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# Clinico-pathological alterations in simple indigestion in buffaloes

**Richa Arora, Arpit Tyagi, S Shekhar, VS Rajora and Niddhi Arora**

**Abstract**
The present study was conducted in the Department of Veterinary Medicine, Teaching Veterinary Clinical Complex and Instructional Dairy Farm, C.V.A.Sc., GBPUA&T, Pantnagar, U.S. Nagar (Uttarakhand, India). A total of 30 buffaloes were included in the study out of which 24 were suffering from simple indigestion whereas 6 healthy buffaloes were chosen to serve as healthy control. In the buffaloes suffering from indigestion, rumen motility significantly reduced, pH of rumen liquor was slightly acidic and rumen liquor examination revealed reduced microbial count and activity, which was indicated by reduced total protozoal count, protozoal motility, bacterial count and amount of Total Volatile Fatty Acids and increase in time values for Methylene Blue Reduction Test (MBRT), Sedimentation Activity Test (SAT) and Nitrate reduction test. Hemogram with leukocytosis, neutrophilia and lymphocytopenia was suggestive of stress like condition. Alterations in biochemical parameters included significant ($P \leq 0.05$) increase in serum Alaninie Amino Transferase (ALT), Aspartate Amino Transferase (AST), Lactate Dehydrogenase (LDH) and Blood Urea Nitrogen (BUN) concentrations and reduced serum glucose levels.

**Keywords:** Indigestion, rumen, blood, biochemical, buffaloes

## Introduction
Buffalo, a triple purpose animal, provides milk, meat and mechanical power to mankind. Due to its highly nutritious milk, leaner meat and best draught power for wet environments buffalo offers immense potential for the improvement of livelihood. Buffalo can efficiently convert low quality feed stuffs like straws and agro-industrial waste into human food, improve soil structure through bio-fertilizer and is a financial asset which can be sold when needs arise. Although, buffalo is the flagship of all agricultural revolutions namely; green, white and red but unfortunately has been neglected in the past [15].

Rumen disorders are of a greater clinical interest as they affect large number of animals and incur great losses in both production and cost of treatment of the affected ruminants [17]. The most common ruminal disorders such as tympany, indigestion and impaction are characterized by poor appetite, altered pH, reduced rumen motility and decreased microbial counts ultimately resulting in decreased milk production. Sudden change in the feed is the most common cause of indigestion in ruminants. Other factors include feeding spoiled and moulded feeds, use of antibiotics especially via oral route, sudden changes in climatic conditions also cause indigestion [16]. Mild simple indigestion is self-limiting in nature. Reduced appetite is one of the early clinical signs seen in case of indigestion while subsequent prominent symptoms include anorexia, depression, reduced or completes cessation of rumination and eructation, decreased reticulo-ruminal movements and significant reduction in bacterial and protozoal counts [5]. Indigestion also leads to hepatic disturbances and reduced milk production in affected animals [19]. The ultimate result of these clinical conditions is production and economic losses to the farmers. With the climate change and global warming ruminants are more likely to suffer from ruminal disorders as the fluctuation in temperature and humidity exerts great effects on animal physiology, performance and production as well as the ruminal bacterial diversity; which altered significantly in response to raised temperature [23]. Temperature has significant effects on ruminal fermentation patterns and gas production. Collection of ruminal fluid is important in disease diagnosis, therapy, and scientific research in ruminants as it contains a mixed community of microorganisms consisting of bacteria ($10^{10}$-$10^{11}$ cells/ml), ciliate protozoa ($10^4$ -$10^6$/ml) and anaerobic fungi ($10^3$-$10^5$zoospores/ml). This study aims at determining clinico-pathological and hemato-biochemical alterations in

buffaloes suffering from simple indigestion and compares these parameters with those in healthy buffaloes.

## Materials and Methods

The present study was undertaken in the Department of Veterinary Medicine, Teaching Veterinary Clinical Complex and Instructional Dairy Farm, C.V.A.Sc., GBPUA&T, Pantnagar, U.S. Nagar (Uttarakhand, India). A total of 30 buffaloes were included in the study out of which 24 were suffering from simple indigestion whereas 6 healthy buffaloes served as healthy control. The alterations in clinical parameters, rumen liquor characteristics and hemato-biochemical parameters were recorded.

