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SHORT COMMUNICATION

Oxidative Status and Some Serum Macro Minerals during Estrus, Anestrous and Repeat Breeding in Cholistani Cattle

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A B S T R A C T

The present study was conducted to determine the macro mineral profile and biomarkers of oxidative stress in Cholistani cattle kept at a public farm and various villages in district Bahawalpur. For this purpose 90 blood samples were collected each from estrual, anestrous and repeat breeding cattle having different age and lactation number. Reproductive tract examination of all the cattle was carried out to determine the reproductive status. Blood samples without EDTA were collected for serum separation at day of estrus (normal cyclic), repeat breeder and anestrous cows. The serum calcium levels were significantly decreased ($P<0.05$) in anestrous (7.31±0.02 mg/dl) cattle as compared to estrus. However, these values were non-significantly different between repeat breeder and cattle having estrus phase. The concentrations of serum phosphorus were significantly higher ($P<0.01$) in normal estrual (4.99±0.08 mg/dl) as compared to repeat breeder (3.90±0.06 mg/dl) and anestrous (3.82±0.04 mg/dl) Cholistani cattle. Mean serum MDA (nmol/ml) levels of repeat breeder (2.68±0.18) and anestrous (2.54±0.22) were significantly ($P<0.01$) higher than the estrous (1.71±0.03) cattle. Moreover, the serum nitric oxide levels (µmol/L) were also increased significantly ($P<0.01$) in repeat breeder (58.28±4.01) and anestrous (61.40±9.40) than the normal estrous (31.67±6.71) cattle. The ratio of Ca: P in normal cyclic animals was lower (1.73:1) as compared to the anestrous animals (1.92:1). It can be concluded from the present study that the level of Ca: P should also be near to 1.5:1 for better reproductive performance.

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INTRODUCTION

Reproduction is essential for continued source of animals, to provide protein in the form of milk and number of animals for meat purpose. Anestrous is the most prevalent form of infertility and drastically affect reproductive performance in dairy cattle (Akhtar *et al*., 2012). Any deviation or prolongation in the breeding rhythm results in a progressive economic loss due to broadening of the dry period, reduces calving and lactations. Repeat breeding and anestrous conditions are recognized as serious problems that increase the calving interval. Different metabolic alterations induced by mismatching of energy requirements leads to negative impacts on fertility. The normal metabolic processes in animal depends on different nutrients including proteins, vitamins and minerals and their deficiency causes hypothalamic-pituitary-ovarian, oogenesis and folliculo-genesis disorders ultimately extend the postpartum anestrous period in cows (Dilshad *et al*., 2012).

The reproductive capability of livestock population is severely influenced by poor management, various diseases and different host/environmental determinants. During different diseases and physiological phases including development, reproductive cycles, pregnancy/calving and lactation animals face physiological stress. In different farm animals, oxidative stress due to the altered redox balance results in numerous pathological conditions including obstruction of air ways, sepsis and pneumonia (Deaton *et al*., 2005). Malondialdehyde (MDA) a lipid peroxidation product is a biomarker of oxidative stress in tissues and cells is known to induce cancer, cellular aging and several infectious diseases. During lipid peroxidation, different intracellular lysosomal enzymes are extensively released that destroy cell membranes leading to tissue damage. Nitric oxide (NO) is a regulator of apoptosis, involves in migration

of lymphocyte, vascular tone, wound repair and different other processes in the body. In macrophages NO production is associated with NO synthase (iNOS) expression and displays its activity against bacteria, parasites and fungi during inflammatory process (Gross *et al.,* 2004). Therefore, the present study was conducted to evaluate the serum mineral profile and the biomarkers of oxidative stress in repeat breeding and anestrous conditions in Cholistani cattle.

MATERIALS AND METHODS

The present study was conducted during 2011-2012 at Govt Livestock Farm, Jugaitpeer, different villages located in (east, west, north and south) radius of 35 km from the main city (Bahawalpur) Pakistan and various Cholistani cattle present in desert conditions.

A total of 300 Cholistani cows without any apparent clinical ailment having 3-10 years of age were included in the present study. The rectal palpation was carried out to diagnose any abnormal condition in the reproductive tract of all animals. Finally out of these animals, 90 cattle were randomly selected were grouped into three categories having 30 animals each including estrous (E): animals in estrus at time of sampling, anestrous (AE): animals having smooth ovaries with no palpable structures and having no clinically abnormalities and repeat breeders (RB): cycling normally, with no clinical abnormalities but failed to conceive after at least two successive inseminations. Groups were based upon their breeding history and current reproductive status ascertained by the clinical examination of their genital organs.

About 15 ml of blood samples without anticoagulant (EDTA) from all the cattle of three different groups were collected aseptically from jugular vein in sterile test. All the blood samples were centrifuged at 3000 rpm for 15 minutes to separate the serum. About 1.5-2 ml serum was collected from each sample in eppendorf tubes and stored at -20°C. The serum calcium was determined using atomic absorption spectrophotometrically while phosphorus was determined by colorimetric method on UV spectrophotometer at 720 nm wavelength against standard and blank (Sitara *et al.,* 2008).

