Investigation on Clinical, Haematological and Serum Biochemical Changes in Experimentally Induced Ruminal Acidosis in Black Bengal Goats

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Abstract: Acute and chronic acidosis, conditions that follow ingestion of excessive amounts of readily fermented carbohydrate are prominent production problems for ruminants fed diets rich in concentrate. Often occurring during adaptation to concentrate-rich diets in feedyards, chronic acidosis may continue during the feeding period. The goal of the experiment was to evaluate the consequences of ruminal acidosis in affected goats. Acidosis was induced in six 12-to14-month-old female Black Bengal goats by feeding them 90 g of rice mixed with khesari (cereal) per kilogram of body weight. Regular observations were made to monitor the progression of clinical symptoms in the animals. Prior to grain feeding (0 h), the respiration rate, heart rate and rectal temperature were registered and blood samples were taken at 24, 48, 72, 96 and 120 h intervals. The respiratory rate, heart rate and rectal temperature all increased significantly ($p \leq 0.05$) during the clinical review. A significant ($p \leq 0.05$) increase in PCV (%), Haemoglobin concentration, RBC values and a significant ($p \leq 0.05$) decrease in blood pH were found in hematological research. There was a change in WBC, Neutrophils and Lymphocytes but it was not important. Significantly increased activities of liver enzymes AST, ALT and bilirubin ($p \leq 0.05$). During ruminal acidosis, serum creatinine was found to be significantly ($p \leq 0.05$) elevated. Triglyceride levels increased significantly ($p \leq 0.05$) while Cholesterol levels remained unchanged, suggesting a functional shift in the vasculature. Lactacidemia, hepatic and renal dysfunction were found to be the primary causes of clinical, haematological and serum biochemical changes in acidotic Black Bengal goats. This study discovered that ruminal acidosis has a significant impact on vital organs such as the liver, kidneys and circulatory system and that the owner should take the required precautions to avoid organ failure.

INTRODUCTION

In the livestock sector, the goat population is very vital to Bangladesh's economy. Veterinary practitioners and farmers are primarily focused on goats because they are one of the key suppliers of animal-derived commodities such as milk, meat and milk products, as well as high-quality skin[1, 2]. Ruminal acidosis is a common carbohydrate fermentation disorder in animals that has a negative impact on animal welfare, productivity and farm profitability. Accidental ingestion of highly fermentable carbohydrates such as maize, wheat, rice and khesari causes an increase in lactic acid content in the rumen[3]. In the etiology of ruminal acidosis, there are two main stages. The first step is characterized by an abrupt rise in the intake of readily fermentable carbohydrates as well as a change in the ruminal microbial population profile and subsequent ruminal acidification. The second step involves acid absorption into the bloodstream which results in systemic and metabolic acidosis[4]. In the mild type, rumen movements are decreased but not completely absent and the affected goats progressively lose weight, suffer from exhaustion, acidemia, diarrhoea, incoordination and eventually die[4, 5]. Farmers suffer a significant financial loss as a result of this and they are discouraged from raising goats or engaging in goat farming.

The degree of prior ruminal microbial adaptation to the carbohydrate substrate as well as the amount and form of carbohydrate-rich feed ingested, influence the severity of ruminal acidosis and disease signs[6]. In goats, clinical symptoms of acidosis include dullness, exhaustion, anorexia, mild dehydration, ruminal stasis and pasty to semi-liquid sporadic diarrhoea. The abdomen is slightly distended and has a doughy feel when palpated. There is also tachycardia and polypnea as well as per-acute clinical symptoms such as extreme vomiting with sunken eyes and the animals do not have diarrhoea but exhibit blindness, salivation and teeth grinding[7].

The biochemical profile of rumen liquor, blood and urine changes dramatically when a significant amount of lactic acid is generated in the rumen[8, 9]. Therapeutic and surgical treatment of bloat or grain engorgement in sheep and goats has been reported[10, 11]. Biochemical changes in different body fluids in acidotic cattle[12] and buffaloes[13] are also recorded.