### (i) Clinical Observations

The clinical observations included recording of rectal temperature, heart rate, respiration rate and ruminal movements. Rectal temperature was measured by using a clinical mercury thermometer and expressed in °C; pulse rate was recorded over the middle coccygeal artery at a point on the underside of the base of the tail by applying gentle digital pressure to feel the arterial pulsation, expressed as per minute. Heart and respiration rates were recorded during auscultation over chest area using a stethoscope. Careful auscultation of chest area was performed to rule out the abnormalities in the lungs and heart. Ruminal motility was detected at the left para-lumbar fossa by using back of the closed fist. Sufficient pressure was applied to overcome the muscular tone. Ruminal movements were carefully recorded for 5 minutes.

### (ii) Rumen Liquor Examination

About 250 ml of ruminal fluid samples were collected from each buffalo under study using stomach tube with a conical flask connected to its free end, while a vacuum pump was connected to the side tube of the flask.

Each sample was divided into three portions. The first one (30ml) was used for estimation of rumen liquor pH, MBRT and evaluation of protozoal activity. The second part of about 200 ml of rumen liquor was used for the estimation of sedimentation time, Nitrate reduction test and VFA estimation. The remaining part (20ml) was used to conduct the microscopic count of ruminal bacteria and protozoa.

Rumen liquor pH was measured immediately after collection of samples using a calibrated electronic digital portable pH meter. Methylene Blue Reduction Test (MBRT), Sedimentation Activity Test (SAT) and Nitrate reduction test were performed by method described by Sharma [18] and their results were expressed in minutes. Estimation of Total Volatile Fatty Acid (T.V.F.A.) was done by method as described by Sharma [18] and results were expressed as millimoles/ml. Total protozoal and bacterial count were performed by method described by Chakrabarti [2] and protozoal motility was described as +++ (abundant and highly motile- 85-95%), ++ (moderate in number and motile- 65-75%), + (low in number and sluggish- 25-45%), ± (no or sporadic alive protozoa) and - (dead protozoa) [4]

### (iii) Haemato- biochemical studies

To study haemato-biochemical alteration in ailing as well as healthy buffaloes, blood samples were collected from selected buffaloes. About 10.0 ml of venous blood sample was collected using dry disposable syringe through the jugular vein from each buffalo and immediately after collection about 3.0 ml blood was transferred to EDTA (@1.5 mg/ml) vials for complete blood count (CBC) that was carried out within two hours of collection. Left over, 7.0 ml of blood was transferred in to a clean and dry test tube without any anticoagulant and was allowed to clot in slanting position for about one hour and then separated serum was harvested gently after centrifugation for 10 minutes at 3,000 rpm. The supernatant serum was collected carefully in a dry eppendorf tubes with the help of micropipette and finally the well labelled sera samples was be preserved at -20$^0$C in a deep freeze for further biochemical and serological estimation.

Haematological studies were conducted to study the effect of indigestion on blood cellular profiles and it was compared with profile of apparently healthy normal buffaloes. Haematological parameters viz. haemoglobin, packed cell volume (PCV), total erythrocyte count (TEC), total leukocyte count (TLC), differential leukocyte count (DLC) and red cell indices viz. MCV, MCH and MCHC were studied as per standard laboratory procedures described by Jain [8]

Biochemical parameters viz. Alaninie amino transferase (ALT), aspartate amino transferase (AST), lactate dehydrogenase (LDH), serum glucose, total serum cholesterol, total serum proteins, blood urea nitrogen (BUN), serum calcium and serum phosphorus were determined using the non-haemolysed serum obtained from the blood samples using diagnostic kits from Erba diagnostics Mannheim, Germany.

### (iv) Statistical analysis

The data was presented as Mean ± standard error (SE). Paired t-test was used for comparing means using Students t-test. Statistical Package for Social Science (SPSS) 17.0 v was used for the analysis. The level of significance was set at $P<0.05$.

