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Research Article

SUPPLEMENTATION EFFECT OF ANTIOXIDANTS ON CERTAIN STRESS MARKERS IN ANOESTRUS SURTI BUFFALOES

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ABSTRACT

The study was carried out on total 12 lactating anoestrus Surti buffaloes maintained at Livestock Research Station, Navsari Agricultural University, Navsari, Gujarat. The buffaloes were randomly and equally divided into two groups *viz.* treatment (n=6) and control (n=6) group. The buffaloes from treatment group were treated with Inj. E-CARE Se (500 mg Vit. E & 15 mg Selenium) @ 10 ml by I/M route along with Cyclomin-7 Bolus @ 3 bolus by oral route on 0, 7, 14 and 21 day while, the control group were not treated and kept as a control to study the effect of antioxidants supplementation on level of certain stress markers. The mean Superoxide Dismutase (SOD), Total Glutathione (GSH), Glutathione Peroxidase (GPx) and Total Antioxidant Status (TAS) levels were differed non-significantly ($p>0.05$) between supplemented and control group at various days intervals except, on day 28 where, mean SOD and GPx levels were significantly ($p<0.01$) lower, whereas, mean TAS and GSH levels were significantly ($p<0.01$) and apparently higher in supplemented as compared to control group. Moreover, mean levels of SOD and GPx revealed decreasing whereas, GSH and TAS levels showed increasing trend within the supplemented group however, no such trend was observed within the control group and the levels of all the above mentioned stress markers differed non-significantly ($p>0.05$) at various days.

Keywords: Buffalo, Glutathione peroxidase, Superoxide dismutase, Total antioxidant status, Total Glutathione

Buffalo is the premier dairy animal in the developing countries of Asia and the main-stay of the Indian dairy industry, contributing over 60 percent of the total milk production (Mondal *et al.*, 2010). Among the various factors that hamper the fertility of buffaloes, the stress is one of them, and among the different stresses, the oxidative stress is one of the most important factor that markedly affects fertility of buffaloes.

As a routine, approximately 95 to 98 per cent of the oxygen consumed is reduced to water during aerobic metabolism, but the remaining fraction may be converted to oxidative byproducts i.e. Reactive Oxygen Species (ROS), that may damage the DNA of genes and contribute to degenerative changes. Antioxidants can be divided into 3 major groups: Enzymatic (SOD, GPX), Non- enzymatic (homocysteine and protein sulfhydryl groups) and Non enzymatic low molecular weight antioxidants (ascorbic acid, glutathione, uric acid, α-tocopherol, β-carotene and retinol). Tissue defense mechanisms against free-radical damage generally include vitamin C, vitamin E, and β-carotene as the major vitamin antioxidant sources. In addition, several metalloenzymes which include glutathione peroxidase (Se bearing), catalase (Fe bearing) and superoxide dismutase (Cu, Zn, and Mn bearing) are also critical in protecting the internal cellular constituents from oxidative damage (Weiss, 2006).

Vitamin E is an important antioxidant that has been shown to play an important role in immuno responsiveness and health in dairy cows (Weiss and Spears, 2006). The α-tocopherol functions as an antioxidant that terminates the chain of events of oxidative processes by donation of its phenolic hydrogen to chain propagating lipid peroxyl radicals, resulting in the enhanced formation of the less reactive α-tocopheroxyl radical (Zhang and Omaye, 2001). In other words, vitamin E functions as an intracellular antioxidant scavenging for free reactive oxygen and lipid hydroperoxidases and converting them to non-reactive forms, thus maintaining the integrity of membrane phospholipids against oxidative damage and peroxidation (Surai, 1999).

Extensive efforts have been put forth into worldwide research to limit the occurrence of anoestrus in buffaloes. Despite all these efforts, postpartum infertility is still an ignition problem in dairy herds. Nonetheless, few advances have been made in reducing the postpartum interval through proper nutrition including supplementation of antioxidant namely vitamin E, selenium and carotenoids in buffaloes. However, meager information regarding supplementation effect of antioxidants as well as its defense mechanisms has been available in anoestruos buffaloes, particularly for Surti breed. Therefore, the present research work was carried out to know the supplementation effect of antioxidants on certain stress markers in anoestrus Surti buffaloes.

