

## Levels of Vitamin A, C, E with Malondialdehyde in Blood and Milk Serum in Subclinical Mastitic Cows

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**Abstract:** The aim of this study was to determine serum and milk concentrations of vitamins A, C and E with Malondialdehyde (MDA) in with and without subclinical mastitic cows. The study was performed on 15 healthy and 23 subclinical mastitic cows, aged 4-9 years. The California Mastitis Test (CMT) was performed. Fifteen udder belonging to 15 cows which gave negative reaction in CMT were considered to be healthy. Thirty two udder quarters belonging to 23 cows which gave positive reaction as CMT +1 and +2 were diagnosed to be affected by subclinical mastitis. Vitamin A, C and E levels in blood serum and milk samples were determined by spectrophotometry, MDA levels were analysed with High Performance Liquid Chromatography (HPLC). There was no significant difference in blood serum vitamin A and E levels between healthy and subclinical mastitic cows. However, vitamin C ( $p \leq 0.01$ ) and MDA ( $p \leq 0.05$ ) levels in subclinical mastitic cows were significantly lower than healthy cows. There was no significant difference in milk samples Vitamins A, E and MDA levels between healthy and subclinical mastitic cows but vitamin C levels in subclinical mastitic cows were significantly lower than healthy cows ( $p \leq 0.01$ ). In conclusion, serum and milk vitamin C with serum MDA levels of healthy cows were determined significantly higher than subclinical mastitic cows but there was no difference in serum and milk vitamin A and E with milk MDA levels between healthy and subclinical mastitic cows.

**Key words:** Cow, mastitis, vitamins, lipid peroxidation, milk samples, Turkey

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### INTRODUCTION

Mastitis can be defined as inflammation of the mammary gland. Mastitis reduces the milk yield, changes milk composition, shortens the productive life of affected cows and is very costly to the dairy farmer. It has been and recognized as one of the major disease problems concerning the dairy industry. Bovine mastitis causes more financial loss to dairy industry than any other disease. Approximately, 70% of this loss can be attributed to reduced milk production caused by subclinical mastitis which dairy producers seldom recognize. The loss is due to irreversibly damaged milk secretory tissue. Manufacturers of dairy products are also losing vast amounts of money due to the adverse processing qualities of mastitic milk. The quality changes of the milk include decreases in the content of fat, lactose and milk proteins such as caseins (Janzen, 1970; Sandholm and Mattila, 1986).

Vitamins are necessary for animals' biological development, reproduction and health condition. One of the compounds is vitamin A. Vitamin A has many functions including maintenance of epithelial cells, vision, gene regulation and immune cell function. Under normal

circumstances, the cattle do not ingest natural vitamin A but enable to produce it from provitamins such as  $\beta$ -carotene contained mostly in green feed.  $\beta$ -carotene, a vitamin A precursor, converted mostly by the mucosa of the small intestine and appears to be the most efficient provitamin (Chew, 1987; Hurley and Doane, 1989).

Vitamin C (ascorbic acid) is synthesized in the body of most mammals except primates and guinea pigs which have a dietary vitamin C requirement (Hurley and Doane, 1989). There are three main roles of vitamin C which is necessary for the reproduction of females and males. These can be listed as follows: biosynthesis of collagen, biosynthesis of steroid and peptide hormones and protection of the gametes against the oxidative harms of the free radicals. Collagen synthesis is required for follicle growth and development of corpus luteum. In addition, it was stated that vitamin C applications during pregnancy reduced congenital defects and that it was also effective in stimulation of ovulation (Luck *et al.*, 1995). Vitamin E is localised in the cell membranes as a biological anti-oxidant and inhibits the formation of lipid peroxides. The most important function of both vitamin E and selenium is the protection of biological membranes (Burk, 1983; Combs and Combs, 1984; Levander, 1987).

Lipid peroxidation is the oxidation reaction of unsaturated fatty acids that are present in phospholipide, glycolipide, triglyceride and sterole in membrane to some products such as peroxides, alcohols, aldehydes, hydroxy fatty acids, ethane and pentane by free oxygen radicals (Nordberg and Arner, 2001). The existing lipid peroxides are smashed quickly and they form reactive carbon compounds. The most important of this reactive carbon compounds is MDA and is generally used as an indicator of lipid peroxidation (Cheeseman and Slater, 1993).

