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RESEARCH ARTICLE

Effect of Rumen Fermentative Disorders on Physiological Parameters in Buffaloes

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ABSTRACT

In this study, the effect of fermentative disorders such as acid and alkaline indigestion was investigated in buffaloes. Six buffaloes each suffering from acid and alkaline indigestion was chosen and various physiological parameters were compared with six healthy buffaloes Clinical examination was carried out to rule out infectious diseases and abomasal displacement. Rumen fluid was evaluated for pH, protozoal motility, iodophilic activity, sedimentation activity time (SAT), methylene blue reduction time (MBRT), total protozoa, gas production, ammonia, total volatile fatty acids (TVFA). Haematological parameters such as haemoglobin, packed cell volume (PCV), erythrocyte, leucocyte and differential counts were performed. In serum, calcium, phosphorus, magnesium, glucose, urea, protein were estimated. The data was analysed using one way ANOVA and Kruskalwallis test.

A significant ($P<0.05$) increase in pulse and respiratory rates and significant decrease in rumen motility was observed in both acidosis and alkalosis. Rumen pH decreased significantly ($P<0.05$) in acidosis and increased in alkalosis. In rumen liquor, SAT and MBRT were significantly ($P<0.05$) increased. The protozoal count, motility and iodophilic activities and gas production were significantly ($P<0.05$) reduced. However, rumen ammonia was only increased in alkalosis and TVFA in acidosis. Increased PCV was seen in both acidosis and alkalosis. Sero-biochemical analysis revealed as significant ($P<0.05$) decrease in calcium, phosphorus and protein content in alkalosis whereas glucose and blood urea nitrogen (BUN) were significantly ($P<0.05$) increased in both acidosis and alkalosis.

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INTRODUCTION

Rumen disorders are responsible for huge economic losses in dairy industry due to decrease in production and increase in expenditure towards treatment. Dysfunctional rumen results in impaired digestion and increased susceptibility to various digestive and metabolic diseases (Kirbas *et al*., 2014; Kahn, 2011; Plaizier *et al*., 2009; Steele *et al*., 2009; Enemark, 2008; Krause and Oetzel, 2005).

Ruminal acidosis is an important nutritional disorder in ruminants which results from feeding highly fermentable feeds to increase productivity (Khafipour *et al*., 2009; Martin *et al*., 2006). Introduction of highly fermentable starch into rumen stimulates the growth of most ruminal bacteria, thereby increasing production of volatile fatty acids if the transition from a forage to cereal grain diet is too abrupt or if the particle size of concentrate ration is too small, microbial population becomes unstable, leading to production of lactic acid and causing acidosis (McAlister *et al*., 1996).

Similarly, feeding of excess of protein-rich concentrates and non-protein nitrogenous compounds such as urea leads to rumen alkalosis (Bencini, 2004; Smith and Sherman, 2009). The disease is characterised by excessive production of ammonia in the rumen which may produce gastrointestinal, hepatic, renal, circulatory and nervous disturbances (Radostits *et al*. 2006). Alkaline digestion develops primarily due to abrupt change in protein content of ration (Hoflund, l967). Other factors contributing to alkaline indigestion are feeding decomposed or putrefied feed and fodder (Hoflund, 1967), drinking contaminated and sewage water (Nagarajan and Rajamani,1973), exclusive feeding of paddy straw (Misra

and Tripathy, 1963) and feeding high doses of urea (Davidovich *et al*., 1977).

Earlier, several attempts were made to study the physiological changes associated with altered ruminal pH (Dong *et al*., 2011; Enemark, 2008; Gozho *et al*., 2005). However, the due to paucity of literature with respective to buffaloes, the present study is taken up to investigate the associated changes in physiology due to rumen acidosis and alkalosis. The understanding of physiological changes during disease helps in formulating rational therapeutic strategies. At present, the use of herbal drugs is a popular therapeutic regime for treating rumen disorders (Embeya *et al*., 2014; Handekar *et al*., 2010; Maphosa and Masika, 2010) as they are cost effective and safe (Sakuntala Devi *et al*., 2012). Further, such knowledge helps in discovering novel drugs for treating important economic diseases of dairy animals (Kumar *et al*., 2013, 2014).

