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Status of Oxidative DNA Damage in Serum and Saliva of Dairy Cows During Lactation and Dry Period

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Abstract: The present study, researchers evaluated for the first time serum and salivary 8-Hydroxy-2'-deoxyguanosine (8-OHdG) and salivary Total Antioxidant Capacity (TAC) during different stages of lactation and also during the dry period in dairy cows. Cows at dairy farms that belong to Morioka City (Iwate, Japan) were classified into five groups according to the month of lactation starting from postpartum till the dry period. Oxidative DNA damage marker (8-OHdG) and TAC were measured in saliva and serum samples using commercial ELISA kits. The results revealed that salivary 8-OHdG levels were higher ($p<0.01$) than its corresponding serum levels during both lactation and dry period. Serum levels of TAC were significantly higher ($p<0.01$) than their salivary levels during lactation and dry period. It could be concluded that oxidative DNA damage was prominent during the dry period. Cows at the dry period have the highest serum level of 8-OHdG and the lowest salivary level of TAC when compared to lactating cows.

Key words: Cows, 8-OHdG, total antioxidant capacity, lactation, dry period

INTRODUCTION

Free radicals are highly reactive substances produced continuously during metabolic processes. Approximately 95% of the oxygen consumed by organism is reduced to water during aerobic metabolism taking place in mitochondrial respiratory chain. The remaining amount may be converted to reactive oxygen species. The most important reactive oxygen species include superoxide anion ($O_2^{\bullet-}$), hydroxyl radical (•OH) and hypochlorous acid (Stohs, 1995; Abd Ellah, 2010). Free radical excess results in impairment of DNA, enzymes and membranes and induces changes in the activity of the immune system and in the structure of basic biopolymers which in turn may be related to mutagenesis and aging processes (Poli, 1993).

The cells contain a variety of antioxidants mechanisms that play a central role in the protection against reactive oxygen species (Par and Javor, 1984; Halliwell, 1991). The antioxidant system consists of antioxidant enzymes (Superoxide Dismutase (SOD), catalase and Glutathione Peroxidase (GSH-Px), glutathione, ancillary enzymes (Glutathione Reductase (GR), glutathione S-transferase and glucose 6-phosphate dehydrogenase, metal-binding proteins (transferrin, ceruloplasmin and albumin), vitamins (α-tocopherol, ascorbate and β-carotene), flavonoids and urate (Halliwell, 1994; Abd Ellah *et al*., 2009). Antioxidants may act by scavenging the radicals and sustaining the activity of antioxidant enzymes or inhibiting the activity of oxidizing enzymes (Schreck and Baeuerle, 1994).

Under physiological conditions, the body usually has sufficient antioxidant reserves to cope with the production of free radicals (Miller *et al*., 1993; Castillo *et al*., 2001). However, when free radical generation exceeds the body's antioxidant production capacity, oxidative stress develops. In dairy cows, the peripartum and early lactation periods are especially critical and present considerable physiological challenges to homeostasis by imposing significant metabolic stressors that may contribute to the onset of diverse disorders (Goff and Horst, 1997).

Oxidative stress has been suggested to contribute to dairy cattle under physiological conditions including

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pregnancy, lactation, estrus cycle, transition period and also associating cattle diseases including mastitis, retained placenta and udder edema (Miller *et al*., 1993; Abd Ellah, 2013). Reportedly, plasma and erythrocyte oxidants as well as antioxidants varied during the transition period in cows and also revealed an alteration of the oxidative status during the early lactation phase in Holstein cows (Miller *et al*., 1993; Formigoni *et al*., 1997; Ronchi *et al*., 2000; Bernabucci *et al*., 2002). Furthermore, Wachter *et al*. (1999) and Castillo *et al*. (2005) reported a progressive decline in antioxidant activity as lactation progresses in dairy cows.

It is well known that reactive oxygen metabolites are produced continuously by normal metabolic processes but the rate of production may be increased markedly under diverse conditions of increased metabolic demand. The metabolic demands imposed on the cow by colostrum production and the onset of lactation far exceeds the demands of the fetus (Castillo *et al*., 2005). In the last few years, the detection of free radical damage and protection against it has become increasingly important in clinical medicine as a complementary tool in the evaluation of the metabolic status (Castillo *et al*., 2003).

