Total oxidant and antioxidant activities in milk with various somatic cell count intervals during discrete cow and buffalo lactation periods

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RESEARCH ARTICLE

Total oxidant and antioxidant activities in milk with various somatic cell count intervals during discrete cow and buffalo lactation periods

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Abstract: This study first investigated the total oxidant and antioxidant capacity (TOC and TAC, respectively) in cow and buffalo milk, with various somatic cell count (SCC) levels, having the same lactation numbers(LN). Second, it determined whether there is an association between the TAC and TOC and the lactation number, for the same SCC level. Quarter milk samples, collected from Holstein cows and Anatolian buffaloes, were separated into SCC levels of $\leq 2\times10^5$, 2×10^5-5×10^5, 5×10^5-1×10^6 and $\geq 1\times10^6$ cells mL^{-1} for cow milk, and $\leq 2\times10^5$, 2×10^5-4×10^5, 4×10^5-1×10^6 and $\geq 1\times10^6$ cells mL^{-1}, for buffalo milk. Next, each group was subdivided, according to the lactation number (cows: 1-2nd, 3-4th, 5-6th, buffaloes: 1-4th, 5-8th, 9-12th), and TOC and TAC of the milk were measured. For the same lactation numbers, TOC increased in the cow and buffalo groups with an SCC $\geq 1\times10^6$ cells mL^{-1} ($p<0.05$). Conversely, TAC decreased in cow milk with an SCC $>5\times10^5$cells mL^{-1}. In buffalo milk, TAC decreased in parallel with the increased SCC. Among the same SCC groups, TOC and TAC were not affected by the lactation number, in cow and buffalo milk. An increased SCC caused an increased TOC and decreased TAC level, for the same lactation number. No relation existed among TOC, TAC and lactation number, for the same SCC level. SCC may be used as an indicator of TOC and TAC in cow and buffalo milk.

Keywords: Buffalo milk, Cow milk, Lactation number, Total antioxidant, Total oxidant

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Introduction

Milk includes somatic cells (SCs) that originate from the mammary epithelial cells (25%) and the macrophages, polymorphonuclear neutrophils cells (PMNs), lymphocytes and erythrocytes from the blood (75%) (Boutinaud and Jammes, 2002). Although SCs have a protective effect against infectious organisms, their accrual alters the raw milk composition. For human consumption, the legal somatic cell count (SCC) threshold in milk and milk products, should be 5×10^5 and 4×10^5 mL^{-1} in raw bovine milk and water buffalo milk, respectively, according to the European Union criteria **(**Anonymous 2006**).** Le Maréchal *et al.* **(**2011**)** indicated that milk composition changes with increased SCC, following mammary infection. After somatic cell destruction, lipase (as lipoprotein lipase), oxidases (catalase [CAT] and lactoperoxidase), glycosidases (e.g. lysozyme) and proteases (e.g. elastase, cathepsins, collagenase), are secreted into the milk (Li *et al.,* 2014).

During mastitis, PMNs migrate from the blood to the mammary epithelium**.** Then, they phagocyte microorganisms and generate ROS and induce oxidative stress**.** One approach to avoid oxidative stress is to prevent the formation of ROS, while the other, is to strengthen the antioxidative defense mechanism **(**Hamed *et al.*, 2008**).**

Milk contains low and high molecular weight compounds that bind to certain metal ions and protect them from prooxidative effects or display an antioxidative effect, by removing free radicals with enzymatic or non-enzymatic activities **(**Kasapoviæ *et al.*, 2005**).**

Total antioxidant capacity is reported to decrease during the lactation period (Castillo *et al.,* 2006). Also, increases in the SCC are associated with increased levels of some antioxidant enzymes, such as CAT (Hamed *et al.,* 2008), glutathione peroxidase (GPX) (Andrei *et al.,* 2009; Hamed *et al.,* 2008), as well as nitric oxide (NO) and TOC (Atakisi *et al.,* 2010) in udder infections. On the contrary, Yang *et al.* (2011) indicated a decrease in GPX in mastitic milk. Suriyasathaporn *et al.* (2006) determined that MDA levels increased because of an increased number of SCs. Also, Hamed *et al.* (2008) documented that PMNs cause oxidative damage and alter the antioxidative status of the animal during mastitis.

Buffalo milk contains more calcium, phosphorus and protein than cow milk (Damé *et al.,* 2010) and also has a very low cholesterol content (Lotfi and Narimani-Rad, 2013). Furthermore, mastitis is mentioned to be less common in buffaloes than cows (Ozenc *et al.,* 2008). Dimri *et al.* (2013) reported that while TOC, NO and CAT increase, TAC and GPX decrease in mastitic buffalo milk.