However, there has been very little research on the systemic effects of grain engorgement or ruminal acidosis, particularly on vital organ function in food animals such as goats. As a result, the aim of this study was to see how induced acidosis affected the clinical and haematobiochemical profiles of Black Bengal goats after functional changes in the liver, kidney and vasculature.

MATERIALS AND METHODS

Animals in research: In this study, two healthy female goats aged 12 and 14 months and weighing 10 and 12 kg were used. Green fodder and concentrate were fed to them in clean, disinfected pens. For adaptation, goats were kept under the same management conditions for two weeks prior to the start of the experiment. The Animal Experiment Ethics Committee (AEEC) of the Department of Surgery and Obstetrics, BAU, Mymensingh has accepted all animal experimentation protocols.

Experimental induction of ruminal acidosis: After a 24-h short, the goats were given 90 g of rice mixed with khesari per kg of body weight. Regular observations were made to monitor the progression of clinical symptoms in the animals.

Diagnosis of ruminal acidosis: Ruminal acidosis was diagnosed by detecting frothiness of rumen contents on needle puncture at the left paralumbar fossa as well as apparent changes in feces consistency.

Before feeding (0 h), the respiration rate, heart rate, rectal temperature and rumen motility were measured and then at 24, 48, 72, 96 and 120 h intervals. The animal's ruminal acidosis progress was tracked on a regular basis. Before recovery, the improvements were recorded.

Collection of blood sample: Using a disposable plastic syringe, 5 mL of blood was drawn from each goat's jugular vein at the indicated time with 2 mL transferred to a vacutainer containing EDTA and used for regular blood tests. The remaining 3 mL of blood was moved to a vacutainer without anticoagulant and used for serum biochemical analysis.

Haematological examination: Total Erythrocyte Count (TEC), Packed Cell Volume (PCV%), Haemoglobin (Hb) concentration, Total Leukocyte Count (TLC) and Differential Leucocytic Count (DLC) were all determined in blood samples obtained from the experimental goats with anticoagulant (EDTA). This was accomplished using a Sysmex XS-1000i digital hematology analyzer (Japan). A portable pH meter was used to calculate the pH of the blood (Lovibond, Senso Direct pH).

Biochemical examination: A clot activator tube was used to extract the blood sample. The serum was collected in an Eppendorf tube using a micropipette for biochemical analysis of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), serum bilirubin, serum creatinine, Blood Urea Nitrogen (BUN), urea and serum cholesterol. The biochemical research was carried out using a kinetic method on a Microlab Biochemistry Analyzer (Germany).

Statistical analysis: The data was measured and interpreted as a mean±SE. The data was analyzed using the Statistical Package for the Social Sciences (SPSS) version 22 and a one-way ANOVA (analysis of variance) was performed. Probability $p<0.05$ or less was considered as statistically significant.

RESULTS

Table 1 shows changes in clinical parameters in goats over the course of the experiment. Normal appetite, shiny coat, shiny eyes and normal defecation in the form of small hard pellets were the most common clinical signs that appeared before feeding (0 h) of rice mixed with khesari.

The respiration rate increased dramatically from 0-48 h (25±1.0) and peaked at 35±0.0. After that, up to 120 h, there was a downward trend in respiration rate. At 24 and 96 h, there was no major difference in respiration rate. At 24 h, a slightly higher heart rate/min (90±1.5) was found which then gradually decreased to about baseline during the experiment.

A reduction in rectal temperature was observed at 24 h (101.2±0.2) as compared to the pre-experimental control value of 0 h (102.1±0.1) which lasted for 48 h before returning to the monitored value and remaining constant during the experiment.

Table 2 shows routine haematology as well as the pre and post experimental periods. Before (0 h) and after inducing acidosis, the haematological parameters were examined. At 0 h, the pH of the blood was 7.3±0.015 which decreased slightly ($p<0.05$) at 24 h (5.83±0.22). After that, from post 48 h to the end of the experiment, there was a growing trend in pH. When compared to the control value, there was no substantial difference in WBC (0 h). At 24 h after acidosis, changes in RBC were important ($p<0.05$). The highest RBC count was 14.015±0.08×106 µL which was obtained after 48 h. In the case of Hb, ruminal acidotic goats had a large ($p<0.05$) increase in Hb content and PCV percent as compared to standard condition (0 h). Hb was 8±.2 gm/dL before acidosis (0 h).