MATERIALS AND METHODS

Six buffaloes each for normal controls, acid and alkaline indigestions were chosen for the study. During clinical examination, rectal temperature, pulse, respiration and rumen motility were recorded. Infectious diseases and abomasal displacement were ruled out. About 100-200 mL of rumen fluid was collected using rumen fluid extractor and the rumen fluid was immediately examined for colour odour, consistency, pH and methylene blue reduction time (MBRT) (Dirksen, 1969). Remaining rumen liquor was preserved in air-tight glass bottles under a thin film of liquid paraffin until further analysis. Whole blood was used for haematological analysis and serum was subjected to biochemical analysis.

Rumen liquor was evaluated for qualitative parameters such as protozoal motility (Misra and Singh, 1974), iodophilic activity (Mishra *et al*., 1972); quantitative parameters such as total protozoal counts (Naga and Elshazly, 1969), sedimentation activity time (SAT) (Nicholus and Penn, 1958), gas production, rumen ammonia nitrogen (Conway, 1957) and total volatile fatty acids (TVFA) (Briggs and Reid, 1957). In serum, calcium, phosphorus, magnesium, glucose, total protein, blood urea nitrogen (BUN) were estimated using standard kits supplied by Span Diagnostics, Pvt, Ltd, Surat.

The data for various quantitative parameters was presented as mean ± standard deviation (S.D) and for qualitative parameters as median (quartile 1 to quartile 3) Quantitative data was analysed by one way ANOVA followed by Tukey's post hoc test. Qualitative data was analysed by using Kruskalwallis test followed b Man-whitney U test. The level of significance was set at $P<0.05$. Statistical package for Social Sciences (SPSS) 17.0 V was used for statistical analysis.

RESULTS

Buffaloes affected with acid and alkaline indigestion exhibited inappetance, anorexia, decreased milk yield, absence of rumination, salivation and diarrhoea with variable severity. A significant ($P<0.05$) increase in pulse and respiratory rates was observed in both acidosis and alkalosis. Rumen motility on the other hand was found to be significantly ($P<0.05$) decreased in both cases (table 1).

Analysis of rumen liquor revealed that pH decreased significantly ($P<0.05$) in acidosis whereas increased significantly ($P<0.05$) in alkalosis. The rumen fluid in acidotic animals was watery, milky grey in colour with pungent sour smell. In alkalosis, the rumen fluid was dark brown, putrefied with ammonical smell. In alkalosis, putrid odour rumen liquor was observed. In this study, Sedimentation activity time (SAT) and methylene blue reduction times (MBRT), were significantly ($P<0.05$) increased in both acidosis and alkalosis. The protozoal count, motility and iodophilic activities and gas production were found to be significantly ($P<0.05$) reduced. However, the decrease in total protozoa was drastic in acidosis. Ammonia content in rumen fluid was significantly ($P<0.05$) higher in alkalosis compared to both control and acidotic animals. The production of

Table 1: Effect of fermentative disorders on clinical parameters in buffaloes

Parameter	Temperature (^{o}C)	Pulse (/min)	Respiration (/min)	Rumen Motility (/5min)
Control	100.00±0.62	49.83 ± 2.93^{a}	20.83 ± 2.48^{a}	7.50 ± 0.84^{b}
Acid Indigestion	100.88±0.30	59.50 ± 3.33^{b}	28.50 ± 1.64^{b}	0.67 ± 0.52^{a}
Alkaline Indigestion	100.62±0.47	56.00 ± 6.07^{ab}	21.67 ± 4.03^{a}	1.17 ± 0.41^{a}
Sig	0.180^{NS}	0.005	0.001	0.000

Values are Mean± SD (n=6); One way ANOVA followed by Tukey's post hoc test using SPSS 17.0 v software; Means or Medians with different superscripts are significantly different ($P<0.05$).

