

Original Research Article

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Assessment of Oxidative Stress by Trace Elements in Pregnant Kankrej Cows

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ABSTRACT

The present study was carried out with the objectives to study oxidative stress in periparturient cows by estimation of the levels of antioxidant trace elements copper, zinc and iron in plasma at 30, 15 days before parturition, on the day of parturition, 15 and 30 days after parturition. During study significantly higher levels of zinc ($P<0.05$) and iron ($P<0.01$) was observed between control and parturited animals. This indicates higher oxidative stress on the day of parturition. Zinc and iron decreased significantly from 30 days before parturition and attained lowest levels on the day of parturition. However, the level increased gradually thereafter up to 30 days after parturition. Significant increase in copper level was observed on the day of parturition, thereafter the levels decreased. Copper is a constituent of SOD enzyme and levels of SOD also increased on the day of parturition. The present study indicates some kind of role of the antioxidant trace elements, zinc and iron in protecting cows from oxidative stress as their levels significantly decreased during pregnancy and parturition.

Keywords

Antioxidant trace elements, Copper, Iron, Oxidative stress, pregnant Kankrej cow, Zinc

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Introduction

A living creature has to survive under the environment created as a result of interactions of internal and external factors. There are many physiological processes namely parturition, milk production, preterm labor, lactation which create a stressful condition in the animal body. As it is very well known that

during the various biochemical metabolic processes nascent oxygen is released and its level is regulated by various antioxidative agents of the body.

Deficiency of any such antioxidative agent will favour the prevailing of the nascent oxygen which will be damaging the cell's metabolic processes or cell itself and produce

reactive oxygen species (ROS), such as hydroxyl radicals, superoxide anion, and hydrogen peroxides. The balance between the rate at which oxidative damage is induced (input) and the rate at which it is efficiently repaired and removed (output) determines the level of oxidative stress. The rate at which damage is caused is determined by how fast the ROS are generated and then inactivated by endogenous defense agents called antioxidants. In a healthy animal, ROS and antioxidants remain in balance, when the balance is disrupted towards an over abundance of ROS, oxidative stress occurs.

Oxidative stress can also lead to the modification of important physiological and metabolic functions. This damage can affect a specific molecule or the entire organism and leads to peroxidative damage of lipids and other macromolecules, with consequent alteration of cell membranes and other cellular components (Toyokuni, 1999).

Trace minerals, such as iron (in catalase), copper, zinc, manganese (in superoxide dismutase) and selenium (in glutathione peroxidase), are essential to the structure and function of many of these antioxidant enzymes (Kleczkowski *et al.*, 2003).

Oxidative stress plays a role in multiple physiological processes from oocyte maturation to fertilization, embryo development during pregnancy, in normal parturition and in initiation of preterm labor (Agrawal *et al.*, 2005).

Pregnancy is a physiological state accompanied by a high energy demand for many bodily functions and an increased oxygen requirement. Because of the increased intake and utilization of oxygen augmented level of oxidative stress would be expected (Gitto *et al.*, 2002). Looking to the immense importance of trace elements in pregnancy we

determined the level of endogenous antioxidant minerals viz. zinc, copper and iron in plasma of pre and post parturient cows.

Materials and Methods

The present study was carried out in Department of Animal Physiology and Biochemistry and in collaboration with Department of Animal Nutrition, College of Veterinary Science and Animal Husbandry, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar. The samples from twelve healthy Kankrej cows were taken from Livestock Research Station, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar. The animals were kept under loose system of housing, in well-ventilated hygienic sheds and fed on standard ration including green fodder. Twelve animals were divided into two groups of six animals each.

Group-I (control group) comprised of six healthy non-pregnant Kankrej cows in their second or third lactation.

Group-II (experimental or periparturient group) comprised of six periparturient Kankrej cows.

