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Alteration of oxidative stress markers, hepatic enzymes and macro-minerals in diarrheic surti buffalo calves

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Abstract
The present study was conducted to study the alteration of oxidative stress markers, hepatic enzymes and macro-minerals in diarrheic Surti buffalo calves. Selection of 30 Surti buffalo calves was done and categorized as diarrheic (n=20) and healthy (n=10) calves. Blood was collected from diarrheic calves as soon as diarrhea was reported along with simultaneous blood collection from healthy calf. Blood was analyzed for hepatic enzymes viz.,Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST); macro-minerals like Calcium (Ca), Phosphorus (P) and Magnesium (Mg) and oxidative stress parameters such as Lipid peroxidation (LPO), Reduced glutathione (GSH), Superoxide dismutase (SOD) and Total antioxidant status (TAS). Results revealed that in diarrheic calves significantly ($p \leq 0.05$) higher levels for ALT, AST, Mg and LPO whereas significantly ($p \leq 0.05$) lower values of Ca, GSH, SOD and TAS were observed as compared to healthy calves. These results led to conclusion that early changes in diarrheic Surti buffalo calves include hepatic and renal impairment, GIT malabsorption and oxidative stress.

Keywords: Oxidative stress, hepatic enzymes, macro-minerals, diarrhoea, Surti buffalo calves

1. Introduction
Buffaloes contribute a sizeable portion to national dairy milk production of India. They are reared both for milk and meat production. However, dairy buffalo rearing can only be rewarding if the buffalo calves are healthy and face less mortality during early life. One of the early causes of ill health and mortality in buffalo calves during early age is diarrhoea especially up to 3 months after birth. The impact of diarrhoea on dairy industry is overwhelming (Elhassan *et al*., 2011, Pourjafar *et al*., 2011) [1, 2]. Even though cause of diarrhoea is multifactorial, early age witnesses more of infectious etiologies and older age of around 2 months has reasons that are more of non-infectious origin such as excess milk intake etc. Early changes that occur in diarrhoea may be transient but may cause stress, metabolic derangement and imbalance of macro minerals. Hepatic involvement during metabolic derangement is tremendous and is also closely associated with oxidative stress. As hepatic enzymes serve as markers indicating metabolic alterations, they are important to be studied along with other changes as oxidative stress and macro mineral imbalance. Surti buffaloes are one of the native breeds of south Gujarat region that is reared for dairy purposes in their native tract of south Gujarat. Therefore, the present study was planned to study effect of diarrhoea on oxidative stress markers, hepatic enzymes and macro-minerals in diarrheic Surti buffalo calves.

2. Materials and Methods
The present study was done at Department of Veterinary Physiology & Biochemistry, COVS& AH, Navsari (KU), Gujarat after approval from IAEC.The study comprised of total 30 Surti buffalo calves aged approximately 2 months from organized livestock farm in Navsari district. The calves that were selected for study were categorized as two groups viz., healthy (n=10) and diarrheic calves (n=20).
Blood was collected from diarrheic calf as soon as diarrhoea was reported along with simultaneous blood collection from a healthy calf of similar age. About 5 ml of whole blood was collected in vacutainers containing anticoagulant K_3EDTA as well as clot activator tubes. Whole blood was used for estimation of GSH, SOD and LPO using methods described by Moron *et al*. (1979) [3], Madesh and Balasubramanian (1998) [4] and Rehman (1984) [5] respectively.

Total antioxidant status (TAS) was measured in plasma using the method of Benzie and Strain (1999) [6] for FRAP (ferric reducing antioxidant power). Serum was harvested from clot activator tubes by centrifugation. Serum was used for analysis of calcium using standard analytical kit of Randox, Magnesium and phosphorus using kits of Lab-Care Diagnostics (India) Pvt. Ltd. Serum was also used for analyzing the concentration of hepatic enzymes Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST).

Result obtained were analyzed statistically to generate mean±SE as well as maximum and minimum values. Group means were compared with the help of student t-test at 5% level of significance ($p \leq 0.05$).

3. Results

3.1 Hepatic enzyme profile

The results for hepatic enzyme profile, macro mineral profile and oxidative stress parameters of healthy (control) and diarrheic (treatment) Surti buffalo calves are mentioned in table 1, 2 and 3 respectively.