Table 2: Effect of fermentative disorders on the characteristics of rumen liquor in buffaloes

Parameter	pH	SAT (min)	MBRT (min)	Gas (mL/h)	Total protozoa ($x10^{5}$)	Rumen Ammonia (mg %)	TVFA (mEq/L)	Protozoal Motility* (Score)	Iodophilic activity * (Score)
Control	7.00 ± 0.13^{b}	7.50 ± 0.84^{a}	5.33 ± 1.21^{a}	13.42 ± 1.32^{b}	3.45 ± 0.51^{c}	12.52 ± 3.22^{a}	86.00 ± 7.69^{b}	3.00^{b} (3.00-3.00)	3.00^{b} (3.00-3.00)
Acid Indigestion	6.31 ± 0.23^{a}	25.33 ± 2.80^{b}	18.67 ± 1.75^{b}	7.02 ± 0.33^{a}	1.03 ± 0.15^{a}	9.52 ± 1.65^{a}	100.83 ± 8.64^{c}	1.00^{a} (0.25-1.00)	1.00^{a} (0.25-1.00)
Alkaline Indigestion	8.03 ± 0.29^{c}	20.50 ± 3.45^{b}	17.17 ± 3.43^{b}	7.83 ± 0.68^{a}	1.82 ± 0.15^{b}	32.89 ± 4.23^{b}	55.17 ± 4.54^{a}	1.00^{a} (1.00-1.00)	1.00^{a} (1.00 -1.00)
Sig	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.001

(SAT=Sedimentation activity time; MBRT = Methylene blue reduction time; TVFA = Total volatile fatty acids); Values are Mean± SD (n=6); One way ANOVA followed by Tukey's post hoc test using SPSS 17.0 v software; * Values are Median (Q1 - Q3); * Kruskal wallis test followed by Man-whitney U test SPSS 17.0 v software; Means or Medians with different superscripts are significantly different ($P<0.05$).

Table 3: Effect of fermentative disorders on hematology in buffaloes

Parameter	Hemoglobin (g%)	PCV (%)	RBC (10^6/µL)	WBC (10^3/µL)	Differential count (%)				
					Neutrophils	Lymphocytes	Eosinophils	Monocytes	Basophils
Control	10.57±0.17[a]	32.67±3.44[a]	5.33±0.58	8.85±0.70	45.67±1.64	49.50±1.64	3.33±1.03	1.33±0.52	0.17±0.41
Acid Indigestion	11.40±0.52[b]	44.50±2.07[b]	5.40±0.46	9.25±0.23	47.33±1.37	47.50±1.38	3.17±0.75	1.50±0.55	0.17±0.41
Alkaline Indigestion	11.60±0.55[b]	44.00±2.10[b]	5.47±0.36	9.13±0.23	46.17±0.75	48.33±0.52	3.50±1.05	1.67±0.52	0.17±0.41
Sig	0.006	0.000	0.888[NS]	0.311[NS]	0.093[NS]	0.408[NS]	0.835[NS]	0.561[NS]	1.000[NS]

(PCV=Packed cell volume; RBC = Red blood cells; WBC = White blood cells; NS = Non-significant); Values are Mean± SD (n=6); One way ANOVA followed by Tukey's post hoc test using SPSS 17.0 v software; Means or Medians with different superscripts are significantly different (P<0.05).

Table 4: Effect of fermentative disorders on sero-biochemical parameters in buffaloes

Parameter	Calcium	Phosphorus	Magnesium	Glucose	Protein	BUN
Control	10.61±1.24[b]	4.80±0.93[b]	2.45±0.33	48.70±3.09[a]	8.04±1.30[b]	10.36±1.34[a]
Acid Indigestion	10.19±0.88[b]	4.15±0.41[ab]	2.39±0.42	62.91±3.70[b]	8.01±0.24[b]	31.17±2.22[b]
Alkaline Indigestion	7.71±0.60[a]	3.50±0.39[a]	2.37±0.74	64.38±5.82[b]	6.22±0.89[a]	28.12±7.20[b]
Sig	0.000	0.009	0.958[NS]	0.000	0.000	0.000

Values are Mean± SD (n=6); One way ANOVA followed by Tukey's post hoc test using SPSS 17.0 v software; Means or Medians with different superscripts are significantly different (P<0.05)

volatile fatty acids (TVFA) was significantly (P<0.05) decreased in alkalosis but was significantly (P<0.05) increased in acidosis.

Haematology (table 3) revealed that haemoglobin and packed cell volume (PCV) were significantly (P<0.05) increased in both acidosis and alkalosis. Sero-biochemical analysis (table 4) revealed as significant (P<0.05) decrease in calcium, phosphorus and protein content in alkalosis whereas glucose and blood urea nitrogen (BUN) were significantly (P<0.05) increased in both acidosis and alkalosis.

