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Investigations of Oxidant/antioxidant Status and Hemoglobin Biophysical Properties in Buffalo Calves with Special Reference to Inferior Prewaning Vitality

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Abstract: The present study aimed to investigate oxidant/antioxidants status and some biophysical properties of hemoglobin in preweaning buffalo calves in relation to their vitality. A total number of 253 buffalo calves (2-3 months old) were clinically examined, the vitality of these animals were recorded and blood samples were collected for determination of some oxidant-antioxidant values, as well as some hemoglobin biophysical properties. Results indicated that 38.74% of the examined calves showed preweaning inferior vitality as indicated by dullness, low growth rate, rough coat and signs of scoring. Inferior vitality calves have high malondialdehyde (MDA, $p < 0.001$) and nitric oxide (NO, $p < 0.001$) and low catalase (CAT, $p < 0.001$), superoxide dismutase (SOD, $p < 0.001$), ascorbic acid ($p < 0.01$), glutathione reduced (GSH, $p < 0.001$), total antioxidant capacity (TAC, $p < 0.001$), zinc (Zn, $p < 0.01$), copper (Cu, $p < 0.01$), iron (Fe, $p < 0.05$) and selenium (Se, $p < 0.001$) in their blood. The electrical conductivity and derivatives of hemoglobin non significantly changed due to calf vitality. In conclusion, there is a tight relationship between oxidant/antioxidant status of buffalo-calves and their preweaning vitality.

Key words: Oxidant, antioxidant, hemoglobin, buffalo calves, weaning, vitality

INTRODUCTION

Buffaloes represent an integral part of the animal production sector in Egypt. The world buffalo population averaged 170 million heads (FAO, 2003). In Egypt, despite buffaloes are the main source of good quality meat and milk, these animals are mainly reared in small holder farms and suffer from a lot of stressful conditions such as mal-nutrition and parasitism and characterized by inferior productive and reproductive potentials (Ahmed *et al.*, 2005).

Calves play an important role in the future of animal wealth either for replacement in the herd or as an important source of good quality protein, necessary to fulfill the requirements of the rapid increasing population. Inferior calves vitality, especially during the first few weeks post natal exposed the animal to variety of pathogens and cause economic losses, both directly through morbidity and cost of treatment and indirectly from reduced future gain (Zaki, 2003).

Free radicals are products of normal body cellular metabolism that have a damaging tissue effect. Cells are protected from such effect by specific endogenous antioxidants system to ensure the removal of such free radicals. However, the oxidative stress of the animal was evaluated by measuring steady state concentration of free radicals in the blood and the level of antioxidant in the

blood is closely related to the health and the nutritional status of the host (Smith *et al.*, 1998; Gaal *et al.*, 2006; Jens and Ove, 2006).

The normal physiological function of hemoglobin is a reversible binding of oxygen with the heme iron in the reduced (ferrous) state. Hemoglobin undergoes inactive form (Methemoglobin) with heme iron in the oxidized (ferric) state depending upon the rate of auto-oxidation (Atef *et al.*, 1995). The oxidation process in erythrocytes following the formation of free radicals affects all cell structures and hemoglobin and membrane. The oxidative hemolysis of erythrocytes can be studied by measuring the variations in the electrical conductivity of hemoglobin to investigate the energy gap (Ibrahim, 1996).

The present research was designed to investigate the oxidant/antioxidants status as well as some biophysical properties of hemoglobin (electrical conductivity, derivatives and absorption spectrum) in blood of low vitality buffaloes calves during the preweaning period in compared to moderate vitality.

MATERIALS AND METHODS

Animals: Buffalo calves reared at small holder farms at Al-Sharkia governorate were used. These animals were 2-3 months old and sucked their dams freely.

Experimental design: A total number of 253 buffalo calves was investigated during the period from September 2004 to August 2006. Calves were clinically examined, owner observations, their gross appearance, health status, weight and reaction were taken in consideration. Calves vitality (Vigor) was determined as outlined by Gwazdauskas *et al.* (1979) and Helal *et al.* (1998) and scored from 1-5 (1 very weak and 5 maximum vitality). Blood samples were collected from jugular veins of inferior vitality calves (score 1-2) and moderate vitality calves (score 3) under possible minimal stress. Two types of samples were collected:

- The first type was collected in tubes containing ethylene diamine tetra acetic acid (EDTA) as anticoagulant to obtain whole blood for assaying hemoglobin derivatives, electrical conductivity and spectrum as well as for assaying Se and GSH.
- The second type was collected without any anticoagulant to separate serum for assaying values of MDA, SOD, CAT, TAC, ascorbic acid, NO, Cu, Zn and Fe.

