# COMPARATIVE STUDIES OF CERTAIN STRESS MARKERS AND TRACE MINERALS IN NORMAL CYCLIC AND ANOESTRUS SURTI BUFFALOES

**N.F. Chaudhari*, C.T. Khasatiya, M.D. Patel, V.S. Dabas, S.S. Chaudhari, J.H. Patel and K.K. Tyagi**

## ABSTRACT

A comparative studies on certain stress markers *viz*. SOD (Superoxide Dismutase), GSH (Total Glutathione) and GPx (Glutathione Peroxidase) as well as trace minerals *viz*. Cu (Copper), Co (Cobalt), Zn (Zinc) and Fe (Iron) were carried out on twenty four (24) lactating Surti buffaloes, divided into two groups *viz*. normal cyclic (n=12) and anoestrus (n=12) groups, maintained at Livestock Research Station, Navsari Agricultural University, Navsari, Gujarat. The mean SOD and GPx levels were found significantly ($P<0.05$) lower as 2.21±0.31 vs. 3.37±0.39 U/ml and 1.94±0.20 vs. 2.81±0.27 mU/ml, respectively whereas, the mean plasma GSH level was observed significantly ($P<0.05$) higher 56.32±1.21 vs. 51.49±1.38 ng/µl in normal cyclic as compared to anoestrus Surti buffaloes. The mean serum Cu and Zn concentration were observed significantly ($P<0.01$) higher 0.58±0.03 vs. 0.42±0.03 and 1.48±0.12 vs. 0.94±0.07 µg/ml, respectively and mean serum Co and Fe concentration were found non-significantly ($P>0.05$) higher 0.65±0.06 vs. 0.58±0.04 and 3.13±0.18 vs. 2.88±0.13 µg/ml, respectively in normal cyclic as compared to anoestrus Surti buffaloes.

**Keywords**: *Bubalus bubalis*, buffalo, Anoestrus, Glutathione peroxidase, normal cyclic, superoxide dismutase, total glutathione

## INTRODUCTION

Buffalo is the premier dairy animal in the developing countries of Asia and the main-stay of the Indian dairy industry, contributing over 60% of the total milk production (Mondal *et al*., 2010). Due to its eminent position among the milk producing animals, buffalo is considered as black diamond but their poor reproductive efficiency affect economy of the farmers in terms of milk yield, net calf crop and additional cost of rearing. Among various reproductive disorders in buffaloes, anoestrus is the most important cause of poor reproductive performance (Devkota *et al*., 2012). The low body weight and poor body condition compounded with lactation stress can further extend the postpartum anoestrous period (Vandeplassche, 1982).

Among the various factors 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. Oxidative stress has

College of Veterinary Science and Animal Husbandry, Navsari Agricultural University, Gujarat, India, *E-mail: naresh_vety@yahoo.co.in

been defined by several workers. As per Dobson and Smith (2000), stress is revealed by the inability of an animal to cope up with its environment, a phenomenon which is often reflected in a failure to achieve genetic potential. Whereas, stress as the cumulative detrimental effects of various factors on health and performance of animals defined by Rosales (1994). Moreover, stress represents the reaction of body to stimuli that disturb normal physiological equilibrium or homeostasis, often with detrimental effects as shown by Khansari *et al*. (1990). According to Stott (1981), stress is the result of environmental forces continuously acting upon animals which disrupt homeostasis resulting in new adaptations that can be either detrimental or advantageous to the animal.

As a routine, approximately 95% to 98% 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. The terms oxidative stress, oxidative damage, free radical and antioxidant have become an integrated part of the variety of scientific discussions in issues related to chemistry, biology and research in biosciences. Free radical reactions are the integral part of normal metabolism and, in order to cleanse cells of ROS antioxidants *viz*., Vitamin E, A and C are required and, in low concentrations they significantly delays oxidation of oxidizable substrates.

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).

Reproductive performance of cattle may not be compromised if copper, cobalt, zinc or iron status is in the marginal to deficient range and most common copper deficiency symptoms in cattle include delayed or suppressed estrus, decreased conception, infertility and embryo death (Phillippo *et al*., 1987; Corah and Ives, 1991). While, cobalt is an important component of vitamin $B_{12}$ and the need of cobalt for thymine synthesis, which is required for DNA synthesis, explains the biological role of cobalt for cell division, growth and reproduction. Similarly, inadequate zinc and iron levels have been associated with decreased fertility and abnormal estrus.