Then it increased to 9.5±.3 gm/dl after 24 h and then to 10.5±.2 gm/dl after 48 h. Following that, it steadily decreased over time until it returned to normal. At 48 h, the PCV level was significantly higher (39.85±0.45%). Following that, values steadily decreased until they were comparable to the 0 h value. There was a non-significant rise in neutrophils and lymphocytes. From a normal state, monocytes had a tendency to decrease. At different intervals, Eosinophils and Basophils were statistically identical.

Table 3 shows the increases in liver enzyme in Black Bengal Goats with experimentally induced ruminal acidosis. The values of SGPT/ALT (IU/L) increased dramatically ($p<0.05$) at 24h (17.29±0.22) and then steadily decreased during the experimental period. AST (IU/L) and Bilirubin (mg/dL) followed a similar pattern. At 24 h, the levels of AST (IU/L) and Bilirubin (mg/dL) peaked at 199.34±6.02 and 0.85±0.05, respectively.

Table 4 shows the effects of ruminal acidosis on enzymes that are characteristic of kidney disease. The serum creatinine and blood urea nitrogen levels were found to have increased significantly from the pre-experimental control value. The highest concentrations of creatinine (1.25±0.05) and BUN (23.50±1.50) were found at 24 hours. Both parameters steadily decreased in value before returning to baseline at 120 h.

Table 5 shows the effects of induced acidosis on cholesterol and triglycerides. Changes in cholesterol

Table 1: Effect of experimentally induced ruminal acidosis on the clinical profile of Black Bengal goats at different intervals (mean±SE) (h)

	Time (h)					
Parameters	0	24	48	72	96	120
Respiration (breath/min)	25±1.0^{a}	29.5±0.5^{b,c}	35±0.0^{d}	32±1.0^{cd}	29.5±1.5^{bc}	27±1.0^{ab}
Heart rate (rate/min)	83±1.0^{a}	90±1.5^{b}	85±1.0^{a}	84.5±1.5^{a}	83±1.0^{a}	81±1.0^{a}
Rectal temperature(^{0}F)	102.1±0.1^{b}	101.2±0.2^{a}	101.2±0.1^{a}	102.05±0.05^{b}	102.1±0.1^{b}	102.1±0.0^{b}

Table 2: Hematological profile in experimentally induced ruminal acidosis in Black Bengal Goats (mean± SE) at various intervals (h)

	Time (h)					
Parameters	0	24	48	72	96	120
pH of blood	7.3± 0.015^{c}	5.83± 0.22^{a}	6.08± 0.14^{ab}	6.33± 0.06^{b}	7.13± 0.035^{c}	7.29± 0.01^{c}
Total WBC (x10^{3}µL)	7.92±.29 ^{a}	8.435± .28 ^{a}	8.755±.19 ^{a}	8.19±.38 ^{a}	8.015±.29 ^{a}	7.95±.30 ^{a}
RBC (x10^{6}µL)	11.61±0.26^{a}	13.095±0.37^{ab}	14.015±0.08^{b}	12.735±0.51^{ab}	11.68±0.07^{a}	11.665±0.24^{a}
Hb (gm/dl)	8±.2^{a}	9.5±.3^{bc}	10.5±.2^{c}	9.05±.15^{ab}	8.5±.4^{ab}	8.1±.2^{ab}
PCV (%)	35.6±0.50^{a}	38.7±0.40^{b}	39.85±0.45^{b}	37.9±0.20^{ab}	35.9±0.60^{a}	35.65±0.45^{a}
Neutrophils (x10^{3}µL)	31±2.00^{a}	31.95±1.85^{a}	32.65±1.25^{a}	31.9±1.60^{a}	31.55±1.45^{a}	31.4±1.60^{a}
Lymphocytes (%)	64.5±1.50^{a}	65.45±1.25^{a}	66.1±0.70^{a}	65.8±0.90^{a}	66.3±1.00^{a}	64.55±1.55^{a}
Monocytes (%)	2±0.00^{c}	1±0.00^{b}	0.6±0.10^{a}	0.6±0.10^{a}	0.65±0.05^{a}	2±0.00^{c}
Eosinophils (%)	2.5±0.50^{a}	1.5±0.50^{a}	0.5±0.50^{a}	1.55±0.55^{a}	1.45±0.45^{a}	1.6±0.40^{a}
Basophils (%)	0±0.00^{a}	0.25±0.05^{a}	0.15±0.05^{a}	0.15±0.05^{a}	0.05±0.05^{a}	0.45±0.45^{a}