## Results and Discussion

Among the clinical parameters, rumen motility reduced significantly ($P<0.05$) in buffaloes suffering from simple indigestion whereas rectal temperature and heart and respiration rates in buffaloes suffering from indigestion were comparable ($P\leq0.05$) to those in healthy buffaloes. In buffaloes suffering from indigestion, pH of rumen liquor was slightly acidic which was significantly ($P\leq0.05$) lower than rumen liquor pH of healthy control buffaloes. Rumen liquor examination of buffaloes suffering from indigestion significantly ($P\leq0.05$) lower total protozoal count, protozoal motility, bacterial count and amount of TVFA in rumen liquor whereas significant ($P\leq0.05$) increase in time values were noted for MBRT, SAT and nitrate reduction test (Table 1). Haemogram of the buffaloes suffering from simple indigestion revealed significant ($P\leq0.05$) increase in total leukocyte count, neutrophil count and significant ($P\leq0.05$) reduction in lymphocyte count. No significant changes were observed in haemoglobin concentration, PCV and erythrocytic count in indigestion affected buffaloes as compared to healthy control group. Consequently, erythrocytic indices in buffaloes suffering from indigestion were comparable ($P\leq0.05$) to those of healthy buffaloes. Alterations in biochemical parameters of the buffaloes suffering from indigestion as compared to healthy buffaloes included significant ($P\leq0.05$) increase in serum ALT, AST, LDH and BUN concentrations and reduction in serum glucose levels. Total serum cholesterol, total serum proteins, serum calcium and phosphorous in buffaloes suffering from simple indigestion were comparable ($P\leq0.05$) to those in healthy buffaloes (Table 2).

**Table 1:** Ruminal parameters [Mean±SE]

| Clinical parameter | Control group (n=6) | Simple indigestion group (n=24) |
|---|---|---|
| Rumen motility (per 5 min.) | 7.00±0.54 | 1.12±0.73[a] |
| Ph | 6.94±0.07 | 6.19±0.10[a] |
| MBRT (min.) | 5.78±0.93 | 10.91±1.63[a] |
| SAT (min.) | 7.08±0.38 | 12.71±1.87[a] |
| NRT (min.) | 22.20±1.26 | 43.62±1.45[a] |
| TVFA (mmol/ml) | 84.67±3.26 | 62.79±2.91[a] |
| Total protozoal count ($10^5$) | 3.27±0.18 | 1.16±0.19[a] |
| Bacterial count ($10^{10}$) | 15.97±0.78 | 6.27±0.42[a] |
| Protozoal motility (+) | 3.00±0.00 | 0.83±0.23[a] |

a=Significant ($P \leq 0.05$) difference as compared to control group within same row.

**Table 2:** Haemato-Biochemical examination [Mean±SE]

| Clinical parameter | Control group (n=6) | Simple indigestion group (n=24) |
|---|---|---|
| Hb (g/dl) | 10.23±0.28 | 11.00±0.56 |
| PCV (%) | 32.00±3.00 | 35.50±2.81 |
| TEC ($10^6$/µL) | 5.76±0.40 | 5.80±0.45 |
| TLC ($10^3$/µL) | 8.71±0.13 | 9.67±0.19[a] |
| Neutrophils (%) | 38.33±3.78 | 49.67±4.31[a] |
| Lymphocytes (%) | 55.50±2.62 | 43.54±5.09[a] |
| ALT (IU/l) | 18.17±2.43 | 23.86±0.48[a] |
| AST (IU/l) | 125.97±8.86 | 186.17±6.78[a] |
| LDH (IU/l) | 1198.18±27.67 | 1409.21±19.64[a] |
| Serum glucose (mg/dl) | 56.41±2.42 | 42.36±0.98[a] |
| Cholesterol (mg/dl) | 50.20±0.94 | 49.96±1.02 |
| Total serum proteins (g/dl) | 9.11±0.17 | 8.96±0.16 |
| BUN (mg/dl) | 23.16±0.63 | 27.53±0.93[a] |
| Serum calcium (mg/dl) | 10.08±0.08 | 10.01±0.12 |
| Serum phosphorus (mg/dl) | 4.97±0.14 | 4.83±0.09 |

a=Significant ($P \leq 0.05$) difference as compared to control group within same row.

