Antioxidant Enzymes in Postpartum Anoestrus Buffaloes Supplemented with Vitamin E and Selenium

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ABSTRACT : The 15 buffaloes were divided into three groups, viz. group 1: normal cyclic buffaloes; group 2: postpartum anoestrus buffaloes and group 3: post partum anoestrus buffaloes supplemented with intramuscular injections of Vit. E.-care Se containing 500 mg α-tocopheryl acetate and 15 mg selenium at weekly intervals for two months. The postpartum anoestrus buffaloes had significantly higher levels of erythrocytic lipid peroxidation, superoxide dismutase and glucose-6 phosphate dehydrogenase activities but lower glutathione peroxidase activity as compared to normal cyclic buffaloes. The supplementation of vitamin E and selenium lowered the level of erythrocytic lipid peroxidation, superoxide dismutase and glucose-6 phosphate dehydrogenase activities but it had no effect on whole blood selenium and erythrocytic gluathione peroxidase activity. All the animals in group 3 became cyclic and showed 60% conception rate. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 5 : 608-611)***

Key Words : Vitamin E, Selenium, Anoestrus, Antioxidant Enzymes

INTRODUCTION

Delay in resumption of ovarian cyclicity after calving is a serious constraint to buffalo reproduction. There is increased requirement for antioxidative vitamins in animals under increased stress of either pregnancy or lactation {Bieleski et al., 1997). Free radical catalysed peroxidation is a continuous biological process causing damage to cellular and intracellular structures. Vitamin E interacts directly with lipid peroxides in plasma lipoprotein and cell membranes to neutralize them (Pironi et al., 1998). Of all the trace elements discovered to be essential selenium has proved to be the most important for reproduction. Vitamin E and selenium share a common role for the body's defense against reactive oxygen species (Brezezinska et al., 1994). The superoxide dismutase, glucose-6-phosphate dehydrogenase and glutathione peroxidase are the important enzymes involved in disposing of reactive oxygen species. Administration of selenium along with vitamin E exerts synergistic effect on the antioxidant status as well as reproductive performance in anoestrus dairy cows (Kim et al., 1997). Keeping this in view, the postpartum anoestrus buffaloes were supplemented with intramuscular injection of vitamin E and selenium and the activities of superoxide dismutase, glucose-6-phosphate dehydrogenase, glutathione peroxidase, selenium concentration and lipid peroxidation were evaluated.

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MATERIAL AND METHODS

Ten postpartum anoestrus and five normal cyclic murrah buffaloes maintained at the dairy farm of Punjab Agricultural University were selected. The animals were kept under semiloose housing conditions with half walled opened sheds and pucca floors. Throughout the experimental period, the animals were given adequate amount of chaffed/unchaffed green fodder depending upon the availability along with wheat bhusa. The ration was supplemented with concentrate mixture containing 70 per cent TDN, 13 to 16 per cent of DCP and 2 per cent mineral mixture. The concentrate was given @ 1 kg per day per animal as a maintenance ration and 2 kg per day per animal/ kg milk yield concentrate as the production ration with water adlibidum.

The fifteen buffaloes after selection were divided into three groups:

Group 1: Normal cyclic buffaloes- lactating buffaloes showing normal estrous as observed earlier during the two proceeding estrous cycles.

Group 2: Postpartum anoestrus buffaloes- lactating buffaloes which has crossed 90 days postpartum without showing the signs of estrus and with smooth ovaries i.e. no palpable structure on the ovaries.

Group 3: Postpartum anoestrus buffaloes supplemented with intramuscular injection of vit. E-care Se (Marketed by VETCARE divn., Bangalore) containing 500 mg α-tocopheryl acetate and 15 mg selenium per animals per week for two months (August and September).

The blood samples were colleted as per the following schedule:

Group 1: 0, 5, 13 and 18 day of estrous cycle

Group 2 and 3: Four sampling at weekly intervals after withdrawal of supplementation

Table 1. Lipid peroxidation of erythrocytes (nmol MDA produced/gHb) in normal cyclic and postpartum anoestrus buffaloes supplemented with vitamin E and selenium (Mean±S.D.)

