PHYSIOLOGICAL AND BIOCHEMICAL CHANGES IN RUMINAL FLUID AND BLOOD OF DIGESTIVE DISORDER GOAT

A clinical report submitted in partial satisfaction of the requirement for the Degree of Doctor of Veterinary Medicine (DVM)

By :

Md. Ariful Islam

Roll No: 18/01

Reg. No: 02057

Intern ID: 01

Session: 2017-18

Faculty of Veterinary Medicine

Chattogram Veterinary and Animal Sciences University

Khulshi, Chattogram – 4225, Bangladesh

PHYSIOLOGICAL AND BIOCHEMICAL CHANGES IN RUMINAL FLUID AND BLOOD OF DIGESTIVE DISORDER GOAT

Approved by:

Dr. S. K. M. Azizul Isalm

Professor

Department of Physiology, Biochemistry & Pharmacology, CVASU

Faculty of Veterinary Medicine

Chattogram Veterinary and Animal Sciences University

Khulshi, Chattogram – 4225, Bangladesh.

Table of Contents

List of Abbreviations

Short form	Full form
S.S.C	Secondary School Certificate
H.S.C	Higher School Certificate
pH	Potential of Hydrogen
MBRT	Methylene blue reduction test
SAT	Sedimentation Activity test
Temp	Temperature
Min	Minute
SD	Standard deviation
Kg	Kilogram
F	Fahrenheit
>	Greater than
<	Less than
≥	Greater than or equal to
mg/dl	Milligrams per deciliter
mmol/L	Milimoles per liter
Min	Minute
ml	Milliliter
CI	Confidence Interval

List of Tables

List of Figures

List of Graphs

Acknowledgements

The author wishes to concede the heartfelt gratitude to the ALMIGHTY for immeasurable propitiousness without it he would never have been able to complete the work successfully.

The author would like to acknowledge with a deep sense of reverence and immense graciousness to him respectful internship supervisor **Professor Dr. S. K. M. Azizul Isalm**, Department of Physiology, Biochemistry & Pharmacology, CVASU, Chattogram Veterinary and Animal Sciences University for his intellectual supervision ,valuable guidance and constant encouragement throughout the period which shaped the present work as its show.

I would like to express my special gratitude to my another respected teacher **Professor Dr. Shahneaz Khan** for providing me Laboratory facilities. I am also grateful to Department of Medicine and Surgery.

I acknowledge to all staffs of SAQTVH, CVASU without whose willingness to provide information this study would not easily be possible to perform.

The author would also like to express his sincere gratitude and thank to **Professor Dr. A. S. M Lutful Ahasan**, honourable vice chancellor of Chattogram Veterinary And Animal Sciences University.

The author paying his respect to **Professor Dr. Mohammad Lutfur Rahman** and **Professor Dr. A. K. M Saifuddin,** Director, External Affairs for the provision of this unique internship program and research exposure.

The author would also extend his appreciation to his family, friends, well-wishes, staffs who helped at the time of questionnaire survey for their kind cooperation in completion of his work.

Abstract

The goal of the six-month case study was to determine the physiological and biochemical status of the ruminal fluid and blood of digestive disorder goats. It was conducted at the Shahedul Alam Quadary Teaching Veterinary Hospital (SAQTVH) of CVASU and household of the Jalalabad Housing Society, Khulshi, Chattogram, from April 2023 to October 2023. Collection of ruminal fluid and blood from 15 goats and performed different test of physiological parameter of rumen fluid (eg. Color, odor, consistency, and pH) as well as sedimentation rate, motility and number of protozoa were observed, and biochemical parameter of ruminal fluid and blood (e.g. glucose, chloride, potassium and sodium) and counted in relation with digestive disorders of goat. Based on pH value 15 samples were divided into two groups. The pH of rumen fluid group from pH value <6 and ≥6 to 8. This study revealed ten (10) colors of rumen fluid viz greenish (13.3%), greenish gray (13.3%), greenish-brown (13.3%), olive (13.3%), milky gray (13.3%), dark brown (6.7%), dark greenish (6.7%), gray (6.7%), grayish brown (6.7%), and grayish-yellow (6.7%). The color of Dark greenish, gray, grayish-yellow, grayish brown, and milky gray correspond to acidosis and the remaining color indicated normal condition respectively. Four(4) type of odors were found like aromatic (46.6%) which indicated normal condition and pungent (26.7%), putrid (13.3%), and sour (13.3%) which indicated the acidosis condition. The movement of rumen protozoa were found as vigorous (26.7%), moderate (6.7%), mild (46.7%), and absent (20%) which indicated animals that in acidosis condition, their protozoan motility were mild and absent. The result was significan ($p \leq 0.01$). The study result, pH value <6, which was Blood Glucose, Ruminal Glucose and electrolyte (Na, Cl, K) was more than pH value ≥6 to 8. pH value <6: Blood Na, Cl, and K were less than pH value ≥6 to 8. The analysis value of pH value <6 which body temperature, sedimentation rate, and methylene blue reduction were more than pH value ≥6 to 8. The result was significant ($p \leq 0.05$) and significant level was 5%. The study also helps to interpret that, pH value have a relation with physiological and biochemical changes of rumen fluid and blood.

Key words : Rumen fluid, Blood, Protozoa, pH, Sedimentation rate.

