

**The Report On Physiological And Biochemical
Changes Of Ruminal Fluid And Blood In Alkalosis Cow,
SAQTVH, Chattogram, Bangladesh.**



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

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**The Report On Physiological And Biochemical
Changes Of Ruminal Fluid And Blood In Alkalosis Cow,
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Statements of Author

I, **Rumi akter**, hereby declare that I have completed all tasks and provided the information in this report. The data was gathered through publications, websites, and other sources both domestically and abroad. All citations have been properly acknowledged. As a result, I am entirely responsible for gathering, combining, preserving, and publishing all of the data that has accumulated in this report.

The Author

October, 2023

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Abbreviation

Short Form	Full Form
CVASU	Chattogram Veterinary and Animal Sciences University
SSC	Secondary School Certificate Examination
HSC	Higher Secondary Certificate Examination
%	Percentage
:	Ratio
mg/dl	Milligrams per deciliter
mmol/L	Milimoles per liter
Min	Minute
ed	Edition
pH	Potential of Hydrogen
C	Celcius
cu.mm	Cubic milimeter
ml	Milliliter

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Honorable

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Abstract

The present investigation was carried out to record the occurrence, causes, clinical findings, Physical and Biochemical changes of rumen fluid and Hematological changes of blood of a cow affected with Alkaline indigestion. There was history of two days ill animal excessive feeding soybean meal, some urea molasses and some pumpkin. Affected cow exhibited clinical signs as complete anorexia, dullness and depression, ruminal atony, constipated pasty faeces, twitching of ears and facial muscle. The parameters like ruminal fluid was found to be altered in alkaline indigestion as pH was 8.5, color changed to dark green color, consistency changed to watery, the odor becomes aromatic and decreased ruminal protozoan activity. Biochemical changes of ruminal fluid were also altered in this condition. The blood test was done by Humalyzer 3000. Serum sodium, potassium, chloride was decreased from normal range, but only serum glucose was increased in blood at alkaline indigestion. Result of serum sodium was 128.7 mmol/L, serum potassium was 2.2 mmol/L, serum chloride was 87.8 mmol/L and serum Glucose was 88.3 mg/dl. Alkaline indigestion was diagnosed finally. Then the animal was treated with suitable medicine (with liquid vinegar, normal saline and vitamin B) and the condition was improved after treatment within 4 days and the abnormal parameters were improved towards normalcy.

Keywords: Ruminal fluid, ruminal ammonia, occurrence, parameters

Introduction

Digestive disorder like indigestion which causes an anorexic condition of the both large and small ruminants is the common case and considerable problem in Bangladesh. It hampers the growth and proper production of animal in the farm and again it causes great economic loss of farmers. A proper rumen environment and microbial activity are more important and necessary factors for good digestion and metabolism of feed for better growth and production of the animal. The members of the rumen microbial ecosystem, a complex consortium of different microbial groups living in a symbiotic relationship with the host, act synergistically for the bio-conversion of lignocellulostic feeds into volatile fatty acids which serves as a source of energy for the animals (Karma, 2005). Rumen mainly consists of rumen fluid and rumen micro-flora. Rumen contains some normal physiological and biochemical parameters like color, odor, consistency, pH, motility, number of rumen micro-flora. Digestion in the rumen is solely dependent upon microbial activity and physio-chemical parameters of rumen fluid (Cristine and Pugh, 1998). Mainly rumen micro-flora digest feed in the rumen, but when all the rumen parameters were changed then rumen dysfunction occur and feed does not digest properly. Some clinical sign is seen then like anorexia, loss of appetite, rumen dysfunction and other abnormal condition of the rumen. Examination of all parameters like pH, color, odor, consistency of rumen fluid, motility and number of rumen protozoa, rumen indigestion can be identified. The physical, microbial and biochemical alteration in the rumen is the important diagnostic tools for any abnormal or disorder of the rumen (Chakraborty, 1994).