8-Hydroxy-2'-deoxyguanosine (8-OHdG) is formed when DNA is oxidatively damaged by Reactive Oxygen Species (ROS). 8-OHdG is one of the most sensitive biomarker for oxidative stress. Although, many studies were established the increased oxidative stress during lactation and pregnancy, none of these studies were concerned with the study of oxidative DNA damage in dairy cows, the current study aimed to evaluate the status of serum and salivary 8-OHdG and Total Antioxidant Capacity (TAC) in dairy cows during lactation and during the dry period and also aimed to determine the stage of lactation or pregnancy during which oxidative DNA damage occurs.

MATERIALS AND METHODS

Animals: The study was carried out using 30 healthy multiparous Holstein cows maintained in dairy farms located in Morioka (Iwate ken, Japan). The animals were divided into six groups. The first group comprised cows during the 1st month of lactation (postpartum). The second group included cows during the second month of lactation with an average milk yield 36.94±16.00 kg/animal/day. The third group included cows during the 3rd month of lactation with an average milk yield 41.10±8.15 kg/animal/day. The fourth group included cows during the 4-5th month of lactation with an average milk yield 37.72±6.48 kg/animal/day. The fifth group comprised cows during the 6-7th month of lactation with an average milk yield 43.07±8.49 kg/animal/day. The sixth group included cows during the dry period. During the study period all animals were kept under identical conditions.

Sample collection and analytical procedures: Blood samples were obtained from the jugular vein in evacuated (Vacutainer) tubes without anticoagulant. Tubes were allowed to clot at room temperature for 30 min before centrifugation at 2000 g for 20 min (Coles, 1986), the serum was frozen at -80°C until analysis.

Saliva samples were collected from all cows in a clean, sterile and dry plastic tube from the buccal cavity. The saliva samples were centrifuged at 10,000 g for 30 min at 4°C and then the supernatant was stored at -80°C until analysis (Rai *et al*., 2010). Serum and saliva samples were used for the quantitative detection of the oxidative DNA adduct 8-OHdG and for measuring TAC.

Measurement of serum and saliva 8-OHdG: Serum samples were filtered using an Ultra-filter (cutoff molecular weight 10 kDa, Nanostep 10K Omega, Pall Corporation, Michigan, USA) to exclude interfering substances.

8-OHdG was quantitatively measured in serum filtrate and saliva using enzyme linked immunosorbent assay kit (New 8-OHdG Check kit, JaiCa, Nikken SEIL Co., Shizuoka, Japan). The absorbance of the standard and samples was measured at 450 nm using Microplate reader (MPR-A4i Tosoh, Tokyo, Japan) a standard curve was generated and used to determine the concentration of 8-OHdG (ng mL^{-1}) present in samples. Assay range was from 0.5-200 ng mL^{-1}.

Measurement of serum and saliva TAC: Serum and saliva TAC were measured using commercial test kits (PAO test kit for total antioxidant capacity, JaiCa, Nikken SEIL Co., Shizuoka, Japan). The absorbance of the standard and samples was measured at 490 nm using Microplate reader (MPR-A4i Tosoh, Tokyo, Japan) a standard curve was generated and used to determine the antioxidants power (μmol L^{-1}) in samples.

Statistical analysis: Statistical significance was carried out using the Statistical Package for the Social Sciences for Windows (SPSS, Version 10.0, Chicago, IL, USA). Groups were tested for difference using analysis of variance LSD Post-hoc test. Statistically significant differences were determined at $p \leq 0.05$.

RESULTS AND DISCUSSION

Serum and salivary 8-OHdG level: Serum 8-OHdG level showed a significant increase ($p<0.05$) during the dry period when compared with its level during the periods

from the 1st-7th month of lactation. No significant changes were observed in serum 8-OHdG levels during the studied lactation periods (Table 1).

