Study on physiological and biochemical parameter analysis on ruminal fluid and blood in acidosis of goat



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List of Abbreviation

Abbreviation	Elaboration
%	Percentage
No.	Number
et. al	And his associate
SSC	Secondary School Certificate
HSC	Higher Secondary School Certificate
CVASU	Chattogram Veterinary and Animal Sciences University
MRT	Methyelene blue reduction test
SAT	Sedimentation activity test
N .P	Number of Protozoa
Na	Sodium
K	Potassium
Cl	Chloride
pH	Potential of Hydrogen
Std	Standard deviation
Min.	Minimum
Max.	Maximum

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Last but not the least, I would want to thank everyone who contributed to the course and apologize for not mentioning each individual contributor individually.

Appendix

	Clinical Case Inve	stigation Record (C	(TV)		
				Date:	
Patients and Owner Detail	is:				
Case Reg. no!					
Category of Cases:Medicin		Vobstetrics			
Diseases: Infectious/non-in					
Types of cases: Fresh/Repo		maile livesteck othe	wa.		
Source of Patient: Farm an	imat/Pet/wild <u>anim</u> at/P	Addre			
Name of the owner:	Education:	Spec	100	Breed: Local/	cross/ND.
Occupation:					BCS:
Age:(M/Y) Sex: M/FB.W:kg 1(cachetic)/2(poor)/3(Fair),		scatus:Pregnancy/ta	cuationyestro	a carrey.	and the second
1(cachetic)/2(poor)/3(Fair), Total No of Sick animal:	Total farm 5iz	e: Total Popu	lation Of the	rt Area:	
Any outbreak in last 6 mor	nth: Y/N Name of thos	e Diseases: Any	previous T	reatment use	ed for curr
illness: Y/N. If yes then wi	hat are those:				
Housing System: Intensi	ve/Semi-intensive/Exte				
ofinsemination/Calving:		available: Y/N. Type	es or grazing	tand: Low/Hit	gh Presence
ofvarious vector/parasites:	Y/N. If yes then what	are those:			
	erosani [†]				
PatientsHistory & Clinica	I Observation				
	ed habit: Normal/Off	fed/Other Setternt	on: V/N The	ination: V/N	
efecation: Normal/Diarrho	a Coughing: Y/N, Vo	mition: Y/N De-we	orming: Y/N	Names of A	athelmen
ed:	Da	te of de-worming:	Vaccination:	Y/N	
ames of vaccine used:	Da	te of vaccination:	Te	mperature:	
esp. rate: /min Pulse		cous membrane: P	ink/Pale/Icto	ric/Red/Othe	er .
ehydration level: No/Mild	Moderate/Severe. R	umen motility:		ath lesion: Y	/N.
espiration: Normal/Dyspn	oca/Shallow/Other. L;	ymph node: Norma	d/Distended	1000 V 100 PM	
kin coat: Normal/Dermatit	tis/Ectoparasites /Alop	ecia /Wound /Absc	ess/Rough &	Stray/Other	
acces: Normal/worm/Bloo	dy/Blackish/Greenish/	Whitish/Mucous/oc	lorous/other		
rine: Normal /Straw color/				other	
enital discharge: Clear/C					
y types of Adventitious s			men):		
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Clinical case investigation record

Abstract

Acidosis is a condition of the rumen's carbohydrate fermentation that can affect goats of all breeds and ages. It is sometimes referred to as lactic acidosis, rumen acidosis, or grain overload. The present investigation was carried out at SAQTVH, Chittagong Veterinary and Animal Science University from August to September 2023, during my internship to examine a variety of rumen fluid parameters in acidotic goats. For the purpose of this investigation, a total of 15 goats were enrolled for the investigation. From them, 10 goats from various age groups, sexes and breeds that had a history of consuming large amounts of highly fermentable, carbohydrate-rich diets, as well as symptoms like anorexia, suspended rumination, and clinical manifestations like diarrhea and distended rumen, were chosen. A control group of 5 healthy goats from households was selected to compare with 10 diseased goats. The current study found substantial differences between healthy and acidosis-affected groups of goats in terms of ruminal pH, color, odor, consistency, and protozoal motility including pH level <6, the color of rumen fluid was milky grey(13.33%), dark brown (6.7%), dark greenish (6.7%), green (6.7%), grayish brown (6.7%), grey(6.7%), greenish yellow(13.33%), greenish (13.33%), brownish yellow(6.7%), olive(13.33%) found.Odors such as putrid (13.3%) aromatic (46.7%), sour (13.33%), and pungent (26.7%) were found. The consistencies result were thick watery(20%), watery(20%), foamy watery(6.7%) found. Biochemistry revealed a large increase in blood glucose level, and serum sodium while serum potassium and chloride level was dramatically lowered. In rumen fluid, the glucose, serum sodium, potassium, chloride levels increase. The analysis of each of the aforementioned indicators led to the conclusion that the goats had ruminal acidosis. From an economic and significant health perspective, ruminal acidosis is a critical nutritional issue in ruminants. According to this study, farmers should refrain from giving ruminants significant amounts of readily digested carbohydrates at one time.