Groups	Days of estrous cycle				
	0 day	5 day	13 day	18 day	Mean
Group 1	177.61±6.12^{ap}	95.11±9.30^{ap}	170.23±3.55^{ap}	108.17±5.41^{ap}	137.78±36.52^{ap}
	Days after withdrawal of supplementation				
	7 day	14 day	21 day	28 day	
Group 2	417.40±10.66^{bq}	447.24±10.19^{bq}	404.18±7.08^{bq}	388.33±8.13^{bq}	414.29±21.62^{bq}
Group 3	160.67±10.15^{ap}	139.48±7.20^{ap}	108.84±11.20^{ap}	95.78±17.44^{ap}	126.19±25.44^{ap}

Group 1: Normal cyclic buffaloes, Group 2: Postpartum anoestrus buffaloes, Group 3: Postpartum anoestrus buffaloes supplemented with vitamin E and selenium. CD (5%): Replicates, NS; Treatments, 59.60. CD (1%): Replicates, NS; Treatments, 90.25.

The values having same superscripts within a column/ row do not differ significantly from each other. The superscripts a, b refer to $p<0.05$. The superscripts p, q refer to $p<0.01$. (CD= Critical difference).

Table 2. Erythrocytic superoxide dismutase activity (U/mg Hb) in normal cyclic and postpartum anoestrus buffaloes supplemented with vitamin E and selenium (Mean±S.D.)

Groups	Days of estrous cycle				
	0 day	5 day	13 day	18 day	Mean
Group 1	5.43±0.29^{ap}	5.58±0.17^{ap}	4.77±0.04^{ap}	4.76±0.33^{ap}	5.14±0.36^{ap}
	Days after withdrawal of supplementation				
	7 day	14 day	21 day	28 day	
Group 2	8.68±0.20^{bq}	8.63±0.29^{bq}	8.77±0.40^{bq}	8.76±0.22^{bq}	8.71±0.06^{bq}
Group 3	6.16±0.49^{cr}	6.15±0.49^{cr}	6.23±0.62^{cr}	6.34±0.53^{cr}	6.22±0.06^{cr}

Group 1: Normal cyclic buffaloes. Group 2: Postpartum anoestrus buffaloes. Group 3: Postpartum anoestrus buffaloes supplemented with vitamin E and selenium. CD (5%): Replicates, NS: Treatments. 0.577. CD (1%): Replicates. NS: Treatments, 0.875.

The values having same superscripts within a column/ row do not differ significantly from each other. The superscripts a, b, c refer to $p<0.05$. The superscripts p, q, r refer to $p<0.01$. (CD= Critical difference).

Preparation of hemolysate

Blood was collected in a heparinized graduated centrifuge tube up to the marked level. Plasma was separated and erythrocytes were washed and centrifuged thrice with normal saline solution. Then distilled water was added to erythrocyte pellet slowly with constant stirring up to the marked level to prepare hemolysate, which was stored in aliquots at -20°C.

Haemoglobin concentration in hemolysate was estimated by cyanmet hemoglobin method using hemoglobin meter (Erma Hb-meter 303 A, Japan). Lipid peroxidation level was assessed in hemolysate by the method of Placer et al. (1966). The activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and glucose-6 phosphate dehydrogenase (G6PD) activities were estimated in hemolysate using the methods of Nishikimi et al. (1977), Hafeman et al. (1974) and Deutsch (1978), respectively. The whole blood selenium concentration was estimated by fluorometric method of Koh and Benson (1983). The data was subjected to analysis of variance (two way ANOVA) as per Singh et al., (1991). There were four replicates and in each replicate there were five individuals treated with three treatments. There were three components of variation. One was due to the variation between the means of replicates, the second was due to the variation between the treatment means and the third was due to the residual random variation.