Chapter 1: Introduction

Ruminants are hoofed herbivorous grazing or browsing mammals that are able to acquire nutrients from plant-based food by fermenting it in a specialized stomach prior to digestion, principally through microbial actions. Commonly known ruminant animals include cows, sheep, goats, camels, deer, and buffaloes. The rumen is a special organ that is used by domestic livestock including cattle, sheep, and goats as the main location for microbial fermentation of ingested plant material. The rumen presents a unique environment for bacteria. Protozoa can significantly affect the ruminal environment, according to measurements of several ruminal parameters in faunated, defaunated, and ciliate free animals (Williams et al., 1992). Large and small ruminants that suffer from digestive disorders or anorexia are the most prevalent and serious issues in our nation, hampering the proper development and productivity of animals. Bangladeshi farmers also suffer significant financial losses as a result. The most significant and vital factors for stable digestion and metabolism, and subsequently to enhance animal development and productivity, are a healthy ruminal environment and microbial activity. Acidosis, occasionally referred to as lactic acidosis, rumen acidosis, or grain overload, is an abnormality of the rumen's fermentation of carbohydrates that can impact animals of all ages. Acidosis, as the name implies, is a condition marked by an acidic pH of the rumen (usually the following ranges: 6.2–6.8). It can be carried on through offering highly fermentable carbohydrates, feeding a diet lacking in fibre, using inadequate management strategies, or a combination of these. The severity of acidosis varies from moderately difficult which is a little decrease in feed intake, to severe, which is mortality rates. The accumulation of acids and glucose in the rumen induces a significant rise in osmolarity and ruminal acidity in acute acidosis. The result can lead to fatal dehydration, injury to the ruminal and intestinal wall, and an overall decrease in blood pH. Acidosis is often accompanied by laminitis, polioencephalomalacia, and liver abscesses (Owens et al., 1998). Lactic acidosis also results in chemical ruminitis, metabolic acidosis, lameness, hepatic abscessation, pneumonia, and, in the most catastrophic situation circumstances, death (Lean and Wade, 2000). Due to excessive lactic acid production in the rumen, digesting considerable quantities of

highly fermentable feeds rich in carbohydrates may result in an acute digestive disease (Hinchcliff et al., 2000). This condition is clinically defined by severe toxaemia, dehydration, ruminal stasis, weakness, and recumbency. Rumen fluid analysis, according to Borges et al. (2002) and Costa et al. (2008), has unquestionable value in the diagnosis of diseases related to ruminant's digestive systems, notably those of pre-gastric compartments, because the microbiota in the rumen is incredibly sensitive to internal as well as external alterations, which the animals are frequently exposed. Ruminal contents can be evaluated for their chemical properties (pH, glucose fermentation, nitrite reduction, and methylene blue reduction test) as well as their physical qualities (colour, odour, consistency, sedimentation, and flotation time). Abnormal fermentative conditions which include rumen acidosis, frequently bring about instances of disease to peri-urban clinics (Donato, 1999) . According to Padmaja et al. (2011), inhabitants whose rear goats in this region adopting a semi-intensive behaviour are more susceptible to suffering from rumen lactic acidosis. Hundreds of different microbial species are present in what is effectively a huge fermentation vat. Indeed, it is estimated that 10–50 billion bacteria, 1 million protozoa, and thousands of yeast or fungi are present in just 1 millilitre of ruminal fluid (Ferber et al., 1929; McAllister, 2000). Therefore, it is possible to detect the microbiological, biochemical, and physical alterations in the rumen as key diagnostic tools for any aberrant or disorder of the rumen (Chakrabarty, 1994). Several studied ruminal activity parameters between healthy and acidotic goats, such as ruminal motolity, ruminal fluid pH, cellulose digestion time, sedimentation activity time, rumen fluid consistency, and protozal motolity (Ram et al., 2007). They discovered that ruminal fluid consistency ranges from watery to viscous and is indicative of health status. In a study on the physical examination of ruminal fluid both the colour and pH of the fluid differed according to the variety of food ingested (Chakrabarty et al., 2002). Normally the colour changes from yellowish brown to greenish, however in the case of concentrate diet, it is grey. Furthermore, they recognized that the smell of the fluid - pungent, putrid, aromatic vinegar is an indication that both the animal's health and dyspepsia. Depending on the feed provided, normal rumen fluid has a pH ranging 6.5 to 7.5, is aromatic, and can vary in color from olive to brownish-green. Protozoal species, both

large and tiny, with active motility and a large proportion of gram-negative rods, represent the population of microorganisms. Normal MBR should be shorter than 6 minutes, and the concentration of rumen chloride should be under 30 mEq/L (Bayne, et al., 2021). During metabolic acidosis, plasma insulin concentrations were lowest and plasma glucose concentrations were greatest. The results presented indicated that minimized insulin secretion happens during metabolic acidosis, which could decrease glucose uptake via the tissue (Bigner et al., 1996). The rise in the level of blood glucose may be the result of increased reabsorption from the amount of sugar that the rumen microbes did not metabolism, in addition to the utilization of absorbed lactic acid for gluconeogenesis (Braun et al., 1992). Animals most impacted Acidosis symptoms include depression and lethargy, anorexia within 8–12 hours of feeding, fluid-filled rumen, sluggish ruminal motility, diarrhoea, and a temperature of the body significantly below normal (Chakrabarty, 2006).

The aim of the study was performed with following objectives:

1) To study the physiological characteristics (color, odor, consistency, ruminal pH, motility, number of protozoa) of ruminal fluid.

2) To study the biochemical parameter of ruminal fluid and blood.

3) To make relationship among the ruminal pH, number of protozoa with methylene blue reduction test and sedimentation activity test.

Chapter 2: Materials and Methods

2.1. Study area and duration:

The investigation was conducted on a total of 15 goats that were treated from April to October 2023 in the Shahedul Alam Quadary Teaching Veterinary Hospital (SAQTVH) of CVASU and from a local farm at the Jalalabad Housing Society, Khulshi, Chattogram. Laboratory tests were performed at Biochemistry Laboratory in CVASU, Chattogram. In particular, the current study included goats brought to the Shahedul Alam Quadary Teaching Veterinary Hospital (SAQTVH) of CVASU who had a history of dietary abnormalities, excessive consumption of a diet high in carbohydrates, that includes wheat and wheat flour, roti, chapatti, boiled rice, vegetables like potatoes, bananas, and jackfruit, and ceremonial wastes. Each of the clinical cases was subjected to a thorough evaluation. Ruminal fluid pH was utilized to select and screen for ruminal acidosis in individuals exhibiting indications of diarrhea, abdominal distension, anorexia, and rumen distension.