MATERIALS AND METHODS

The study was carried out on total 12 lactating anoestrus Surti buffaloes maintained at Livestock Research Station, Navsari Agricultural University, Navsari, Gujarat over a period of seven months from June, 2014 to December, 2014. The climate of the region is sub-humid tropical with heavy rainfall. The average minimum

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and maximum ambient temperatures of the zone ranged from 14.0° to 27.9° C and 30.0° to 35.9° C and relative humidity ranged from 81 to 93 per cent in the morning and 38 to 79 per cent in the evening, respectively, during the period of study. The selected buffaloes were randomly and equally divided into two groups viz. treatment (n=6) and control (n=6) group. The buffaloes from treatment group were treated with Inj. E-CARE Se (Vetcare Pharma; 500 mg Vit. E & 15 mg Selenium) @ 10 ml by I/M route along with Cyclomin-7 Bolus (Pfizer Ltd.) @ 3 bolus by oral route on 0, 7, 14 and 21 day while, animals in the control group were not treated and kept as a control. Selection of anoestrus buffaloes was carried out on the basis of history and repeated per-rectal examination. The buffaloes that had not shown any sign of estrus since last three months and having no palpable structure over the ovaries were considered as anoestrus.

All the selected animals were fed green fodder, hay and compounded concentrate, as per the standard feeding schedule followed on the farm and had free access to drinking water. The animals were appropriately vaccinated against Foot and Mouth Disease and Hemorrhagic Septicemia. As a routine, all animals were dewormed before and after monsoon.

Approximately 10 ml of blood was collected from all the animals on days 0, 7, 14, 21 and 28 by jugular vein puncture into two separate vaccutainer, without anticoagulant for serum separation and EDTA vaccutainer for plasma separation, respectively. Both the serum and plasma samples were kept in a 4.5 ml plastic storage vial, and stored at –20 °C in deep freezer until analysis.

Plasma SOD, GSH and Serum GPx levels were determined by using a commercial kit (BioVision Research Product, according to the manufacturer's instruction. Plasma total antioxidant activity was measured by ferric reducing antioxidant power (FRAP) assay (Benzie and Strain, 1999). The values were expressed as µM by comparing the test samples to that of standard solution of known FRAP value. FRAP value of ascorbic acid is 2.

The test of significance between treatment and control groups were analyzed by Standard student's paired "t" test. The week-wise variation within the treatment and control groups were tested for each hormone by using completely randomized design as well as the mean differences between and within the groups were tested using Duncan's New Multiple Range Test (DNMRT) at 1 per cent and 5 per cent levels of significance.

RESULTS AND DISCUSSION

The levels of various stress markers (SOD, GSH. GPx and TAS) are presented in table 1.

Plasma Superoxide Dismutase (SOD)

The mean level of plasma SOD differed non-significantly between supplemented and control group on 0, 7 and 14 days, while the values were significantly ($p<0.05$ & $p<0.01$) lower at 21 day and 28 day in supplemented as compared to control group of anoestrus buffaloes.

Further, in the supplemented group, the mean plasma SOD level shown a decreasing trend with significantly ($p<0.05$) lowest value at 28 day as compared to all other days. Whereas, no such steady decreasing trend was observed within the control group and mean plasma SOD level was non-significantly fluctuated at various days interval.

The findings of present study was in close agreement with the results of Kahlon and Singh (2003) who reported, the declining trend of SOD activity in vitamin E and selenium supplemented anoestrus buffalo heifers. However, no such trend was observed in corresponding control group.

Similarly, Anita *et al.* (2004) and also reported, significantly ($P<0.05$) declined mean SOD activity 6.34±0.53 vs. 8.76±0.22 U/mg Hb after 28 days of supplementation in vitamin E and selenium supplemented as compared to control group. However, they did not observed any decreasing trend of mean SOD activity in either supplemented or control group of anoestrus buffaloes.