Analyses

Oxidant/antioxidant status: The following oxidant/antioxidant markers were analyzed using chemical kits obtained from Biodiagnostic Co, Egypt.

Malondialdehyde: MDA was assayed according to Satoh (1987). The assay depends on the colorimetric reaction of MDA with thiobarbitric acid giving a pink complex that can be measured at 532 nm.

Nitric oxide: NO was measured by a method depends on the addition of Griess reagent which convert nitrate into a deep purple azo compound which can be measured at 540 nm (Montgomery and Dymock, 1961)

Catalase: CAT reacts with a known quantity of hydrogen peroxide and the reaction stopped by CAT inhibitor, the remaining hydrogen peroxide react with 3, 5-dichloro-2-hydroxybenzene sulfonic acid and 4-aminophenazone to form a chromophore with a color intensity proportional to the CAT in the sample and measured at 510 nm (Aebi, 1994).

Superoxide dismutase: SOD assay relies on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye and measured at 560 nm (Nishikimi and Yogi, 1972).

Ascorbic acid: Ascorbic acid was measured by a method depends on redox reaction of ascorbate with 2, 6-dichlorophenol indophenol in acid solution involves

reduction of this dye to a colourless leucobase while ascorbate is oxidized to dehydroascorbate and measured at 520 nm (Harris and Ray, 1935).

Glutathione reduced: GSH assay is based on the reduction of 2-nitrobenzoic acid with glutathione to produce a yellow compound measured at 405 nm (Beutler *et al.*, 1963).

Total antioxidant capacity: Total antioxidant react with a defined amount of hydrogen peroxide provided and the residual hydrogen peroxide is determined colorimetrically at 505 nm (Koracevic *et al.*, 2001).

Trace elements: Zinc, copper and iron (diluted serum) selenium (whole blood) concentrations were determined by atomic absorption spectrophotometry (Varly *et al.*, 1980).

Biophysical properties of hemoglobin

Derivatives: Four Hb derivatives were determined using simultaneous multicomponent spectrophotometry (Atef *et al.*, 1995).

Electrical conductivity: Electrical conductivity of Hb was measured using conductivity digimeter as outlined by Nicolau (1973).

Absorption spectrum: Absorption spectrum of Hb was recorded in the range between 200 and 700 nm at 25±1°C by Shimadzu UV-visible 240, double beam automatic recording spectrophotometer.

Data were computed and statistically analyzed using Student t-test (Snedecor and Cochran, 1980).

RESULTS

Calves vitality: Table 1 shows that out of the examined buffalo-calves, 38.74% animals have very low to low preweaning vitality (scored 1-2), 47.04% have moderate vitality and 14.23% have high to maximum vitality. Inferior vitality calves revealed signs of dullness (as indicated by their reaction with surrounding stimuli), rough coat and signs of scouring (on the hind limbs and tails), respiratory disorders (in forms of nasal discharges and coughing) and low growth rate.

Oxidant/antioxidant status: Values of some oxidant/antioxidant markers in the blood of inferior vitality preweaning buffalo-calves are shown in Table 2. The results revealed that MDA and NO significantly increased ($p < 0.001$), while CAT ($p < 0.001$), SOD ($p < 0.001$), ascorbic

Table 1: Monitoring of buffalo calves vitality during the preweaning period (%)

Vitality (Score)	No.	Percentage
Very low (1)	38	15.02
Low (2)	60	23.72
Moderate (3)	119	47.04
High (4)	25	9.88
Maximum (5)	11	4.35
Total	253	100.00

Table 2: Some oxidant/antioxidants markers in the blood of preweaning buffalo calves in relation to vitality (Mean±SE)

Parameters	Moderate vitality (Score 3; N = 15)	Inferior vitality (Score 1-2; N = 15)
Malondialdehyde (MDA, mmol mL ⁻¹)	1.97±0.06	3.36±0.11***
Nitric oxide (NO, µmol L ⁻¹)	19.65±1.38	29.34±2.20***
Catalase (CAT, U mL ⁻¹)	2.97±0.04	0.871±0.06***
Superoxide dismutase (SOD, U mL ⁻¹)	417.50±10.12	336.53±2.23***
Ascorbic acid (µg L ⁻¹)	159.85±7.62	114.33±7.18**
Glutathione reduced (GSH, mmol L ⁻¹)	7.92±0.12	4.87±0.19***
Total antioxidant capacity (TAC, mmol L ⁻¹)	1.72±0.08	0.87±0.07***

p<0.01, *p<0.001

Table 3: Concentrations of some trace elements in relation to vitality in preweaning buffalo calves (Mean±SE)