Keywords: Anorexia, acidosis, breeds, carbohydrate, ruminal fluid, protozoa, motility.

Introduction

A complex ecology found in ruminants is home to a wide range of bacteria that can cause various forms of fermentation. The rumen, the biggest compartmental stomach in ruminants, functions as a closed fermentation vat where the microbiota targets the feed that has been consumed. Protozoa, fungi, and bacteria make up the majority of the rumen microflora. Few flagellates play a significant role in providing nutrients to the host species, while the majority are ciliate protozoa (Ogimoto and Imai, 1981). Rumen protozoa are responsible for regulating the fermentation of starch and soluble carbohydrates, as well as for maintaining acidity within the rumen (Mackie et al., 1978)

The most common and serious issue that impairs the healthy growth and productivity of animals is digestive disorders, or anorexia conditions, in both large and small ruminants. For Bangladeshi farmers, it also results in significant financial losses. To ensure optimal digestion and metabolism, as well as the growth and productivity of the animal, two of the most important and critical components are a healthy ruminal environment and microbial activity. (Sen et al., 2010)

Worldwide, goats are raised primarily for their meat and milk (Escareno et al., 2012). Pure or mixed wheat grains or their byproducts are fed to encourage efficient growth and rapid weight gain. According to (Kamra et al., 2005), these grains are highly fermentable in the stomach's rumen. Overfeeding on fermentable grains consistently results in the development of metabolic diseases, especially lactic acidosis. However, due to primary or secondary symptoms including the development of liver abscesses, laminitis, and rumenitis, ruminal acute and sub-acute lactic acidosis have a major economic impact (Penner et al., 2007). Any diet that is highly fermentable in excess can lead to the development of lactic acidosis.

There are several rumen bacteria present, but Lactobacillus and Streptococcus are the most common. Acid-resistant bacteria can accelerate the fermentation of carbohydrates and alter rumen function by producing more lactate and volatile fatty acids and multiplying; this lowers the pH of the rumen to less than 5.00 (Gozho et al., 2005; Gonzalez et al., 2010). Depending on the kind and quantity of feed high in carbohydrates ingested, lactic acidosis might vary in severity (Gentile et al., 2004).

Acidosis has two primary causes: systemic and metabolic acidosis, which results from the absorption of acids into the bloodstream, and fermentable carbohydrate consumption and a fast rise in the ruminal microbial population (Radostits et al., 2007). The morbidity rate of ruminal acidosis in clinically affected animals ranges from 10 to 50 percent. The condition is characterized by an abrupt increase in heart rate, breathing rate, body temperature, abdominal distension, pain, anorexia, constipation or pastry diarrhea, depression, weakness, dehydration, and if left untreated, death (Radostits et al., 2000).

The above abnormalities of the rumen are caused by any change in ruminal physiology. Thus, a key predictor of anorexia, decreased feed intake, and other digestive diseases in animals is the analysis of these characteristics, such as pH, color, odor, consistency of rumen fluid, motility, and quantity of ruminal protozoa. Numerous infectious and non-infectious affect livestock. One of the non-infectious issues facing all ruminant species is ruminal acidosis. Acidosis is a condition characterized by the improper fermentation of carbohydrates in the rumen that can impact animals of all ages. Acidosis, which is brought on by giving easily fermentable carbohydrates, providing a low-fiber diet, using inadequate management techniques, or a combination of these, causes the rumen's pH to become acidic (it should normally be between 6.2 and 6.8). According to Tufani et al., (2013) symptoms of the acute type of ruminal acidosis in ruminants include dyspepsia, rumen stasis, toxemia, incoordination, collapse, and frequent mortality. The ruminal pH typically drops to less than 5.00 due to the rapid fermentation of carbohydrates, which is caused by the growth of acid-resistant bacteria, an increase in the production of volatile fatty acids, D and L lactate (Gozho et al., 2005), and (Gonzalez et al., 2012). Due to the build up of acids and glucose in acute acidosis, ruminal acidity and osmolarity significantly increase. They cause dehydration, which can be fatal, harm the ruminal and intestinal walls, and lower blood pH. Goats raised in a free-grazing environment are more susceptible to unintentionally consuming excessive amounts of diets high in carbohydrates (Valmik et al., 2017).

