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Yilkal Manaye
Faculty of Veterinary Medicine, University of Gondar, Ethiopia

Murali Manohar B
Faculty of Veterinary Medicine, University of Gondar, Ethiopia

Ramaswamy Velappa
Department of Clinical Studies, Faculty of Veterinary Medicine,

Mulat Asrat
School of veterinary Medicine, Wollo University, Ethiopia

Correspondence
Yilkal Manaye
Faculty of Veterinary Medicine, University of Gondar, Ethiopia

Analysis of rumen fluid in apparently healthy slaughtered cattle at Gondar Elfora abattoir

Yilkal Manaye, Murali Manohar B, Ramaswamy Velappa and Mulat Asrat

Abstract
A cross sectional study was conducted from November 2013 to May 2014 in Gondar University, Amhara region, Northwest of Ethiopia to determine the physical, chemical and microbial characteristics of rumen fluid and to develop a base line data on healthy parameters of rumen fluid for therapeutic transfusion in clinical ruminal disorders from apparently healthy indigenous cattle of Ethiopia. Analysis was performed by physical, chemical and microscopical methods. Of the 384 rumen fluid samples of indigenous cattle examined color was found 54.7% yellowish brown, 25% brownish green, 11.2% greenish and 9.1% milky; consistency 88.8% Viscous, 4.7% watery and 6.5% frothy; odour 89.6% aromatic and 10.4% sour; sedimentation 95.6% were completed within 4-8 minute and 4.4% no appreciable sedimentation or floatation; pH 49% ranges from 5.5-6.5, 34.6% from 5.6 to7 and 6% greater than 7; complete cellulose digestion were observed 89.6% within 48-56hrs, 10.4% more than 56 hrs; methylene blue reduction achieved 65.4% within 3 minute, 30.2% within 6 minute and 4.4% more than 6 minute; glucose fermentation test revealed that 89.6% had gas formation and 10.4% with no gas formation; protozoan motility observed 13.8% as excellent motility, 75.4% with medium motility, 7.8% having sluggish motility and 6.0% with no motility; proportion of bacteria 92% with dominant gram negative bacteria and the remaining 8% of samples collected had dominant positive bacteria. This is the basic base line data for rumen content transfusion and for future study.

Keywords: Analysis, cattle, Gondar, healthy, rumen fluid

1. Introduction
The majority of the world's rural poor, and a significant proportion of the urban poor, keep livestock and use them in a variety of ways that extend far beyond income generation. In many cases, livestock are a central component of smallholder risk management strategies (Bailey *et al*., 1999).

Rumen micro flora plays important role in converting plant fibers into products like milk and meat and if this rumen digestion by microbes is affected due several rumen disorders, the production and productivity of animals are reduced. Rumen is a fermentative vat which allows the continuous development of microbial population, acting as a fermentation chamber due to having, anaerobiosis, buffer mean pH near neutral, presence of bacteria, protozoa and fungi, nutrient supplement and continuous removal of digesta and fermentation products, and constant osmotic pressure (Ruckebush, 1988) [30]. Rumen fluid is a mixture of all fluids present in rumen chamber as a consequence of digestive process and ingestion from the esophagus i.e. swallowing. The ingested fluids include saliva, water, and mucus that mix with all the digestive components, enzymes, carbohydrates, nitrogen and other suspended gases (Dehority, 2003) [10].

Rumen contents can be examined for the physical aspects like; color, odor, consistency and sedimentation activity test and the chemical characteristics such as; pH, glucose fermentation, cellulose digestion and methylene blue reduction test and also as for microbial tests like protozoan motility test and gram staining for rumen bacteria (Rosenberger, 1979). This examination of rumen fluid is often essential to assist in determination of the state of rumen environment and digesta after rumen fluid 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 (Radostitis *et al*., 2007) [28].

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 (Thauer, 1977) [33].