The first aim of this study was to investigate the association between the SCC and the oxidant and antioxidant system in the milk of Holstein cows and Anatolian buffaloes. Second, to compare the lactation number, TOC and TAC of the milk, displaying the same SCC intervals.

Materials and Methods

The experiments were approved by the Local Ethics Committee of Laboratory Animals (2014/43). Quarter milk samples were collected from healthy Holstein cows (n=222) and Anatolian buffaloes (n=167), each of which was fed with the same ration. For each milk sample, the SCC was determined by an automated fluorescent microscopic somatic cell counter (Bentley IBC-M; Bentley Instruments Inc., Chaska, MN, USA). Ethidium bromide dye was used for specific binding to DNA in cell nuclei.

Each milk was classified according to the SCC into $\leq 2\times10^5$, 2×10^5-5×10^5, 5×10^5-1×10^6 and $\geq 1\times10^6$ per mL for cows and $\leq 2\times10^5$-4×10^5, 4×10^5-1×10^6 and $\geq 1\times10^6$ per mL for buffaloes. Each of these groups was then subdivided, according to the lactation number, as 1-2nd, 3-4th, and 5-6th for cows; and 1-4th, 5-8th, and 9-12th for buffaloes. The milk was defatted by centrifugation (15000 rpm at 4 °C), due to interference resulting from the turbidity caused by the milk fat, and then, stored at -80 °C, until analysis. Before analysis, the defatted milk was filtered (45 µm), to improve the milk clarification. Then, TOC and TAC were measured, using commercial kits (Cat no.: RL0024 and RL0017 respectively, Rel Assay Diagnostics, Turkey). The number of samples is provided in Table 1.

Statistical analysis was done, using GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA) software for Windows. All data were assessed by one-way analysis of variance (ANOVA). Post-hoc multiple comparison tests were performed on the least-square (LS) means, by using Tukey's test for P-values. The results represent the LS means ± standard error (SE). $P<0.05$ conferred statistical significance.

Results and Discussion

Within each lactation number group, TOC was significantly higher in all groups with $\geq 1\times10^6$ SCC than the other groups ($p<0.05$). Conversely, within the groups with the same SCC, TOC values were not significant until $\geq 1\times10^6$ SCC and the highest TOC values were measured in cow milk with 1-2nd, 3-4th and 5-6th lactation number, respectively (Figure 1).

As shown in Figure 2, TAC was higher in $\leq 2\times10^5$ and 2×10^5-5×10^5 groups than 5×10^5-1×10^6 and $\geq 1\times10^6$ SCC in bovine milk. When the groups with the same SCC were compared with respect to TAC, no difference was observed, according to the lactation number.

Figure 3 reveals that TOC decreased with decreased SCC, while TOC values were the highest with $\geq 1\times10^6$ SCC in all lactation groups in Anatolian buffaloes. The highest TOC values were determined in the milk of animals with $\geq 1\times10^6$ SCC and 9-12th lactation, in the groups with the same SCC. Also, TOC activity in milk with 4×10^5-1×10^6 SCC at 9-12th lactation, was higher than the 1-4th and 5-9th lactation. No difference was observed in the groups

Table 1 Number of quarter milk samples in Holstein cows and Anatolian buffaloes

		Number of somatic cell count			
Number of	Lactation age (LA)	$\leq 2\times10^5$	2×10^5-5×10^5	5×10^5-1×10^6	$\geq 1\times10^6$
quarter milk	1-2th	31	11	15	12
samples in	3-4th	31	24	15	12
Holstein cows	5-6th	33	10	12	16
Number of		$\leq 2\times10^5$	2×10^5-4×10^5	4×10^5-1×10^6	$\geq 1\times10^6$
quarter milk	1-4th	35	9	15	19
samples in	5-8th	20	10	10	12
Anatolian buffaloes	9-12th	17	7	6	7