The objectives of the study are:

- 1. To observe the demographic characteristics(age, weight, parity, feeding habit) and physical characteristics of the rumen fluid(color, odor, consistency) of the goat.
- 2. To compare the rumen fluid and rumen microbiota parameters(motility, number of protozoa) between animals that are in good condition and those that are anorexic.
- 3. Collection of rumen fluid and blood to do an evaluation of rumen and blood electrolyte.
- 4. To determine how rumen motility, rumination, body temperature, and feeding schedule relate to pH, motility, number of protozoa, and sedimentation activity test.

Chapter 2: Materials and Methods

1.Study Area and Population:

The present study was carried out on 15 goats of different breeds, age, sex where 10 goats with clinical history having accidental ingestion of wheat, grain, pumpkin, bran developed clinical signs comprising of inappetite, anorexia, distended abdomen, diarrhoea were brought to the SAQTVH, Chattogram Veterinary and Animal Sciences University, Chattogram during my internship rotation from 1st August to 30th September. The 5 healthy goats were selected from household for comparative study.

1.1 Sampling procedure

The rumen liquor was collected aseptically through inserting stomach tube into oesophagus and collected in the beaker then marking was done using marker pen for further identification.

1.2 Sample preservation and storage

The rumen fluid was centrifuged at 3000rpm for 20 minutes to remove the feeding materials. After centrifugation, the supernatant was collected in a eppendorf tube through micropipette. The supernatant was stored in eppendorf tube for biochemical analysis of glucose, sodium, potassium and chloride of rumen fluid.

2. Physical examination of rumen fluid

2.2.1 Color

The rumen fluid's color was compared to that of healthy and acidosis affected animals using visual inspection.

2.2.2 Odour

An organoleptic test was used to detect and document the odour of collected rumen fluid.Rumen fluid's odour is influenced by the rumen's health.Unusual circumstances provide varying scents.

2.2.3 Consistency

A sample of rumen fluid was collected and it's consistencies were examined right away.Various forms of consistency were discovered in various samples.Every kind of consistency were noted.

2.2.4 Sedimentation activity test

Sedimentation activity test provides a rapid evaluation of microfloral activity. Put a sample of rumen fluid in test tube and let to stand. Measure the time needed for completion of sedimentation, which is referred to as the sediment activity time. Normal time is 4-8 minutes. Abnormal time may be very rapid sedimentation with no floatation or 4-8 min< occurs in rumen acidosis, prolonged anorexia, inactive microflora from indigestible roughage.

3.Biochemical evaluation of rumen fluid

3.1 Rumen fluid pH

A wide range pH indicator paper was used to measure the pH of the rumen fluid. Rumen liquor was applied to the paper, and the pH was measured by comparing the colour change of the indicator paper to the standard colours of the indicator paper. Since rumen liquor might raise in pH when exposed to air, the pH was measured as soon as it was collected.

3.2 Methylene blue reduction test

Biochemical test which is methylene blue reduction test that reflects the anaerobic fermentation metabolism of bacterial population. A 20 ml of rumen content was drawn in testube with 1 ml of 0.03% methylene blue and let to stand at room temperature. The time was measured for color of the mixture to be changed.Normal rumen fluid from herbivorous fed on a hay and grain diet needs 3 minutes to decolorize. Abnormal reduction of time up to 15 minutes indicates indigestible roughage, anorexia of several days or rumen acidosis.

3.3 Nitrate reduction test

Nitrate reduction test provides an idea on activity of microbes that synthesize nitrogen compounds.

3.4 Rumen Electrolyte

Following the physical and biochemical examination, a biochemical analyzer was used to determine the electrolyte content (Glucose, sodium, potassium, chloride) of the rumen fluid (Humalyzer, 3000).