2.2. Study population:

For the purpose of this research, rumen fluid and blood were obtained from 15 goats. The group categorized as either healthy or unhealthy is based on the pH value. A pH value was used to code two groups. The pH value range was assigned on levels pH ≥6 to 8 and on levels <6. Goat populations in Chittagong district are relatively modest. So the sample size is tiny.

2.3 Sample Collection:

For the purpose of this research, rumen fluid was obtained from 15 goats according a stomach tube was used for collecting rumen fluid. Following collection, it was brought to the laboratory in a jar sealed with aluminum foil in order to preserve the anaerobic environment. Then, significant laboratory testing was carried out there. Due to the animosity experienced by different owners, blood samples were collected for hematological examination and blood collection. With the support of a disposable syringe, about 5 ml of whole blood was aseptically collected from the jugular vein of each studied goat. Following collection, it was brought to the laboratory in a vacutainer without EDTA.

2.4. Sample Preparation & Preservation:

Blood: Load a vacutainer tube with whole blood. The researcher should use the red vacutainer tubes if commercially available tubes are to be used. Allow the blood to clot by storing it undisturbed at room temperature immediately the whole blood has been collected 15 to 30 minutes are typically required for this. In a centrifuge, remove the clot by centrifuging at 3000 rpm for 10 minutes. The resulting supernatant is designated serum. Following centrifugation, it is important to immediately transfer the liquid component (serum) into a clean polypropylene tube (preferably a minimum Eppendorf tube holding 1.5 ml) using a Micropipette. The serum should be separated into 100 µl portions, and each tube should be clearly labeled with the animal ID and the collection date. Following handling the samples, these should be maintained on wet ice. The serum sample should be refrigerated at –80°C if it is not examined immediately following collection (Texas Biomedical Research Institute). Following that, the serum potassium, serum sodium, serum chloride, and glucose were determined using the Humalyzer 3000.

Ruminal Fluid: The ruminal fluid was centrifuged at the rate of 3000 rpm for 15 minutes to remove suspended particular materials (Sen et al., 2010). After centrifugation, the supernatant must be quickly transferred using a Micro-pipette into a clean Eppendorf tube. The Humalyzer-3000 for determining the percentages of sodium, potassium, chloride, and glucose in the rumen fluid.

2.5. Performing Test:

Physical, chemical, and biochemical methods were used to test ruminal fluid. The sedimentation activity test, color, consistency, odor, and protozoa were the physical tests. The nitrate test and the MBRT test were the chemical examination. The electrolyte balance of the ruminal fluid and blood was evaluated by the biochemical procedures (https://www.slideshare.net/vetvinodh/rumen-fluid-examination). In the CVASU biochemistry lab, a number of chemical and biochemical experiments were performed, which included the Methylene Blue Reduction Test (MBRT), the Nitrate Reduction Test (NRT) using Reagent and the Humalyzer 3000 to evaluate the concentrations of glucose, potassium, chloride, and sodium in rumen fluid. For the

purpose of determining the concentrations of serum sodium, potassium, and chloride in addition to the amount of glucose, a hematological test was conducted.

Methylene Blue Reduction Test (MBRT): The anaerobic fermentation metabolism of the bacterial population is reflected in this analysis. In a test tube, mix 20 ml of rumen content with 1 ml of 0.03% methylene blue, and allow it to stand at room temperature. Estimate the amount of time it will take for the mixture's coloration to change. Cattle on a hay and grain diet typically take three minutes for their rumen fluid to decolorize, leaving an opaque blue ring at the top of the decolorizing mixture. A reduction in time of up to 15 minutes is abnormal and indicates indigestible roughage, several-day anorexia, or rumen acidosis (Bayne et al., 2021).

Sedimentation Activity Test (SAT): This type of test provides an immediate evaluation of microflora activity. Insert a test tube with a sample of rumen fluid and leave it there. Estimate the sediment activity time or the duration of time required for sedimentation to be completed four to eight minutes is usual. Abnormal time may include the following: In cases of rumen acidosis, protracted anorexia, and inactive microbiota from indigestible roughage, very fast sedimentation without flotation happens. There is negligible flotation and sedimentation in foamy bloat, with certain cases of vagal dyspepsia (Bayne et al., 2021).

Color: Colored of the rumen fluid was observed by the naked eye and compared with the rumen fluid of a healthy goat.

Odor: Odor of the collected rumen fluid was observed by the sense organ (nose). It varied with rumen physiological condition of the rumen.

pH: Rumen pH is an essential factor for good digestion and microbes' activity. It was determined by pH paper. The pH of rumen fluid changes according to healthy and diseased animals.

Consistence: As soon as after collection of rumen fluid consistency was observed and its variable according to physical condition.

Examination of protozoa activity and motility: A drop of fresh rumen fluid was taken in a clear glass slide and examined under a microscope 40X. The motility was graded as vigorous, moderate, mild, and absent.

Counting of Rumen Protozoa:

Preparation of working solution: working solution was made by admixture of 1ml 10% formal saline (8.5g normal sodium chloride in 900ml of distilled water+ 100ml of 40% formaldehyde), 20.5ml 5% Lugol's iodine and 1.5ml 30% Glycerine.

Method of Counting: Working solution was first used to dilute freshly collected ruminal fluid at a ratio of 1:10. Following that, 0.1 ml was pipetted into a counting slide and covered with a cover slip after a gentle shake. By using the 40X objective, differential total counts were conducted. Eight square millimetres were counted in the case of a hemocytometer with Neubar ruling, and the average was then multiplied by a dilution factor of 50,000. The outcome was given as total counts per millilitre (n × 105) (Sen, et al., 2010).