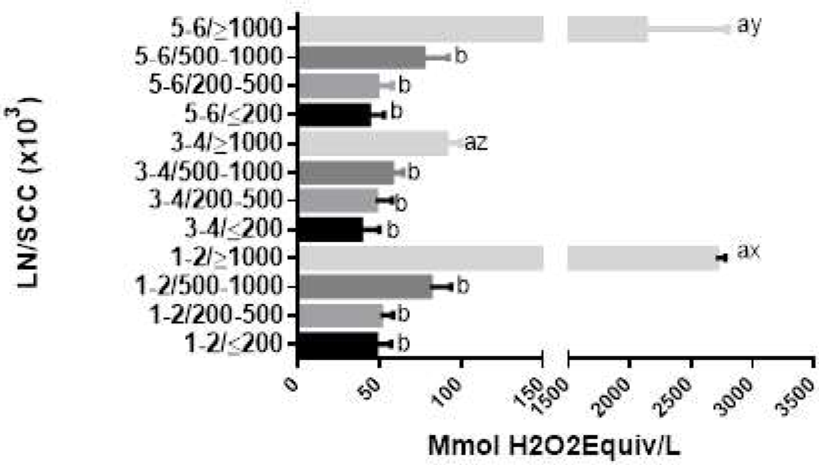


Figure 1 Total oxidant capacity of Holstein milk with various somatic cell count (SCC) at 1-2nd, 3-4th and 5-6th lactation number. {Values with different letters above bars (a, b, c) among the same lactation number are significantly different (p<0.05). Values with different letters above bars (x, y, z) among the same SCC are significantly different (p<0.05)}.

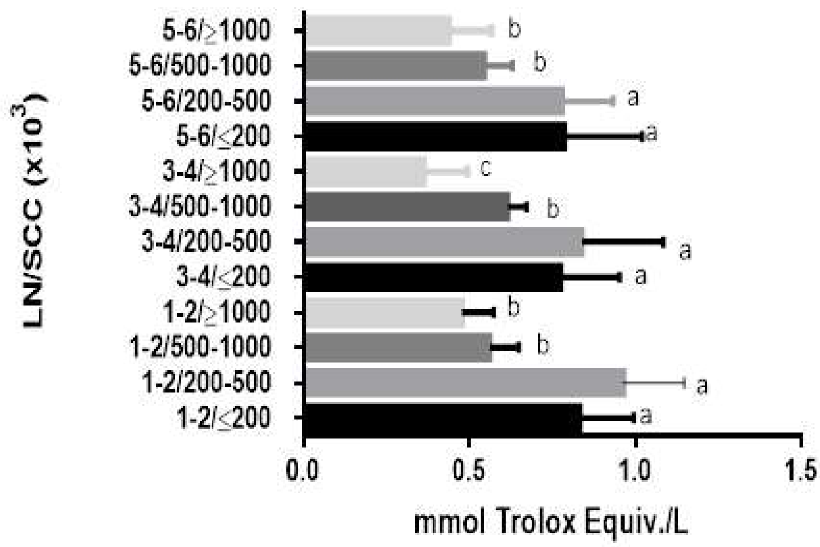


Figure 2 Total antioxidant capacity of Holstein milk with various somatic cell count (SCC) at 1–2nd, 3–4th and 5–6th lactation number {Values with different letters above bars (a, b, c) among the same lactation number are significantly different (p<0.05).Values with different letters above bars (x, y, z) among the same SCC are significantly different (p<0.05)}.

with 2×10^5-4×10^5 and $\leq2\times10^5$ SCC, according to the lactation number.

In Anatolian buffaloes, while TAC was the highest in the $\leq2\times10^5$ SCC group, it was the lowest in the $\geq1\times10^6$ SCC group in the 1-4th lactation number. In the 5-8th lactation number, TAC was higher in the $\leq2\times10^5$, 2×10^5-4×10^5 and 4×10^5-1×10^6 SCC groups than the $\geq1\times10^6$ SCC group. In the 9-12th lactation number, TAC was the highest in the $\leq2\times10^5$ SCC group and it was the lowest in the $\geq1\times10^6$ SCC group ($p<0.05$) (Figure 4).

Milk, which is an indispensable food, is composed of casein, milk serum proteins (pepsin, trypsin), peptides (and amino acids formed by their breakdown), vitamins A, E and C, enzymes (superoxide dismutase, CAT, GPX, glutaryl reductase, lactoperoxidase, ceruloplasmin), carotenoids and lactic acid bacteria, in addition to numerous antioxidant substances, such as lactoferrin and NO. Lindmark-Månsson and Åkesson (2000) stated that the content of the antioxidant substances of the milk, may depend on the race, nutritional status, feed, geographical location, lactation period, and the processing and presentation of the products. The mechanisms of action of antioxidant compounds vary. Possible antioxidant mechanisms of milk proteins are based on the neutralization of the free radicals by the release of lactoferrin and whey albumin, and the amino acids, such as tyrosine and cysteine (Jiménez *et al.,* 2008; O'Connell and Fox, 2001). Antioxidant activities of serum proteins depend on the presence of sulfhydryl groups and, when these groups are absent, the activity is lost (Shahidi, 2000). Identification of the TAC of the milk and characterization of these active compounds is important for health and particularly for protection against free radicals. In this context, it is also valuable to know the potential health-promoting properties, as well as the nutritional attributes of milk consumption. Milk antioxidants also play a key role in inhibiting lipid peroxidation and maintaining milk quality (Sönmez *et al.,* 2010).