4. Microscopic Examination

A fresh drop of ruminal fluid was put on a spotless glass slide, coated with a cover glass and studied using a low-power microscope magnification. The active rumen liquor increases with the quantity of protozoa. based on the classification of protozoal activity as mild(+), moderate (++) or vigorous (+++). (Sen et al., 2010)

Density	Symbol	Grade
None	-	1
Mild/Few	+	2
Moderate	++	3
Vigorous	+++	4

4.1 Ruminal Protozoa Counting

Creating a functional solution:

The following ingredients was used to make it:1)Formal saline(0.85% NS with formalin in equal amount)-1ml 2)5% Lugol's iodine-2.5ml 3)30% Glycerin-1.5ml

4.2 Method of counting

First, a working solution was used to dilute freshly collected ruminal fluid at a ratio of 1:10. Subsequently,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 ($n \times 10^{5}$). The obtained sample's ruminal protozoa were calculated, and the results were compared to those of earlier research and a standard study.

Diet / Condition	No. of protozoa	Motility
Mixed ration	1 x10 ⁵ /ml	+++
Concentrate ration	1x10 ⁶ /ml	+++
Acid ingestion	No or less in number	0 to +
Alkali indigestion	No or less in number	0 to +

5.Collection of blood and examination

Using a syringe, blood was drawn from the jugular vein of the same animal that provided the ruminal fluid. The blood was then stored in a test tube for serum separation and blood was maintained using EDTA, and anticoagulent.

5.1 Determination of blood pH

Blood pH was measured by using pH paper, pH of blood was determined.

5.2 Serum separation for mineral content determination

The syringe was left in an inclined position at room temperature for one to two hours following blood collection. The syringe was then stored for the night at 4C in a freezer. After that, centrifuged for 15 minutes at 3000 rpm. Then the serum was transferred into eppendorf tube through micropipette and stored for biochemical analysis.

5.3 Blood serum electrolyte

Following the physical examination, a biochemical analyzer was used to determine the electrolyte content (Glucose, sodium, potassium, chloride) of the Blood. (Humalyzer, 3000).

6. Data input and analysis

The acquired data were exported to STATA 7.0 (Stata Corporation, College Station, Texas, USA) for analysis after being entered into MS Excel 2000. To represent the findings in terms of mean, standard deviation and percentage, a descriptive analysis was conducted. To determine the level of significance, the student "t" test was employed.

Chapter 3: Results

Table-1:Physiological parameters of healthy goats

Breed	Feedin g history	color	Tempe rature(F)(101 -104.5)	Respir ation(2 0- 30/min)	Ruminati on	PH (6.2- 6.8)	Number of protozoa/ ml	Motility type	Methylene blue(3- 4min)
Hariana	Normal	Greenish yellow	103.9	22	Present	7	5.32×10 [^]	Vigorous (+++)	3
Hariana	Normal	olive	102	24	Present	7	5.88×10^6	Vigorous (+++)	3.3
Totapari	Normal	greenish	104.1	21	Present	7.5	5.98×10^ 6	Vigorous (+++)	3.5
Black Bengal	Normal	Brown yellow	102.4	24	Present	6.5	3.36×10^ 6	Moderate (++)	2
Black Bengal	Normal	olive	101.6	22	Present	7	5.06×10^ 6	Vigorous (+++)	3

Breed	Feed	Color	Tempe rature(F)(101 -104.5)	Respiratio n (20- 30/min)	Rumination	PH (6.2- 6.8)	Number of protozoa/ ml	Motility type	Methylene blue(3- 4min)
Totapar i cross	Pumpk in	Grayis h yellow	105.6	18	Absent	4.5	0	Absent	20
Black bengal	Wheat bran	Milky gray	104.3	21	Absent	5	1.57×10^ 3	Mild(+)	14
Cross	Green grass,g rain	Greeni sh grey	104	17	Absent	5.5	2.03×10^ 4	Mild(+)	12
Jamuna Cross	Off fed	Greeni sh brown	106.2	19	Absent	5	1.30×10^ 3	Mild(+)	15
Black bengal	grass, jackfru ite leaf	Dark greenis h	103.2	23	Absent	6	3.7×10^4	Mild(+)	11
Jamuna	Off fed	Dark green	102.6	21	Absent	4	0	Absent	21
Black bengal	wheat, milk,gr ass	Gray	105.7	20	Absent	5	1.88×10^ 3	Mild(+)	15
Jamuna	Rice mixed with bran	Grayis h yellow	104.1	16	Absent	5	1.66×10^ 3	Mild(+)	10
Cross	Off fed	Milky gray	103.8	19	Absent	4.5	0	Absent	14
Black bengal	jackfru it	Dark browni sh	101.1	21	Absent	6	2.2×10^4	Mild(+)	10