There is no or little information is available regarding levels of various stress markers in Surti buffaloes hence, the present study was designed for comparative studies of certain stress markers and trace minerals in normal cyclic and anoestrus Surti buffaloes.

## MATERIALS AND METHODS

The study was carried out on twenty four (24) lactating Surti buffaloes, comprising of 12 normal cyclic and 12 anoestrus Surti buffaloes, maintained at Livestock Research Station, Navsari Agricultural University, Navsari, Gujarat. The climate of the region is sub-humid tropical with heavy rainfall. The average minimum and maximum ambient temperatures of the zone ranged from 14.0$^{o}$C to

27.9°C and 30.0°C to 35.9°C and relative humidity ranged from 81 to 93% in the morning and 38 to 79% in the evening, respectively, during the period of study.

Selection of normal cyclic and anoestrus buffaloes was carried out on the basis of history and per-rectal examination. The buffaloes having history of regular estrus cycle and either follicle or CL on ovary during per-rectal palpation were considered as normal cyclic while, the buffaloes that were not shown any sign of estrus since last three months and having no any palpable structure over ovary 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 Haemorrhagic Septicemia. As a routine, all animals were dewormed before and after monsoon.

Approximately 10 ml of blood were collected from all the animals aseptically by jugular vein puncture into two separate vaccutainer, without anticoagulant for serum and EDTA vaccutainer for plasma, respectively and serum as well as plasma was separated by centrifugation at 3000 rpm for 5 minutes and stored at -20°C in deep freezer until analysis.

Plasma SOD, GSH and Serum GPx were determined by using a commercial kit (BioVision Research Product, Catalog #K335-100, #K261-100 and #K762-100, respectively) according to the manufacturer's instruction.

The blood serum samples (0.5 ml each) were digested with 4.5 ml volume of tri-acid mixture (perchloric acid: sulphuric acid: nitric acid; 1:2:1) on a hot plate according to the method of Krishna and Ranjhan (1980). The clear transparent residues were diluted in double glass-distilled water and the final volume was made to 25 ml. These aliquots were then used for estimation of trace elements, *viz*. Cu, Co, Zn and Fe on an Atomic Absorption Spectrophotometer.

The test of significance for various parameters between normal cyclic and anoestrus group were made by Standard Student's paired 't' test.

## RESULTS AND DISCUSSION

The mean plasma SOD, GSH and serum GPx levels in normal cyclic and anoestrus Surti buffaloes were presented in Table 1 and Figure 1. The mean plasma SOD and serum GPx levels were found significantly ($P<0.05$) lower whereas, mean plasma GSH level was observed significantly ($P<0.05$) higher in normal cyclic as compared to anoestrus Surti buffaloes.

The findings of present study, concerning SOD was in close agreement with the results of Kahlon and Singh (2003) and Anita *et al.* (2004) who reported, significantly ($P<0.05$ and $P<0.01$, respectively) lower mean erythrocytic SOD activity as 6.93±0.04 vs. 10.20±0.06 and 5.14±0.36 vs. 8.71±0.06 U/mg Hb, respectively in normal cyclic as compared to anoestrus Murrah buffalo heifers. While, contrary to the present findings, Ghosh *et al.* (2015) reported significantly ($P<0.01$) higher mean erythrocytic SOD activity as 224.679±24.800 vs. 132.18±19.48 U/g Hb in cyclic as compared to postpartum anoestrus buffaloes. Significantly elevated mean plasma SOD activity in anoestrus as compared to normal cyclic Surti buffaloes might be attributed to the up-regulation of this enzyme in an attempt to mitigate the superoxide radical challenge.

Scanning through the available literature revealed no such kind of comparative studies of GSH in between normal cyclic and anoestrus buffaloes. However, from the findings of present study it can be postulated that, the low mean level of GSH in anoestrus buffaloes is responsible for the stressful condition in these animals and resulted into anoestrus condition. Moreover, the synthesis of intracellular glutathione is a critical part of oocyte cytoplasmic maturation (Eppig, 1996). Low level of GSH found in anoestrus buffaloes was responsible for the stressful condition and resulted into anoestrus.