Lipid peroxidation in mammary glands, causes mastitis (Suriyasathaporn *et al.,* 2006). During mastitis, PMNs migrate from the blood to the mammary epithelium**.** Then, they phagocyte

Figure 3 Total oxidant capacity of Anatolian buffalo milk with different somatic cell count (SCC) at 1–4th, 5–8th and 9–12th lactation number {Values with different letters above bars (a, b, c) among the same lactation number are significantly different ($p<0.05$). Values with different letters above bars (x, y, z) among the same SCC are significantly different ($p<0.05$)].

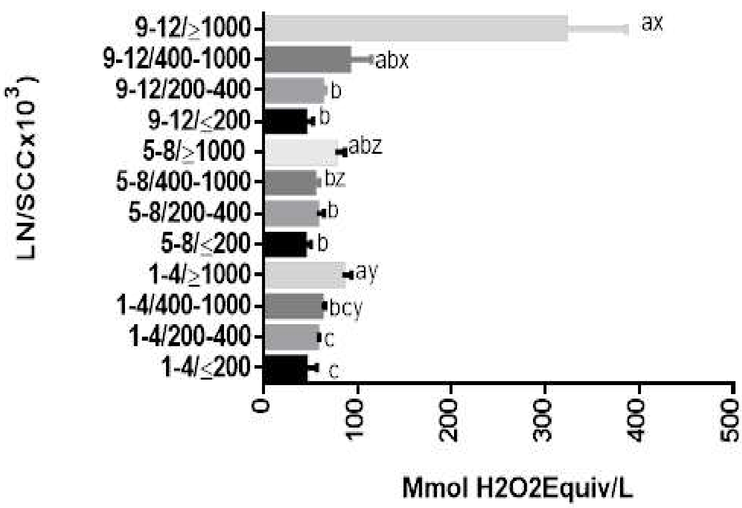
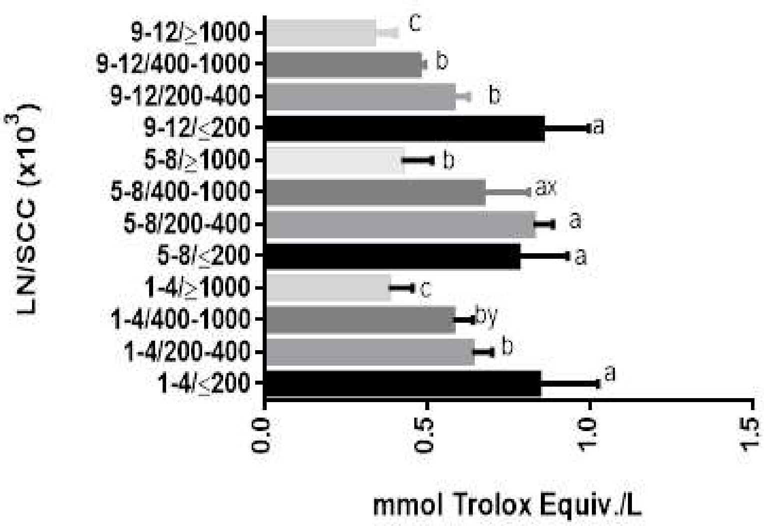


Figure 4 Total antioxidant capacity of Anatolian buffalo milk with different somatic cell count (SCC) at 1–4th, 5–8th and 9–12th lactation number {Values with different letters above bars (a, b, c) among the same lactation number are significantly different ($p<0.05$). Values with different letters above bars (x, y, z) among the same SCC are significantly different ($p<0.05$)}.



microorganisms, generating ROS and inducing oxidative stress (Hamed *et al.,* 2008), thus, the SCC and MDA level in milk increases (Andrei *et al.*, 2010). Also, TOC and NO increase as SCC increases (Atakisi *et al.,* 2010). In the current research, the group with $\geq 1\times10^6$ SCC had the highest TOC, among all the lactation number groups in cows, which concurs with the above-mentioned findings that report oxidative stress increases the SCC. The TOC decreased in the order 1-2nd> 5-6th> 3-4th lactation number in the groups with $\geq 1\times10^6$ SCC and this may indicate that oxidative stress does not depend on the number of lactation.