 Table-2: Physiological parameters of Acidosis affected goats

Table-3: Physical Characteristics of rumen fluid of healthy group and diseased	
goats	

Parameters	Healthy mean±std (n=5)	Diseased mean±std (n=10)	P Value
Age /year	$1.24{\pm}0.40$	1.04±0.38	0.38
Weight(kg)	26.00±8.27	29.02±7.05	0.50
parity	.80±0.83	.60±0.69	0.66
PH	7.00±0.35	5.05±0.64	0.00
Number of protozoa/ml	5120000.00±1055746.18	8571.00±13084.82	0.00
Sedimentation Rate(min)	5.30±1.20	12.30±3.46	0.00
Body temperature(F)	102.80±1.13	1.42±1.42	0.17

Parameters	Min-Max	Healthy mean±std (n=5)	Diseased mean±std (n=10)	P Value
Blood Glucose(mg/dl)	63.5-206.6	80.30±30.44	111.63±63.95	0.41
Blood sodium(mmol/l)	120.5-149.3	133.94±11.46	136.85±8.79	0.68
Blood chloride(mmol/l)	85.3-105.7	92.74±6.28	89.40±11.51	0.62
Blood potassium(mmol/l)	2.2-3.9	3.38±0.39	3.08±.66	0.45
Ruminal Glucose(mg/dl)	12.7-33.6	17.96±4.13	21.00±7.63	0.33
Ruminal sodium(mmol/l)	68.8-119.8	90.02±16.62	95.44±18.55	0.58
Ruminal Chloride(mmol/l)	20-46.9	33.10±5.80	36.06±7.95	0.43
Ruminal potassium(mmol/l)	28.4-46.6	35.26±2.65	37.00±5.08	0.40
Methylene Blue(min)	2-21	2.96±0.57	14.20±3.82	0.00

Table-4: Haemato-biochemical findings in apparently healthy control group and affected goats

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

MRT_SAT	MRT_N.P	N.P_SAT	
.644**	841**	734**	
.000	.000	.002	

MRT= Methylene blue reduction test, SAT= Sedimentation activity test, N.P= Number of protozoa, *=P<0.05, **=P<0.01

Chapter 4: Discussion

Colour: For this investigation, we used goat rumen fhuids of four distinct colours. The equivalent percentages for these are 13.33%, 6.7%, 6.7%, 6.7%, 6.7%, 6.7%, 6.7%, 13.33%, 13.33%, 6.7%, and 13.33%. They are milky grey, dark brown, dark greenish, green, greyish brown, grey, greenish yellow, greenish, brownish yellow, and olive. The food and abnormal rumen conditions affect these colours. According to earlier studies, when there is acid reflux, the pH turns milky grey; when there is alkaline reflux, the pH turns dark brown or greenish brown. Thus, this research offers compelling evidence to support the earlier study.(Chakrabarty A et al., 1994)

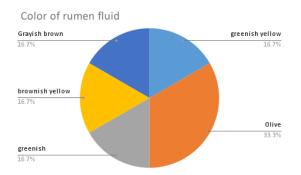


Fig: Pie chart showing different kinds of color of rumen fluid in healthy goats

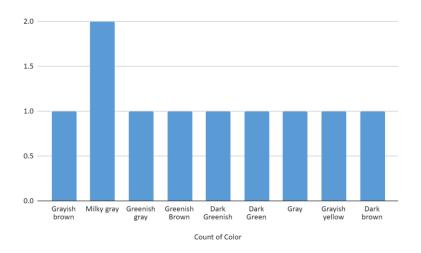


Fig: Bar chart showing different kind of colors of rumen fluid in acidotic goats

Odor: Various types of odor were found in this study. Such as putrid (13.3%) aromatic (46.7%), sour (13.33%), pungent (26.7%) found. In an earlier study, putrid, aromatic, sour and stale indicates respectively the protein over feeding or sub acute indigestion, normal condition, acid indigestion and acidosis or inactive gastric juice. (Srivansan and Gnanaprakasam, 2005)

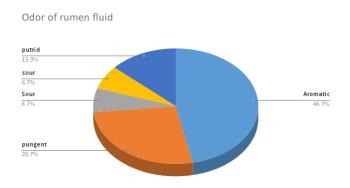


Fig.: Pie chart displaying the rumen fluid odor data for both healthy and sick goats.