Salivary 8-OHdG level showed a significant increase ($p<0.01$) during the dry period when compared with its level during the 1st month of lactation. The highest salivary 8-OHdG level was observed during the 4-5th month of lactation (116.00±44.8 ng mL^{-1}) which was significantly higher than its level during other months of lactation but was insignificant when compared to its level (89.50±37.23 ng mL^{-1}) during the dry period (Table 1). Generally, salivary 8-OHdG levels were higher ($p<0.01$) than its corresponding serum levels during all months of lactation and during the dry period (Table 2).

Serum and salivary TAC: Serum TAC showed a significant increase ($p<0.05$) at 2nd-6th month of lactation compared to its level during the 1st month after parturition. Furthermore, serum TAC was significantly decreased ($p<0.01$) during the dry period compared to its level during the 2nd-6th months of lactation (Table 1).

The highest level for salivary TAC was observed during the 2nd month of lactation (693.50±153.34 μmol L^{-1}) which was significantly higher ($p<0.01$) than its level during other months of lactation. The lowest value for salivary TAC was observed during the dry period (105.29±57.45 μmol L^{-1}) during which its level was significantly decreased when compared with levels measured in other months of lactation except the 3rd month. Starting from the 4th month of lactation, salivary TAC levels showed a linear decrease (Table 1). However, a steady decrease in serum TAC was started at the 4th month of lactation.

Generally, serum TAC levels were significantly higher ($p<0.01$) than their salivary levels during all months of lactation except during the 2nd month of lactation. Also, serum TAC level was significantly higher ($p<0.01$) than its corresponding saliva level during the dry period (Table 2).

The present study constitutes the first that investigated the serum and salivary levels of 8-OHdG in dairy cows. Serum TAC was measured during lactation in some studies (Cao and Prior, 1998; Niki and Noguchi; 2000; Castillo *et al*., 2005) however, the current study represents the first record for measurement of salivary TAC in dairy cows.

The role of oxidative stress in health and disease was studied extensively in both human and animal medicine (Valko *et al*., 2007). Dairy cows undergo massive metabolic changes during lactation and pregnancy which may have a negative impact on the health and productive performance of the dairy cows (Sordillo *et al*., 2009). It is well known that reactive oxygen metabolites are produced continuously by normal metabolic processes but the rate of production may be increased markedly under diverse conditions of increased metabolic demands. Metabolic adaptations to lactation are initiated in late pregnancy, especially during the dry period. Nevertheless, these adaptations vary widely among individual cows (Bell, 1995) especially after calving which lead to inter-individual variation in metabolic activities with variable tissue consumption of O_2 and hence variable production of lipoperoxides.

Oxidative stress can result in DNA damage including the oxidation of nucleosides. 8-hydroxy-2'-deoxyguanosine is an oxidized nucleoside that is excreted in the body fluids with DNA repair. Several studies have demonstrated that 8-OHdG in body fluids can act as a

Table 1: Serum and salivary 8-OHdG and TAC levels in dairy cows

	Serum		Saliva	
Time period	8-Hydroxy-2'-deoxyguanosine (ng mL^{-1})	Total antioxidants capacity (μmol L^{-1})	8-Hydroxy-2'-deoxyguanosine (ng mL^{-1})	Total antioxidants capacity (μmol L^{-1})
1st month of lactation (n = 6)	1.40±0.73^{b}	602.12±47.990^{a}	18.60±0.630^{b}	304.47±93.130^{b}
2nd month of lactation (n = 4)	1.24±0.43^{b}	688.62±39.210^{b}	104.40±62.61^{a}	693.50±153.34^{c}
3rd month of lactation (n = 5)	0.66±0.41^{b}	743.58±66.070^{bdc}	54.47±21.02^{ab}	181.44±77.810^{ab}
4-5th month of lactation (n = 6)	1.21±0.81^{b}	802.34±116.09^{dc}	116.00±44.80^{c}	368.60±178.60^{d}
6-7th month of lactation (n = 5)	1.52±0.81^{ab}	774.71±62.210^{bc}	62.13±51.63^{abc}	273.16±103.88^{bd}
Late pregnancy (dry period) (n = 4)	2.35±0.89^{a}	609.56±53.050^{a}	89.50±37.23^{ac}	105.29±57.450^{a}