TAC is documented to be a general indicator of measurable antioxidants in plasma and body fluids (Ghiselli *et al.,* 2000) and the plasma TAC levels in cows can change, according to the species and the season (Mandebvu *et al.,* 2003). Lipko-Przybylska and Kankofer (2012) informed that milk antioxidant activity was the highest in the 2-5th lactation numbers, in female pigs that have 1-8 lactation periods. Also, according to the same report, antioxidant activity started to decrease towards the 5th lactation and some antioxidants should be added to the rations of pregnant pigs. In the present study, it was found that TAC activity decreased in all groups with $>5\times10^5$ SCC, which is the acceptable limit for consumption of raw cow milk ($p<0.05$). Furthermore, TAC was not affected by the lactation number ($p>0.05$) within the same SCC groups. Our results show that the increase in SCC in the milk, which is an important indicator of udder health, alters the composition of the milk (Le Maréchal *et al.,* 2011) because, following the breakdown of SCs, some antioxidant factors are released into the milk (Li *et al.,* 2014). This reason might be the cause of the decreasing TAC as SCC increases. Although buffalo milk contains more fat (7-8% on average) than cow milk, the cholesterol level (0.65 mg kg^{-1}) of buffalo milk is considerably lower (3.14 mg kg^{-1}). The protein content of buffalo milk (3.5-4%) is higher compared to cow milk and contains fewer bacteria, due to the antibacterial effect of lactoferrin protein. It has also been reported that the amount of tocopherol and peroxidase activity, which are natural antioxidants in buffalo milk, is 2-4 times higher than in cow milk and also contains higher levels of vitamins A, C, E, B1 and B2. Hence, it has been suggested that buffalo milk may be superior to cow milk, in terms of taste and health benefits (Akgün and Yazici, 2012; Çetinkaya *et al.,* 2012; Küçükkebabçý and Aslan, 2002).

It has been documented that mastitis in buffaloes, corresponds to an SCC >2×10^5 cells mL^{-1} (Tripaldi *et al.,* 2010). Ozenc *et al.* (2008) determined that there is an infection in milk with an SCC >130×10^3 cells mL^{-1}, and after the 5th lactation, the SCC increases in Anatolian buffaloes. According to Dimri *et al.* (2013), while TOC, NO and CAT increase, TAC and GPX decrease in mastitic buffalo milk. A previous study (Bansal *et al.,* 1995) has mentioned that the mastitis rate is similar in cows and buffaloes. In the current study, it is thought that the highest TOC level corresponded to the groups with an SCC $\geq1\times10^6$ cells mL^{-1} in the Anatolian buffaloes, among the same lactation group, because of the severity of mastitis. These findings were similar to those of Uallah *et al.* (2005), who informed that subclinical mastitis affects the milk composition. Also, the TOC level of milk with 4×10^5- 1×10^6 cells mL^{-1} SCC was higher in the 1-4th and 9-12th lactation groups than the 5-8th. There was no difference among the other lactation groups, at the same SCC level. These findings agree with the notification that if the udder or mammary gland is not infected, there is no correlation between lactation period, age, season, and the effects of various stresses with SCC (Harmon, 1994). Hence, the TOC value is not expected to be affected by the lactation number.

Rajesh *et al.* (2010) reported that the antioxidant activities of casein fractions were higher in buffalo than cow milk. In accordance with the severity of the mastitis. Sharif *et al.* (2007) stated that the casein content, which possesses the main TAC of buffalo milk, was reduced. In the current study, the probable reason of decreased TAC activity in the groups with $\leq2\times10^5$ cells mL^{-1} SCC compared to the groups with $\geq1\times10^6$ cells mL^{-1} SCC, for the same lactation number, may be a decreased casein level. A comparison of the lactation number, revealed that TAC was higher in the group at 5-8th than the group at 1-2nd in the groups with a SCC of $4\times10^5 1\times10^6$ cells mL^{-1}. There was no statistically significant difference observed in the 9-12th lactation period. These findings support previous reports that the milk SCC is not influenced by the lactation number, age, season and various stresses, if the mammary gland is not infected and, consequently, there are only a few factors that affect the milk SCC (Harmon, 1994).

Conclusions

Although TOC and TAC could be associated with the SCC in cow and buffalo milk, it was observed that the lactation number was not linked to oxidant and antioxidant activity. These results proved that the milk, that have acceptable limit of SCC in cow and buffalo, has more TAC activity and beneficial for health, and raw milk antioxidant capacity may be an indicative parameter for consumption.

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