The objective of this study was to determine serum and milk concentrations of vitamins A, C and E with MDA in with and without subclinical mastitic cows.

**MATERIALS AND METHODS**

**Animals and nutrition:** Thirty eight cows (21 Swiss-Brown, 12 Holstein and 5 Simmental) between 4 and 9 years of age were used in the study. The study was performed on 15 healthy and 23 subclinical mastitic cows at Research and Implementation Farm of the Firat University. All the animals were under the same care and feeding conditions. The chemical composition of feed concentrates given to the animals was as follows: dry matter-93.75%; ash-5.09%; crude fiber-9.75%; crude protein-15.18%; ether extract-5.8%; organic matter-88.66%; barley-70.5%; sunflowerseed meal-17.5%; soybean-7.5%; limestone-3.0%; DCP- (Dicalcium Pohosphate) 0.5%; salt-0.5%; vitamins-0.25% and trace elements-0.25%. Composition of forage feed was as follows: dry matter-95.2%; ash-9.47%; crude fiber-35.0%; crude protein-3.3%; ether extract-3.2% and organic matter-85.73%. The ration was given in the morning and evening.

**Experimental design:** The milk samples were collected once between the 3rd and 4th month after calving. Before collection of blood and milk samples, the CMT was performed. CMT results were evaluated as described by Schalm *et al.* (1971). Fiveteen udder belonging to 15 cows which were CMT negative were considered healthy. Thirty two udder quarters belonging to 23 cows which gave positive reaction as CMT +1 and +2 were considered being affected by subclinical mastitis. Blood samples

(10 mL) from all the animals were collected from the jugular vein under aseptic conditions. They were kept for separation of serum at room temperature for 2 h. Then, they were centrifuged at 3000 rpm for 15 min. Milk samples (10 mL) were collected into tubes after the udder was thoroughly washed with clean warm water and dried with clean paper towel. The samples were centrifuged at 3000 rpm for 20 min and then transferred into fresh tubes. Both blood and milk samples were stored at -20°C until analysis.

**Vitamins and MDA assay:** Vitamin A values were determined by using Suzuki and Katoh and vitamin E levels were determined spectrophotometrically according to Martinek (1964)'s Method. The level of vitamin C was measured by Phosphotungstic Acid Method of Kyaw (1978) with Shimadzu UV-1208, UV-VIS spectrophotometer. MDA values were determined by high performance liquid chromatography according to Karatas *et al.* (2002).

**Statistical analysis:** Differences in milk and blood serum vitamin A, C, E and MDA concentrations between healthy and subclinical mastitic cows were analyzed with ANOVA. Differences were considered as significant when  $p < 0.05$ . Healthy and subclinical mastitic cows were compared by unpaired student t-test (SAS/STATM, 1999).

**RESULTS AND DISCUSSION**

In this study, the mean concentrations of blood serum vitamins A, C and E with MDA in healthy and subclinical mastitic cows were shown in Table 1. There was no significant difference in vitamin A and E levels between healthy and subclinical mastitic cows. However, vitamin C ( $p \leq 0.01$ ) and MDA levels ( $p \leq 0.05$ ) in subclinical mastitic cows were significantly lower than in healthy cows.

The mean levels of milk serum vitamins A, C and E with MDA in healthy and subclinical mastitic cows were shown in Table 2. There was no significant difference in vitamins A, E and MDA levels between healthy

Table 1: The mean blood serum levels of vitamins A, C and E with MDA in healthy and subclinical mastitic cows (Mean±SE)

Animal groups	Vitamin A ( $\mu\text{g dL}^{-1}$ )	Vitamin C ( $\text{mg dL}^{-1}$ )	Vitamin E ( $\text{mg dL}^{-1}$ )	MDA ( $\text{nmol mL}^{-1}$ )
Healthy cows (n = 15)	0.32±0.26	3.66±0.83	2.46±0.36	1.81±0.64
Subclinical mastitic cows (n = 20)	0.30±0.26	2.75±0.52	2.76±0.84	1.51±0.61
p-value	NS	$\leq 0.01$	NS	$\leq 0.05$

Table 2: The mean milk serum levels of vitamins A, C and E with MDA in healthy and subclinical mastitic cows (Mean±SE)