Consistency: Various samples exhibited different types of consistency. These were, in the following proportions, thin watery, thick watery, watery, foamy watery, less watery, and foamy percentages 20%, 20%, 20%, 6.7%, 20%, 6.7%, and 6.7% showed respectively. It shows several aberrant conditions of the sheep's rumen data from an earlier study (Dehority and Grubb, 1975) indicates that the consistency of the rumen fluid changes from gruel-like porridge during ruminal acidosis.

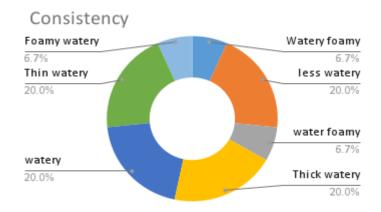


Fig: The pie chart showing consistencies of rumen fluid in healthy and diseased goats

pH : The pH of the rumen fluid, motility, and quantity of ruminal protozoa are highly correlated. The study indicated that the pH of the rumen fluid varied from 4 to 5.5, 6 to 7, and 7.1 to 7.5. These values correspond to 26.7%,6.7% and 20% of acidic, normal, and alkaline pH, respectively. pH typically ranged from 4.0 to 5.5 during acid reflux disease and from 7 to 8.5 during alkalosis (Chakrabarty A et al, 1994 and Srinivasan S.R., 2005)

Motility and number of protozoa: Protozoal motility and total count greatly vary when the pH is altered because of the aberrant state of the rumen. This investigation motility was present in mild(46.7%), demonstrates that moderate(6.7%),vigorous(26.7%),), and absent (20%) cases. Additionally, there were 1×10^{6} to 1x10⁵1x10⁴ to 1x10³ and none were discovered, making up 46.7%, 6.7%, 26.7%, 20% of the total number of protozoa. The p value is zero, indicating significance (p < 0.05). The significant level was 5%. Thus, in this investigation, we found a correlation between the rumen fluid pH and the motility and quantity of ruminal protozoa. Because ruminal protozoa either had reduced motility or were missing when the ruminal pH was lower. According to Dehority (1975), goats with acid indigestion and low ruminal pH are likely acidotic due to consuming too much high-carb green grass or hay, such as bromegrass, alfalfa, and orchad grass.(Meyer and Bryant 1960)

In order to conduct this investigation, blood and ruminal fluid from 15 goats were collected; five of the goats were healthy and ten were sick. The samples underwent physical and biological testing. Age, weight, body temperature, and parity were the physical attributes, as Table 3 demonstrates. The recorded values in a healthy goat were 1.24 ± 0.404 , 26.00 ± 8.276 , 102.80 ± 1.134 , and 0.80 ± 0.837 . According to Table 3, the ill goat's physiological values were 1.04 ± 0.381 , 29.02 ± 7.059 , 1.428 ± 1.428 , and $.60\pm0.699$, in that sequence. The result was p < 0.05, which indicates statistical significance. There was a 5% significance threshold.

Table 4 demonstrated that the analytical values of Ruminal Glucose, Ruminal Na, Ruminal Cl, and Ruminal K in ill goats were higher than those of healthy goats. A healthy goat had the following values: glucose (17.96±4.139), Na (90.02±16.62), CL (33.10±5.806), and K (35.26±2.653), in that order. However, compared to healthy goats, the biochemical analysis of goats affected by acidosis was higher. The results were as follows: K(37.00±5.088), Na(95.44±18.557), CL(36.06±7.953), and glucose (21.00±7.631). Blood glucose levels may have increased due to both the use of absorbed lactic acid for gluconeogenesis and increased reabsorption from the quantity of sugar that the rumen bacteria could not metabolise (Braun et al., 1992).

The biochemical result of p value was (p > 0.05). The significant level was more than 5%.So, it is non-significant.

Table 4 further demonstrated that the analytic values of the blood glucose, blood sodium, blood chloride, and blood potassium of the ill goat were higher than those of the healthy goat. In the blood, the healthy goat had the following values: glucose (80.30 ± 30.444), Na (133.94 ± 11.464), CL (92.74 ± 6.282), and K (3.38 ± 0.396), in that order. However, compared to healthy goats, the biochemical analysis of goats affected by acidosis was higher. Glucose (111.63 ± 63.950), Na (136.85 ± 8.793), CL (89.40 ± 11.510), and K ($3.08\pm.660$) were the values. P value's biochemical outcome was (p > 0.05). There was a considerable level of above 5%. Thus, it is not important. According to the biochemical analysis, sedimentation rate and methylene blue reduction were related. The table shows that in healthy goats sedimentation rate and methylene blue value were $5.30\pm1.204, 2.96\pm0.577$ found. But in ten acidosis affected diseased goat there was a significant rise found. The values of sedimentation rate and methylene blue was 12.30 ± 3.466 , 14.20 ± 3.824 results found. It means there is a proportional relation between sedimentation rate and methylene blue reduction test. The result was statistically significant (p < 0.05). The significance level was 5%.