The present study results pertaining to GPx were corroborated well with the findings of Kahlon and Singh (2003) who, reported significantly ($P<0.05$) lower mean erythrocytic GPx activity as $11.61\pm0.19$ vs. $14.09\pm0.54$ U/mg Hb in normal cyclic as compared to anoestrus Murrah buffalo heifers. Likewise, Ghosh *et al.* (2015), also reported non-significantly lower mean values of GPx as $1.779\pm0.125$ vs. $1.939\pm0.128$ IU/g in cyclic as compared to postpartum anoestrus Murrah buffaloes. Furthermore, the association between increased activity of mean blood $GP_X$ and incidence of anoestrus or subestrus was reported by Jukola *et al.* (1996). However, contrary to this, Anita *et al.* (2004) reported significantly ($P<0.05$) higher mean erythrocytic GPx activity as $13.14\pm2.97$ vs. $10.34\pm1.00$ U/mg Hb in normal cyclic as compared to anoestrus Murrah buffaloes. Significantly elevated mean serum GPx activity in anoestrus as compared to normal cyclic buffaloes might be attributed to the up-regulation of this enzyme for effective removal of $H_2O_2$ and ROOH radical.

The mean serum Cu, Co, Zn and Fe concentrations in normal cyclic and anoestrus Surti buffaloes were presented in Table 2 and Figure 2. The mean serum Cu and Zn concentrations were observed significantly ($P<0.01$) higher whereas, mean serum Co and Fe concentration were found non-significantly higher in normal cyclic as compared to anoestrus Surti buffaloes.

Similarly many research workers reported significantly/non-significantly higher level of Cu, Zn and Fe in normal cyclic as compared to anoestrus buffaloes (Dutta *et al.*, 2001; Singh *et al.*, 2006; Yadav *et al.*, 2006; Hedaoo *et al.*, 2008). Further, the findings of present study related to serum Co concentration was well corroborated by the findings of Khasatiya *et al.* (2005); Singh *et al.* (2006) who reported non-significantly higher level of Co as $0.61\pm0.02$ ppm and $0.022\pm0.002$ µg/ml vs. $0.52\pm0.02$ ppm and $0.018\pm0.001$ µg/ml in fertile and normal cyclic as compared to infertile and anoestrus buffaloes.

However, contrary to the results of present study, significantly lower level of Zn ($1.66\pm0.06$ vs. $1.95\pm0.06$ ppm) and non-significantly lower level of Fe ($3.48\pm0.11$ vs. $3.69\pm0.11$ ppm) in fertile as compared to infertile groups of buffaloes were reported by Khasatiya *et al.* (2005). Further, Yadav *et al.* (2006) reported significantly ($0.283\pm0.028$ vs. $0.950\pm0.077$ µg/dl, $P<0.05$) whereas, Hedaoo *et al.* (2008) reported non-significantly ($0.44\pm0.08$ vs. $0.47\pm0.06$ ppm) lower level of Co in normal cyclic as compared to anoestrus buffaloes which was contradictory to the present findings.

Significantly higher mean serum Cu and Zn and apparently higher Co and Fe levels in normal cyclic compared to anoestrus buffaloes indicated their importance in normal reproduction.

Table 1. Levels of stress markers in normal cyclic and anoestrus Surti buffaloes (Mean ± SEM).

| Stress markers | Groups | | 't' -value | P-value |
|---|---|---|---|---|
| | Normal cyclic (n=12) | Anoestrus (n=12) | | |
| SOD (U/ml) | $2.21 \pm 0.31_x$ | $3.37 \pm 0.39_y$ | 2.35 | 0.03 |
| GSH (ng/µl) | $56.32 \pm 1.21_x$ | $51.49 \pm 1.38_y$ | 2.63 | 0.02 |
| GPx (mU/ml) | $1.94 \pm 0.20_x$ | $2.81 \pm 0.27_y$ | 2.59 | 0.02 |

Means bearing different subscripts within a row (between the groups) differ significantly (P<0.05).

Table 2. Levels of trace minerals in normal cyclic and anoestrus Surti buffaloes (Mean ± SEM).

| Stress markers | Groups | | 't' -value | P-value |
|---|---|---|---|---|
| | Normal cyclic (n=12) | Anoestrus (n=12) | | |
| Copper (µg/ml) | $0.58 \pm 0.03_x$ | $0.42 \pm 0.03_y$ | 4.25 | 0.00 |
| Cobalt (µg/ml) | $0.65 \pm 0.06_x$ | $0.58 \pm 0.04_x$ | 0.99 | 0.33 |
| Zinc (µg/ml) | $1.48 \pm 0.12_x$ | $0.94 \pm 0.07_y$ | 3.80 | 0.00 |
| Iron (µg/ml) | $3.13 \pm 0.18_x$ | $2.88 \pm 0.13_x$ | 1.18 | 0.25 |

Means bearing different subscripts within a row (between the groups) differ significantly (P<0.01).