Alanine aminotransferase (ALT) in healthy calves was 23.93±1.57 U/l and diarrheic calves was 28.59±1.27 U/l. Minimum and maximum alanine aminotransferase (ALT) in healthy calves ranged from 18.25 U/l to 32.59 U/l and in diarrheic calves from 17.34 U/l to 36.45 U/l. Alanine aminotransferase (ALT) was significantly ($p \leq 0.05$) higher in diarrheic calves as compared to healthy calves.

Aspartate aminotransferase (AST) in healthy calves was 102.20±2.22 U/l and diarrheic calves was 109.05±1.45 U/l. Minimum and maximum aspartate aminotransferase (AST) in healthy calves ranged from 94.00 U/l to 114.00 U/l and in diarrheic calves from 100.00 U/l to 124.00 U/l. Aspartate aminotransferase (AST) was significantly ($p \leq 0.05$) higher in diarrheic calves as compared to healthy calves.

Table 1: Hepatic enzyme profile of healthy and diarrheic Surti buffalo calves

Parameter	Group	Mean±SE	Range
Alanine aminotransferase, ALT (U/l)	Healthy calves (n=10)	23.93^{b}±1.57	18.25-32.59
	Diarrheic calves (n=20)	28.59^{a}±1.27	17.34-36.45
Aspartate aminotransferase, AST(U/l)	Healthy calves (n=10)	102.20^{b}±2.22	94.00-114.00
	Diarrheic calves (n=20)	109.05^{a}±1.45	100.00-124.00
Means with alphabetical superscripts (a, b) differ significantly ($p \leq 0.05$) between groups			

3.2 Mineral profile

Table 2: Macro mineral profile of healthy and diarrheic Surti buffalo calves

Parameter	Group	Mean±SE	Range
Calcium, Ca (mg/dl)	Healthy calves (n=10)	12.90^{a}±0.38	11.22-14.57
	Diarrheic calves (n=20)	10.55^{b}±0.20	8.88-11.97
Phosphorus, P (mg/dl)	Healthy calves (n=10)	6.70±0.34	5.26-7.93
	Diarrheic calves (n=20)	6.25±0.20	5.12-8.39
Magnesium, Mg (mg/dl)	Healthy calves (n=10)	3.03^{b}±0.26	2.04-4.22
	Diarrheic calves (n=20)	3.99^{a}±0.15	2.55-4.98
Means with alphabetical superscripts (a, b) differ significantly ($p \leq 0.05$) between groups			

Calcium (Ca) in healthy calves was 12.90±0.38 mg/dl and diarrheic calves was 10.55±0.20 mg/dl. Minimum and maximum calcium (Ca) in healthy calves ranged from 11.22 mg/dl to 14.57 mg/dl and in diarrheic calves from 8.88 mg/dl to 11.97 mg/dl. Calcium (Ca) was significantly ($p \leq 0.05$) lower in diarrheic calves as compared to healthy calves.

Phosphorus (P) in healthy calves was 6.70±0.34 mg/dl and diarrheic calves was 6.25±0.20 mg/dl. Minimum and maximum phosphorus (P) in healthy calves ranged from 5.26 mg/dl to 7.93 mg/dl and in diarrheic calves from 5.12 mg/dl to 8.39 mg/dl. Phosphorus (P) was slightly lower in diarrheic calves but the difference was not significant.

Magnesium (Mg) in healthy calves was 3.03±0.26mg/dl and diarrheic calves was 3.99±0.15mg/dl. Minimum and maximum magnesium (Mg) in healthy calves ranged from 2.04 mg/dl to 4.22 mg/dl and in diarrheic calves from 2.55 mg/dl to 4.98 mg/dl. Magnesium (Mg) was significantly ($p \leq 0.05$) higher in diarrheic calves as compared to healthy calves.

3.3 Oxidative stress parameters

Lipid peroxidation (LPO) in healthy calves was 3.16±0.08 nM of MDA produced/ml of packed cells and diarrheic calves was 3.57±0.06 nM of MDA produced/ml of packed cells. Minimum and maximum lipid peroxidation (LPO) in healthy calves ranged from 2.77 nM of MDA produced/ml of packed cells to 3.52 nM of MDA produced/ml of packed cells and in diarrheic calves from 3.05 nM of MDA produced/ml of packed cells to 4.03 nM of MDA produced/ml of packed cells. Lipid peroxidation (LPO) was significantly ($p \leq 0.05$) higher in diarrheic calves as compared to healthy calves.