Element	Moderate vitality (Score 3; N = 15)	Inferior vitality (Score 1-2; N = 15)
Zinc (Zn, µg dL ⁻¹)	155.31±14.43	109.77±2.38**
Copper (Cu, µg dL ⁻¹)	125.31±7.96	97.11±5.13**
Iron (Fe, µg dL ⁻¹)	225.62±9.06	197.18±8.11*
Selenium (Se, µg L ⁻¹)	153.65±6.32	119.17±5.87***

*p<0.05, **p<0.01, ***p<0.001

acid (p<0.01), GSH (p<0.001) and TAC p<0.001) were significantly decreased, as compared to calves of moderate vitality.

Concentrations of some trace elements: Concentrations of some trace elements such as Zn (p<0.01), Cu (p<0.01), Fe (p<0.05) in serum and Se (p<0.001) in whole blood were significantly decreased in low vitality buffalo calves as compared to moderate vitality group (Table 3).

Hemoglobin biophysical properties: Some hemoglobin biophysical properties of buffalo calves in relation to their vitality were recorded in Table 1. Electrical conductivity was slightly decreased in inferior vitality group as compared to moderate vitality calves (Table 4). Low concentration of oxyHb and high concentration of metHb, carboxyHb and sulfHb were detected in inferior vitality calves as compared with the control group. Moreover, the absorption spectrum of hemoglobin at 578 nm (Fig. 1) showed that calves with inferior vitality revealed

Table 4: Electrical conductivity and derivatives of hemoglobin in relation to preweaning calf vitality in buffaloes (Mean±SE)

Parameters	Moderate vitality (Score 3; N = 15)	Inferior vitality (Score 1-2; N = 15)
Electrical conductivity (µs cm ⁻¹)	84.61±2.52	79.44±2.03
Oxyhemoglobin (oxyHb, mg dL ⁻¹)	79.36±5.12	78.08±5.03
Methemoglobin (metHb, mg dL ⁻¹)	6.38±1.87	7.84±1.89
Carboxyhemoglobin (carboxyHb, mg dL ⁻¹)	3.17±0.64	5.62±0.98
Sulfhemoglobin (sulfHb, mg dL ⁻¹)	2.16±1.47	3.75±1.76

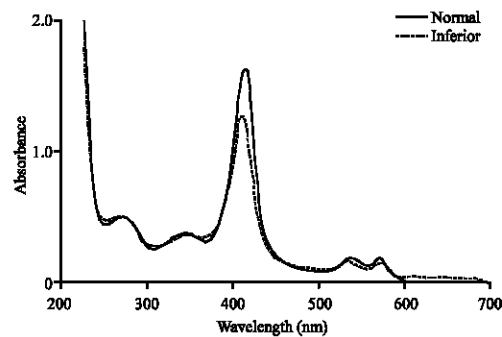


Fig. 1: Hemoglobin absorption spectrum of normal and inferior vitality buffalo calves

increased globin band at 275 nm and a decreased in the half width of the area under Soret band as compared with the control calves.

DISCUSSION

Buffalo is the back bone of animal production in Egypt, especially, calves which are the future of animal wealth. This study was design to investigate the oxidant/antioxidant status as well as some biophysical properties of hemoglobin in preweaning buffalo-calves in relation to their vitality.

In this study, a considerable percent of examined calves (38.74%) showed inferior preweaning vitality as indicated by clinical signs. In this respect, it was reported that weak calf syndrome before weaning accounts for almost a third of calf crop losses and are higher in subtropical and tropical regions and. influenced by a variety of environmental factors, occurrence of dystocia and parturient anoxia and there is a genetic cause for reduced vigor or vitality (Riley *et al.*, 2004). On the other hand, the reported signs here in are in accordance with the findings of Romeo and Simon (1999) who reported that dullness and scouring are the most common signs of morbidity in buffalo calves and that calf hood dullness results in significant long term effects on subsequent survival as replacement heifers as well as

productivity. The risk of inferior preweaning vitality was significantly increased with those calves that were manually assisted at birth and those that were perceived by owners to have received insufficient milk during the first month following birth. Wells *et al.* (1996) found that dystochia increases the stress on the new born calf and causes a delay in the ingestion of colostrums and subsequent transfer of immunoglobulin within the first 24 h after birth. Also, suboptimal nutrition is likely to predispose calves to a range of infections and other conditions. Also, Abeni *et al.* (2004) added that calves born to cows with dystocia and a calf too light at birth have more neonatal problems of vitality.