Nitrate is absent from both healthy and diseased goats. The outcome was statistically significant (p < 0.05). The significance level was 5%.

Table-5 shows that correlation between methylene blue reduction, number of protozoa and sedimentation test :

As presented in table, all biochemical tests showed a significant relationship between MRT and SAT, MRT and N.P and N.P and SAT. The methylene blue reduction test showed a significant relationship between Sedimentation rate and number of protozoa.Number of protozoa showed a significant relationship with sedimentation activity test. The methylene blue reduction test was proportional to sedimentation activity test. But number of protozoa showed inverse relationship with methylene blue reduction test and sedimentation activity test (Shah et al., 2013). The correlational was statistically significant (p<0.05).

Chapter 5: Conclusion

Conclusions from this study are that animals with anorexia, chronic acidosis, or indigestion showed clear changes in the color, odor, consistency, and pH of their rumen fluid, as well as changes in the motility and quantity of numinal protozoa. Rumination is associated with changes in the physiological parameters of rumen fluid and motility, as well as the number of rumen protozoa in anorexic or acidosis-affected goats in addition to body temperature, glucose levels increased in the body due to excess indigestible carbohydrates and rumen motility decreased. The findings of this study revealed that there is a proportional relation between pH, number of protozoa and methylene blue reduction test, sedimentation activity test, and inversely relation between pH and methylene blue reduction test. Ruminal and systemic alkalizers are used to correct ruminal acidosis and provide a quick solution in goats. Proper feeding practices and management should be suggested to the farmers to lower the risk of ruminal acidosis in goats.

Limitation

- There were few acidosis cases found in SAQTVH.
- Time was short to follow up with patients.
- Sometimes owners don't cooperate to collect samples from goats.
- The biochemical tests were very costly to perform.

Recommendations

The biochemical tests should be less costly in order to make a proper diagnosis. The owner should be aware of their feeding of goats. The goat should not be fed excess grass, wheat, bran, or rice. The samples should be collected as soon as possible and brought to the lab.

List of Figures





Collection of rumen fluid by stomach tube

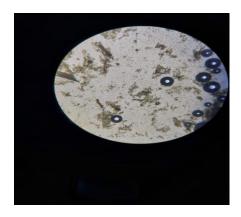
Determination of pH



Milky grey color



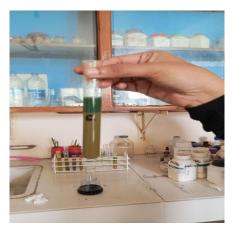
Methylene blue reduction test



Counting the number of protozoa in microscope



Sedimentation activity test



Methylene blue reduction test

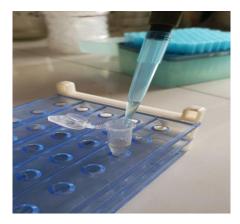




Fig: Serum after centrifugation of blood



Centrifugation of rumen fluid



Serum was taken in eppendorf tube by micropipette

References

- Alam, M., Das, B. C., Hassan, M. M., Ahaduzzaman, M., Al Faruk, M. S., & Hasanuzzaman, M. (2014). Ruminal acidosis-A case compilation study in SAQ Teaching Veterinary Hospital, Bangladesh. Veterinary World, 7(1), 38.
- Baran Sen, A. (2009).Study on analysis of physiological parameters rumen of rumen fluid in digestive disordered sheep. Chattogram Veterinary and Animal Sciences University Khulshi, Chattogram-4225, Bangladesh.
- Basak, D. N. Pan, S. and Chakrabarti, A.(1993) Physico-chemical and microbial changes in rumen liquor of experimentary induced lactic acidosis in goats. Indian. Journal of Animal Science 63: 263.
- Constable, P. D., Hinchcliff, K. W., Done, S. H., & Grünberg, W. (2016). Veterinary medicine: a textbook of the diseases of cattle, horses, sheep, pigs and goats. Elsevier Health Sciences. 10th ED. Saunders:Edinburg 2007, 169-250
- Das P, K., Mishra S,K., Effect of sudden change of feed ruminal activities of dairy cows and the results of stomach therapy on these animals. Indian Veterinary Journal 1972;49(10):1035-1040.
- Escareño, L., Salinas-González, H., Wurzinger, M., Iñiguez, L., Sölkner, J., & Meza-Herrera, C. (2012). Dairy goat production systems: status quo, perspectives and challenges. Tropical animal health and production, 45, 17-34.
- Gnanaprakasan , V. Prathaban, S and Srinivasan, S. R. (1990) A key role view on ruminant medicine . Department of Clinical Medicine, Madras p.22