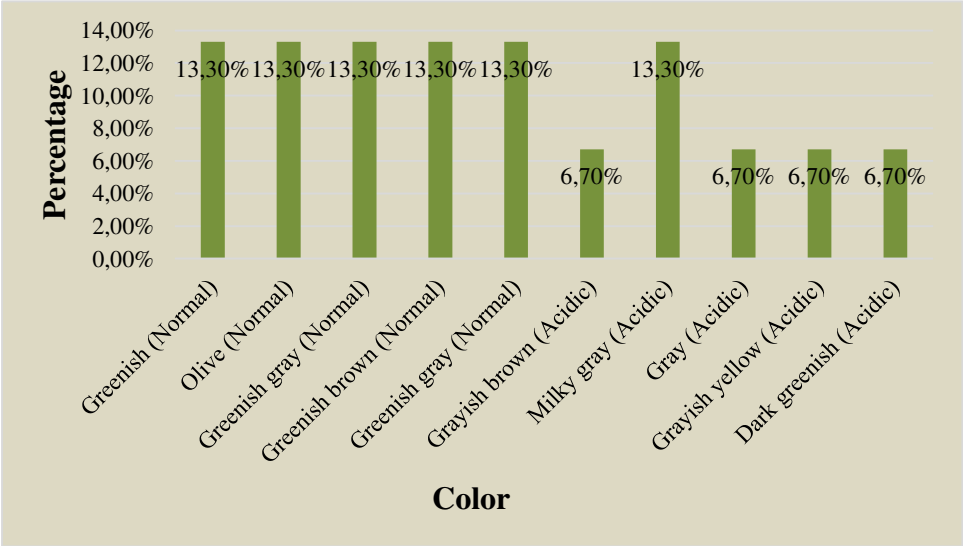
Data input and analysis :

Data were collected , sorted out , coded, and entered into MS Excel 2010, and exported to the SPSS-26 (Statistical Package for the Social Sciences) for statistical analysis, descriptive statistics including percentage, mean ,standard deviation, graphs were performed. The t- test was performed between two groups with 95% confidence interval. The significant difference was express as $p \leq 0.05$.

Chapter 3: Results

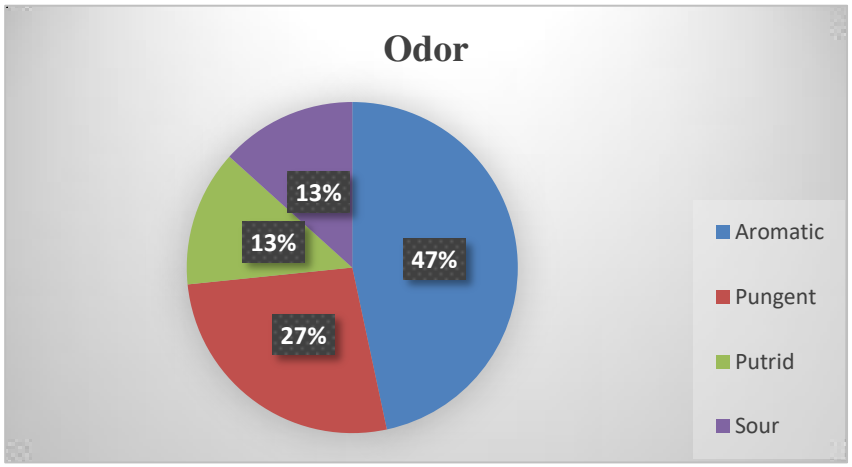
Color of rumen fluid: The percentage of 10 different colors of rumen fluid of goat which included each of greenish (13.3%), greenish gray (13.3%), greenish-brown (13.3%), olive (13.3%), and milky gray (13.3%), and the remaining color of dark brown (6.7%), dark greenish (6.7%), gray (6.7%), grayish brown (6.7%), grayish-yellow (6.7%).

Graph 1: Graphically presented the colors of rumen fluid.



Odor: The maximum percentage (46.6%) of aromatic, followed by pungent (26.7%), putrid (13.3%), and sour (13.3%).

Graph 2: Graphically presented the odor of rumen fluid.



Consistency: There were 7 types of consistency in collected rumen fluid respectively watery (20%), thick watery (20%), thin watery (20%), less watery (20%), less foamy watery (6.7%), watery foamy (6.7%), and foamy (6.7%).

pH: The study was done based on rumen fluid pH. The value of pH <6 and ≥6 to 8 respectively 60.4% & 39.6%. The study showed pH ranged from 4 to 4.5, 5 to 5.5, 6 to 6.5, 7 to 7.5 were found respectively 20%, 33.4%, 20%, and 26.6%. The pH range from 4 to 5.5 which indicated acidic condition, pH from 5.5 to 6 indicated indigestion and pH from 6 to 7.5 indicated normal pH of rumen fluid.

Sex: There were 2 types of sex. The frequency of males and females respectively 7 and 8 and the percentage was 46.7% and 53.3%.

Illness duration: In this study, duration counted by 0 to 7 days which 0, 1, 2, 3, 4, and 7 days frequency and percentage were 5 & 33.3%, 1 & 6.7%, 4 & 26.7%, and 7 & 6.7%.

Motility and number of protozoa: In the study, motility were graded as vigorous, moderate, mild, and absent which percentages respectively 26.7%, 6.7%, 46.7% & 20%. The p-value was 0 which was significant ($p \leq 0.01$)

Table 1: Grade of number of protozoa in rumen fluid.

Grade	N	Mean ± SD	P value
Vigorous	4	$5.56\times10^{6} \pm 4.42\times10^{5}$	0.00
Moderate	1	$3.36\times10^{5} \pm 00$	
Mild	7	$1.22\times10^{3} \pm 1.42\times10^{4}$	
Absent	3	00 ± 00	

Significant level: 1%, (p≤ 0.01)

Overall :

In this study, blood and ruminal fluid were collected from a total of 15 goats which was performed to physical and biochemical test. The oveeall parameter were age, weight, sedimentation rate, number of protozoa, and body temperature, which respectively contained 1.11±.39, 28.01±7.33, 28.01±7.33, $1.71\times10^6\pm2.55\times10^6$ and 103.46±1.38.

Table 2: Physiological and Biochemical parameter of ruminal fluid and blood.