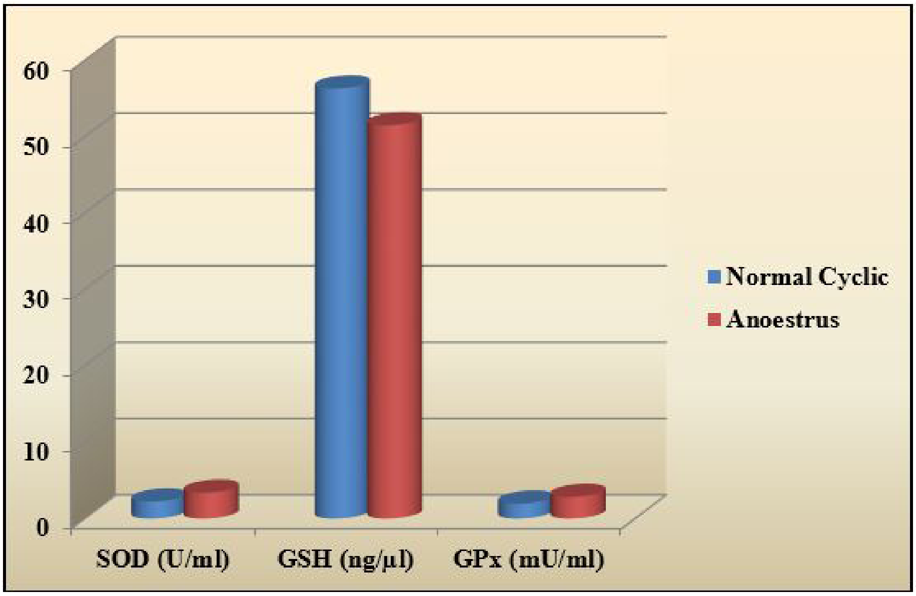


Figure 1. Levels of stress markers in normal cyclic and anoestrus Surti buffaloes.

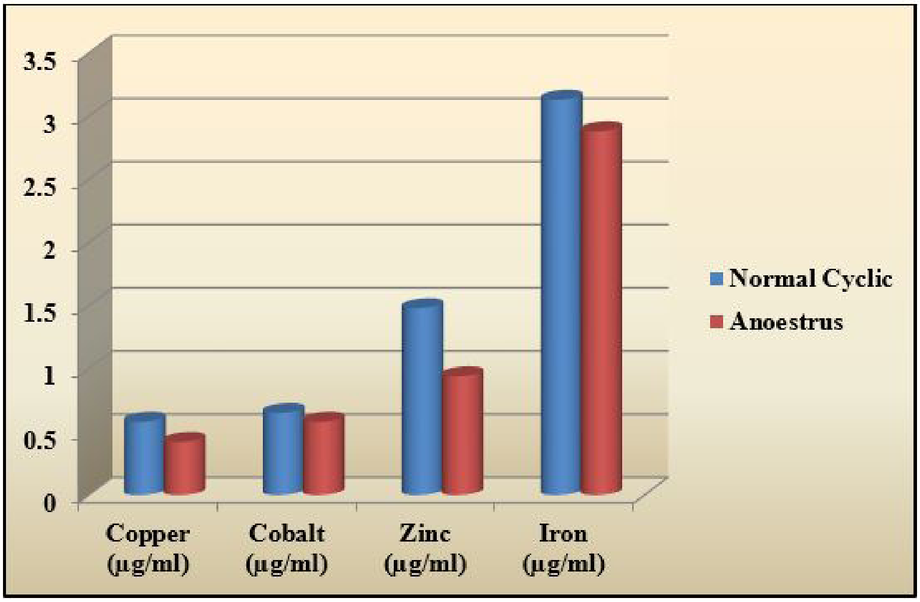


Figure 2. Levels of trace minerals in normal cyclic and anoestrus Surti buffaloes.

## ACKNOWLEDGEMENT

The authors are highly thankful to the Dean, College of Veterinary Science and Animal Husbandry and Research Scientist, Livestock Research Station, Navsari Agricultural University, Navsari, for providing research facilities and experimental animals for conducting the present experiment.


## REFERENCES


Anita, A., S.P.S. Singh, K.S. Dhillon and S. Nayyar. 2004. Vitamin E and selenium influence the blood biochemical constitutes in postpartum anoestrus buffaloes. *Indian J. Anim. Sci*., **74**(4): 376-378.