- González, L. A., Manteca, X., Calsamiglia, S., Schwartzkopf-Genswein, K. S., & Ferret, A. (2012). Ruminal acidosis in feedlot cattle: Interplay between feed ingredients, rumen function and feeding behavior (a review). Animal feed science and technology, 172(1-2), 66-79.
- Gozho, G. N., Plaizier, J. C., Krause, D. O., Kennedy, A. D., & Wittenberg, K. M. (2005). Subacute ruminal acidosis induces ruminal lipopolysaccharide endotoxin release and triggers an inflammatory response. Journal of dairy science, 88(4), 1399-1403.
- Grubb, J. A., & Dehority, B. A. (1975). Effects of an abrupt change in ration from all roughage to high concentrate upon rumen microbial numbers in sheep. Applied Microbiology, 30(3): 404-412.
- Kamra, D. N. (2005). Rumen microbial ecosystem. Current science: 124-135.
- Lorenz, I., & Gentile, A. (2014). D-lactic acidosis in neonatal ruminants. Veterinary Clinics: Food Animal Practice, 30(2), 317-331.
- Mackie, R. I., Gilchrist, F. M., Robberts, A. M., Hannah, P. E., & Schwartz, H. M. (1978). Microbiological and chemical changes in the rumen during the stepwise adaptation of sheep to high concentrate diets. The Journal of Agricultural Science, 90(2), 241-254.
- Meyer, N. F., & Bryant, T. C. (2017). Diagnosis and management of rumen acidosis and bloat in feedlots.Veterinary Clinics: Food Animal Practice,33(3):481-498
- Nichols, R. E., & Penn, K. E. (1958). Simple methods for the detection of unfavorable changes in ruminal ingesta. Journal of the American Veterinary Medical Association, 133, 275-277.

- Ogimoto, K., & Imai, S. (1981). Atlas of rumen microbiology. Japan Scientific Societies Press.
- Penner, G. B., Beauchemin, K. A., & Mutsvangwa, T. (2007). Severity of ruminal acidosis in primiparous Holstein cows during the periparturient period.Journal of dairy science,90(1):365-375.
- Saravanan, S., Ramprabhu, R., Mohanapriya, T., & Chitra, R. (2021). Ruminal lactic acidosis and its haematobiochemical alterations in free ranging goats. Journal of Entomology and Zoology Studies, 9:1773-1777.
- Shah, O., Shaheen, M., Gupta, G., LATHER, A., Nabi, S. U., Wani, A. R., & Hassan, M. (2013). Clinical and haemato-biochemical changes in rumen acidosis in south down breed of sheep in Kashmir valley.Haryana Veterinarian,52:60-62
- Tufani, N. A., Makhdoomi, D. M., & Hafiz, A. (2013). Rumen acidosis in small ruminants and its therapeutic management.
- Udainiya, S., Tiwari, A., Singh, B., & Gawai, P. (2020). Diagnosis and treatment of ruminal acidosis affected goat.International Journal Of Life Sciences and Applied Sciences, 2(1):13-13.
- Valmik, S., Padmaja, K., Nagaraj, P., & Reddy, A. (2017). Studies on rumen fluid analysis in ruminal acidotic goats. International Journal of Livestock Research, 7(9), 250-258.

Biography

My name is Proma Roy. My home town is Chattogram. I graduated from Dr. Khastogir Govt. Girls' High School in Chattogram with a Secondary School Certificate (SSC) in 2015 and from Chattogram College with a Higher Secondary Certificate (HSC) in 2017. In the 2017-18 academic year, I enrolled at Chattogram Veterinary and Animal Sciences University, Bangladesh to pursue a Doctor of Veterinary Medicine (DVM) Degree.

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