	Traits	N	Mean ± SD	Min.	Max.	Normal range
Demo-graphic	Age (year)	15	1.11 ± 0.39	0.3	1.8	-
	Weight (kg)	15	28.01 ± 7.33	13	45	-
	Parity	15	0.67 ± 0.72	0	2	-
Physio-logical	pH	15	5.70 ± 1.09	4	7.5	6.2-6.8
	MBRT(min)	15	10.45 ± 6.30	2	21	3-9
	Number of protozoa	15	$1.71\times10^6\pm2.55\times10^6$	0	5.98×10^6	-
	SAT (min)	15	9.97 ± 4.45	4	17	4-8
	Body Temp (°F)	15	103.46 ± 1.38	101.1	106.2	101.5-104.5
Blood	Glucose (mg/dl)	9	94.22 ± 47.64	63.5	206.6	50-75
	Na (mmol/L)	9	135.23 ± 9.85	120.5	149.3	142-155
	Cl (mmol/L)	9	91.26 ± 8.52	78.8	105.7	99-103
	K (mmol/L)	9	3.24 ±. 52	2.2	3.9	3.5-6.7
Rumen fluid	Glucose (mg/dl)	15	19.99 ± 6.67	12.7	33.6	-
	Na (mmol/L)	15	93.63 ± 17.53	68.8	119.8	-
	Cl (mmol/L)	15	35.07 ± 7.24	20	46.9	-
	K (mmol/L)	15	36.42 ± 4.40	28.4	46.6	-

Max= Maximum, Min= Minimum, N= Number, min= Minute.

Biochemical analysis:

Using the Humalyzer 3000, glucose, potassium, chloride, and sodium concentrations in rumen fluid are evaluated. The table 3 showed that the analytical result, pH value <6, which was Blood Glucose, Ruminal Glucose, Ruminal Na, Ruminal Cl, and Ruminal K, was more than pH value ≥6 to 8. pH value <6: Blood Na, Cl, and K were less than pH value ≥6 to 8. The biochemical result was not significant ($p \geq 0.05$).

Table 3: Biochemical parameter of ruminal fluid and blood according to pH.

Sample	Test	pH	N	Mean ± SD	P value	95% CI	
						Upper	Lower
Blood	Glucose (mg/dl)	<6	3	120.80±75.02	0.262	117.21	-37.48
		≥6 to 8	6	80.93 ± 27.27			
	Na (mmol/L)	<6	3	132.70 ± 3.55	0.619	13.48	-21.08
		≥6 to 8	6	136.50±12.01			
	Cl (mmol/L)	<6	3	90.77 ± 13.69	0.936	29.49	-30.96
		≥6 to 8	6	91.50 ± 6.38			
	K (mmol/L)	<6	3	3.07 ± .80	0.633	1.52	-2.05
		≥6 to 8	6	3.33 ± .37			
Ruminal fluid	Glucose (mg/dl)	<6	7	22.31 ± 8.70	0.218	11.62	-2.92
		≥6 to 8	8	17.95 ± 3.72			
	Na (mmol/L)	<6	7	98.11 ± 20.40	0.387	28.97	-12.16
		≥6 to 8	8	89.71 ± 14.83			
	Cl (mmol/L)	<6	7	36.81 ± 9.35	0.429	12.20	-5.68
		≥6 to 8	8	33.55 ± 4.90			
	K (mmol/L)	<6	7	38.01 ± 4.98	0.215	7.99	-2.01
		≥6 to 8	8	35.03 ± 3.56			

Physiological and Chemical test analysis :

The table showed that that the analysis value of pH value <6 which body temperature, sedimentation rate, and methylene blue reduction were more than pH value ≥6 to 8. pH value <6 number of protozoa was less than pH value ≥6 to 8. Nitrate absent in both pH. The result was significant ($p \leq 0.05$).

Table 4: Physiological and chemical test of ruminal fluid according to pH value.

Test	pH	N	Mean±SD	P value	95% CI	
					Upper	Lower
pH	<6	7	4.71 ± .39	00	-1.23	-2.47
	≥ 6 to 8	8	6.56 ± .67			
Body Temp (°F)	<6	6	104.35±1.16	0.03	2.95	0.17
	≥6 to 8	8	102.79±1.17			
Sedimentation Rate (min)	<6	7	12.43 ± 3.95	0.04	8.98	0.26
	≥6 to 8	8	7.81 ± 3.85			
Number of protozoa	<6	7	$9.15\times10^{2}\pm8.73\times10^{2}$	0.01	9.49×10^{5}	-5.47×10^{6}
	≥6 to 8	8	$3.2\times10^{6}\pm2.75\times10^{6}$			
MBRT (min)	<6	7	15.57±3.78	0.00	14.09	5.10
	≥6 to 8	8	5.98±4.21			

Significant level: 5%, ($p \leq 0.05$).

Correlation between pH, methylene blue reduction, number of protozoa and sedimentation test:

As presented in table, all physiological and biochemical tests showed a significant relationship between methylene blue reduction test and pH, pH and sedimentation rate, pH and number of protozoa. The rumen fluid pH showed a significant relationship between methylene blue reduction test, sedimentation rate, and number of protozoa. The rumen pH was inversely proportional to methylene blue reduction test and sedimentation rate. The pH and the number of protozoa were proportional relationships. The correlation was significant ($p \leq 0.01$) and the significant level was 1%.

Table 5: Correlation between pH, methylene blue reduction, number of protozoa and sedimentation rate:

MRT& pH	pH & SAT	pH & N.P
-.934**	-.587*	.880**
.00	.02	.00

MRT= Methylene blue reduction test, SAT= Sedimentation activity test, N.P= Number of protozoa, pH= Potential of Hydrogen, *=$P \leq 0.05$, **=$P \leq 0.01$.

Significant level: 1%, ($p \leq 0.01$).

Chapter 4: Discussion

Greenish, greenish gray, greenish brown and milky gray, dark brown, dark greenish, gray, grayish brown, and grayish yellow were the colors of the goat ruminal fluid found in this study. The colors mentioned above essentially depend on the diet and abnormal rumen conditions. An earlier study shown that the color of rumen fluid changed following acid consumption from milky grey to dark brown or greenish brown, and that it also changed color due to persistent bloating from dark brown to grayish green (Chakrabarty, 1996). The current research results aligned with the previous investigation.

