk significantly altered the Hp and SAA levels in cows with chronic subclinical mastitis (Grönlund *et al*., 2005). Our Hp and MAA levels findings showed a difference between the healthy milk group and milk with various clinical and subclinical mastitis groups. However, there was no statistical significance ($p>0.05$), incompatible with the literature data. This may be due to the stage and severity of the disease because it has been reported that the disease's being acute or chronic may affect the milk Hp and MAA values (Nielsen *et al*., 2004; Kumar *et al*., 2017)

Our study could not establish a significant relationship between the SCC, mHp and the MAA values. In contrast to our findings, Akerstedt *et al*. (2007) reported a significant relationship between Hp and SCC in dairy cattle with subclinical mastitis 4-udder groups and the composite milk levels. Similar results have also been reported previously (Kovac *et al*., 2007; Safi *et al*., 2009). Gultiken *et al*. (2012) reported that although the SCC results and the CMT scores decreased in the third week of mastitis, they could not find a significant relationship between these parameters and the plasma Hp and mHp. Based on these findings, it can be said that studies in cattle with clinical and subclinical mastitis should be carried out, taking into account some details such as the disease stage and the severity in order to establish a clear relationship between the parameters studied.

It has been reported that the MAA levels in milk can only function as a sign of mastitis in the initial stage of asymptomatic mastitis in dairy cows. The MAA levels were evaluated as an indicator of mastitis (Vasil' *et al*., 2012). The Hp and MAA levels and the SCC values in the milk of cows with clinical mastitis were significantly higher than those of healthy cows and cows with subclinical mastitis (Gerardi *et al*., 2009; Dalanezi *et al*., 2020). Similarly, in our study, the SCC and MAA values were detected higher in clinical mastitis than in healthy and subclinical mastitis group, but while the increase in SCC values was found to be statistically significant ($p<0.001$), the difference in the MAA values was not statistically significant ($p>0.05$).

In conclusion, mHp and MAA levels may not be valuable parameters for diagnosing subclinical mastitis in dairy cattle, as well as monitoring the efficacy of the

treatment. In future studies, it is thought that APP-based diagnostic tests that are useful for cattle and that will yield results in a short time following the collection of blood or milk samples should be developed and optimized to be used in the field. Besides, details such as the stage and course of the disease, whether the previous treatment has been applied or not should be determined in more detail by monitoring more than one APP and considering these parameters.

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Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Akerstedt, M., Persson Waller, K. and Sternesjö, A., 2007. Haptoglobin and serum amyloid A in relation to the somatic cell count in quarter, cow composite and bulk tank milk samples. *J. Dairy Res.,* **74**: 198–203. https://doi.org/10.1017/S0022029906002305

Akerstedt, M., Persson Waller, K. and Sternesjö, A., 2009. Haptoglobin and serum amyloid A in bulk tank milk in relation to raw milk quality. *J. Dairy Res.,* **76**: 483–489. https://doi.org/10.1017/S0022029909990185

Alaçam, E., Tekeli, T., Erganiş O. and Nedim İzgi, A., 1989. İnek ve mandalarda subklinik mastitislerin tanısı, etkenlerin izolasyonu ve bunlara karşı etkili antibiyotiklerin belirlenmesi. *Eurasian J. Vet. Sci.,* **5**: 92–101.

Alkan, H., Baştan, A., Salar, S., Özdal, M. and Kaymaz, M., 2014. Kuru döneme çıkarken enfekte sağlıklı meme loblarında california mastitis test ve somatik hücre sayısı ile bakteriyolojik muayene sonuçlarının karşılaştırılması. *Ankara Ünv. Vet. Fak. Derg.,* **61**: 179–183. https://doi.org/10.1501/Vetfak_0000002626

Alsemgeest, S.P., Kalsbeek, H.C., Wensing, T., Koeman, J.P., van Ederen A.M. and Gruys, E., 1994. Concentrations of serum amyloid-A (SAA) and haptoglobin (HP) as parameters of inflammatory diseases in cattle. *Vet. Q.,* **16**: 21–23. https://doi.org/10.1080/01652176.1994.9694410

Ashraf, A. and Imran, M., 2018. Diagnosis of bovine mastitis: from laboratory to farm. *Trop. Anim. Hlth. Prod.,* **50**: 1193–1202. https://doi.org/10.1007/s11250-018-1629-0

Coşkun, A. and Şen, İ., 2011. Sığırlarda akut faz proteinlkeri ve klinik kullanım alanları. *Sağlık Bilim. Derg.,* **20**: 240–246.