Color of rumen fluid of apparently healthy cattle varies 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. Abnormal color like are milky grey can be observed in the time of acidosis (Rosenberger, 1979). 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 (Smith, 1996) [32]. Odor is normally aromatic and not repellent, depending on the odor of the particular feed eaten (hay, grass, roots, kale, silage, and so on). Abnormal odors include the repulsive musty, foul odor of protein decomposition, the penetrating acid odor of lactic acid from over eating readily-digestible carbohydrate, and the stale, indifferent odor of inactive rumen juice and the smell of abomasal contents indicative of pyloric obstruction (Rosenberger, 1979). The sedimentation activity time or sedimentation or floatation test provides a rapid evaluation of microbial activity. But the abnormal time; very rapid sedimentation with no floatation occurs in rumen acidosis, prolonged anorexia, and in time of inactive micro flora from indigestible roughages (Smith, 1996) [32]. 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 (Fubini, 2004) [15].

Cellulose digestion test is used to evaluate the cellulose digesting ability of rumen micro flora. The rumen harbors various types of cellulolytic bacteria which are active in degradation
of the components of the feed. These bacteria identified in the rumen plays an important role in cellulose digestion and have been reported in high roughage diets (Church, 1993). The common features of bacteria found in the rumen of animals fed on high roughage are; majority of the bacteria are gram negative but the number of gram positive bacteria tends to increase on increasing high energy diets in the ration (Weimer *et al*, 1999). The ability of micro flora to ferment glucose is assessed by glucose fermentation test through measuring the volume of gas formed (Rosenberger, 1979).

The rumen microbial ecosystem is a complex environment pre dominated by the bacterial population which is estimated to be between 10^9-10^{11}cells per gram of ruminal contents based on direct microscopic counts (Lee *et al*., 2000). Among these large bacterial populations majority of the bacterial species are obligate anaerobes or facultative anaerobes (Church, 1993).

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 (Williams and Coleman, 1997) [36]. An air dried smear of rumen fluid is stained by Gram's stain, or others are used for bacteria identification. Then the results of this staining will be interpreted based on the characteristics of a normal rumen flora the so called leading bacteria, the dominancy of one group of bacteria over the other can be determined (Smith, 1996) [32].

Rumen fluid of apparently healthy cattle has a motile fauna w hile the abnormal fluid have sluggish or no movement in case of acidosis (Smith, 1996) [32]. 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).

Transfaunation of inactive rumen can be possible by providing ruminal fluid from a healthy ruminant of the same species.Cross species transfaunation may be of some benefit a s some species of ciliates are common to different ruminants. Ruminal contents may be obtained at a slaughtered house or from another animal that has been fitted with a ruminal canula. A potential to transmit certain disease exists, therefore, a tested donor or a herd mate of the patient is desirable (Smith, 1996) [32]. Considering the importance of rumen microbial digestion in ruminants in converting agricultural waste by products into valuable animal products, the present work is designed with the following objectives:

- To study the physical, chemical and microbial status of rumen fluid
- To develop a base line data on healthy parameters of rumen fluid for therapeutic transfusion in clinical ruminal disorder cases.

2. Materials and Methods

2.1 Study Area: A cross sectional study was conducted for the analysis of rumen fluid in apparently healthy slaughtered cattle from November 2013 to May 2014 at Gondar ELFORA Abattoir in North Gondar Administrative Zone, Amhara Regional State. Gondar ELFORA Abattoir is located in Gondar town. Gondar town is found about 740 km northwest from the capital city, Addis Ababa at latitude, longitude and altitude of 12.3-13.8^{0}N, 35.3-35.7^{0}E and 2200 meters above sea level respectively. The annual mean minimum and maximum temperature of the area vary between 12.3-17.7 and 22-30^{0C}, respectively. North Gondar zone has an estimated human population of 2,398,291 of which 201,958 are found in Gondar town. The livestock population of North Gondar is estimated to be 1,936,514 cattle (exotic, cross and local), 524,083 sheep, 682,264 goats, 36,828 horses, 12,473 mules, 223,116 donkey and 3,165,068 poultry (MoA, 2013)

2.2 Study animals

The study included 384 indigenous breed cattle brought for sl aughter to Gondar ELFOR A battoir from November 2013 up to May 2014. These animals originated from different areas of Gondar and its surroundings. All the cattle slaughtered were males, adult and belonged to local breeds.