Plasma Total Glutathione (GSH)

The mean level of plasma GSH differed non-significantly between supplemented and control group at various days intervals. However, the values of GSH were found apparently higher on 7, 21 and 28 days and lower on 0 and 14 days in the treatment as compared to control groups.

Further, an increasing trend was observed in the mean plasma GSH level in supplemented group with significantly ($p<0.05$) highest value found on 28 day as compared to all other days. However, no such constant trend was observed within the control group and the mean level of plasma GSH was found non-significantly fluctuated between 50.86±3.96 and 56.56±1.18 ng/µl at various days intervals.

The results of present study was well supported by Rodrigues (2014) who reported that cows supplemented with dietary antioxidants had increased total glutathione compared to control cows. While, contrary to the findings of present study, Sunil Kumar *et al.* (2011) reported

Table 1

Level of various stress markers in treatment and control groups of anoestrus Surti Buffaloes at various days interval (Mean±SEM)

Stress Markers	Days of blood collection	Treatment Group	Control Group	't' -Value	P-Value
SOD (U/ml)	0	$3.42\pm0.40_{x}^{b}$	$3.33\pm0.71_{x}^{a}$	0.12	0.91
	7	$3.37\pm0.69_{x}^{b}$	$3.10\pm0.50_{x}^{a}$	0.31	0.76
	14	$3.14\pm0.25_{x}^{b}$	$3.81\pm0.27_{x}^{a}$	1.80	0.10
	21	$2.93\pm0.40y^{ab}$	$3.94\pm0.23_{x}^{a}$	2.19	0.05
	28	$1.72\pm0.28_{y}^{a}$	$3.71\pm0.34_{x}^{a}$	4.56	0.00
GSH (ng/ml)	0	$49.82\pm1.54_{x}^{a}$	$53.16\pm2.20_{x}^{a}$	1.24	0.24
	7	$53.71\pm2.70_{x}^{ab}$	$50.86\pm3.96_{x}^{a}$	0.59	0.57
	14	$53.80\pm1.59_{x}^{ab}$	$55.04\pm1.55_{x}^{a}$	0.56	0.59
	21	$58.39\pm1.83_{x}^{bc}$	$54.66\pm1.33_{x}^{a}$	1.65	0.13
	28	$61.58\pm3.34_{x}^{c}$	$56.56\pm1.18_{x}^{a}$	1.42	0.19
GPx (mU/ml)	0	$3.03\pm0.25_{x}^{b}$	$2.58\pm0.48_{x}^{a}$	0.84	0.42
	7	$3.11\pm0.29x^{b}$	$2.54\pm0.22_{x}^{a}$	1.55	0.15
	14	$2.57\pm0.35_{x}^{ab}$	$2.93\pm0.25_{x}^{a}$	0.83	0.42
	21	$2.54\pm0.40_{x}^{ab}$	$3.39\pm0.53_{x}^{a}$	1.29	0.23
	28	$1.99\pm0.27_{y}^{a}$	$3.27\pm0.30_{x}^{a}$	3.18	0.01
TAS (mm/L)	0	$1597.22\pm107.40_{x}^{a}$	$1541.67\pm199.54_{x}^{a}$	0.25	0.81
	7	$2062.50\pm172.72_{x}^{ab}$	$1833.33\pm233.98_{x}^{a}$	0.79	0.45
	14	$2319.44\pm266.51_{y}^{b}$	$1534.72\pm115.08_{x}^{a}$	2.70	0.02
	21	$2416.67\pm219.16_{x}^{b}$	$1833.33\pm170.44_{x}^{a}$	2.10	0.06
	28	$2743.06\pm276.26_{y}^{b}$	$1791.67\pm84.71_{x}^{a}$	3.29	0.01

^{a,b,c} Means bearing different superscripts within a column (between days of treatment) differ significantly ($p<0.05$) and $_{x, y}$ Means bearing different subscripts within a row (between the groups) differ significantly ($p<0.01$ & $p<0.05$)

significantly ($p<0.05$) lower mean GSH concentration in antioxidants supplemented (sodium bicarbonate, potassium carbonate ascorbic acid polyphosphate and zinc oxide) as compared to control group of buffaloes during hot-dry condition whereas, in the hot-humid condition, mean GSH concentration was differed non-significantly ($p>0.05$) between supplemented and control group of buffaloes.