Dalanezi, F.M., Schmidt, E.M.S., Joaquim, S.F., Guimarães, F.F., Guerra, S.T., Lopes, B.C., Cerri, R.L.A., Chadwick. C. and Langoni, H., 2020. Concentrations of acute-phase proteins in milk from cows with clinical mastitis caused by different pathogens. *Pathogens,* **9**: 706. https://doi.org/10.3390/pathogens9090706

Darbaz, İ., Salar, S., Sayiner, S., Baştan, İ., Ergene, O. and Baştan, A., 2019. Evaluation of milk glutathione peroxidase and superoxide dismutase levels in subclinical mastitis in Damascus goats. *Turk. J. Vet. Anim. Sci.,* **43**: 259–263. https://doi.org/10.3906/vet-1810-60

Deb, R., Kumar, A., Chakraborty, S., Verma, A.K., Tiwari, R., Dhama, K., Singh, U. and Kumar, S., 2013. Trends in diagnosis and control of bovine mastitis: A review. *Pakistan J. biol. Sci.,* **16**: 1653–1661. https://doi.org/10.3923/pjbs.2013.1653.1661

Dhama, K., Latheef, S.K., Dadar, M., Samad, H.A., Munjal, A., Khandia, R., Karthik, K., Tiwari, R., Yatoo, M.I., Bhatt, P., Chakraborty, S., Singh, K.P., Iqbal, H.M.N., Chaicumpa, W. and Joshi, S.K., 2019. Biomarkers in stress related diseases/disorders: Diagnostic, prognostic, and therapeutic values. *Front. Mol. Biosci.,* **6**: 91. https://doi.org/10.3389/fmolb.2019.00091

Duarte, C.M., Freitas, P.P. and Bexiga, R., 2015. Technological advances in bovine mastitis diagnosis: An overview. *J. Vet. Diagn. Invest.,* **27**: 665–672. https://doi.org/10.1177/1040638715603087

Eckersall, P.D., 2004. The time is right for acute phase protein assays. *Vet. J.,* **168**: 3-5. https://doi.org/10.1016/S1090-0233(03)00165-5

Eckersall, P.D., Young, F.J., McComb, C., Hogarth, C.J., Safi, S., Weber, A., McDonald, T., Nolan, A.M. and Fitzpatrick, J.L., 2001. Acute phase proteins in serum and milk from dairy cows with clinical mastitis. *Vet. Rec.,* **148**: 35–41. https://doi.org/10.1136/vr.148.2.35

Eckersall, P.D., Young, F.J., Nolan, A.M., Knight, C.H., McComb, C., Waterston, M.M., Hogarth, C.J., Scott, E.M. and Fitzpatrick, J.L., 2006. Acute phase proteins in bovine milk in an experimental model of staphylococcus aureus subclinical mastitis. *J. Dairy Sci.,* **89**: 1488–1501. https://doi.org/10.3168/jds.S0022-0302(06)72216-0

Gerardi, G., Bernardini, D., Azzurra Elia, C., Ferrari, V., Iob, L. and Segato, S., 2009. Use of serum amyloid A and milk amyloid A in the diagnosis of subclinical mastitis in dairy cows. *J. Dairy Res.,* **76**: 411–417. https://doi.org/10.1017/S0022029909990057

Grönlund, U., Hallén Sandgren, C. and Persson Waller, K., 2005. Haptoglobin and serum amyloid A in milk from dairy cows with chronic sub-clinical mastitis. *Vet. Res.,* **36**: 191–198. https://doi.org/10.1051/vetres:2004063

Grönlund, U., Hultén, C., Eckersall, P.D., Hogarth, C. and Persson Waller, K., 2003. Haptoglobin and serum amyloid A in milk and serum during acute and chronic experimentally induced *Staphylococcus aureus mastitis*. *J. Dairy Res.,* **70**: 379–386. https://doi.org/10.1017/S0022029903006484

Gultiken, N., Ay, S., Yarim Gul, F., Anadol, E. and Fındık, M., 2012. Evaluation of plasma and milk haptoglobin concentrations in the diagnosis and treatment follow-up of subclinical mastitis in dairy cows. *Acta Vet.,* **62**: 271–279. https://doi.org/10.2298/AVB1203271G