2.3 Study Design

A cross sectional study design was used in order to determine the physical, chemical and microbial characteristics of rumen fluid and to develop a base line data for normal rumen fluid for indigenous breed so that later it can be used for therapeutic purposes to treat those cases with ruminal disorders. Each week a two days visit was made and after ante mortem and postmortem examination rumen fluid was

collected from the slaughtered cattle which was immediately transported to the laboratory for examination.

2.4 Sample size determination

The sample size required for this study was determined according to Thrusfield (2005). Since there was no previous work done in this study area, 50% prevalence was taken as expected prevalence for sample size determination of this study. The other determinants considered in sample size determination were 95% confidence interval and 5% desired absolute precision. Hence the sample size is estimated as follows:

$$N=\frac{1.96^2\, p_{exp}\,(1-p_{exp})}{d^2}$$

Where, N=required sample size

Pexp=expected prevalence

d^2=desired absolute precision

$$n=\frac{1.96^2\, 0.5(1-0.5)}{(0.5)^2}=384$$

From the confidence interval d=5%=0.5

Using the above formula, the minimum sample size calculated as 384.

2.5 Sampling methods

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

2.6 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 animal was marked for the identification by writing a code number on its gluteal region by using unwashable ink. The animals which passed ante mortem examination were then selected for rumen fluid collection.

Post mortem rumen fluid collection : In the post mortem examination all the animals were examined for lesions. Those animals which did not reveal any gross lesion were selected for 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 fluid was taken by gentle squeezing of ingesta from the rumen manually. The rumen fluid so collected was immediately transported to pathology laboratory for further analysis.

2.7 Statistical Analysis

All data collected during the study period were entered and stored in Ms excel worksheet. Before subjected to statistical analysis, the data were thoroughly screened for errors and properly coded. For analysis SPSS Microsoft software version 16.0 was used. Descriptive statistical analysis such as table was used to summarize and present the data collected.

3. Results

3.1 Physical characteristics

3.1.1 Color

Of 384 samples collected from apparently healthy cattle, the color of rumen fluid were yellowish brown in 210 (54.7%), brownish green in 96 (25%), greenish in 43 (11.2%) and milky in 35 (9.1%) animals, subjected for rumen fluid examination.

Table 1: Color of rumen fluid examined

Color	Frequency	Percent
brownish green	96	25.0
deep green	43	11.2
yellowish	210	54.7
milky	35	9.1
Total	384	100.0

3.1.2 Consistency

Out of 384 samples of rumen fluid examined, 341 samples (88.8%) were viscous in consistency, 18 samples (4.7%) were watery in consistency and 25 samples (6.5%) were frothy in nature.

Table 2: Results of Consistency test

consistency	Frequency	Percent
viscous	341	88.8
watery	18	4.7
frothy	25	6.5
Total	384	100.0

3.1.3 Odor

The odours of rumen fluid collected from 384 slaughtered cattle on examination were found to be aromatic in 344 (89.6%) and sour in 40 (10.4%) samples tested.

Table 3: Results of odor test

Type of odor	Frequency	Percent
aromatic	344	89.6
sour	40	10.4
Total	384	100.0

3.1.4 Sedimentation activity test

Out of 384 samples of rumen fluid examined, sedimentation activity was completed within 4-8 minute in 367 (95.6%) samples while no appreciable sedimentation or floatation was observed in 17 (4.4%) samples examined.

Table 4: Results of Sedimentation activity test

Sedimentation activity test within 4-8 min	Frequency	Percent
no sedimentation	17	4.4
sedimentation	367	95.6
Total	384	100.0

3.2 Chemical characteristics

3.2.1 pH

The pH of the rumen fluid was examined in 384 cases which varied from 5.5 to greater than 7 .0. The acidic pH of less than 5.5 was observed in 10.4% of cases; pH from 5.5-6.5 in 49.0%, and pH from 5.6 to7 in 34.6% of cases examined. the alkaline pH of greater than 7 was seen in 6% of animals.