Approximately 10 ml of blood was collected aseptically in heparinized vials by puncturing the jugular vein of animals under study. Blood was collected only once at diestrous stage from Group I animals. Blood samples were collected at approximately 30, 15 days before parturition, on the day of parturition, 15 and 30 days after parturition from Group II animals. The plasma samples were separated from whole blood by centrifugation at 3000 rpm for 15 minutes, and stored at -20⁰C for further analysis. Biochemical analyses of plasma samples were carried out for trace elements.

Trace elements

0.2 ml of blood plasma samples were digested with 1.8 ml of triple acid mixture (concentrated sulphuric acid: perchloric acid: nitric acid in the ratio of 1:2:1) till it becomes colorless. After digestion the final volume was made to 10 ml with triple glass distilled water. Copper, zinc and iron concentration from the digested samples were determined by atomic absorption spectrophotometer (Model AAS 4141, Electronic Corporation of India Ltd. make) method as described by (Oser, 1979).

Statistical analysis

The significance of difference between means for different biochemical parameters were tested by applying paired t-test for dependant samples and unpaired t-test for independent samples assuming equal variances (Snedecor and Cochran, 1994).

Results and Discussion

Zinc

Control group with periparturient group

The mean concentration of zinc in blood plasma of non-pregnant Kankrej cows was 1.24 ± 0.02 ppm, while that in periparturient cows at 30 and 15 days before parturition, on the day of parturition, 15 and 30 days after parturition were 1.06 ± 0.06 , 1.05 ± 0.07 , 1.02 ± 0.07 , 1.19 ± 0.07 and 1.23 ± 0.06 ppm, respectively and the same has been presented in Table.

Periparturient group

The results indicate that the plasma zinc concentration was significantly lowest on the day of parturition (1.02 ± 0.07 ppm) compared to 30 and 15 days before

parturition, 15 and 30 days after parturition (1.06 ± 0.06 , 1.05 ± 0.07 , 1.19 ± 0.07 and 1.23 ± 0.06 ppm, respectively) of the experiment. A decreasing trend in levels was observed from 30 day before parturition, while reverse trend was observed after the day of parturition.

Copper

Control group with periparturient group

Copper concentration in blood plasma of non-pregnant Kankrej cows was observed to be 1.19 ± 0.01 ppm, and the same has been presented in Table.

Highly significant differences ($p < 0.01$) were observed for copper level between non-pregnant group with periparturient group at 30, 15 day prepartum and 30 day postpartum. Non-significant differences were observed between non-pregnant group with periparturient group on the day of parturition and 15 day after parturition.

Periparturient group

The result indicated that the plasma copper concentration was highly significantly higher on the day of parturition (1.17 ± 0.03 ppm) compared to 30 and 15 days before parturition, 15 and 30 days after parturition (0.62 ± 0.03 , 0.71 ± 0.03 , 1.13 ± 0.03 and 0.82 ± 0.03 ppm, respectively) of the experiment.

Iron

Control group with periparturient group

The average level of iron in blood plasma of non-pregnant Kankrej cows was 2.00 ± 0.08 ppm. The mean concentration of iron in blood plasma of control and periparturient group is given in Table.

Periparturient group

The plasma iron concentration at 30, 15 days before parturition, on the day of parturition, 15 and 30 day after parturition were 1.69 ± 0.05 , 1.66 ± 0.05 , 1.62 ± 0.05 , 1.77 ± 0.11 and 1.99 ± 0.10 ppm, respectively of the experiment.

Iron levels were lower in prepartum animals than postpartum. The lowest concentration of iron was observed on the day of parturition.

These micronutrients are cellular antioxidants, preventing peroxidative damage, in the cytoplasm (trace elements), and are essential for a well-functioning immune system (Weiss 2002). The variation observed in the blood content of minerals is largely in response to physiological changes that account during the periparturient period in dairy cows. Trace minerals, such as iron (in catalase), copper, zinc, manganese (in superoxide dismutase) and selenium (in glutathione peroxidase), are essential to the structure and function of many of the antioxidant enzymes (Kleczkowski *et al.*, 2003) and reduces oxidative stress.