The pH result if more than normal range then the condition will be considered as Alkalosis or alkaline indigestion and if less than normal range then it will be considered as Acidosis. Indigestion resulting from a change in pH of ruminal fluid exceeding 7.5 is referred to as alkaline indigestion or ruminal alkalosis (Vijayakumar *et al.*, 2010). Alkaline indigestion occurs usually due to excess and sudden intake of protein rich feed, urea feeding, abrupt change of ration, drinking of contaminated and sewage water and excessive feeding of straw over a prolonged

period of time (Misra and Tripathy, 1963). Most of the time soybean meal is commonly fed to dairy cows and growing cattle due to its high energy and nitrogen content leading to alkalosis (Sauvant *et al.*, 2002). In case of alkalosis excessive ammonia produces in the rumen due to excess protein diet. Rumen microbes cannot utilize excessive ammonia and these ammonia absorbed beyond the gastrointestinal tract (Kumbharet *al.*, 2018).The increased level of ruminal ammonia results into increased rate of absorption (Huntington, 1986).

When too much ammonia enters the portal circulation, it is carried to the liver where it is transformed into urea. After that, the urea is eliminated by the kidneys in the form of urine or recycled into the rumen through blood or saliva. An excessive protein intake will cause the ruminal fluid's pH to become alkaline due to the excessive buildup of ruminal ammonia, nitrogen, and a decrease in the VFA. Excessive alkaline pH inhibits ruminal contraction and cause paresis of rumen musculature. The range of pH in alkaline indigestion remains between 7.5 to 9.5. When the pH exceeds 7.5, there is a decrease in the number of ruminal microflora and rumen fluid appears watery and dark brown in colour, odour is changed also. It may become slimy and having putrid odour. Impotence and difficulties with the digestion of cellulose are caused by the paresis of the rumen muscles and the high pH of the ruminal fluid (Chakrabarti, 2014). The principal signs of alkaline indigestion include anorexia, moderate decrease in milk yield, depression, increased pulse and respiratory rate and passage of semi-solid or pasty faeces, atony of rumen along with hepatic, renal, circulatory and nervous disturbances along with leucocytosis (Stocker *et al.*, 1999; Mode *et al.*, 2006; Radostits *et al.* 2006).Alkaline indigestion in cattle is relatively poorly studied compared to other forms of indigestion (Radostits *et al.*, 2006).

For these reasons the investigation was conducted with the following objectives:

1. To examine the incidence, aetiology, clinical manifestations of ruminal alkalosis cattle.
2. 2. To record bio-chemical and hematological changes of ruminal alkalosis cattle.

MATERIALS AND METHODS

Selection of animal

The study was carried out in one cow which was brought to the Shahedul Alam Quadary Teaching veterinary hospital(SAQTVH) of Chattogram veterinary and Animal Sciences University (CVASU) for treatment. The cow was suffering from digestive problems which had the occurrence of Alkaline indigestion from two days. The cow was selected for the investigation on the basis of the occurrence of digestive problem (Alkaline indigestion) irrespective of age, breed, sex. It was a 4.5 months female Sahiwal breed. The animal came with clinical signs like complete anorexia, dullness and depression, ruminal atony, constipated pasty faeces, twitching of the ears and facial muscle. There was history of two days illness with excessive feeding soybean meal, some urea molasses and some pumpkin. Laboratory tests were performed at Biochemistry laboratory in CVASU, Chittagong, Bangladesh. Rumen fluid was collected with a stomach tube. After collection, it was transported to Laboratory through jar with a layer of aluminum foil to maintain anaerobic environment. Then further Laboratory tests were done in the Lab.

Physical and biochemical parameters in Sahiwal breed

The clinical examination of cattle suffering from alkaline indigestion included anamnesis, recording of clinical parameters like rectal temperature (RT), heart rate (HR), respiration rate (RR), ruminal motility and hydration status of animals (Kelly, 1984). All the physiological parameters of the affected cow were recorded.

Color

The color of the rumen fluid was observed by naked eye. Then the abnormal color was compared with normal healthy animal's rumen fluid.

Odor

The odour of the collected rumen fluid was observed by organoleptic test and record accordingly. It varies with the normal physiological condition of the rumen.