Table 5: pH of rumen fluid examined

Ph range	Frequency	Percent
less than 5.5	40	10.4
5.5-6.5	188	49.0
6.6-7	133	34.6
greater than 7	23	6.0
Total	384	100.0

3.2.2 Cellulose digestion test

Complete cellulose digestion was observed within 48-56 hrs in 344 samples (89.6%) and the cellulose digestion time was more than 56 hrs in 40 (10.4%) samples examined.

Table 6: Results of Cellulose digestion

Cellulose digestion time	Frequency	Percent
more than 56 hours	40	10.4
48-56 hours	344	89.6
Total	384	100.0

3.2.3 Methylene blue reduction test

Methylene blue reduction was positive within 3 minutes in 251 (65.4%) samples, within 6 minutes in 116 (30.2%) samples and it was more than 6 minutes in 17 (4.4%) samples examined.

Table 7: Results of Methylene blue reduction test

Methylene blue reduction time	Frequency	Percent
less than or equal to 3	251	65.4
less than 6	116	30.2
greater than 6	17	4.4
Total	384	100.0

3.2.4 Glucose Fermentation test

A total of 344 (89.6%) rumen fluid samples were positive for Glucose fermentation test which was characterized by gas formation and gas formation could not be seen in the remaining 40 (10.4%) samples collected from 384 healthy animals.

Table 8: Results of Glucose Fermentation test

Glucose fermentation	Frequency	Percent
no gas formation	40	10.4
gas formation	344	89.6
Total	384	100.0

3.3. Microscopic Examination of Protozoa and Bacteria

3.3.1 Protozoan motility

Protozoan motility was found to be varying, with excellent motility (+++) in 13.8%, medium motility (++) in 22.1%, sluggish motility (+) in 7.8% of samples examined while no motility (0) could be observed in the remaining 6.0% of samples.

Table 9: Results of Protozoan motility observed in rumen fluids examined.

Motility status	Frequency	Percent
No motility	23	6.0
Sluggish motility	30	7.8
Medium motility	278	72.4
Excellent motility	53	13.8
Total	384	.100

3.3.2 Gram's stained rumen fluid smears

Gram staining test revealed 92% of samples revealed predominantly gram negative bacteria whereas the remaining 8% of samples examined, had mostly gram positive bacteria.

Table 10: Results of Gram's stained rumen fluid smears

Type of bacteria	Frequency	percent
Dominant gram negative bacteria	354	92
Dominant gram positive bacteria	30	8
Total	384	100

4. Discussion

Color: In most of the cases the colors of rumen 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 examination of ruminal contents revealed 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 rumen fluid from healthy slaughtered animals was observed to be mostly viscous in consistency. This finding concurred with Smith (1996) [32] who has stated that normal consistency of rumen fluid of cattle was slightly viscous in consistency and watery fluid was indicative of inactive bacteria and protozoa and in such abnormal cases sample may be frothy. The consistent viscous consistency observed in the present result is in the agreement with Smith (1996) [32] 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 respectively which indicate the normal rumen environment containing adequate population of ruminal flora, ingesta and varying quantities of digestive fluids.

In most of the cases of slaughtered cattle characteristics of rumen fluid observed had aromatic odors. Rosenberger (1979) has reported that the odor of ruminal fluid from healthy cattle would be normally aromatic and not repellent which supports the present result**.** This observation of aromatic odor in the ruminal fluids recorded in the present study may be presumed as normal odor for the indeginous breed of Ethiopia.

The present study the rumen fluid from majority of cases revealed sedimentation within 4-8 minutes. Smith (1996) [32] has recorded no sedimentation in rumen fluid from cases of indigestion, anorexia and vagal indigestion In the present study also a few samples did not show any sedimentation which could be due to anorexia and indigestion prior to slaughter. However most of the samples showed sedimentation within 4-8 minutes. Hence it may be construed that the normal time required for sedimentation of rumen fluid is 4-8 minutes in local breeds.

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 to 7.0 which may be considered as normal base line value for the local breeds on available feeds.

In the present study the results indicated complete cellulose digestion within 48-56 hrs in 344 samples, while in 40 samples it was more than 56 hrs. The present study showed that most of the samples had active population of cellulose digesting rumen microflora which could digest the cellulose thread with the given time. However in 40 samples more time was taken for digestion of cellulose which may be due either inactivity or loss of partial population before examination of samples. Smith (1996) [32] opined that rumen fluids having fully active rumen micro flora would digest the cellulose within 48-56 hours.