The maximum percentage of aromatic, followed by pungent, putrid and sour. Putrid, aromatic, sour, and stale denotes subacute or protein overfeeding, normal state, acid indigestion, and chronic bloat or inactive gastric juice, respectively, in accordance with (Chakrabarty, 1996), which is similar to the current findings.

Within the collected rumen fluid, there were seven distinct types of consistency: most likely watery, thick watery, thin watery, less watery, less foamy watery, watery foamy, and foamy. In accordance with Gnanaprakasan et al. (1990), rumen fluid becomes gruel-like during rumen acidosis and frothy with chronic bloat.

In the research using the pH of rumen fluid. The pH values are <6 and ≥ 6 to 8, representing 60.4% and 39.6% respectively. In general, pH ranged from 4.0 to 5.5 during acidosis and from 7 to 8.5 during alkalosis (Chakrabarty, 1996 and Gnanaprakasan et al., 1990).

The study classified the motility as follows: Vigorous (26.7%), moderate(6.7%), mild (46.7%), and absence (20%). The p value was zero, indicating significance ($p \leq 0.01$). The significant level was 1%. According to the current investigation, there was a decrease in the protozoa count per ml of rumen fluid at pH values between 4.5 to 5.9. Furthermore, there was a decrease in the protozoal count during alkalosis, which is consistent with previous findings (Purser and Moir, 1959). According to the study's findings, rumen motility decreased or disappeared when pH changed beyond a normal range, which is consistent with previous research (Blood and Radostits,

1989). In this investigation, 15 goat's blood and ruminal fluid were collected and the samples were evaluated physically and biochemically. Age, weight, sedimentation rate, number of protozoa, and body temperature were the physical traits; the values were 1.11±.39, 28.01±7.33, $1.71\times10^{6}\pm2.55\times10^{6}$, and 103.46±1.38 respectively. The table-3 showed that the analytical value of pH <6, which was Blood Glucose, Ruminal Glucose, Ruminal Na, Ruminal Cl, and Ruminal K was more than pH value ≥6 to 8. The pH value <6; Blood Na, Cl, and K were less than pH value ≥6 to 8. The rise in the level of blood glucose may be the result of increased reabsorption from the amount of sugar that the rumen microbes did not metabolize, in addition to the utilization of absorbed lactic acid for gluconeogenesis (Braun et al., 1992). The biochemical result was not significant ($p \geq 0.05$). The significant level was not satisfactory.

The table-4 showed that that the analysis value of pH value <6 which body temperature, sedimentation rate, and methylene blue reduction were more than pH value ≥6 to 8. pH value <6 number of protozoa was less than pH value ≤6 to 8. Nitrate absent both pH. The result was significant ($p \leq 0.05$). The significant level was 5%.

Table-5, all physiological and biochemical tests showed a significant relationship between methylene blue reduction test and pH, pH and sedimentation rate, pH and number of protozoa. The rumen fluid pH showed a significant relationship between methylene blue reduction test, sedimentation rate, and number of protozoa. The rumen pH was inversely proportional to methylene blue reduction test and sedimentation rate. The pH and the number of protozoa were proportional relationships. The correlation was significant ($p \leq 0.01$) and the significant level was 1%. In accordance with previous research (Blood and Radostits, 1989), the study found that rumen motility decreased or disappeared when pH was beyond its normal range.

Chapter 5: Conclusion

Based on the current results, it is evident that each of the goats had acidosis or indigestion. The result was significantly correlated with physical changes in the rumen fluid (color, odor, consistency, and pH), and additionally with changes in the biochemical parameters of the blood and rumen fluid (serum glucose, sodium, potassium, and chloride). Considering the rumen fluid's pH change to be an indication, one could treat the goat..

Chapter 6: Limitation

Despite my best efforts, this study has certain limitations, including:

1. There are an extremely fewer goats in Chittagong. Consequently, only fifteen goat’s samples were gathered.

2. Furthermore, there were also fewer reports of digestive disorders.

3. The owner rarely permitted the collection of the animal's rumen fluid.

4. Sample preservation were difficult in sometimes.

References

Bayne, J. E., & Edmondson, M. A. 2021. Diseases of the gastrointestinal system. Sheep, goat, and cervid medicine, Third edition, pp. 63-96.

Bigner, D. R., Goff, J. P., Faust, M. A., Burton, J. L., Tyler, H.D., & Horst, R. L. 1996. Acidosis effects on insulin response during glucose tolerance tests in Jersey cows. Journal of Dairy Science, 79(12), 2182-2188.

Blood, D. C., Radostits, O. M., & Henderson, J. A. 1989. A textbook of the diseases of cattle, sheep, pigs, goats and horses. London, Baillière Tindall, 7.

Borges, N. C. Silva, L. A. F. Fioravanti, M. C. S. Cunha, P. H. J. Moraes, R. R. Guimaraes, P. L. Martins, M. E. P. 2002 Availiacao do suco ruminal de bovinos "a fresco" e apos 12 horas de conservacao. Ciencia Animal Brasilieira, volume. 3, no. 2, pp. 57-63.

Braun, U., Rihs, T., & Schefer, U. (1992). Ruminal lactic acidosis in sheep and goats. The Veterinary Record, 130(16), 343-349.

Chakrabarty, A. 1996. A text book of Veterinary Clinical Medicine, Mechanism of Rumen Digestion. pp. 312-313, 323-336.

Chakrabarty, A. 2006. Text Book of Clinical Veterinary Medicine 3rd revised and enlarged edition pp. 291-295.

Costa, D. P. B. Silva, J. C. G. Mourao, R. C. Rodrigues, V. C. Costa, Q. P. B. Lima, E. S. 2008. Microrganismos do rumen de bovinos e bubalinos castrados e inteiros. Pubvet, Publicacoes em Medicina Veterinaria e Zootecnia, volume. 2, no. 34, pp. 01-11.