±: Standard error; Values with different superscript letter in same row varied significantly at 5% level of significance

Table 3: Changes in enzyme indicative of functional alteration of liver due to experimentally induced ruminal acidosis in Black Bengal Goats (mean± SE) at various intervals (h)

	Time (h)					
Parameters	0	24	48	72	96	120
SGPT/ALT (IU/L)	11.38±0.73^{a}	17.29±0.22^{b}	14.54±0.37^{c}	13.94±0.92^{cd}	12.01±0.78^{ad}	11.48±0.76^{a}
AST(IU/L)	173.25±3.00^{a}	199.34±6.02^{b}	185.27±1.32^{a}	177.18±1.67^{a}	175.11±2.89^{a}	174.18±3.02^{a}
Bilirubin (mg/dL)	0.45±0.05^{a}	0.85±0.05^{b}	0.75±0.05^{bc}	0.60±0.10^{ac}	0.50±0.00^{a}	0.45±0.05^{a}

Table 4: Changes in enzyme indicating functionality of kidney due to ruminal acidosis in Black Bengal Goats (mean±SE) at various intervals (h)

	Time (h)					
Parameters	0	24	48	72	96	120
Creatinine (mg/dL)	0.95±0.05^{a}	1.25±0.05^{b}	1.20±0.00^{bc}	1.05±0.05^{abc}	1.00±0.10^{ac}	0.95±0.05^{a}
Blood Urea Nitrogen (mg/dL)	18.50±0.50^{a}	23.50±1.50^{b}	23.50±1.50^{b}	20.50±0.50^{ab}	19.00±1.00^{a}	18.75±0.75^{a}

Table 5: Changes in cholesterol and triglyceride in experimentally induced ruminal acidosis in Black Bengal Goats (mean±SE) at various intervals (h)

	Time (h)					
Parameters	0	24	48	72	96	120
Cholesterol (mg/dL)	72.50±7.50	65.00±5.00	65.00±5.00	66.50±8.50	70.50±0.50	67.50±2.50
Triglyceride (mg/dL)	29.50±2.50^{a}	46.50±3.50^{b}	58.50±4.50^{c}	32.50±0.50^{a}	31.00±3.00^{a}	29.75±2.25^{a}

±: Standard error; Values with different superscript letter in same row varied significantly at 5% level of significance

levels were non-significant during the experiment but triglycerides were found to be significantly higher at various time points, as shown in this graph. The highest count was reported at 48 h (58.50±4.50), after which it steadily decreased until 120 h. At 24 h ($p<0.05$), there was a significant improvement that lasted until 48 h. The value steadily decreased before returning to the pre-experimental control value (0 h).

DISCUSSION

The aim of this study was to look into the clinical, haematological and serum biochemical changes in Black Bengal Goats that were subjected to experimentally induced ruminal acidosis.

Effects of ruminal acidosis on clinical parameters: The acidotic goat's respiration and heart rate were significantly higher than the control value in this analysis. Similar results have been reported by Gozho *et al*.[14]. The distended rumen moves the diaphragm cranially, compressing the thoracic cavity and causing respiratory embarrassment, according to Aschenbach *et al*.[15]. The heart rate rises dramatically in this situation to compensate for the anoxic state of the blood which is the immediate cause of death.