Oxidative stress was determined by the estimation of lipid per oxidation product malondialdehyde (MDA) concentration in serum spectrophotometrically (Suriyasathaporn *et al.,* 2006) and serum NO level was determined (Nisebet *et al.,* 2007).The data were analyzed statistically using analysis of variance technique (ANOVA) and the differences between means were compared by t-test.

RESULTS AND DISCUSSION

Various expenses like veterinary treatment cost, decreased milk production, increased labor charges and various reproductive diseases are compound conditions where culling become necessary. Therefore, there is a need to control and eradicate the important reproductive disorders in animals by altering management practices those are related with risk of reproductive diseases. The animals in Pakistan suffer from various disease problems from time to time and among them reproductive tract disorders are important and multifactorial problems hindering the development of dairy sector (Akhtar *et al.,* 2012).

The mean serum calcium level was significantly reduced in anestrous (7.31±0.02 mg/dl) cattle (Table 1). However, these values were non-significantly different between repeat breeder (7.82±0.06 mg/dl) and anestrous (7.31±0.02) cattle. The results also revealed that the serum calcium level was non-significantly higher in normal cyclic than repeat breeder cattle. The decrease in calcium level in blood serum of anestrous and repeat breeder cattle could be due to the involvement of pathogens or deleterious effects of increased reactive oxygen species which damage the junctional complex in blood vascular channels or disrupt the normal homeostatic mechanisms resulting in poor absorption or increased losses from blood. Previously similar results also have been studied in anestrous, repeat breeder and in normal cyclic Sahiwal cows (Aslam and Tucker, 1998). The serum phosphorus levels were significantly ($P<0.01$) decreased in repeat breeder (3.82±0.04 mg/dl) and anestrous (3.90±0.06 mg/dl) Cholistani cattle as compared to normal estrual cattle. Phosphorus is essential for transfer of biological energy (ATP) and its deficiency may arrest the phenomenon of fertilization, may cause early embryonic death resulting in the repeat breeder and anestrous condition of cattle. These results are in agreement with who recorded significantly lower phosphorus levels in anestrous as compared to normal cyclic and repeat breeder animals. Similar results also have been reported in buffaloes (Kumar *et al.,* 2010). From the results of present study it was revealed that the repeat breeding and non-cyclic conditions in bovines could be due to the lower serum profile of calcium and phosphorus. The decreased values of serum macro minerals in repeat breeding and anestrous cattle can also be related to the inflammatory process and oxidative stress upon reproductive system due to invasions of pathogens in these animals as the values of MDA and NO were significantly increased during these phases (Table 1). Previously, it has been determined that deficiency and imbalance ratio of calcium to phosphorus in the blood of cattle is one of the causes of anestrous and repeat breeding (Jayachandran *et al.,* 2013).

The results of present study showed that the serum malondialdehyde (MDA) levels in repeat breeder and anestrous cattle differed significantly from estrual animals. Mean serum MDA levels in repeat breeder (2.68±0.18) and anestrous (2.54±0.22) were significantly higher than the normal estrous (1.71±0.03) cattle. The serum nitric oxide (NO) levels were increased significantly in repeat breeder (58.28±4.01) and anestrous (61.40±9.40) when compared to estrous (31.67±6.71) cattle (Table 1). The increased level of serum MDA and NO in present study could be due to the increase production of free radicals (ROS) during decrease in antioxidant defense and various infections (Turk *et al.,* 2013). The free radicals released from phagocytes react with DNA, lipid and different other macromolecules such as proteins which acts as natural targets of oxidation (Poulsen, 2005) and alters the normal functions of proteins, malfunctioning of enzymes and amino acids present in different enzymes results in oxidative damage. The increased levels of serum MDA and NO in present study could be due to the inflammatory process induced by different pathogens and are important biomarkers of inflammatory disease (Kullisaar *et al.,* 2013).

Table 1: Serum calcium (Ca) and phosphorus (P) profile in different reproductive phases in Cholistani cattle

Parameters	Reproductive Phases		
	Estrual	Repeat breeder	Anestrous
Mineral profile			
Ca (mg/dl)	8.9±0.05A	7.82±0.06AB	7.31±0.02C
P (mg/dl)	4.99±0.08A	3.82±0.04B	3.90±0.06B
Ca:P	1.73:1	1.92:1	1.96:1
Oxidative status			
MDA nmol/ml)	1.71±0.03A	2.68±0.18B	2.54±0.22B
NO (µmol/L)	31.67±6.71A	58.28±4.01B	61.40±9.40B

Values (mean±SD) in each row with different letters differ significantly (P≤0.05).

Conclusion: From the findings of present study it can be concluded that the dietary supplement of calcium and phosphorus is crucial to fulfill requirement of minerals and also help to attain the maximum conception rate along with reducing the calving interval.

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