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Study on Digestive Physiology of Rumen in Selected Cattle Slaughtered at Addis Ababa Abattoir, ETHIOPIA

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Abstract
A cross sectional study was conducted from November 2009 to April 2010 on 210 cattle in Addis-Ababa abattoir, originating from every corner of the country including Addis Ababa for the aim of quantifying functional rumen microbial groups and looking at the factors affecting the ruminal environment. During the study, the randomly selected animals were restrained by the assistants and the specimen was collected by inserting the stomach tube until it reaches the rumen. Likewise collecting the rumen content was done immediately after the animals get slaughter. Finally the rumen content were examined for their color, consistency, cotton thread breaking ability and their activity (denoted by motion) of the protozoa on microscopic glass slide. Up on doing the above examinations prevalence of 77.8% (87/210), 82.8% (30/210) was recorded from color content of green and yellow-brown respectively with statistically significant difference ($P<0.05$). Prevalence of 75.9% on slight viscous, 71.4% extremely and 2% milky were also recorded when looked on the consistency. Furthermore, the pH and Cellulose (Cotton breakage ability) were statistically significant ($P<0.05$) with the prevalence of 75.1% (basic) and 4.8% (acidic); and 82.4% (unbroken cotton) and 7.5% (broken) recorded respectively. In conclusion, this study has indicated that there is a serious decrease in the motile protozoa number, especially those of medium sized and large sized with over dominance of the small sized protozoa. This is the basic base line data for rumen digestion environment and for future further studies.
Keywords: Abattoir, Cattle, Rumen.

1. INTRODUCTION

The bovidae family (3,000 million heads) is the most diverse group (155 species) and includes the African antelope, buffalo, cattle, sheep and goats. Nutritional strategy and adaptation to feed resources have played a major role in the evolution of modern herbivores.[33]

Rumen contains a vast number of anaerobic eukaryotic and prokaryotic microorganisms, which break down ingested feed materials to short chain fatty acids that are absorbed, to be used by the host animal for energy.[23] It has a major influence on the health of the animal, so understanding the composition and function of the ruminal microbiota will also help to improve the health and welfare of the livestock.[34,26,9]

The rumen microbiome consists of a complex microbial community composed of bacteria, archaea, protozoa, and fungi. The metabolic activity of these microbial symbionts converts complex fibrous substrates into volatile fatty acids (VFA) and microbial protein that are used by the ruminant host for maintenance, growth and lactation.[22] Although the rumen is one of the most effective systems for degrading plant cell walls, less than 50% of cell wall carbohydrates are digested in low quality forages such as straw.[12]

Microbiome of rumen is a dynamic system that rapidly changes with diets. The type of forage alters the rumen microbial community composition mainly due to its specific fiber structure, which determines fermentation products and ruminal pH. [5,25] Change in microbial community could provide us a clear understanding of interaction between forage and runinal microbes.[37]

Rumen protozoa may represent up to 50% of microbial biomass and play a key role in ruminal N and carbohydrate metabolism.[35] In contrast to bacteria, protozoa have been shown to have very little host specificity.[14,15]

The ruminant animals play an important role especially in the livelihood of farmers in the developing world, providing substances as milk and meat, animal traction, manure for crop production, energy, cash income from sales of their products and a safety net of capital assets to face risks and misfortune in harsh environment.[4]

Examination of rumen content is often essential to assist in determination of the state of rumen environment and digesta after rumen content was obtained mainly from slaughtered animal and sometimes by rumen pump via stomach tube. The major relevant notice during collection is avoiding contamination of the sample with saliva.[27]

The microbial protein synthesis in the rumen depends on the growth of microorganisms and on the efficiency of using energy and nitrogen substrates, which is the main constituent of animal's body and, therefore, vital for maintenance, growth and reproduction processes. The net result of these reactions going on in the rumen is responsible for the bioconversion of feed in to such form that is utilizable by the animal as a source of energy.[31]

Color of rumen fluid of apparently healthy cattle varies according to feed type, from gray and olive to brownish green; green in grazing cattle, grey in those given fodder beet, yellowish-brown in those given maize

silage or straw. Rosenberger (1979) reported that abnormal color like are milky grey can be observed in the time of acidosis. Normal consistency of rumen fluid of cattle is slightly viscous. Extremely viscous samples may be composed chiefly of saliva, in which case another sample will have to be taken.[28]