In the present study, the buffaloes suffering with simple indigestion showed reduced rumen motility, protozoal and bacterial counts, protozoal motility, and TVFA production. There was an increase in SAT, MBRT and nitrate reduction test. The rumen pH was slightly acidic. Even though, primary atony caused by dietary abnormality is difficult to explain. Changes in the pH of rumen contents markedly affect the motility of the rumen and in cases caused by overeating on grain an increase in acidity is probably of importance. The toxic amides and amines produced may include histamine, are known to cause ruminal atony [16]. Reduction in rumen liquor pH was earlier reported by Kasaralikar [9] and Mohan [13]. Earlier, Garry [5] stated that in simple indigestion, pH changes were mild and tend towards acidosis or alkalosis. The decrease in the rumen liquor pH was due to production of lactic acid in rumen, as a result of increase in lactic acid fermenting bacteria and regeneration microflora. Rumen pH had been reported to be one of the most valuable ecological factors influencing the microbial population in the rumen. pH is one of the most important factors responsible for protozoal motility and activity. The protozoan motility decreases whenever there is a reduction in rumen pH [21] and during underfeeding or starvation [3]. Large entodiniomorphs are more sensitive to change in pH. Small trichostomatids (holotrichs) tend to be most tolerant. However, below pH 5.0, all protozoa die [1]. It had been shown that the efficiency of growth of predominant rumen bacteria vary considerably with changes in rumen pH. Cellulolytic and methanogenic bacteria were severely affected once the rumen pH falls below 6.0 [12]. Increase in time values for SAT, MBRT and nitrate reduction test indicates decreased microbial fermentation in rumen due to inactive microflora and fauna [5], suppressed microbial fermentation [14] and reduced ability of microbes to digest proteins [18].

Hematological studies revealed leukocytosis, neutrophilia and lymphocytopenia in the buffaloes suffering from simple indigestion. Stocker [22] also reported leukocytosis in animals suffering from indigestion. Leukocytosis can be attributable to inflammatory response to gastrointestinal stasis leading to absorption of toxins into circulation [5]. The results of present investigation corroborate with those of Hussain and Uppal [7] who also reported significant neutrophilia and lymphocytopenia in animals suffering from ruminal disorders. Neutrophilia might have resulted from chronic irritation of the forestomach wall by impacted feed materials, leaving the wall exposed to secondary infection, which resulted in inflammation [6]. Decreased lymphocytes could be due to release of corticosteroid as a result of stress [8].

Results of serum biochemical examination revealed increase in ALT, AST, LDH, BUN and reduction in serum glucose levels. Increased levels of ALT, AST and LDH are indicative of necrosis of liver due to toxaemia from the damaged rumen mucosa or disturbed liver functions and hepatic ischaemia [5]. Madan *et al.* [11] had also reported an increase in AST, LDH and BUN concentrations in the buffaloes suffering from indigestion. Higher BUN levels could be attributed to the failure of urea recycling process and its neutralization by rumen microbes [10]. Moreover, during ruminal disorders there is failure of urea cycling process and urea is not utilized by rumen microbes [20]. This could also be correlated with anorexia and decreased ruminoreticular activity as catabolism is accelerated under such conditions [11]. Kasaralikar *et al.* [9] also reported significant reduction in blood glucose levels of animals suffering from indigestion when compared with healthy animals. Low blood glucose levels were suggestive of a state of hypoglycemia which could be due to prolonged

anorexia [9] or it might have been due to less volatile fatty acid production in the rumen [11].

## Conclusion

In the buffaloes suffering from indigestion, rumen motility substantially reduced pH of rumen liquor was slightly acidic in and rumen liquor examination revealed reduced microbial count and activity, which was indicated by reduced total protozoal count, protozoal motility, bacterial count and amount of TVFA in rumen liquor and increase in time values for MBRT, SAT and Nitrate reduction test. Hemogram with leukocytosis, neutrophilia and lymphocytopenia is suggestive of stress like condition. Alterations in biochemical parameters included significant ($P \leq 0.05$) increase in serum ALT, AST, LDH and BUN concentrations and reduced serum glucose levels.

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