Donato, I. V. Sores, P. C. Batista, A. M. V. Silva, E. P. da Costa, J. N. da. Marques, C. T. Maia, F. C. L Teixeira, M. N. 1999. Aspectos fisicoquimicos do fluido ruminal de dietas compostas de vagemde algaroveira (Prosopis fuliflora D. C.) e capim elefante (Penisetum purpureum shum.) em diferentes proposicoes. Recife, Ciencia Veterinaria Tropical, volume. 2, no. 1, pp. 01-06.

Ferber, K. E., & Winogradowa-Fedorowa, T. 1929. Zahlung und Teilungsquote der Infusorien im Pansen der Wiederkauer. Biological Zentralbl, 49, 321-328.

Gnanaprakasan, V., S. Prathaban and S. R. Srinivasan. 1990. A key role view on ruminant medicine. Department of Clinical Medicine, Madras.

Lean. I. J. Wade, L. K., Curtis, M. A., & Porter, J. 2000. New approaches to control of ruminal acidosis in dairy cattle. Asian Australasian Journal of Animal Science, 13,pp. 266-269.

McAllister, T. 2000. Learning more about rumen bugs, Genetic and Environmental factors affecting rumen bugs. Southern Alberta Beef Review. 2(1).

Owens, F. N., Secrist, D. S., Hill, W. J., & Gill, D. R. 1998. Acidosis in cattle: a review. Journal of Animal Science, 76(1), pp. 275-286.

Padmaja, K., & Praveena, G. 2011. Rumen acidosis in goats. Intas polivet, 12(2), pp. 318-319.

Radostits O. M., Gay C. C., Blood D. C., Hinchcliff K. W. 2000. In Veterinary Medicine, A Text Book of Disease of Cattle, Sheep, Pig and Horses. 9th edition., W. B. Sounders, Har-court Publisher Ltd. London.

Sen, A. B., Islam, S. K. M. A., Kibria, A. S. M. G., Hassan, M. M., & Uddin, M. S. 2010. Rumen physiology in digestive disordered sheep. International Journal of Sustainable Agricultural Technology, 6(10), pp. 13-17.

Williams, A. G., Coleman, G. S., Williams, A. G., & Coleman, G. S. 1992. Role of protozoa in the rumen. The Rumen Protozoa, pp. 317-347.

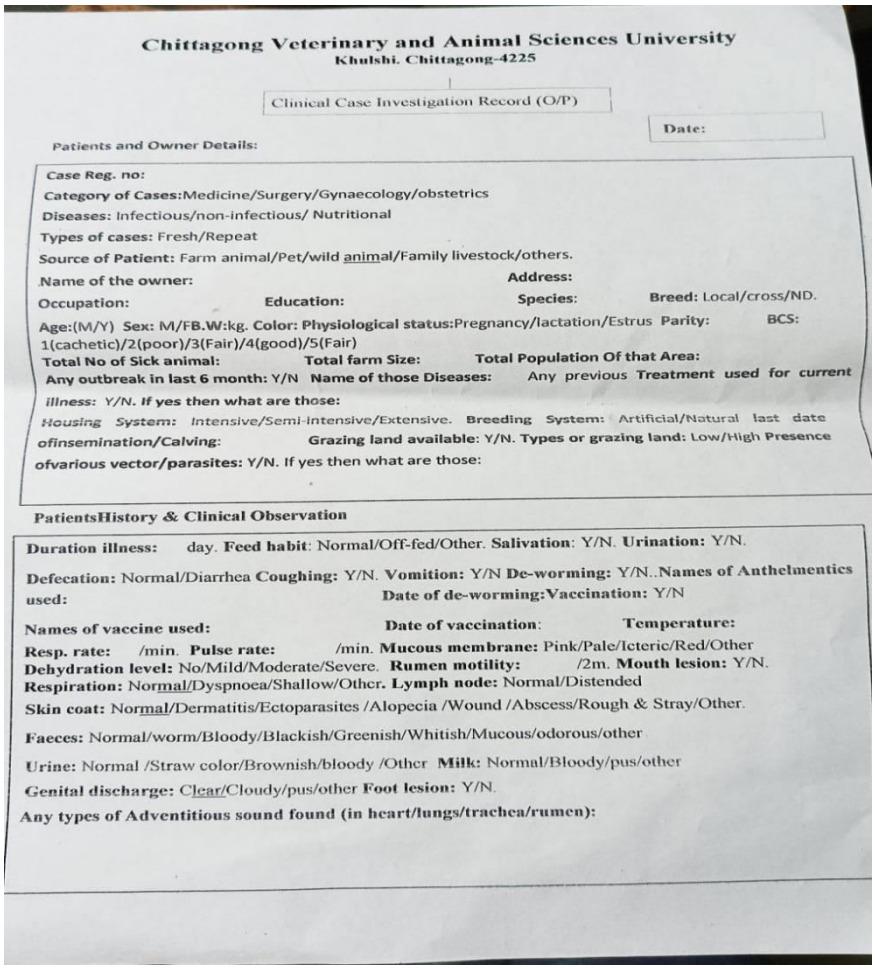
Biography of Author

I am Md. Ariful Islam, Son of Md. Shajahan and Jesmin Akter. I passed Secondary School Certificate Examination from St. Placid's High School & College, Chattogram in 2014 (G.P.A-5) followed by Higher Secondary Certificate Examination from Govt. Hazi Mohammad Mohsin College, Chattogram in 2016 (G.P.A-5). Now I am an intern veterinarian under the Faculty of Veterinary Medicine And Animal Sciences University, Bangladesh.