Data were expressed as Mean±SD; In each column, different letters means significant

Table 2: Comparison of Serum and salivary 8-OHdG and TAC levels in dairy cows

	8-Hydroxy-2'-deoxyguanosine (ng mL^{-1})		Total antioxidants capacity (μmol L^{-1})	
Time period	Serum	Saliva	Serum	Saliva
1st month of lactation (n = 6)	1.40±0.73	18.60±0.630**	602.12±47.990	304.47±93.130**
2nd month of lactation (n = 4)	1.24±0.43	104.40±62.61**	688.62±39.210	693.50±153.34
3rd month of lactation (n = 5)	0.66±0.41	54.47±21.02**	743.58±66.070	181.44±77.810**
4-5th month of lactation (n = 6)	1.21±0.81	116.00±44.87**	802.34±116.09	368.60±178.60**
6-7th month of lactation (n = 5)	1.52±0.81	62.13±51.63**	774.71±62.210	273.16±103.88**
Late pregnancy (dry period) (n = 4)	2.35±0.89	89.50±37.23**	609.56±53.050	105.29±57.450**

Data were expressed as Mean±SD; **highly significant ($p<0.01$)

biomarker of oxidative stress (Chiou *et al*., 2003; Liu *et al*., 2004; Wu *et al*., 2004). Salivary 8-OHdG level was extensively studied in human medicine (Takane *et al*., 2005; Canakci *et al*., 2006) and in rat (Yoshino and Nakagawa, 2011). However, there is no published study on salivary 8-OHdG in animals. In the present study, salivary 8-OHdG level was significantly higher ($p<0.01$) than its corresponding level in serum in all cows under study. The highest value (116.00±44.87 ng mL^{-1}) was reported during the 4-5th month of lactation. On the other hand, the highest serum 8-OHdG level (2.35±0.89 ng mL^{-1}) was reported during the dry period. Serum and salivary 8-OHdG levels in rat were recorded as 6.59±4.33 and 16.33±4.33 ng mL^{-1}, respectively (Yoshino and Nakagawa, 2011). In human, levels of 8-OHdG levels in serum and saliva were varied from one study to another. Canakci *et al*. (2009) reported that salivary 8-OHdG level was 1.41±0.22 ng mL^{-1} in healthy human. However, Takane *et al*. (2005) reported that 8-OHdG level was 2.35±0.18 ng mL^{-1} in human saliva. Rai *et al*. (2010) found that serum and salivary 8-OHdG levels in human were 2.21±1.08 and 0.45±0.02 ng mL^{-1}, respectively. On the other hand, Su *et al*. (2009) reported that 8-HOdG in saliva of control group was 42.7 ng mL^{-1} which is much higher than other studies done on human. They attributed the discrepancy to the differences in how saliva was collected. Serum level of 8-OHdG level from the present study is in accordance with that reported in some studies on human (Takane *et al*., 2005; Rai *et al*., 2010). However, salivary 8-OHdG level is higher than all levels that reported in previous studies that were done on human and on rats. It seems that normal salivary 8-OHdG level is higher in dairy cows than its level in human. It was suggested that 8-OHdG is secreted from the salivary gland (Yoshino and Nakagawa, 2011) which may constitute the cause that stand behind its high level in saliva that recorded in the present study.

In Table 2, the significant increase in serum TAC than its level in saliva indicated that the secretion of antioxidants into saliva is controlled as the body needs the antioxidants to overcome the increased oxidative stress. Results of the present study revealed that during the dry period there were significant decreases in serum and salivary TAC and significant increases in serum and salivary 8-OHdG levels which indicated that oxidative DNA damage was evident during the dry period. The increased oxidative DNA adduct (8-OHdG) in serum and saliva during the dry period may be attributed to increased oxidative stress due to the high metabolic demands.

CONCLUSION

It could be concluded that salivary 8-OHdG level is higher than its corresponding serum level during all months of lactation and during the dry period. Serum TAC is higher than its salivary level during lactation and dry period. Oxidative DNA damage was prominent during the dry period. Cows at the dry period have the highest serum level of 8-OHdG and the lowest salivary level of TAC when compared to lactating cows.

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