Consistency

Immediately after collection of rumen fluid consistency was observed and it varied from the normal physiological consistency of rumen fluid.

pH

Rumen pH is an important indicator for diagnosing indigestion problems. It is an essential factor for good digestion and microbial activity. The pH can be determined with pH Paper and digital pH meter in the Laboratory. The pH of rumen fluid changes due to different indigestion problems like rumen acidosis, alkalosis which ceases the microbial activity of the rumen. The sample pH was tested with pH paper. One pH paper soaked in the rumen fluid and then waited for color changing and then compared to the standard.

Examination of rumen protozoan activity and motility

Examination of rumen protozoan activity and motility need a drop of fresh rumen fluid which was taken in a clear glass slide. A cover slip was placed over the glass slide and then examined under microscope. The motility of rumen protozoan was graded arbitrarily as -, +, ++, +++, i.e.-none, mild, moderate and vigorous respectively depending on the motility. Moderate (++) to vigorous (+++) motility indicates normal protozoan activity.

Counting of rumen protozoa

This test was done with the help of working solution. So, Working solution needs to prepare at first. Working solution was made by a mixture of 1 ml formal saline (0.85% normal saline with equal part of formalin), 20.5 ml 5% Lugol's iodine and 1.5 ml 30% Glycerine.

Method of counting

Counting of protozoa need freshly collected rumen fluid which was diluted with a working solution (1:10). Then shook the mixture gently and 0.1 ml was placed on a counting slide with a pipette. Then the slide covered with a cover slip. Differential total counts were carried out by very low power (10x) objectives. Every side as upper left, upper right, middle one, lower left & lower right to be counted but have to ignore touching the bottom & right border for proper counting. Average count is taken as No.of protozoa / cu.mm. The number of microorganisms in per ml of rumen liquor, we multiplied the count of protozoa by 5000 (50x1000) dilution factor. The result of the test was expressed as total count per ml ($n \times$). Then it was graded as vigorous (+++), moderate (++) , few (+) and none (-). The result then compared with previous studies and standard value.

Sedimentation test

Rumen content was mixed properly at the beginning of the test. Then 10 ml rumen fluid was taken into test tube and kept it undisturbed until the sample sedimented.

Biochemical parameters

Some biochemical tests were done like Methylene blue reduction test (MBRT), Nitrate reduction test (NRT) by using reagent and determination of glucose, potassium, Chloride and Sodium in rumen fluid by using Humalyzer 3000 in the biochemistry Lab of CVASU.

Hematological analysis

Blood collection had to proceed at first to the hematological test. The blood sample was collected from the cow. About 5 mL whole blood was collected aseptically with the help of a disposable syringe from the jugular vein and transferred into the red screw capped tubes without containing 0.5 ml of 1% ethylene diamine tetra acetate (EDTA) solution as an anticoagulant. Allowed the blood to clot by storing it at room temperature. Immediately the whole blood had been collected, and 15 to 30 minutes are required for this. Then Centrifugation was done at 3000 rpm for 10 minutes and collected the serum from the supernatant. Serum was collected into a

clean polypropylene tube (preferably a minimum Eppendorf tube holding 1.5 ml) using a Micro- pipette. The serum sample should be refrigerated at -80°C if it is not examined immediately following collection (Texas Biomedical Research Institute). Then test was done by using Humalyzer 3000 to test serum sodium, serum potassium, serum Chloride and serum Glucose.

Treatment

The affected cow was treated with Liquid Vinegar, Normal saline and Vitamin B. The treatment was given by duty doctor Dr. Md. Ahaduzzaman, Department of medicine and surgery, CVASU. Liquid vinegar used orally, 20 ml mixed with equal amount water for two days, 1L normal saline gave in intravenously for two days and 4ml Injection Bional Forte (Vitamin B1, B2, B6, B12) gave in intramuscularly for 5 days. The follow-up of this treatment was taken daily during treatment continued. And within 4 days the animal recovered. No further sign was occurred.