DISCUSSION

In this study, animals affected with acid indigestion had a history of suddenly ingesting easily fermentable foods such as cooked rice, excessive grains or root crops (sugar beets and potatoes). Abnormal fermentation of simple carbohydrates by the acidogenic aerobic microbes results in the production of organic acids such as formic, valeric and succinic acids leading to reduction of rumen pH (Dirksen, l970). Due to the production of acid, amines and toxins, the rumen motility is inhibited (Singh *et al*., 2003; Huber, 1976). Further, high concentrations of volatile fatty acids reflexly inhibit rumen motility through sensory epithelial receptors (Garry, 2002). Feeding of paddy straw was the primary cause of alkaline indigestion. Feeding poorly digestible roughages like paddy straw decreases rumen microflora and consequently volatile fatty acid production. Due to decreased VFA, buffering of alkaline saliva by rumen is hampered, resulting in alkalosis. Further, the bicarbonate ions generated due to absorption of VFA across rumen also contributes to alkalosis, with acetate produced from roughages being the highest generator of bicarbonate ions.

Rumen protozoan are sensitive to changes in pH as the growth, multiplication and motility of the protozoa is dependent on hydrogen ion concentration. The protozoan concentration and motility was sluggish in both acidosis and alkalosis due to lack of nutrients and optimal pH. Further, excessive acid generated in acidosis and toxic amines generated in alkalosis due to putrefaction are responsible for decreased motility and death of protozoa (Hoflund, 1967). Consequent to the decreased activity of microflora and fauna, in both acid and alkaline indigestion SAT and MBRT were increased and gas production time was reduced.

Rumen ammonia nitrogen increased in alkalosis due to the destruction of normal microflora and the absence of cellulolytic bacteria due to production of ammonia nitrogen in toxic concentrations in rumen liquor (Ahuja *et al*., 1989). Saprophytic bacteria (coliforms and proteus species) entering through spoiled and wet feed are also reponsible for increase in ammonia production (Dirksen and Smith, 1987). Further, non-protein nitrogen compounds and ammonium fertilizers also cause dramatic increase in ammonia generation (Ahuja *et al*., 1989; Randhawa *et al*., 1991; Randhawa and Singh, l982). Total volatile fatty acid increased in acidosis due to rapid fermentation of easily fermentable foods generating VFA along with lactic acid (Randhawa *et al*., 1981). However, in chronic cases of acidosis, VFA are reduced suggesting that VFA content is dependent on the stage of the disease (Sinha *et al*., 1985).

Haematological parameters such as haemoglobin and PCV were increased in both alkalosis and acidosis as a result of haemo-concentration due to increased osmolarity of rumen contents which withdraws fluid from intravascular compartments (Huber, l971).

Glucose levels were found to be significantly increased in both acidosis and alkalosis. Similar observations were made by Bide *et al.* (1973) and Dirksen (1970) in moderate acidosis and by Venkateswarlu *et al.* (1998) and Singh *et al.* (2003) in alkalosis. Hyperglycaemia is a result of decreased peripheral utilization of glucose coupled with hepatic glycogenolysis under the influence of corticosteroids released digestive stress (Nauriysal and Baxi, 1981; Randhawa *et al*., l981 and 1991). Similarly, blood urea nitrogen (BUN) is increased in both acidosis and alkalosis due to increased conversion of ammonia to urea. Further, decreased renal function and hepatic insufficiency is also a contributing factor (Radostits *et al*. 2006; Patra *et al*., 1996). In alkalosis, a significant decrease in calcium and phosphorus was observed due to decreased absorptive capacity of intestine (Choudhuri *et al*., l980). Similar findings were observed by Gupta *et al.* (1995). Acidosis being acute in its onset is unable to produce changes in serum biochemical profile.

Conclusions

Acid and alkaline indigestion hampers the fermentative capacity of rumen by inhibiting microflora and fauna. Putrefactive changes due to saprophytes are characteristic of alkalosis. Acid indigestion has more drastic effects on survival of rumen microflora and fauna than alkalosis. Though both acidosis and alkalosis reduce volatile fatty acid production, in acidosis it is dependent on the stage of disease. Alkalosis produced sero-biochemical changes which are absent in acidosis due to acute onset.

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