Hillerton, J.E. and Walton, A.W., 1991. Identification of subclinical mastitis with a hand-held electrical conductivity meter. *Vet. Rec.,* **128**: 513–515. https://doi.org/10.1136/vr.128.22.513

Hiss, S., Mielenz, M., Bruckmaier, R.M., and Sauerwein, H., 2004. Haptoglobin concentrations in blood and milk after endotoxin challenge and quantification of mammary Hp mRNA expression. *J. Dairy Sci.,* **87**: 3778–3784. https://doi.org/10.3168/jds.S0022-0302(04)73516-X

Hussein, H.A., El-Razik, K.A.A., Gomaa, A.M., Elbayoumy, M.K., Abdelrahman, K.A. and Hosein, H.I., 2018. Milk amyloid A as a biomarker for diagnosis of subclinical mastitis in cattle. *Vet. World,* **11**: 34–41. https://doi.org/10.14202/vetworld.2018.34-41

Ismail, Z.B., Muhaffel, M.M. and Abu-Basha, E., 2018. The effect of dry cow therapy using systemic tylosin in combination with common intramammary medications on mastitis rate, cull rate, somatic cell count, and milk production in dairy cows affected with subclinical mastitis. *Vet. world,* **11**: 1266–1271. https://doi.org/10.14202/vetworld.2018.1266-1271

Jain, S., Gautam, V. and Naseem, S., 2011. Acute-phase proteins: As diagnostic tool. *J. Pharm. Bioallied Sci.,* **3**: 118–127. https://doi.org/10.4103/0975-7406.76489

Kovac, G., Popelková, M., Tkáčiková, Ľ., Burdová, O. and Ihnát, O., 2007. Interrelationship between somatic cell count and acute phase proteins in serum and milk of dairy cows. *Acta Vet. Brno,* **76**: 51–57. https://doi.org/10.2754/avb200776010051

Kováč, G., Tóthová, C., Nagy, O. and Seidel, H., 2011. Milk amyloid a and selected serum proteins in cows suffering from mastitis. *Acta Vet. Brno,* **80**: 3–9. https://doi.org/10.2754/avb201180010003

Kumar, N., Manimaran, A., Kumaresan, A., Jeyakumar, S., Sreela, L., Mooventhan, P. and Sivaram, M., 2017. Mastitis effects on reproductive performance in dairy cattle: A review. *Trop. Anim. Hlth. Prod.,* **49**: 663–673. https://doi.org/10.1007/s11250-017-1253-4

Mahmood, I., Nadeem, A., Babar, M., Ali, M.M., Javed, M., Siddiqa, A., Hussain, T. and Pervez, M., 2017. Systematic and Integrated Analysis Approach to Prioritize Mastitis Resistant Genes. *Pakistan J. Zool.,* **49**: 103–109. https://doi.org/10.17582/journal.pjz/2017.49.1.101.106

Nazifi, S., Khoshvaghti, A. and Gheisari, H.R., 2008. Evaluation of serum and milk amyloid A in some inflammatory diseases of cattle. *Iran. J. Vet. Res. Shiraz Univ.,* **9**: 222–226.

Nielsen, B.H., Jacobsen, S., Andersen, P.H., Niewold, T.A. and Heegaard, P.M.H., 2004. Acute phase protein concentrations in serum and milk from healthy cows, cows with clinical mastitis and cows with extramammary inflammatory conditions. *Vet. Rec.,* **154**: 361–365. https://doi.org/10.1136/vr.154.12.361

Pedersen, L.H., Aalbaek, B., Røntved, C., Ingvartsen, K., Sorensen, N.S., Heegaard, P.M.H. and Jensen, H.E., 2003. Early Pathogenesis And Inflammatory Response In Experimental Bovine Mastitis Due to *Streptococcus uberis*. *J. comp. Pathol.,* **128**: 156–164. https://doi.org/10.1053/jcpa.2002.0620

Pir Yağcı, İ., 2008. Koyunlarda subklinik mastitis: etiyoloji, epidemiyoloji ve tanı yöntemleri. *Kafkas Üniv. Vet. Fak. Derg.,* **14**: 117–122.