The pH of rumen fluid ranges from 5.5-7.0 in apparently healthy cattle on a balanced ration. A pH paper with half unit sensitivity is sufficient to diagnose ruminal acidosis or alkalosis. Cattle on high carbohydrate diets have lower pH than those on roughage diets. Acid pH less than 5.5 in ruminants indicates ruminal acidosis while ruminal pH greater than 7 indicates ruminal alkalosis. Simple ruminal inactivity or anorexia results in ruminal alkalosis.[8]

Gram stained smears from rumen fluid samples can be prepared for the identification of rumen bacteria. Mainly gram negative bacteria will dominate in physiologic rumen fluid but in ruminal acidosis gram positive streptococci and lactobacilli predominate.[36]

Rumen fluid of apparently healthy cattle has a motile fauna while the abnormal fluid have sluggish or no movement in case of acidosis (Smith, 1996)[31]. And hence this motility of ciliate protozoa is examined in a fresh film under magnifying microscope. And their motility is judged as highly mobile and very crowded (+++), motile and crowded (++), sluggish motility and low number (+), no or alive infusoria (0) (Rosenberger, 1979).

The rumen is an open, dynamic, stable, a very diverse and complex microbial ecosystem and is highly specialized, pre-peptic adaptation of the digestive tract that facilitates the storage and microbial processing of a large quantity of plant material.[13] The digestion of plant material and subsequent conversion of primary ruminal products such as volatile fatty acids (VFA) for energy requirements to the host ruminant and compounds incorporated into microbial cells are performed through synergistic act and a complex symbiotic relationship of billions of microbes within the rumen.[21] As of the genotypic and phenotypic mounting evidence, diversified major functional groups of ruminal micro-organisms are hosted in different ruminant species and geographical regions.[10]

Highly diversity rumen microbial ecosystem consisting of bacteria (more than 50 genera); ciliated protozoa (more than 25 genera); an aerobic fungi (representing 5 genera) and bacteriophages are found in the rumen.[11] A comparison of numbers and relative volumes of bacteria and protozoa indicated that while protozoa are far less numerous than bacteria, they are so much larger than the bacteria that they may occupy a volume nearly equal to that occupied by the bacteria. The most important ruminal protozoa are anaerobic ciliates that are differentiated on the basis of morphology.[17]

These microbial symbionts are adapted to survival under conditions of anaerpbiosis, high dilution rates, high cell densities and protozoa predation, and have evolved the capacity for efficient utilization of complex plant polymers such as cellulose and hemi-cellulose.[7]

Microbial digestion and synthesis of microbial components in the rumen requires certain conditions provided by the host. These include retention of digesta and ruminal microbes for prolonged periods of time, anaerobiosis, constant temperature (39^0C), neutral pH (7.0), and removal of end products.[29] In most circumstances, this environment is closely controlled by mechanisms such as the type and quantity of food consumed, saliva secretion during eating, mixing via ruminal contractions, absorption of end products (VFA, NH_3) and passage of undigested residues and microbial cells out of the rumen.[20]

Aiming at improving efficiency of post-ingestion digestion of feeds and ultimately to improve productivity in ruminants, several direct physico-chemical treatment of the feed[2] and various indirect biotechnological manipulation of rumen fermentation are currently being employed to modify the composition and activity of rumen microbial population, increasing propionate concentration in the rumen, depression of methane production and decreasing-dietary protein degradation by bacteria.[16,32,6] To bring such interventions into effect, characterization of microbes and various ruminal parameters under different dietary conditions was crucially important task.[38]

Knowledge about microbial (Protozoa) populations was obtained by microscopic observations, pH of the sample collected and by performing some tests. Microscopic observation helps in evaluating the number and activity of rumen flora and was used in monitoring the severity of the indigestion. The activity of rumen flora was mainly looked by their motility. Accordingly for normal activity of rumen flora ciliates and flagellates were found at rate of >6 organisms per field with small, large and medium sized protozoa being the large once dominant. However, in condition of indigestion the large protozoa start dying primarily following medium and small sized protozoa. Molecular based approaches have been adapted recently and resulting in much more comprehensive description of micro-organisms.[30,18] The objectives of this study were: To quantify functional rumen microbial groups (Protozoa's) in cattle at Addis Ababa abattoir and to assess factors affecting the ruminal environment.