Reduced glutathione, (GSH) in healthy calves was 6.90±0.10

mg/dl and diarrheic calves was 6.57±0.07 mg/dl. Minimum and maximum reduced glutathione, (GSH) in healthy calves ranged from 6.45 mg/dl to 7.32 mg/dl and in diarrheic calves from 6.18 mg/dl to 7.12 mg/dl. Reduced glutathione (GSH) was significantly ($p \leq 0.05$) lower in diarrheic calves as compared to healthy calves.

Superoxide dismutase, (SOD) in healthy calves was 2.87±0.08U/mg of Hb and diarrheic calves was 2.46±0.06U/mg of Hb. Minimum and maximum superoxide dismutase (SOD) in healthy calves ranged from 2.42U/mg of Hb to 3.26U/mg of Hb and in diarrheic calves from 2.09U/mg of Hb to 2.83U/mg of Hb. Superoxide dismutase (SOD) was significantly ($p \leq 0.05$) lower in diarrheic calves as compared to healthy calves.

Table 3: Oxidative stress parameters of healthy and diarrheic Surti buffalo calves

Parameter	Group	Mean±SE	Range
Lipid peroxidation, LPO (nM of MDA produced/ml of packed cells)	Healthy calves (n=10)	3.16^{b}±0.08	2.77-3.52
	Diarrheic calves (n=20)	3.57^{a}±0.06	3.05-4.03
Reduced glutathione, GSH (mg/dl)	Healthy calves (n=10)	6.90^{a}±0.10	6.45-7.32
	Diarrheic calves (n=20)	6.57^{b}±0.07	6.18-7.12
Superoxide dismutase, SOD (U/mg of Hb)	Healthy calves (n=10)	2.87^{a}±0.08	2.42-3.26
	Diarrheic calves (n=20)	2.46^{b}±0.06	2.09-2.83
Total antioxidant status, TAS (µM)	Healthy calves (n=10)	317.34^{a}±8.56	272.76-352.18
	Diarrheic calves (n=20)	276.86^{b}±4.67	242.64-320.37
Means with alphabetical superscripts (a, b) differ significantly ($p \leq 0.05$) between groups			

Total antioxidant status (TAS) in healthy calves was 317.34±8.56 µM and diarrheic calves was 276.86±4.67 µM. Minimum and maximum total antioxidant status (TAS) in healthy calves ranged from 272.76 µM to 352.18 µM and in diarrheic calves from 242.64 µM to 320.37 µM. Total antioxidant status (TAS) was significantly ($p \leq 0.05$) lower in diarrheic calves as compared to healthy calves.

4. Discussion

4.1 Hepatic enzyme profile

Transfer of α - amino groups from alanine to α -ketoglutaric acid to form pyruvic and glutamic acid is mediated by enzyme Alanine aminotransferase (ALT) and from aspartic acid to alpha-ketoglutaric acid to form oxaloacetic acid and glutamic acid occurs through enzyme Aspartate aminotransferase (AST). ALT and AST enzymes of the diarrheic calves were significantly elevated as compared to healthy calves. ALT is not of much importance in large ruminant but for pre-ruminant calves that is considerably monogastric it holds some relevance. AST in not specific to liver and also has extrahepatic sources.

As per Chernecky (2013) [7] increased level of ALT and AST may be seen during inflammation of GIT and pathological affections of liver. Generally, injury or change in permeability of cell membrane of liver, intestine and muscle fibers are responsible for higher activities of AST and ALT. Damage or increase in permeability of cell membrane causes these enzymes to leak and enter plasma as reported by Benjamin (2013) and Kaneko (1997) [8, 9].

These enzyme levels in the diarrheic calves of present study might have been elevated due to diarrhoea per se, dehydration or metabolic stress due to change in pattern of utilization of energy substrate. Results of higher concentration of theses enzymes is in agreement with rise of same enzymes as reported by Ozkan *et al.* (2011) and El-Seadawy *et al.* (2020) [10, 11].

Thus higher serum levels of ALT and AST enzymes are important changes that are observed in diarrheic calves.

4.2 Mineral Profile

Calcium is important for cell structure and signalling at cellular levels. It is most prevalent cation that is present mainly in bone and ECF and is critical for neuromuscular functions. Calcium that is protein bound is about 40% of the total bound calcium. Amongst protein bound calcium $1/5^{th}$ part is bound to globulin and $4/5^{th}$ portion is bound to albumin. Phosphate has several roles ranging from structural support, presence in soft tissue and bones. Its organic form has important roles in DNA, phospholipids, and high-energy compounds such as ATP. Calcitonin and parathormone hormones antagonistically regulate their levels to maintain their optimal ratio. Magnesium is second most abundant ion found intracellularly. It plays major role in intracellular metabolism. Only 1% of the total magnesium is present in ECF.