In this study, it was clear that calves in the inferior vitality group are under oxidative stress as indicated by increased free radical (MDA and NO) and decreased antioxidants markers (CAT, SOD, Ascorbic acid, GSH and TAC) in their blood. Newly born animals are mostly hyperactive and consumed increased amount of oxygen with consequent generation of increased amount of free radicals. Therefore, sufficient antioxidants contents in their body and those supplied by maternal route are essential to blunt the excessive production of free radicals. In this respect, it was reported that antioxidant defense temper the negative influence of free radical and associated reaction and keep them in check (Priscilla and Heather, 2000; Jens and Ove, 2006). Also, Sikka *et al.* (2002) found that exposure of pregnant dam to excessive membranes lipid peroxidation significantly reduce the transferring of immunoglobulin from dams to calves and affect their immune function. CAT is responsible for break down of hydrogen peroxide, an important reactive oxygen species produced during metabolism. Reduced activity of CAT in low vitality calves could be correlated to increase generation of hydrogen peroxide (Patra and Swoup, 2000; Gaal *et al.*, 2006). On the other hand, the oxidation of oxyhemoglobin to methemoglobin in inferior vitality group generate superoxide ions, which in turn can diminish the activity of SOD followed by induction of its biosynthesis as a protective mechanism against free radical toxicity (Patra and Swoup, 2000; Jens and Ove, 2006). Ascorbic acid is used in the process of quenching free radicals produced by polymorphonuclear neutrophils in the blood (Camus *et al.*, 1994; Smith *et al.*, 1998; Priscilla and Heather, 2000) and its supplement is useful in reducing the effects of stress (Priscilla and Heather, 2000). Changes in glutathione in the blood indicate increased oxidative reactions and it mobilizes from tissue stores to combat oxidative stress elsewhere in the body (Priscilla and Heather, 2000; Gaal *et al.*, 2006; Jens and Ove, 2006).

In this study, concentrations of some studied trace elements in the blood (Zn, Cu, Fe and Se) of low vitality calves were lower as compared to moderate vitality group. The concentration of these elements depends upon the nutritional status of these calves as well as that of their dams (Zaki, 2003). Sikka *et al.* (2002) reported that micronutrients such as Zn, Cu, Fe and Mn improve the efficiency of antioxidant system in lipid peroxidation prevention. Moreover, it was recorded that decreased blood copper and zinc concentrations in calves result in general weakness, stunted growth and macrocytic hypochromic anemia (Damir *et al.*, 1988; Brzezinska-Slebodzinska *et al.*, 1994) Se decreases lipid peroxidation (Paulla-lobes *et al.*, 2003) and support body defense mechanism (Malbe *et al.*, 2003).

The studied biophysical properties of hemoglobin in present study indicated that buffalo calves with inferior vitality had slightly changes in electrical conductivity, hemoglobin derivatives and its spectrum. These changes in hemoglobin properties will be reflected on its proper function and consequently tissues are supplied by low concentration of oxygen. The decreased electrical conductivity were attributed to the changes in folding and unfolding mechanism of hemoglobin molecules and broken hydrogen bonds between hydrophobic nonpolar group (SaadEldin and Kamal, 1996). In the same time, the decreased oxyHb and increased methHb in the low vitality calves could be attributed to changes in the activity of methemoglobin reductase system which transfer methHb to oxyHb during increased oxidative stress (Mal and Challerjee, 1991). In the inferior vitality calves, it is clear from hemoglobin spectrum that the half width of area under Soret band decreased as compared to moderate vitality calves and this is due to the decrease of the strength in the bond between heme iron and nitrogen of pyrole in porphrin of the hemoglobin molecule which has an effect on the function of hemoglobin molecule (Abdel-Baset *et al.*, 1994).

In conclusion, inferior vitality calves during the preweaning period are suffering from oxidative stress with disturbed hemoglobin function. Therefore, late pregnant and newly born animals should be supplied by a mixture of antioxidants to raise the vitality of newly born calves and to maintain significantly higher immune bodies, disease resistance, growth rate and long term productivity.

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