Animal groups	Vitamin A ( $\mu\text{g dL}^{-1}$ )	Vitamin C ( $\text{mg dL}^{-1}$ )	Vitamin E ( $\text{mg dL}^{-1}$ )	MDA ( $\text{nmol mL}^{-1}$ )
Healthy cows (n = 20)	0.12±0.26	10.48±2.10	0.43±0.48	6.30±1.47
Subclinical mastitic cows (n = 32)	0.11±0.22	6.65±1.73	0.34±0.50	5.83±1.70
p-value	NS	$\leq 0.01$	NS	NS

NS: Not Significant

and subclinical mastitic cows but vitamin C levels in subclinical mastitic cows were significantly lower than in healthy cows ( $p \leq 0.01$ ).

The mean levels of vitamin C and MDA in milk samples were higher than in blood serum (Fig. 1 and 2). Vitamin C value in milk samples of healthy cows were higher than in subclinical mastitic cows'. Similarly that value in blood serum of healthy cows were higher than mastitic cows (Fig. 1). The mean MDA levels in milk and serum samples of healthy cows were higher than in mastitic cows' (Fig. 2).

In this study, vitamin A levels serum and milk samples of healthy cows were higher than subclinical mastitic cows' but the difference statistically was not important (Table 1 and 2).

A number of studies (Johnston and Chew, 1984) reported that plasma vitamin A level in healthy cows were higher than in subclinical mastitic cows but the difference statistically was not important. Also, there was no different milk vitamin A concentrations between mastitic and nonmastitic cows (Johnston and Chew, 1984). In a similar study, Simsek and Aksakal stated that plasma and milk vitamin A levels of healthy cows were higher than subclinical mastitic cows. Also, the differences statistically were important ( $p \leq 0.001$ ). However in another study, Kaya and Guven reported that the levels of plasma vitamin A in healthy cows were lower than in mastitic cows' ( $p < 0.01$ ). It was noted that level of vitamin A content in plasma during oestrus cycle of cows was not

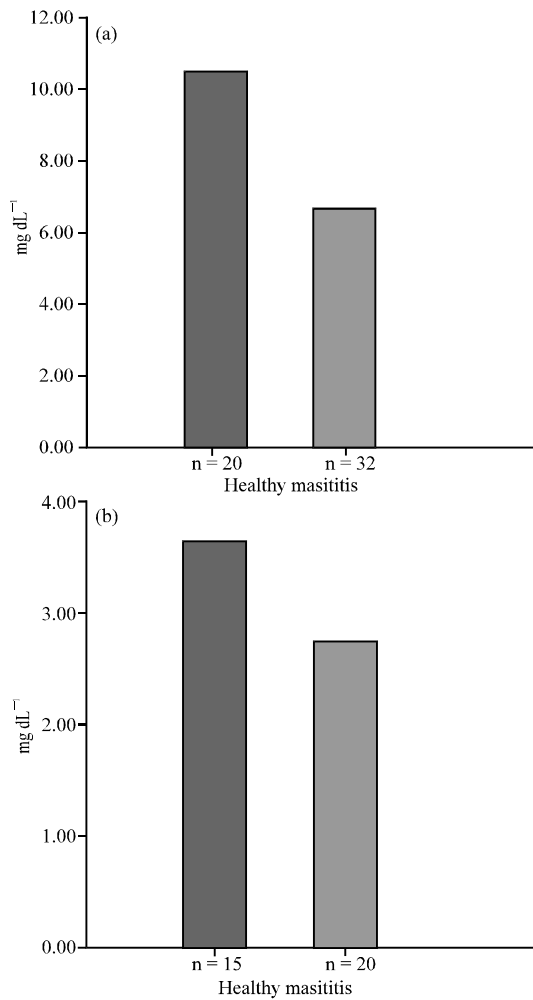


Fig. 1: Milk and serum levels of vitamin C in healthy and subclinical mastitic cows; a) milk level; b) Serum level