Figure



Fig 1: Examination of color and pH

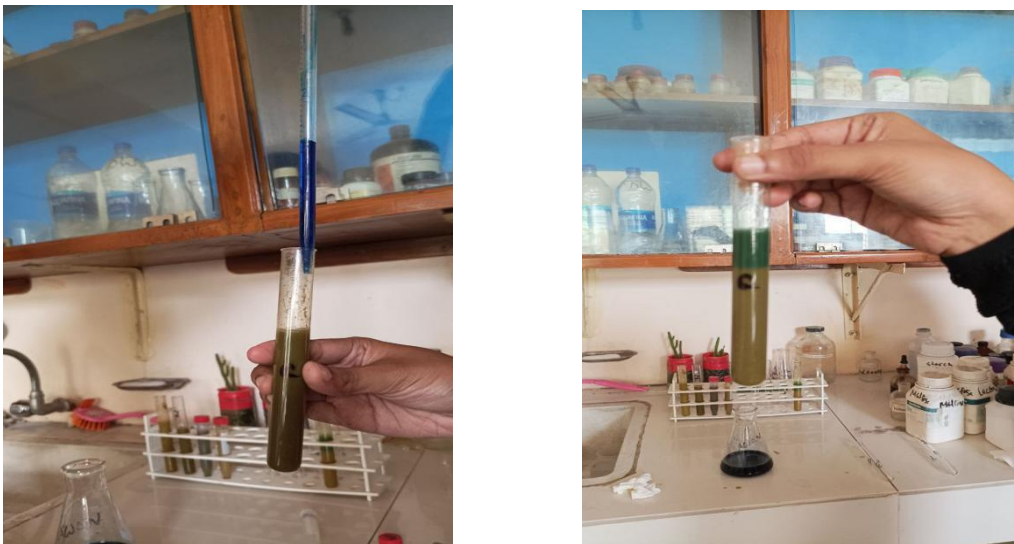


Fig 2: Methylene Blue Reduction Test (MBRT)



Fig 3: Examination of biochemical parameter (blood)

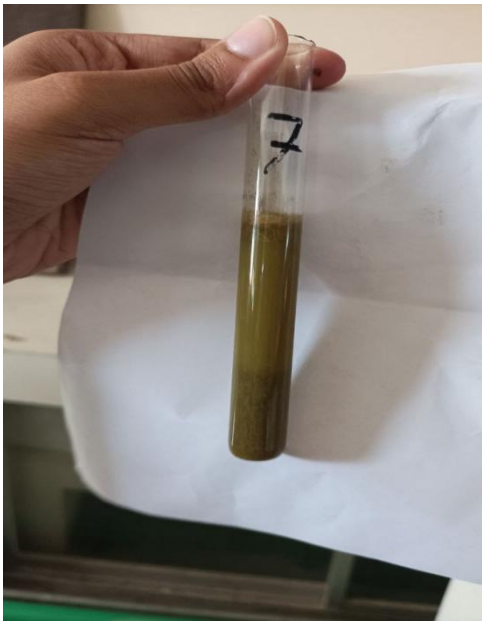


Fig 4: Sedimentation activity test and Rumen protozoa



Fig 5: Treatment time

RESULT AND DISCUSSION

In the present study, the case of digestive disorder was reported and was diagnosed as alkaline indigestion based on high ruminal fluid pH (≥ 7.5), history of ingestion of protein-rich feed. The test result was discussed in details.

Color of the rumen fluid

The color of rumen fluid depends on the diet and abnormal condition of the rumen. The test sample color changed to dark green color which indicates alkaline indigestion. While the normal rumen fluid is olive to brownish-green (Bayne, J. E. *et al.*, 2021). Previous workers demonstrated that the colour of rumen liquor turned dark brown or greenish brown in alkaline indigestion, while the colour of rumen fluid turned milky grey in acid indigestion (Chakrabarty, 1996). So, a similar observation was found in the color of rumen fluid in alkaline condition with this researcher.

Odour :

Normal rumen fluid is aromatic in odour (Bayne, J. E. *et al.*, 2021). The test result was putrid odour which was different from normal fluid.