The Author

November, 2023

Appendix



Chittagong Veterinary and Animal Sciences University
Khulshi. Chittagong-4225

Clinical Case Investigation Record (O/P)

Date:

Patients and Owner Details:

Case Reg. no:
Category of Cases: Medicine/Surgery/Gynaecology/obstetrics
Diseases: Infectious/non-infectious/ Nutritional
Types of cases: Fresh/Repeat
Source of Patient: Farm animal/Pet/wild animal/Family livestock/others.
Name of the owner: **Address:**
Occupation: **Education:** **Species:** **Breed:** Local/cross/ND.
Age:(M/Y) **Sex:** M/F**B.W:**kg. **Color:** **Physiological status:**Pregnancy/lactation/Estrus **Parity:** **BCS:** 1(cachetic)/2(poor)/3(Fair)/4(good)/5(Fair)
Total No of Sick animal: **Total farm Size:** **Total Population Of that Area:**
Any outbreak in last 6 month: Y/N **Name of those Diseases:** Any previous **Treatment used for current illness:** Y/N. **If yes then what are those:**
Housing System: Intensive/Semi-intensive/Extensive. **Breeding System:** Artificial/Natural last date **ofinsemination/Calving:** **Grazing land available:** Y/N. **Types or grazing land:** Low/High **Presence ofvarious vector/parasites:** Y/N. If yes then what are those:

PatientsHistory & Clinical Observation

Duration illness: day. **Feed habit**: Normal/Off-fed/Other. **Salivation**: Y/N. **Urination:** Y/N.
Defecation: Normal/Diarrhea **Coughing:** Y/N. **Vomition:** Y/N **De-worming:** Y/N..**Names of Anthelmentics used:** **Date of de-worming:** **Vaccination:** Y/N
Names of vaccine used: **Date of vaccination**: **Temperature:**
Resp. rate: /min. **Pulse rate:** /min. **Mucous membrane:** Pink/Pale/Icteric/Red/Other
Dehydration level: No/Mild/Moderate/Severe. **Rumen motility:** /2m. **Mouth lesion:** Y/N.
Respiration: Normal/Dyspnoea/Shallow/Other. **Lymph node:** Normal/Distended
Skin coat: Normal/Dermatitis/Ectoparasites /Alopecia /Wound /Abscess/Rough & Stray/Other.
Faeces: Normal/worm/Bloody/Blackish/Greenish/Whitish/Mucous/odorous/other
Urine: Normal /Straw color/Brownish/bloody /Other **Milk:** Normal/Bloody/pus/other
Genital discharge: Clear/Cloudy/pus/other **Foot lesion:** Y/N.
Any types of Adventitious sound found (in heart/lungs/trachea/rumen):

Fig 1: Clinical case investigation record (O/P)

O = Owner

P = Patient

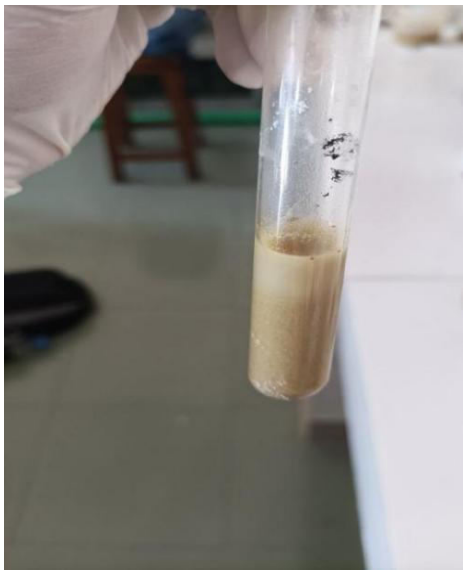


Fig 2: Ruminal fluid : Grayish brown & dark greenish and milky white.

Fig 3: Serum collection.

Fig 4: Blood serum.

Fig 5: Sample stored in anaerobic condition.

Fig 6: Collection of sample.

Fig 7: Supernatant of ruminal fluid.

Fig 8: Methylene blue reduction test.

Table 6: Demographic data of overall sample.

Sample No	Feeding habit	Illness duration (day)	Age (year)	Weight (Kg)	Sex	Temp. (°F)
1	Normal	0	1.8	32	Female	103.9
2	Normal	0	1.4	34	Female	102
3	Normal	0	1.1	24	Female	104.1
4	Normal	0	1.2	27	Female	102.4
5	Normal	0	0.7	13	Male	101.6
6	Off-fed	2	1.5	45	Female	105.6
7	Off-fed	2	1.2	32.7	Male	104.3
8	Less feeding	4	1.3	30.4	Female	104.0
9	Less feeding	7	1.4	34.2	Female	106.2
10	Off-fed	4	1	27.5	Male	103.2
11	Off-fed	3	0.8	26.4	Female	102.6
12	Less feeding	2	0.3	23.6	Male	105.7
13	Less feeding	3	0.6	22.4	Male	104.1
14	Off-fed	1	1	21	Male	103.8
15	Less feeding	2	1.3	27	Male	101.1

Table 7: Physiological characteristics of ruminal fluid.

N	pH	Color	Odor	Consistency	Sedimentation (min)	Motility of protozoa
1	7	Greenish yellow	Aromatic	Watery foamy	4.5	Vigorous (+++)
2	7	Olive	Aromatic	Less watery	5	Vigorous (+++)
3	7.5	Greenish	Aromatic	Less watery	6	Vigorous (+++)
4	6.5	Brownish yellow	Aromatic	Less watery	4	Moderate (++)
5	7	Olive	Pungent	Less foamy watery	7	Vigorous (+++)
6	4.5	Grayish brown	Sour	Thick watery	11	Absent (-)
7	5	Milky gray	Aromatic	Watery	10	Mild (+)
8	5.5	Greenish gray	Pungent	Thin watery	10.5	Mild (+)
9	5	Greenish brown	Sour	Thick watery	19	Mild (+)
10	6	Dark greenish	Aromatic	Thick watery	13	Mild (+)
11	4	Dark green	Aromatic	Watery	10	Absent (-)
12	5	Gray	Pungent	Foamy watery	17	Mild (+)
13	5	Grayish yellow	Putrid	Thin watery	11	Mild (+)
14	4.5	Milky gray	Putrid	Watery	12	Absent (-)
15	6	Dark brown	Pungent	Thin watery	14	Mild (+)