Zinc

Control group with periparturient group

Significantly higher level of zinc was found between non-pregnant cows and 15 day prepartum cows, between non-pregnant cows and recently parturited cows. On the day of parturition zinc levels were lowest. However non-significant differences were observed between non-pregnant group with 30 day prepartum, 15 and 30 day postpartum.

Zinc has a critical role in repair and maintenance of uterine lining following parturition and early return to normal reproductive function and oestrus. Zinc has also been shown to increase plasma beta-carotene, which has been directly correlated

to improved conception rate and early embryonic development. Our findings are in agreement with Pavlata *et al.*, (2005), Akhtar *et al.*, (2009), Cahit *et al.*, (1999). However Dhama *et al.*, (2003) found significantly lower zinc level in lactating animals than in cyclic and pregnant heifers. However, non-significant difference was observed for zinc level by Asif *et al.*, (1996) and Gosai, N.M., (1998).

Periparturient group

Plasma zinc concentration was highly significantly lower on the day of parturition as compared different days of intervals during pre and post parturient period. There were highly significant differences ($p < 0.01$) among all the five zinc levels measured on different days before and after parturition except significant differences were observed between 30 day before parturition with 15 day after parturition, 30 day before parturition with 30 day after parturition.

Similar results were observed by Dufty *et al.*, (1977), Goff and Stable (1990), Meglia *et al.*, (2001, 2004), Mehre *et al.*, (2002), Jacob *et al.*, (2003), Pankajkumar and Sharma (2005) and Ram Pravesh (2006). Non-significant difference was observed for zinc level by Asif *et al.*, (1996). Cahit *et al.*, (1999) found significantly higher concentration of zinc in early pregnant cows than in non-pregnant cows and concluded that zinc has an important role in early pregnancy in cows from the observed higher levels in early period of pregnant cows than non-pregnant cows.

Rapid need for zinc in synthesis of colostrum may explain why Zn concentration is 22 % lower in blood of cows on day of calving (Kincaid, 2008). Zinc, is responsible for normal functions of certain antioxidant enzymes. The blood concentration of zinc decreased dramatically at calving mainly in

response to colostrum formation (Goff and Stabel, 1990), and to redistribution to other tissues such as, liver.

The peripartum in dairy cows is considered a stressful period, and stress can induce synthesis of metallothionein, a protein associated with zinc metabolism, making zinc less available (Spears *et al.*, 1991; Xin *et al.*, 1993).

Copper

Control group with periparturient group

Highly significant differences ($p < 0.01$) were observed for copper level between non-pregnant group with periparturient group at 30, 15 day prepartum and 30 day postpartum. However, non-significant differences were observed between non-pregnant group with periparturient group on the day of parturition and 15 day after parturition.

Increased copper concentration during parturition may be required to trigger the endocrine glands related to the physiology of initiation of labor pain and process of parturition. Copper is an important cofactor of superoxide dismutase, an enzyme, which protects cells from the pro oxidative influence of free radicals (Kleczkowski, 2003).

Our findings are an agreement with Pavlata *et al.*, (2005), Akhtar *et al.*, (2009). However non-significant difference was reported by Asif *et al.*, (1996), Gosai *et al.*, (1999) and Cahit *et al.*, (1999) for copper concentration. Dhama *et al.*, (2003) found low copper in parturient and lactating cows compared to young calves, pregnant heifers and repeat breeding cows.

Periparturient group

Significantly higher level of plasma copper was found on the day of parturition as

compared to 30, 15 days before parturition, 15 and 30 days after parturition. Copper is constituent of SOD enzyme and level of SOD also increased on the day of parturition.