RESULTS AND DISCUSSION

Lipid peroxidation

The lipid peroxidation level of normal cyclic buffaloes in group 1 (Table 1) did not vary significantly during 0, 5, 13 and 18 day of estrous cycle ($p<0.05$). The mean lipid peroxidation level was higher in case of postpartum anoestrus buffaloes (group 2) as compared to the normal cyclic buffaloes. The supplementation of vitamin E and selenium brought down the lipid peroxidation to the level comparable to that of normal cyclic buffaloes. The level of lipid peroxidation in group 3 buffaloes did not vary significantly during 1-4 weeks after the withdrawal of supplementation. Increased lipid peroxidation in postpartum anoestrus buffaloes indicated the oxidative stress in these animals, which was lowered by the vitamin E-selenium supplementation.

Enzymes

The erythrocytic SOD activity (Table 2) did not vary significantly in normal cyclic buffaloes (group 1) during 0, 5, 13 and 18 day of estrous cycle.The mean activity of SOD was higher in postpartum anoestrus buffaloes (group 2) as compared to normal cyclic buffaloes. The SOD activity decreased significantly after supplementation of vitamin E and selenium in group 3. Although it was higher in comparison to group I. The cattle fed on diets deficient in vitamin E and selenium can upregulate the activity of some

Table 3. Erythrocytic glucose-6-phosphate dehydrogenase activity (U/mg Hb) in normal cyclic and postpartum anoestrus buffaloes supplemented with vitamin E and selenium (Mean±S.D.)

Groups	Days of estrous cycle				
	0 day	5 day	13 day	18 day	Mean
Group 1	29.50±6.50^{a}	25.22±6.75^{a}	13.82±2.45^{ap}	22.50±8.04^{a}	22.76±5.72^{a}
	Days after withdrawal of supplementation				
	7 day	14 day	21 day	28 day	
Group 2	31.60±1.85^{ap}	42.35±2.61^{bp}	40.63±2.34^{bq}	37.33±4.87^{bp}	37.97±4.08^{bp}
Group 3	13.28±2.63^{bq}	12.05±4.76^{cq}	13.19±8.42^{ap}	18.54±12.38^{aq}	14.27±2.50^{aq}

Group 1: Normal cyclic buffaloes, Group 2: Postpartum anoestrus buffaloes, Group 3: Postpartum anoestrus buffaloes supplemented with vitamin E and selenium.

CD (5%): Replicates, NS; Treatments, 11.39. CD (1%): Replicates, NS; Treatments, 17.26

The values having same superscripts within a column/ row do not differ significantly from each other. The superscripts a, b, c refer to $p<0.05$. The superscripts p, q refer to $p<0.01$. (CD=Critical difference).

Table 4. Erythrocytic glutathione peroxidase activity (U/mg Hb) in normal cyclic and postpartum anoestrus buffaloes supplemented with vitamin E and selenium (Mean±S.D.)

Groups	Days of estrous cycle				
	0 day	5 day	13 day	18 day	Mean
Group 1	13.14±2.97^{a}	14.00±1.16^{a}	14.82±1.40^{a}	14.73±2.90^{a}	14.17±0.68^{a}
	Days after withdrawal of supplementation				
	7 day	14 day	21 day	28 day	
Group 2	10.34±1.00^{b}	10.01±1.18^{b}	10.29±1.11^{b}	10.12±1.22^{b}	10.19±0.12^{b}
Group 3	12.59±1.94^{a}	11.15±2.57^{b}	11.24±2.01^{b}	10.45±3.04^{b}	11.36±0.78^{b}

Group 1: Normal cyclic buffaloes. Group 2: Postpartum anoestrus buffaloes. Group 3: Postpartum anoestrus buffaloes supplemented with vitamin E and selenium.