Pumipuntu, N., Tunyong, W., Chantratita, N., Diraphat, P., Pumirat, P., Sookrung, N., Chaicumpa, W. and Indrawattana, N., 2019. *Staphylococcus* spp. associated with subclinical bovine mastitis in central and northeast provinces of Thailand. *PeerJ.,* **7**: e6587. https://doi.org/10.7717/peerj.6587

Rişvanlı, A. and Kalkan, C., 2002. Sütçü İneklerde Yaş ve Irkın Subklinik Mastitisli Memelerin Sütlerindeki Somatik Hücre Sayıları ile Mikrobiyolojik İzolasyon Oranlarına Etkisi. *Van Vet. J.,* **13**: 84-87.

Safi, S., Khoshvaghti, A., Jafarzadeh, S.R., Bolourchi, M. and Nowrouzian, I., 2009. Acute phase proteins in the diagnosis of bovine subclinical mastitis. *Vet.*

clin. Pathol., **38**: 471–476. https://doi.org/10.1111/j.1939-165X.2009.00156.x

Schalm, O.W. and Noorlander, D.O., 1957. Experiments and observations leading to development of the California mastitis test. *J. Am. vet. med. Assoc.,* **130**: 199–204.

Schröder, A., Hoedemaker, M. and Klein, G., 2005. Resistance of mastitis pathogens in northern Germany. *Berl. Munch. Tierarztl. Wochenschr.,* **118**: 393–398.

Shitandi, A. and Kihumbu, G., 2004. Assessment of the California mastitis test usage in smallholder dairy herds and risk of violative antimicrobial residues. *J. Vet. Sci.,* **5**: 5–9. https://doi.org/10.4142/jvs.2004.5.1.5

Souza, F.N., Cunha, A.F., Rosa, D.L.S.O., Brito, M.A.V., Guimarães, A.S., Mendonça, L.C., Souza, G.N., Lage, A.P., Blagitz, M.G., Della Libera, A.M.M.P., Heinemann, M.B. and Cerqueira, M.M.O.P., 2016. Somatic cell count and mastitis pathogen detection in composite and single or duplicate quarter milk samples. *Pesqui. Vet. Bras.,* **36**: 811–818. https://doi.org/10.1590/s0100-736x2016000900004

Suojala, L., Orro, T., Järvinen, H., Saatsi, J. and Pyörälä, S., 2008. Acute phase response in two consecutive experimentally induced *E. coli* intramammary infections in dairy cows. *Acta Vet. Scand.,* **50**: 18. https://doi.org/10.1186/1751-0147-50-18

Taghdiri, M., Karim, G., Safi, S., Rahimi Foroushani, A. and Motalebi, A., 2018. Study on the accuracy of milk amyloid A test and other diagnostic methods for identification of milk quality. *Vet. Res. Forum,* **9**: 179–185.

Thielen, M.A., Mielenz, M., Hiss, S., Zerbe, H., Petzl, W., Schuberth, H.-J., Seyfert, H.-M. and Sauerwein, H., 2007. Cellular Localization of haptoglobin mRNA in the experimentally infected bovine mammary gland. *J. Dairy Sci.,* **90**: 1215–1219. https://doi.org/10.3168/jds.S0022-0302(07)71609-0

Thomas, F.C., Waterston, M., Hastie, P., Parkin, T., Haining, H. and Eckersall, P.D., 2015. The major acute phase proteins of bovine milk in a commercial dairy herd. *BMC Vet. Res.,* **11**: 207. https://doi.org/10.1186/s12917-015-0533-3

Tomazi, T., Gonçalves, J.L., Barreiro, J.R., Arcari, M.A. and V dos Santos, M., 2015. Bovine subclinical intramammary infection caused by coagulase-negative staphylococci increases somatic cell count but has no effect on milk yield or composition. *J. Dairy Sci.,* **98**: 3071–3078. https://doi.org/10.3168/jds.2014-8466

Vasil', M., Elečko, J., Farkašová, Z. and Zigo, F., 2012. Diagnostic importance of the concentration of milk amyloid A in quarter milk samples from dairy cows with mastitis. *Acta Vet. Brno,* **81**: 133–138. https://doi.org/10.2754/avb201281020133

Wenz, J.R., Fox, L.K., Muller, F.J., Rinaldi, M., Zeng, R. and Bannerman, D.D., 2010. Factors associated with concentrations of select cytokine and acute phase proteins in dairy cows with naturally occurring clinical mastitis. *J. Dairy Sci.,* **93**: 2458–2470. https://doi.org/10.3168/jds.2009-2819

Wiley, A., 2008. Transforming milk in a global economy. *Am. Anthropol.,* **109**: 666–677. https://doi.org/10.1525/aa.2007.109.4.666