2. MATERIALS AND METHODS

2.1. Study area

The study was conducted in Addis Ababa area from November 2009 to April. Addis Ababa has topography of

540km^2 and it is 2500m above sea level and receives rainfall of 1800m^2 in bi-modal pattern. Its long rainy season extends from June to September. Its annual average minimum and maximum temperatures are 10.7^{0}C and 23.4^{0}C respectively.[24]

2.2. Study population

The study animals were 210 local breeds of cattle. The exact age and previous history of management or health status of the cattle were not known. However, all of them were clinically normal during ante mortem examination. These animals originated and brought to the abattoir from different area of the country including Addis Ababa. It was impossible to trace back individual animals to their source of origin because of lack of identification and reluctance of cattle dealers and owners to provide information. Some of the cattle dealers don't even know the initial origin of their animals. All the cattle slaughtered were males, adult and belonged to indigenous breeds.

2.3. Study design and sampling strategy

2.3.1 Study design

A cross sectional was under taken to identify the quantity the normal flora from the stomach of the cattle and to develop a base line data for normal rumen fluid for indigenous breeds and later all it can be utilized for further therapeutic purposes. Each week a two days visit was made and after ante mortem and postmortem examination rumen contents was collected from different randomly selected slaughtered cattle. Samples were labeled using the animal code number and the specimens were immediately transported in an ice box to the laboratory for examination.

2.3.2. Sample collection

Each study animal was individually identified and restrained by an assistant and kept fixed. After keeping fixed the mouth gag was put inside the mouth of the animal. Then after the stomach tube was inserted until rumen odor come out. The sample was replaced in to the sterile container to which 0.3 ml of 10 % glucose was added.[1] After labeling, the container was kept in an ice box and transported to the laboratory.

Likewise following the slaughter of the identified animal, collection of the specimen was held. After slaughter, immediate collection of the rumen content was also done by cutting the rumen. The rumen was churned to have an equal distribution of the floras and collected to labeled contains. Again the same to the above 0.3 ml of 10 % glucose was added to the specimens. In the abattoir samples were immediately examined the color of the rumen content and the pH using the litmus paper. Again in the laboratory the samples were immediately examined by direct microscopic smear preparation to look at the motile protozoa and prepare cellulose digestion tests.

2.4. Sampling methods

The animals were selected using systematic random sampling using regular interval to study rumen flora analysis of cattle presented in Addis Ababa abattoir for slaughter**.**

2.5. Study methodology

Ante mortem examination: In this abattoir ante mortem examination was performed before slaughter and during this time body condition scoring, tick infestation and any other abnormalities were observed and recorded. During ante mortem examination each study animal was marked for the identification by writing a code number on its gluteal region by using permanent (not washable) ink. The animals which passed ante mortem examination were then selected for rumen content collection.

Post mortem rumen content collection: In the post mortem examination all study cattle were examined for lesions. Those animals which did not reveal any gross lesion were selected for sample material collection. Immediately after slaughter in the evisceration stage, the fore stomachs were carefully removed from the abdominal cavity and opened and explored for abnormal contents as well as for the presence of any foreign materials by visualization and palpation. Then rumen content was taken by gentle squeezing of ingesta from the rumen manually. The rumen content so collected was immediately transported to laboratory for further analysis.

2.4. Protozoa examination

2.4.1. Microscopically examination

The rumen content in the container were thoroughly agitated and mixed. Then a drop of specimen were smeared on the microscope glass slide, covered with cover slip and examined under 10x magnification power microscope. From the slide we are going to look the motile protozoa dominating the slide. Accordingly we were identified small medium and large sized protozoa on the microscopic glass slide.

2.4.2. Color of the content

Since color of the rumen content is among the indicative for some conditions, it was examined by naked eye

after collection. The color of the specimen varies on their feeding behavior and disease condition.