Corah, L.R. and S. Ives. 1991. The effects of essential trace minerals on reproduction in beef cattle. *Veterinary Clinics of North America: Food Animal Practice*, **7**: 41-57.

Devkota, B., T.P. Bohara and N. Yamagishi. 2012. Seasonal variation of anestrus conditions in buffaloes (*Bubalus bubalis*) in southern Nepal. *Asian J. Anim. Vet. Adv*., **7**: 910-914.

Dobson, H. and R.F. Smith. 2000. What is stress and how does it affect reproduction. *Anim. Reprod. Sci*., **60**: 743-752.

Dutta, A., B.C. Sarmah and K.K. Baruah. 2001. Concentrations of serum trace elements in cyclic and heifers in lower Brahmaputra Valley of Assam. *Indian Vet. J*., **78**(4): 300-302.

Eppig, J.J. 1996. Co-ordination of nuclear and cytoplasmic oocyte maturation in eutherian mammals. *Reprod. Fert. Develop.*, **8**: 485-489.

Ghosh, M., M. Gupta, R. Kumar, S. Kumar, A.K. Balhara and I. Singh. 2015. Relation between antioxidant status and postpartum anoestrous condition in Murrah buffalo. *Vet. World*, **8**(10): 1163-1166.

Hedaoo, M.K., K.P. Khllare, M.D. Meshram, S.K. Sahatpure and M.G. Patil. 2008. Study of some serum trace minerals in cyclic and non-cyclic Surti buffaloes. *Vet. World*, **1**(3): 71-72.

Jukola, E., J. Hakkarainen, H. Saloniemi and S. Sankari. 1996. Blood selenium, vitamin E, vitamin A and β-carotene concentrations and udder development, fertility treatments and fertility. *J. Dairy Sci*., **79**(5): 838-845.

Kahlon, R.S. and R. Singh. 2003. Status of antioxidant enzymes in normal cycling and α-tocopherol supplemented anestrus buffalo heifers (*Bubalus bubalis*). *Asian Austral. J. Anim. Sci*., **16**(2): 217-221.

Khansari, D.N., A.J. Murgo and R.E. Faith. 1990. Effect of stress on the immune system. *Immunol. Today*, **11**: 170-175.

Khasatiya, C.T., A.J. Dhami, V.P. Ramani, F.P. Savalia and F.S. Kavani. 2005. Reproductive performance and mineral profile of postpartum fertile and infertile Surti buffaloes. *Indian J. Anim. Reprod*., **26**: 145-148.

Krishna and S.K. Ranjhan. 1980. *Laboratory Manual for Nutrition Research*, Vikash Publishing House Pvt. Ltd., New Delhi, India. p. 83-84.

Mondal, S., K.P. Suresh and S. Nandi. 2010. Endocrine profiles of oestrous cycle in buffalo: a meta-analysis. *Asian Austral. J. Anim. Sci*., **23**(2): 169-174.

Phillippo, M., W.R. Humphries, T.G. Atkinson, D. Henderson and P.H. Garthwaite. 1987. The effect of dietary molybdenum and iron on copper status, puberty, fertility and estrous cycles in cattle. *J. Agr. Sci.*, **109**: 321-336.

Rosales, A.G. 1994. Stress syndrome in birds. *J. Appl. Poultry Res*., **3**: 199-203.

Singh, B., C.V.S. Rawal and J.P. Singh. 2006. Serum zinc, copper and cobalt level in normal cyclic, anestrous and repeat breeder buffaloes. *Indian Journal of Animal Reproduction*, **27**: 34-36.

Stott, G.H. 1981. What is animal stress and how is it measured*. J. Anim. Sci*., **52**: 150-153.

Vandeplassche, M. 1982. Reproductive efficiency in cattle: A guideline for projects in developing countries. *FAO Animal Production and Health Paper No.* 25. Food and Agriculture Organization of the United Nations (FAO), Rome, Italy. 118p.

Weiss, W.P. 2006. Antioxidant nutrients, cow health and milk quality, p. 11-18. *In Proceeding of Penn State Dairy Cattle Nutrition Workshop*, Grantvill, Canada.

Yadav, K.V.S., M.R. Ansari and A. Kumaresan. 2006. Profile of macro, microelement, total protein and cholesterol in serum of cyclic and acyclic Murrah buffaloes. *Indian J. Vet. Res*., **15**: 10-13.