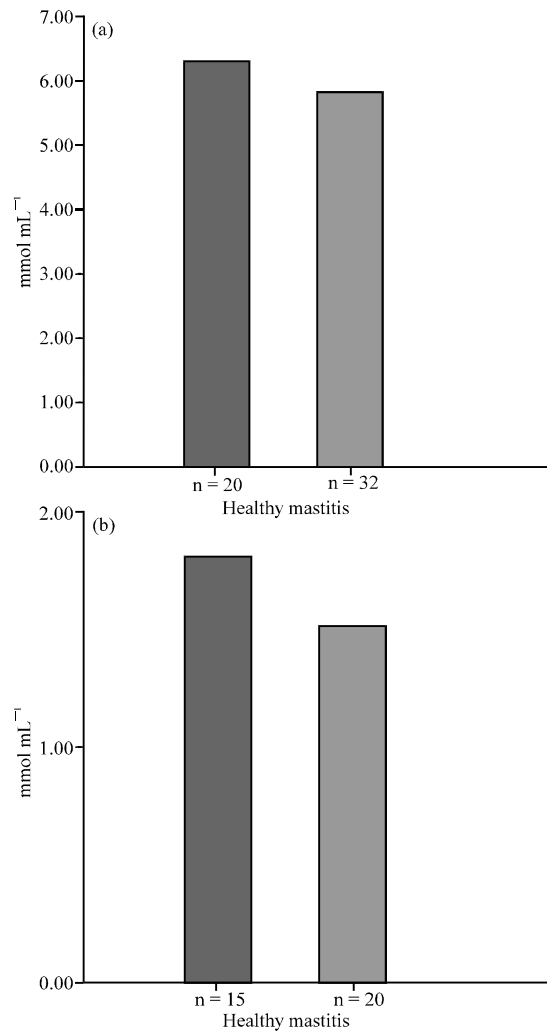


Fig. 2: Milk and serum levels of MDA in healthy and subclinical mastitic cows; a) milk level; b) Serum level

any significant change (Karatas *et al.*, 2001). Yildiz *et al.* (2005) found that levels of serum vitamin A in the pregnant cows were measured to be higher compared to the nonpregnant cows ( $p < 0.001$ ) during oestrus cycle. In similar a study, Ataman *et al.* (2010) found that the mean vitamin A level was higher in pregnant cows than that in nonpregnant cows 21 days after AI ( $p < 0.05$ ) but there was no significant difference at the time of and 3 and 12 days after AI. In another study, Ceylan *et al.* (2007) revealed that mean serum vitamin A levels in healthy cows were higher than repeat breeder and anestrus cows. It was demonstrated in this study that the serum vitamin A level was similar to the results obtained by other researchers (Johnston and Chew, 1984).

In the present study, mean serum vitamin C levels of the healthy and subclinical mastitic cows were  $3.66 \pm 0.83$  and  $2.75 \pm 0.52$  mg dL<sup>-1</sup>, respectively. Also, average milk vitamin C levels of healthy and subclinical mastitic cows were  $10.48 \pm 2.10$  and  $6.65 \pm 1.73$  mg dL<sup>-1</sup>, respectively. Mean serum and milk vitamin C levels in healthy cows were significantly higher than in subclinical mastitic cows' ( $p < 0.01$ ) (Table 1-2 and Fig. 1). The results of a study reported that plasma vitamin C levels were higher at metoestrus and dioestrus in pregnant cows ( $3.96 \pm 0.19$  and  $4.81 \pm 0.21$  µg mL<sup>-1</sup>) than that in nonpregnant cows ( $3.04 \pm 0.30$  and  $1.67 \pm 0.44$  µg mL<sup>-1</sup>) (Ataman *et al.*, 2010). It was found that plasma vitamin C value was  $5.03 \pm 0.28$  µg mL<sup>-1</sup>. In another study, Yildiz *et al.* (2005) demonstrated that the post-pregnancy vitamin C levels were lower than those in the pregnancy period. Similarly in a study, Kizil *et al.* (2005) stated that there was no difference in the average serum vitamin C levels pregnant and nonpregnant cows during the cycle. This findings were in agreement with that reported by some researchers (Ataman *et al.*, 2010).

In this research, serum vitamin E levels of the healthy and subclinical mastitic cows were  $2.46 \pm 0.36$  and  $2.76 \pm 0.84$  mg dL<sup>-1</sup> and milk vitamin E levels of the healthy and subclinical mastitic cows were  $0.43 \pm 0.48$  and  $0.34 \pm 0.50$  mg dL<sup>-1</sup>, respectively. The mean levels of vitamin E in blood serum were higher than in milk samples. There was no difference in serum and milk average vitamin E levels between healthy and subclinical mastitic cows (Table 1 and 2). It was found that in healthy animals mean plasma vitamin E value was  $6.00 \pm 0.23$  and in mastitic animals  $4.84 \pm 0.14$  mg L<sup>-1</sup>, the difference being significant ( $p < 0.05$ ) (Atroshi *et al.*, 1986).