Consistency :

The consistency of rumen fluid in 'normal', healthy cattle is slightly viscous. Anomalies in the consistency of the rumen fluid when rumen function and inactivity occur, the viscosity of rumen fluid changes from somewhat viscous in healthy, normal cattle too watery (Petrovski, K. R. 2017). Consistency of the test sample was found watery which indicate dysfunction of the rumen.

pH :

Usually the normal pH of rumen fluid ranges 6.2–6.8 (Blood, D. C. *et al.*, 1989). The test result was 8.5 which was greater than normal value. Generally, during alkaline indigestion, pH ranged from 7 to 8.5 (Chakrabarty, 1996 and Gnanaiprakasam *et al.*, 1990). The test result indicated alkaline indigestion which was similar to the research work of Chakrabarty, 1996 and Gnanaiprakasam *et al.*, 1990.

Motility and number of rumen protozoa :

In the study, the motility of the rumen protozoa was nil and number of the rumen protozoa were zero. It indicated that, in alkaline indigestion rumen protozoa were died due to excess ammonia production and due to high pH production. There is a correlation between protozoan motility and rumen pH which is concluded with the Dchority and Scott (1967). The study showed that, during the alkalosis rumen protozoal count less or zero which is in agreement with the earlier findings (Purser and Moir, 1959).

Sedimentation test (SAT)

The test sample was taken 12 minutes to sediment which indicated the abnormal rumen condition. The normal sedimentation time of rumen fluid is 5-10 minutes. The observed result was similar to the earlier findings (KR Petrovski - Journal of Dairy Veterinary Science, 2017).

Biochemical parameters

The test sample was taken 12 minutes for methylene blue reduction which indicated for abnormal rumen condition. The normal methylene blue reduction time of rumen fluid is 2-6 minutes (KR Petrovski - Journal of Dairy Veterinary Science, 2017). The more time required for reduction of methylene blue dye in rumen fluid might be due to inactivity or death of normal micro- flora and fauna (Vasu and Nagarajan, 1985). In the study, the motility of the rumen protozoa was nil and number of the rumen protozoa were zero which was similar to the research. So, the result of the test indicated the indigestion problem.

The result of the NRT was negative. This meant that nitrate had been present and had been reduced to form nitrite.

Hematological analysis

The result of hematological test Were: Serum sodium was 128.7 mmol/L which was decreased than normal value. The normal value of serum sodium is 132-154 mmol/L. Serum potassium was 2.2 mmol/L which was also decreased than normal value. The normal value of serum potassium is 3.9-5.8 mmol/L. Then serum Chloride was 87.8 mmol/L which was also decreased from the normal value. The normal value of serum chloride is 100.4- 108.2 mmol/L. The test showed the increased value of serum glucose in blood from the normal value. The normal range of serum glucose is 45-75 mg/dl and the result was 88.3 mg/dl in blood.

Table 1: Clinical observations in healthy and alkaline indigestion affected cattle.

K. Jerry, Jhon, Clinical Biochemistry of Domestic animals, 5th edition.)

Clinical parameter	Result	Normal range
Rectal temperature (F)	101.5	101.5-103.5
Heart rate(beats/min)	77	55-80
Respiration rate (breaths/min)	22	10-30
Skin fold test	3	2-4

Table 2: Physical and Biochemical parameters of healthy and alkaline indigestion affected cattle. (Blood, D. C and O.M. Radostits. 1989. Veterinary Medicine 7th edition., Bayne, J. E., & Edmondson, Purser and Moir, 1959., M. A. 2021, Petrovski, K. R. 2017, Vasu and Nagarajan, 1985, ER Duarte. 2018, KR Petrovski - Journal of Dairy Veterinary Science, 2017

Physical and Biochemical parameters	Result	Normal range
Color	dark green color	Olive to brownish green
Odour	Putrid	Aromatic
Consistency	Watery	Slightly viscous
pH	8.5	6.2 to 6.8
Motility	Nil	Moderate to vigorous
Number of rumen protozoa	0	10 ⁴ to 10 ⁶
SAT (time)	12 min	5-10 min
MBRT (time)	12 min	2- 6 min
NRT	Negative	

Table 3: Electrolyte of rumen fluid in alkaline indigestion affected cattle.