There was a substantial reduction in mean rectal temperature values ($p<0.05$) in this analysis. A decrease in body temperature was noted by Bach *et al*.[16] and Bevans *et al*.[17] who registered a similar finding. It may be a reflection of the decreased metabolism and dehydration caused by the loss of intravenous cellular fluid into the rumen and osmotic diarrhea and it may also explain the slight rise in heart rate caused by hemoconcentration[14].

At 24 h, the mean rectal temperature was slightly lower ($p<0.05$) than the control value. Following focus feeding, the rumen pH drops, compromising the reticulo-ruminal wall's integrity. Endotoxin content in the rumen increases and it is easily absorbed through the rumen wall[13]. Increased endotoxin levels in the blood can cause peripheral vasodilation. The successful circulatory rate, tissue metabolism and hypothermia can all be affected[11].

Effects of ruminal acidosis on haematological profile: The blood pH of goats was found to be lower after 24 h of acidosis in this study. This is due to an excess of lactic acid being produced which is absorbed through the rumen and then circulated across the body, resulting in lacticaemia[18] which causes a drop in blood pH.A rumen acidosis haematological review revealed a substantial rise in PCV and Hb concentrations. Similar findings were also reported by Mauro *et al*.[9] and Baraka *et al*.[11].

A substantial rise in RBC was also seen in rumen acidosis haematological research (Table 2). This is most likely due to pathological changes in osmotic equilibrium and the stress induced by experimental acidosis, since in cases of ruminal acidosis, the efflux of liquid from intra- and extracellular compartments to the rumen in order to preserve intra-rumen balance results in an increase in hematocrit[6]. The stress caused by acidosis, on the other hand, induces splenic contraction due to the action of epinephrine; haemoconcentration can occur as a result of the amount of red blood cells released into the peripheral bloodstream and as a result, an increase in the hematocrit value[16]. When Gozho *et al*.[14] induced ruminal acidosis in goats, they found similar effects, albeit more extreme hematocrit activity. The highest increase in Hb at 0 h is

most likely due to dehydration caused by a higher hematocrit value which maintained a more stable plasma volume than the control value. Due to reduced plasma volume and splenic contraction, there have been many reports of a rise in hemoglobin rates in ruminants with acute indigestion[11,13].

Our WBC changes are similar to those recorded by Nikolov[6] who looked at acidosis in goats, cattle and buffalo. The mobilization of neutrophils is linked to the inflammation of the rumen mucosa triggered by a high concentration of lactic acid in the ruminal fluid which acts as an irritant to the epithelium and initiates the ruminitis phase. The subsequent recovery of leukocyte count was attributed to an improvement in the animal's clinical state, as the evolution of induced lactic acidosis was moderate, as shown by Brown *et al*.[19] in cows with acute ruminal acidosis.

Effect of ruminal acidosis on liver enzyme: Hepatocellular damage such as sub-lethal degeneration or necrosis, is reflected in increased ALT activity, while major increases in AST are reflected in hepatocellular damage or released from degenerated skeletal muscles[20]. An excessive amount of RBC destruction (hemolysis) or an inability of the liver to process bilirubin could cause an increase in bilirubin[19].

Effect of ruminal acidosis on renal enzyme: The large increase in serum urea and creatinine in acidotic goats is an indicator of decreased glomerular filtration volume which is caused by renal damage or a reduction in successful renal flow as well as a drop in arterial blood pressure, resulting in subnormal renal function[21]. Dijkstra *et al*.[22] found no increase in blood urea nitrogen (BUN) concentration in acidosis in cattle, despite the fact that higher BUN levels have been identified in long-term acidosis in cattle and buffaloes.

CONCLUSION

Ruminal acidosis causes a rise in heart rate and respiration rate in goats but it lowers rectal temperature within the first 1 to 2 days. It also affects hematological profile and, more significantly, essential organ function as measured by enzymes indicative of the liver, kidneys and vasculature with the affection being more intense after 48 h.

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Author contributions: The research was carried out by Moktadin Md. Shihab. Sumaia Jahan Tamanna helped with data collection and sampling. Mst. Antora Akter assisted with the manuscript preparation. Md. Mahmudul Alam planned the thesis, oversaw the report and reviewed the manuscript critically. The final manuscript was read and accepted by all contributors.

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