These bacteria identified in the rumen plays an important role in cellulose digestion and have been reported in high roughage diets (Church, 1993). In the present study the rumen fluid samples examined had microflora which could digest the cellulose within 48-56 hrs, which may be considered as normal value for the ruminal fluid of the local breed cattle.

Methylene blue reduction time for the most of the ruminal fluids examined was less than or equal to 3 min. This result concurred with Bouda *et al*, (2000) who have reported that under normal conditions of ruminal functioning the methylene blue reduction time must be of three minutes at most. Dirksen *(*1969*)* also reported similarly and he stated that it was an indirect measure of the redox potential and bacterial activity of rumen fluid. Bouda *et al*, (2000) and Dirksen (1969) attributed this due to presence of physiological anaerobic fermentative bacteria in the rumen fluid samples. Booder *et al,* (2010) observed an increase in the MBRT in animals affected with rumen impaction due to plastics and other foreign body. Since most of the samples examined in the present study took 3 min and less for methylene blue reduction, this value may be considered as normal.

In the present study a total of 344 rumen fluid samples were positive for Glucose fermentation test which was characterized by gas formation. However in 40 samples no gas formation could be observed. This gas formation could be attributed to the presence of fully active glucose fermenting rumen bacteria in the rumen fluid. Rosenberger (1979) has also stated that in physiologically healthy cattle the normal rate of gas formation was 1-2ml per hour which was in accordance with the results of the present study. Hence, the results of present study may be considered as normal.

The results of the present study revealed excellent and medium motility in most of the samples tested. Sluggish motility and no motility were also observed in a few samples. The excellent and medium motility observed in the study may be due to varying level and types of concentrate fed to animals. Franzolin and Dehority (1996) and Dehority and Orpin (1997) have also opinied that the concentration of protozoa in rumen contents generally decreased with the addition of concentrates to diets. Protozoan numbers typically increased when grain was included in forage-based diets (Hristov *et al*., 2001) and their number also may be sensitive to the type of grain fed or if mixed grains were included in the diet (Mendoza *et al.*, 1999). The reason for low and no motility noticed in the present study may be due to reduction in number of protozoa following increased concentrate feeding. Inclusion of very high concentrate or grain in the diet might cause the diversity and number of protozoa to decline, a factor that may exacerbate a low ruminal pH and increase the risk of acidosis in cattle fed these types of diets. Based on the results of the present study, the base line value for motility of protozoa in the normal rumen fluid, may be considered as either excellent or medium motility.

The examination of gram stained rumen fluid smears revealed mostly gram negative bacteria in the rumen fluid of apparently healthy slaughtered indigenous cattle. Williams and Coleman, (1997) [36] stated that gram negative bacteria would normally dominate in physiologic rumen fluid. Hence, the predominance presence of Gram negative bacteria may be considered as normal bacterial flora of rumen fluid collected from healthy local cattle.

5. Conclusion and Recommendations

Based on the results of the present study, it may be concluded that the rumen fluids collected from normal, healthy, local breed cattle slaughtered at abattoir, on analysis revealed that the colors were yellowish and brownish green, viscous in consistency, aromatic in odor, sedimentation within 4-8 minutes, pH ranged from 5.5 to 7, complete cellulose digestion occurred within 48-56 hours, methylene blue reduction by rumen bacteria within 3 minutes, glucose fermentation by fermentative rumen bacteria characterized by the presence of gas formation, medium protozoan motility and predominant gram negative bacteria in most of rumen fluid samples. These finding may be considered as base line data for assessing the quality of rumen fluid before transfusion to treat ruminal disorders and to restore normal rumen environment after evacuation of ruminal contents following rumenotomy.

Based on the above conclusions the following recommendations are forwarded:

- Further research should be undertaken to develop base line data for different breeds and in different agro climatic regions, as the type of feed, environmental micro flora may vary.
- The quality of rumen fluid should be examined before transfusion to other animals.
- The health status of donor animals should be checked to avoid transmission of diseases.

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