Serum Glutathione Peroxidase (GPx)

The mean level of serum GPx differed non-significantly between supplemented and control group at different days interval except, on 28 day, where, it was found significantly ($p<0.05$) lower (1.99±0.27 vs. 3.27±0.30 mU/ml) in the treatment as compared to control group of anoestrus buffaloes.

Moreover, on day 7 onwards declining trend in the level of GPx was observed in supplemented group with significantly ($p<0.05$) lowest value (1.99±0.27 mU/ml) on 28 day as compared to various days intervals. On the other hand, no such declining tendency was observed in the control group.

Anita *et al.* (2004) also reported that, the supplementation of vitamin E and selenium significantly ($p<0.05$) decreased the mean erythrocytic GPx activity in supplemented group and lowest activity (10.45±3.04 U/mg Hb) was observed on 28 day post supplementation whereas, the mean erythrocytic GPx activities in non-supplemented anoestrus group were non significantly fluctuated.

However, according to Kahlon and Singh (2003) the erythrocytic GPx activity in vitamin E and selenium supplemented and control anoestrus buffalo heifers differed non-significantly ($p>0.05$) suggesting that, supplementation of α-tocopherol @ 3000 mg per week in anoestrus buffalo heifers had not any significant ($p>0.05$) effect on the erythrocytic GPx activity.

Plasma Total Antioxidant Status

The mean plasma TAS values differed non-significantly between supplemented and control group at various days interval except on 14 and 28 days, where it was found

significantly ($p<0.05$) higher in treatment as compared to control group (Table 1).

Moreover, in supplemented group, increasing trend was observed in the level of plasma TAS and highest value (2743.06±276.26 µm/L) was recorded at 28 days interval. However, no such trend was recorded in the control group and plasma TAS value was non-significantly fluctuated at various days intervals.

Similarly, Kumar *et al.* (2015) studied the sustained delivery of exogenous melatonin on total antioxidant capacity (TAC) in summer stressed anoestrous water buffalo and reported higher concentration of mean serum TAC in the treatment as compared to control group, with a significant ($p<0.05$) increasing trend within the treatment group and lowest concentration (1.40±0.09 mmol/L) observed on 8 day before treatment was significantly ($p<0.05$) increased (2.80±0.69 mmol/L) on the 28 day post treatment. Whereas, no significant difference was observed in mean TAC level within the control group. Moreover, Gupta *et al.* (2011) analyzed the TAC with respect to follicle size, and reported that, the TAC of follicular fluid increased as follicle size increased with the large follicles (1168 mM Trolox equivalents) exhibited significantly ($p<0.01$) while, medium follicles (1157 mM Trolox equivalents) non-significantly higher TAC compared with the small follicles (966 mM Trolox equivalents). Further Miller *et al.* (1993) reported that feeding 1,000 IU vitamin E/ head/day during dry period to cows, led to significant ($p<0.05$) increase in the mean plasma total antioxidant activity at parturition.

However, contrary to the results of present experiment, Panda *et al.* (2006) reported non-significant ($p>0.05$) difference in the mean level of TAS at different days interval in Murrah buffaloes supplemented with 0, 1000, 1500 and 2000 IU vitamin E during periparturient period.

In conclusion, the administration of vitamin E and selenium as antioxidants was responsible for reducing the oxidative stress due to its remedial effect, hence decline in SOD and GPx activity, whereas increased level of GSH and TAS observed in supplemented group of anoestrus buffaloes.

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