2.4.3. Cellulose digestion test

This test is among the useful procedures in examination of the rumen environment. 10ml specimen was added into test tube together with 0.3ml of 10% glucose. Then a cotton thread was immersed in to the test tube containing the specimen. Finally introduce into the 37^0C incubator for 48hr to look at the breaking power of the protozoa on the cotton.

2.4.4. Viscosity of the content

The contents viscosity was examined after collecting the specimen either after slaughter of the animal or by using stomach tube. Accordingly we can appreciate the content as slightly viscous, extremely viscous or watery.

2.5. Data analysis

All the results were recorded for each study animal. All data collected during the study period were entered and stored in Ms excel worksheet. Before subjected to statistical analysis, were analyzed using SPSS Microsoft software versions 16.0. Descriptive statistical analysis was used to summarize the data and present the data collected.

3. RESULTS

In this study, a total of 210 cattle were examined by taking their rumen content. Different results was recorded when we were looking the motile protozoa in relation to consistency, color of content, pH of the content and the test held. In general from the 210 samples 143 (68.1%) contain small sized protozoa's 5 (2.4%) contained medium sized; 22 (10.5%) large sized, when observed in green, milk, yellow-brown and brown color rumen contents respectively. The prevalence of influence of the factors (color content) is displayed in table 1. Statistically significant difference was recorded (P<0.05) among different color content of the feed consumed by the animal, the highest being 77.8% followed by 82.8% from green and yellow-brown respectively.

Table 1: Prevalence of color content in rumen environment in cattle

Color of content	**Number animal (n)**	**%**	**Number of motile protozoa (%)**				
			Small size	Medium	Large	All die	Small & medium
Green	20	9.5	87(**77.8%**)	0(1%)	10(8.8%)	3(2.7%)	11(9.7%)
Milky	18	8.6	2(11.1%)	0(%)	0(0%)	16(88.9%)	0(0)
Yellow-brown	113	53.8	30(**82.8%**)	5(10%)	10(20%)	2(4%)	3(6%)
Brown	59	28.1	24(60%**)**	0(0%)	2(6.9%)	1(3.4%)	2(6.9%)
Total	210	100	**143(68.1%)**	**5(2.4%)**	**22(10.5%)**	**22(10.5%)**	**16(7.6%)**

P-value=0.000, df=15

The study has also revealed that statistically significant difference (P<0.05) in consistency of the content, the highest being in slight viscous (75.9%) followed by extremely viscous (71.4%). The prevalence of consistency was described in table 2.

Table 2: Prevalence of consistency in motile protozoa in cattle rumen content

Consistency	**Total examined animal (n)**	**Number of motile protozoa (%)**				
		Small sized	Medium	Large	All die	Small & medium
Slight viscous		**88(75.9%)**	2(1.7%)	10(8.8%)	3(2.7%)	11(9.7%)
Extremely	210	**55(71.4%)**	3(3.9%)	10(20%)	2(4%)	3(6%)
Watery		0(0%)	0(0%)	2(6.9%)	1(3.4%)	2(6.9%)
Total	210	143(68.1%)	5(2.4%)	22(10.5%)	22(10.5%)	16(7.6%)

P-value=0.000, df=10

The study has also revealed that statistically significant difference (P<0.05) in pH of the rumen content occur. The prevalence was highest being in basic (75.1%) followed by acidic (4.8%) (Table 3).

Table 3: Summary of PH in motile protozoa in cattle rumen content

PH	**Total number examined (n)**	**Number of motile protozoa (%)**				
		Small sited	Medium	Large	All die	Small & medium
Acidic		**1(4.8%)**	0(0%)	1(4.8%)	17(81%)	2(9.5%)
Basic		**142(75.1%)**	5(2.6%)	21(11.1%)	5(2.6%)	14(7.4%)
Total	210	143(65.1%)	5(2.4%)	22(10.5%)	22(10.5%)	16(7.6%)

P-value=0.000, df=5

Likewise statistically significant association was recorded (P<0.05) for cellulose digestion test, the highest record being in un-broken of cotton (82.4%) and the least in broken (7.5%). Summary of the cellulose digestion in relation to motile protozoa was given below (table 4).