Kaya and Guven reported that levels of plasma vitamin E in healthy and mastitic cows were  $24.74 \pm 9.83$  and  $14.23 \pm 4.47$  µg dL<sup>-1</sup>, respectively. Also, the difference statistically were important ( $p < 0.001$ ). In another study,

Simsek and Aksakal found that level of milk vitamin E in healthy cows ( $2.34 \pm 0.33$  mmol L<sup>-1</sup>) were higher than mastitic cows ( $2.04 \pm 0.38$  mmol L<sup>-1</sup>) and the difference statistically were important ( $p < 0.01$ ). Also, levels of plasma vitamin E in healthy and mastitic cows were  $5.79 \pm 1.34$  and  $4.09 \pm 0.90$  mmol L<sup>-1</sup>, respectively, the difference statistically were important ( $p < 0.001$ ). Ndiweni *et al.* (1991) stated that levels of plasma vitamin E in low incidence and high incidence clinical mastitis were  $7.57 \pm 1.86$  and  $7.74 \pm 1.69$  µg mL<sup>-1</sup>, respectively. Similarly in a study, Braun *et al.* (1991) revealed that in control herds serum vitamin E concentrations were  $6.60 \pm 3.90$  and in chronic mastitis herds  $5.70 \pm 3.50$  mg L<sup>-1</sup>, the difference was not significant.

Atroshi *et al.* (1986) reported that the concentrations of vitamin E in blood plasma were higher than those in milk samples. Similar findings were also observed in the present study. Mean vitamin E level in milk was similar to that reported by Atroshi *et al.* (1986) but less than those reported by some researchers (Braun *et al.*, 1991). In this study, average vitamin E level in serum less than those reported by researchers (Atroshi *et al.*, 1986; Braun *et al.*, 1991). In this study, serum MDA levels of the healthy and subclinical mastitic cows were  $1.81 \pm 0.64$  and  $1.51 \pm 0.61$  nmol mL<sup>-1</sup> and milk MDA levels of the healthy and subclinical mastitic cows were  $6.30 \pm 1.47$  and  $5.83 \pm 1.70$  nmol mL<sup>-1</sup>, respectively. The mean levels of MDA in milk samples were higher than in blood serum. There was no difference in milk samples mean MDA levels between healthy and subclinical mastitic cows but in blood serum average MDA levels in healthy cows were significantly higher than subclinical mastitic cows ( $p < 0.05$ ).

In a study, Dundar *et al.* (2000) found that serum MDA levels of the healthy and mastitic cows were  $3.79 \pm 0.38$  and  $6.69 \pm 0.59$  nmol mL<sup>-1</sup> and milk MDA levels of the healthy and mastitic cows were  $4.19 \pm 0.55$  and  $4.08 \pm 0.46$  nmol mL<sup>-1</sup>, respectively. Also, the MDA level in blood of subclinical mastitic cows was significantly higher ( $p < 0.001$ ) from that of healthy cows. In another study, Simsek and Aksakal reported that plasma MDA levels in healthy and mastitic cows ( $0.61 \pm 0.06$  and  $0.75 \pm 0.07$  nmol mL<sup>-1</sup>) and milk MDA levels in healthy and mastitic cows ( $0.50 \pm 0.07$  and  $0.66 \pm 0.07$  nmol mL<sup>-1</sup>). Also, plasma and milk MDA levels in healthy cows were significantly lower ( $p < 0.001$ ) from that of subclinical mastitic cows.

In this study, serum MDA levels detected in healthy and mastitic cows were less than those reported by Dundar *et al.* (2000), higher than those reported by Simsek and Aksakal but milk MDA levels detected in healthy and mastitic cows were higher than those reported by others.

## CONCLUSION

As a result, serum and milk vitamin C with serum MDA levels of healthy cows were determined significantly higher than of subclinical mastitic cows but there was no difference in serum and milk vitamin A, E with milk MDA levels between healthy and subclinical mastitic cows.

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