Electrolyte name	Result
Glucose	31.3 mg/dl
Potassium	31 mmol/L
Chloride	30.3 mmol/L
Sodium	68.8 mmol/L

Table 4: Hematological parameters (electrolytes) in healthy and alkaline indigestion affected cattle. (J. Jerry, Jhon, Clinical Biochemistry of Domestic animals, 5th edition.)

Electrolyte name	Result	Normal range
Serum Sodium	128.7	132-152 mmol/L
Serum potassium	2.2	3.9- 5.8 mmol/L
Serum chloride	87.8	100.4-108.2 mmol/L
Serum glucose	88.3	45-75 mg/dl

Diagnostic procedure of the cow was done properly by using different methods and the result diagnosed the clinical case as Alkaline indigestion or alkalosis. There was some variation in hematological parameters like serum sodium, potassium and chloride were decreased with this indigestion. Every test result was nearly similar to the findings of the research work. The physical and clinical observation was recorded before and after recovery. The animal took 4 days to recovery. Due to feed excess amount high protein-rich diet this condition occurred. Treatment was continued until it recovery and finally the animal was cured.

CONCLUSION

In Bangladesh, there are thousands of village where rural people rear cattle in their home They feed their cattle traditional cattle feed and commercial feed also. Sometimes unsafely they feed their cattle protein rich feed like soybean meal, fish meal, urea molasses etc. These feed cause production of excess amount of ammonia in rumen and then rumen flora are failing to metabolize this excess amount ammonia and then cause alkaline indigestion. Most of the time soybean meal is commonly fed to dairy cows and growing cattle, due to its high energy and nitrogen content leading to alkalosis. This condition changes normal rumen environment and indigestion occur. Alkalosis causes high rumen pH (more than 7.5), abnormal color, odour, consistency, electrolyte of rumen fluid. Excessive alkaline pH inhibits ruminal contraction and cause paresis of rumen musculature. Impotence and difficulties with the digestion of cellulose are caused by the paresis of the rumen muscles and the high pH of the ruminal fluid. The principal signs of alkaline indigestion include anorexia, moderate decrease in milk yield, depression, increased pulse and respiration rate and passage of semi-solid or pasty faeces, atony of rumen along with hepatic, renal, circulation and nervous disturbances along with leucocytosis. The present case shows clinical signs as complete anorexia, dullness and depression, ruminal atony, constipated pasty faeces, twitching of the ears and facial muscle. The parameters like ruminal fluid was found to be altered in alkaline indigestion as pH was 8.5, color changed to dark green color, consistency changed to watery, the odor becomes aromatic and decreased ruminal protozoan activity. The animal was treated with Liquid vinegar, Normal saline and vitamin B for 5 days. The dose of treatment was completed properly. The animal was cured within 4 days. Due to feed high protein feed cause this alkaline indigestion which changed the biochemical property of rumen fluid and also changed the electrolyte of blood and progressive onset of clinical symptoms after long-term use of such feed. Due to lake of balance feeding knowledge of farmers, this type of situations has been occurred. To lessen the incidence of alkaline indigestion in cattle, careful planning about balanced feeding with an accessible amount of feed and a decent source of carbohydrates during the period of shortage is required.

Limitations

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

1. Previously, no study on biochemical parameter of ruminal fluid
2. Furthermore, there were also fewer reports of digestive disorders.
3. The owner rarely permitted the collection of the animal's blood.
4. Sample preservations were difficult in sometimes.

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Biography

This is Rumi Akter, the second child of Md. Enamul Haque and Farida yasmin, doing her graduation in Doctor of Veterinary Medicine (DVM) at Chattogram Veterinary and Animal Sciences University under the Faculty of Veterinary Medicine. She passed the Secondary School Certificate Examination (SSC) in 2017 from Saraipara city corporation high school and the Higher Secondary Certificate Examination (HSC) in 2017 from Govt. Chattogram college, Chattogram. Currently, she is doing her yearlong internship. She has a great interest in pet practice and wanted to be a veterinary surgeon or any Government senior officer in future.