Pregnancy is a copper dependant process and the body demands additional copper from conception onwards. Reduction in zinc and copper availability in the early postpartum period (Muehlenbein *et al.*, 2001) of dairy cows might explain the reduction of SOD activity (Michiels *et al.*, 1994). The level of copper during parturition was higher than pre parturition or early lactation. This might be due to conservation of copper by increasing absorption and preventing losses via excretory pathways in order to accommodate high demands of fetus. At parturition, increased circulatory levels of estrogen stimulate ceruloplasmin synthesis by the liver and thereby resulting in increased concentration of copper. Our finding were similar to those observed by Meglia *et al.*, (2001), Mehere *et al.*, (2002), Jacob *et al.*, (2003). Pankajkumar and Sharma (2005), Asif *et al.*, (1996), Cahit *et al.*, (1999) and Ram Pravesh (2006) found non-significant difference for Cu level, contradictory to our findings. Devraj (1982) reported copper level was low up to 3 day postpartum which rose in subsequent stages.

Iron

Control group with periparturient group

The average level of iron in blood plasma of non-pregnant Kankrej cows was significantly higher than 30, 15 day pre-partum. Highly significant difference were observed between control animals and on the day of parturition, while non-significant differences were observed between non-pregnant group with 15 and 30 day post-partum group. Iron being an integral part of cytochrome oxidase/peroxidase and catalase enzymes, is responsible for biological oxidation. Our findings are an agreement with Akhtar *et al.*,

(2009), Setia *et al.*, (1994). The non-significant difference was found by Asif *et al.*, (1996), for iron concentration. The

opposite result was observed by Dhama *et al.*, (2003).

Table.1 Mean and standard error of zinc, copper and iron in blood plasma of Kankrej cows

Group	Animal	Zinc (ppm)	Copper (ppm)	Iron (ppm)
Group-I	Non-pregnant Kankrej cows (Control)	1.24 ±0.02 ¹	1.19 ±0.01 ¹	2.00 ±0.08 ¹
Group-II	Peri parturient cows			
1.	30 day before parturition	1.06 ±0.06 ^{1,a}	0.62 ±0.03 ^{1**,a}	1.69 ±0.05 ^{1*,a}
2.	15 day before parturition	1.05 ±0.07 ^{1*,a**,b}	0.71±0.03 ^{1**,a**,b}	1.66 ±0.05 ^{1*,a**,b}
3.	On the day of parturition	1.02 ±0.07 ^{1*,a**,b**,c}	1.17 ±0.03 ^{1,a**,b**,c}	1.62 ±0.05 ^{1**,a**,b**,c}
4.	15 day after parturition	1.19 ±0.07 ^{1,a*,b**,c**,d}	1.13±0.03 ^{1,a**,b**,c**,d}	1.77 ±0.11 ^{1,a,b,c,d}
5.	30 day after parturition	1.23±0.06 ^{1,a*,b**,c**,d**}	0.82±0.03 ^{1**,a**,b**,c**,d**}	1.99±0.10 ^{1,a**,b**,c**,d**}

Means bearing same numerical superscript within a column did not differ significantly (P≤0.05).

Means having same alphabetical superscript within a column did not differ significantly (P≤0.05)

from each other for Group-II. * =significant (P<0.05) and ** =significant (P<0.01)

Periparturient group

Present study revealed that the plasma iron was significantly lower on the day of parturition as compared to different days of intervals during pre and post parturient period. Moreover, highly significant differences (p<0.01) for iron concentration were observed between all periparturient groups except between 30 day prepartum with 15 days postpartum, 15 day prepartum with 15 days postpartum and on the day of parturition with 15 days postpartum differences were non-significant.

Similar results were observed by Jacob *et al.*, (2003) and Ram Pravesh (2006). Pankajkumar and Sharma (2005) found adequate level of iron in pregnant cattle of specific area. Asif *et al.*, (1996) found non-

significant difference for Fe level. Mehre *et al.*, (2002) found almost same values of serum iron levels from day of calving till 4 weeks postpartum. The serum iron levels did not show significant variation among the weeks.

Iron is the most abundant trace element in the body. Iron is a cellular antioxidant and prevents peroxidative damage (Powell, 2000). The decreased level of iron during pregnancy and on the day of parturition might be due to erythropoiesis of the fetus. However increasing values of this element after parturition may be partly due to their binding with the protein and poor excretion along with milk (Kumar *et al.*, 2000).

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