Table 4: Cellulose digestion test based prevalence in cattle

Cellulose digestion test	**Total number examined animal (n)**	**Number of motile protozoa (%)**				
		Small sited	Medium	Large	All die	Small and medium
Breakage of cotton	210	**3(7.5%)**	1(2.5%)	22(55%)	1(2.5%)	11(27.5%)
Unbroken cotton		**140(82.4%)**	4(2.4%)	0(0%)	21(12.4%)	5(2.9%)
Total	210	143(68.1%)	5(2.4%)	22(10.5%)	22(10.5%)	16(7.6%)

P-value=0.000, df=5

4. DISSCUSSION

There is luck of documented report in this study area in order to compare the obtained results and relative proportion of most functional ruminal microbes.

Despite the standardization of counting procedures, variations in protozoa counts among animals were high. Morphologically based speciation and enumeration were also very difficult as enormous but functionally minor microbial species can be found this method. Some protozoa species may simply have only morphological variants of the same proportional taxonomic unit.[3]

Type and status of feed influences composition and proportion of microbial community. Microbial organism may found in rumen, predominating one another when animal feed at a particular feed type. Some may be isolated from animals fed hay or cluster of organisms may be found in animal fed a grain based diet. It was also found that increased frequencies of occurrence of lactate-producing and lactate utilizing bacteria three days after a shift to a high-grain diet. Likewise quantity of these fibrolytic microbes was high during fibrous feed and lower in shifting to concentrate rich feed stuffs.[19]

Statistically significant difference was recorded ($P<0.05$) among different color content of the feed consume. Therefore, the occurrence in number of small sized motile protozoa, as indicated in the work was dominantly found in green (9.5%), yellow-brown (53.8%) and brown-green (28.1%) color contents. However in milky colored rumen content only 8.6% of prevalence was evident. The green, yellow-brown and brown color contents may be the result feeding at pasteur, straw and hay respectively which have large volume of fiber. However, the milky color may arise from concentrate feed or grain-diets. Concentrate and grain-diets are highly responsive for lactic acidosis, then after giving the feed a milky color. [15]

In most of the cases the colors of rumen content fluid observed were yellow and brownish green which were in agreement with Rosenberger (1979) who has reported that color of rumen fluid of apparently healthy cattle varied according to feed type, from gray and olive to brownish green; pure green in grazing cattle, grey in those given fodder beet, yellowish-brown in those given maize silage or straw and the color may be abnormally milky in grain over feeding. In the present study, the test of ruminal contents showed that mostly these animals that were slaughtered must have been fed with grass, hay and concentrate. However, Jasmin *et al.* (2011) reported that ruminal color was milky with rumen acidosis. The differences in the color of ruminal fluid observed may be due to the different proportion of these feeds, fed to the animals which were healthy in condition at the time of slaughter.

The isolation of medium sized and large sized protozoa get decrease when looked in the cattle with different color content, for instance green (8.8%, large), yellow brown (20% for large and 10% for small). This may arise from the condition of starvation as the animals arrive at the abattoir after traveling long distance without feed and water. Death of all protozoa where encountered in rumen content colored milky (88.9%). Milky colored rumen content may arise from feeding of the animal with grain-diet or concentrate feed. Since grain-feeds (with carbohydrate) are highly fermentable they markedly change the microbial environment in to acidic (<5.5 pH) causing death of protozoa and paradoxically flare up of lactobacilli.

The phase of rumen content, being extremely viscous, watery or slightly viscous also influences distribution of some microbes due to substrate affinity.[30] Accordingly the consistency was found significantly correlated with distribution of microbes (protozoa) being slightly viscous (71.4%) and watery (2%). As the viscosity is normal the microbes may get an access to the substrate and move free, however in the watery consistent the number of motile protozoa gets zero that may be due to death of all microbes by the lactic acid produced in the rumen. The rumen fluid from healthy slaughtered animals was observed to be mostly viscous in consistency. The consistent viscous consistency observed in the present result is in the agreement with Smith (1996) [28] who reported that it could be due to the presence of active rumen micro flora. The viscous consistency was observed in most of the cases followed by frothy and watery rumen fluid which indicate the normal rumen environment containing adequate population of ruminal flora, ingesta and varying quantities of digestive fluids.