CD (5%): Replicates, NS; Treatments, 1.62. CD (1%): Replicates, NS; Treatments, 2.46. The values having same superscripts within a column/ row do not differ significantly from each other. (CD=Critical difference).

antioxidant enzymes including SOD to mitigate the effects of peroxidative challenge (Walsh et al., 1993a). In the present study, lower vitamin E and higher level of lipid peroxidation in postpartum anoestrus buffaloes may be the reason for higher SOD activity. Supplementation of vitamin E and selenium might have relieved the oxidative stress in postpartum anoestrus buffaloes, thus lowering the erythrocytic SOD activity. The activity of erythrocytic G6PD (Table 3) in normal cyclic buffaloes (group I) did not vary significantly during 0, 5, 8 and 13 day of estrous cycle. The mean level of G6PD activity was higher in postpartum anoestrus (group 2) buffaloes as compared to normal cyclic buffaloes. After supplementation of vitamin E and selenium in postpartum anoestrus buffaloes (group 3), the G6PD activity declined significantly and it was comparable to group 1. The conversion of glucose-6-phosphate to D-glucose-δ-lactone by G6PD is the major metabolic source of NADPH, which is required for the maintenance of intracellular reduced glutathione concentration (Walsh et al., 1993b). Thus the increased activity of G6PD in vitamin E deficient animals represents an attempts or physiological adaptation to maintain the intracellular reduced glutathione concentration required for the effective removal of 4-hydroxynonenal, the toxic end product of lipid peroxidation. Supplementation of vitamin E and selenium suppressed the activity of SOD and lipid peroxidation in erythrocytes, which led to the decreased generation of H_2O_2, ROOH and 4-hydroxynonenal. Hence a decreased requirement of intracellular reduced glutathione after vitamin E and selenium supplementation might have a sparing effect on intracellular NADPH which resulted in lowering of erythrocytic G6PD activity in group 3 buffaloes. The mean erythrocytic GSH-Px activity (Table 4) was significantly higher in normal cyclic buffaloes (group 1) than that in postpartum anoestrum buffaloes (group 2).

There was no significant variation in activity of GSH-Px during 0, 5, 13 and 18 day of estrous cycle. Supplementation of vitamin E and selenium caused no significant variation in the GSH-Px activity of group 3 buffaloes in comparison to group 2. the level of whole blood selenium (Table 5) was not significantly different in group1 and 2. After the supplementation of vitamin E and selenium, there was no significant effect on the mean level of selenium in postpartum anoestrus buffaloes. In group 2 and 3, the level of selenium remained almost the same till 4 weeks after the withdrawal of supplementation and GSH-Px activity too showed no increase. The one reason may be that the animals were lactating and the level of selenium in the milk was not evaluated in the present study. The activity of GSH-Px is directly related with its component i.e. selenium concentration. Kim et al., (1997) also observed no increase in plasma selenium concentration in cows injected with combination of vitamin E and selenium. In conclusion, the postpartum anoestrus buffaloes had significantly higher

Table 5. The blood selenium concentration (μg/dL) in normal cyclic and postpartum anoestrus buffaloes supplemented with vitamin E and selenium (Mean±S.D.)

Groups	Days of estrous cycle				
	0 day	5 day	13 day	18 day	Mean
Group 1	6.90±1.18[a]	7.47±0.76[a]	8.16±0.82[a]	7.61±1.74[a]	7.53±0.44[a]
	Days after withdrawal of supplementation				
	7 day	14 day	21 day	28 day	
Group 2	7.04±1.38[a]	7.88±0.73[a]	6.29±0.91[a]	7.48±1.49[a]	7.17±0.58[a]
Group 3	7.26±1.43[a]	6.34±1.18[a]	6.24±0.64[a]	8.41±0.55[a]	7.06±0.88[a]

Group 1: Normal cyclic buffaloes. Group 2: Postpartum anoestrus buffaloes. Group 3: Postpartum anoestrus buffaloes supplemented with vitamin E and selenium.

CD (5%): Replicates. NS: Treatments, NS. The values having same superscripts within a column/ row do not differ significantly from each other. (CD= Critical difference)

level of lipid peroxidation and the activities of SOD and G6PD in erythrocytes but lower GSH-Px activity as compared to the normal cyclic buffaloes. Administration of vitamin E and selenium reduced the level of lipid peroxidation and the activity of SOD and G6PD with no alternation in the GSH-Px activity and whole blood selenium. The relief from oxidative stress was reflected in the reproductive performance as all the five animals in group 3 showed the signs of estrus by the day 30.8±7.2 since the beginning of supplementation and three animals become pregnant by the day 19.7±2.1 since beginning of supplementation.

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