Hypocalcaemia and hypophosphatemia may occur in cases of anorexia and intestinal malabsorption as seen during diarrhoea. Reduced levels could also be due to lesser binding with albumin and globulin as their levels were lower in diarrheic calves discussed earlier. This can probably explain the significant lowering of calcium level in blood of diarrheic calves as compared to healthier counterparts. Similar findings have been reported by Zilaitis *et al.* (2015) and Galbat *et al.* (2015) [12, 13]. Ghanem and Abd El-Raof (2006) [14] reported malabsorption as one of the reasons for hypocalcaemia. Walker *et al.* (1998) [15] have also reported hypophosphatemia during diarrhoea in calves.

Hypermagnesemia found in present calves could not be reviewed due to lack of such studies for calf diarrhoea. Moreover, its reason for increased levels could not be ascertained except for few reports that suggest poor kidney functioning is responsible for its elevation in blood. In support to this it can be noted that diarrheic calves in present study were dehydrated and might have reduced renal blood flow as compromised renal function. Apart from this, hypocalcaemia induced release of parathormone causes an increase in renal threshold of calcium and magnesium therefore resulting in higher magnesium levels in serum of in diarrheic calves. Based on present study findings, lower calcium and higher magnesium can be considered as important changes in diarrheic Surti buffalo calves.

4.3 Oxidative Stress Parameters

Diarrhoea like disorders (both infectious and non-infectious origin) causes GIT inflammation, metabolic stress and dehydration. These in turn may result in generation of excess free radicals and oxidative damage (Valko *et al*, 2007) [16]. Characteristically during oxidative damage, free radicals interact with PUFA of cell membrane and cause lipid peroxidation resulting in Malondialdehyde (MDA) production that acts a marker of oxidative stress. MDA levels were significantly high in diarrheic calves of present study as compared to healthy calves of present study.

Excess free radicals and other entities with potential of causing oxidative stress such as reactive oxygen species are frequently combated with the help of antioxidants of endogenous origin. Enzymatic antioxidants such as SOD act by scavenging superoxide anion (dismutation) and converting it to O_2 and H_2O_2 (Pryor *et al*, 2006) [17] whereas non-enzymatic antioxidants such as GSH is oxidized to GSSG simultaneously furnishing reducing equivalents to antioxidant enzymes, hydroxyl radicals and ROS. Thus to prevent oxidative stress whenever these antioxidants will be utilized their concentration will decrease. Therefore, even in the present study there were significantly lower levels of SOD and GSH in diarrheic calves than control healthy calves.

Total antioxidant status represents collective antioxidant defence present in blood and is sum total of several antioxidant parameters. In order to negate the oxidative stress present in diarrheic calves of present study their antioxidants might have been utilized and therefore depleted to make their levels significantly low as compared to healthy control calves.

Oxidative stress associated with enteric diseases has been reported by many workers like Ranjan *et al.* (2006) and Samiran *et al.* (2015) [18, 19]. Other studies are in consonance with present study i.e. increase in MDA reported by Ahmed and Hassan (2007), Mahran and Abd Allah (2020)[20, 21]; decrease in SOD levels were reported by Ghanem *et al.* (2012), Ahmed and Hassan (2007),Kumar and Jakhar (2020) and Kumar *et al*. (2018) [14, 20, 22, 23] and decrease in total antioxidant capacity was reported by Ahmed and Hassan (2007), Mahran and Abd Allah (2020) and Akyuz and Kukurt (2021) [20, 21, 24]. Due to lack of study assessing GSH levels in diarrheic calves, present study findings of reduced GSH levels in diarrheic calves could not be reviewed however reduction in glutathione reductase as well as glutathione-s-transferase have been observed during calf diarrhoea (Kumar and Jakhar, 2020) [22] and reduced glutathione peroxidase in diarrheic neonatal goat kids (Cheng *et al.*, 2021) [25].

Thus in Surti buffalo calves, increased lipid peroxidation and lower levels of GSH, SOD and TAS may act as oxidative stress markers during early stage of diarrhoea.

5. Conclusion

It was concluded from the present study that early changes in diarrheic Surti buffalo calves include hepatic and renal impairment, GIT malabsorption and oxidative stress as manifested by elevated alanine aminotransferase and aspartate aminotransferase; lower calcium and higher magnesium; increased lipid peroxidation whereas decrease in levels of lowered reduced glutathione, superoxide dismutase, total antioxidant status.

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