The frequency of isolation of large and medium sized protozoa gets decreased during examination of the consistency. For instance, in slight viscous 8.6% and 1.7% for large and medium protozoa respectively as

compared to the 75.9% for small sized protozoa. This may be due to the reason that the animals mostly prohibited from taking feed until they get reach to the abattoir, so death of the large sized followed by medium sized protozoa will be evident. Likewise as Tajima et al. (2001)[30] indicated, starved animals produce large volume of saliva hinder the protozoa from their substrate affinity.

Microbial digestion and synthesis of microbial components in the rumen requires certain conditions provided by the host. These include retention of digest and ruminal micros for prolonged period of time, constant temperature (39^0C) and neutral pH (7.0) to slightly acidic (5.5).[29] The study was significant as showed in the table 3 above that the PH was significantly correlated with the presence of motile protozoa. Accordingly, 4.8% acidic and 75.1% basic pH was found. This may be due to the animal coming to abattoir was a little bit starved, and there were a lot of production of saliva. This produced saliva enters to rumen changing the rumen environment to basic because saliva contains large amount of $NaHCO_3$. The remain 4.8% may be due to the feeding of the coming animal with concentrate feed or grain diets which precipitate development of lactic acid production by the developing lactobacilli bacteria.

The distribution of protozoa with the pH, frequent death of all protozoa in acidic (81%) and in basic (75.1%) predomination of the small-sized protozoa was evident. Acidic ruminal environment (pH <5.5) was not favorable for the growth of protozoa rather for some gram positive bacteria. However, in case of the basic media the decrease in isolation of the large and medium sized protozoa were may be due to starvation of the animal and therefore death of mainly large and medium sized protozoa. In the present study, the pH of the rumen fluids examined was found to be mostly between 5.5-7.0. It has been reported that the pH of rumen fluid ranges from 5.5-7.0 in apparently healthy cattle on a balanced ration (Rosenberger, 1979). Slyter *et al*. (1970) have also stated that rumen microflora prefered a rumen pH between 6-6.7.2. A pH of 5.50 was considered as the cut-point between normal and abnormal by Nordlund and Garret and Garret *et al*, (1999) who reported it as the best cut-point to distinguish normal and fiber-deficient rations. Nordlund and Garret (1994) and Owens (1998) reported that the rumen fluid pH was dropped in animals when fed with large quantities of concentrate. In the present study, the rumen fluid pH was found to range from 5.5-7.0 which may be considered as normal base line value for the local breeds on available feeds.

The prevalence of occurrence of cellulose digestion by the ruminal microbes was also found significant. From the 210 taken sample only 7.5% was seen with breakage of by the small sited protozoan, 55% large sited protozoa. But the majority of test was found with unbroken cotton with prevalence of 82.4%.

The breakage of the cotton may mainly occur by the presence of large sited protozoa which can destruct the feed consumed mechanically or by the production of enzyme. However, in the unbroken, the small sized protozoa were found dominating the specimen. So, their size and their immaturity to produce enzyme may retard them from cutting the cotton containing high cellulose.

5. CONCLUSION AND RECOMMENDATIONS

The result of this study revealed that small-sized motile protozoa found dominant on cattle. Most of the small sized motile protozoa were identified from rumen content of green color and with small amount from yellowish brown and brown respectively. Likewise, rumen content with slight viscous was found to contain large amount of small sized protozoa (mainly) than extremely viscous and watery. Moreover measuring the rumen content's pH indicated, basic and with rear percentage of acidic, being small-motile protozoa dominantly found in the basic medium. Finally the result also showed the small-motile protozoa dominating the rumen content were with power of breaking of the cotton thread. Based on the above conclusion, the following points are forwarded:

- Further research should be carried out in different breeds of cattle.
- Characterization of rumen ecology and microbial diversity works should be performed using standardized methods.
- Useful forage species that enhance efficiency of rumen fermentation and reduce methane emission should be further screened and properly utilized.

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