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Studies on rumen liquor analysis in goats affected with ruminal acidosis

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Abstract
The present study was conducted to study various rumen liquor parameters in acidotic goats in Jaipur, Rajasthan during June, 2020 to December, 2020. A total of 50 clinical cases of ruminal acidosis of different age group, sex and irrespective of breed having the history of ingestion of large quantity of highly fermentable carbohydrate rich diet, in appetence to anorexia, suspended rumination and clinical manifestations of distended rumen and diarrhoea and acidic pH of rumen liquor were selected for present investigation and divided into five different groups (group 1,2,3,4 and 5) consisting of 10 goats in each. Ten healthy goats were also included as healthy control group in the study.
Ruminal liquor analysis was undertaken and compared against ten apparently healthy goats (group 6) from the same area with similar husbandry practices which served as healthy control. Ruminal liquor parameters such as acidic rumen liquor pH, grey to milky grey color, semisolid or slightly watery consistency, sour or pungent odour, sluggish or nil motility of protozoa, and dominance of gram positive bacteria were observed in present investigation. Based on study of all above parameters, the goats were confirmed as suffering from ruminal acidosis.

Keywords: dietary abnormalities, ruminal acidosis, rumen liquor pH, protozoa, grampositive bacteria

Introduction
Acidosis also known as lactic acidosis, rumen acidosis or grain overload is a carbohydrate fermentation disorder of the rumen that can affect all animals of all ages. Acidosis results in acidic pH of rumen (normal being 6.2-6.8) which is caused by feeding of readily fermentable carbohydrates, feeding of low fiber diet, poor management practices or a combination of these. Degree of acidosis varies from seriousness, a slight drop in feed intake (mild) to death (severe). Acute form of the ruminal acidosis in ruminants is manifested by indigestion, rumen stasis, toxemia, in-coordination, collapse and frequent death (Tufani *et al*., 2013) [17].
Clinically it is manifested by anorexia, depression, dehydration, ruminal stasis, profuse diarrhea sweet-sour odor of feces that may contain undigested kernels, weakness, and ataxia leading to recumbency. Rumen may or may not feel full, but atonic and fluid splashing sounds are audible on ballottement (Radostits *et al*., 2007) [14].
Rapid fermentation of carbohydrates alters the ruminal function through proliferation of acid resistant bacteria and an increase in the production of volatile fatty acids and D and L lactate, which cause a marked drop in ruminal pH to < 5.00 in most cases (Gozho *et al*., 2005) [7] and Gonzalez *et al.*, 2012) [6].
In acute acidosis, ruminal acidity and osmolarity increases markedly due to accumulation of acids and glucose. These damages the ruminal and intestinal wall, decreases blood pH, and cause dehydration which proves fatal. The goats reared under free grazing system are more vulnerable for accidental excessive ingestion of carbohydrate-rich diets (Valmik *et al.*, 2017) [18].
The grain overload in goats is an emergency medical condition and requires a prompt therapeutic approach and good supportive care.

Materials and Methods
The work was conducted at the Department of Veterinary Medicine in collaboration with Department of Veterinary Clinical Complex (VCC), Department of Veterinary Physiology and Biochemistry and Veterinary Clinical Diagnostic Laboratory Section Veterinary Clinical Complex, Post Graduate Institute Veterinary Education and Research (PGIVER) Jaipur Rajasthan during June, 2020 to December, 2020.

Screening of animals

In the present investigation, total 50 goats of different age, sex, irrespective of breed having the history of ingestion of large quantity of highly fermentable carbohydrate rich diet were screened to find out the occurrence of ruminal acidosis in goats. The diet varied between goat to goat which mostly included barley, wheat, amla leaves, groundnut fodder, stale chapattis or other leftover cooked human food. The clinical examination was carried out and rumen liquor samples were collected from such cases for immediate estimation of pH. Those cases having rumen liquor pH below 5.5 were included in this study. 10 healthy goats were also selected as healthy control group.

Sampling Procedure

The rumen liquor was collected aseptically through rumenocentesis using 18 gauge sterile needle and 10 ml disposable syringe the puncture site was the ventral rumen at left para-lumbar fossa.

Rumen liquor Evaluations

The rumen liquor examination of goats was carried out for the diagnosis of ruminal acidosis. It included pH, colour, odour, consistency, as per the method described by (Nichols and Penn 1958) [11] rumen protozoal motility/activity as per the method described by (Dash and Misra 1972) [4] and gram's staining for ruminal bacteria.

pH of rumen liquor

The rumen liquor pH was measured using wide range pH indicator paper. The rumen liquor was put on the paper and pH was recorded by change in the colour of indicator paper and matching it with the standard colours of the indicator paper. The pH of rumen liquor was determined immediately after collection before exposure to air as it can cause increase in pH if exposed to air.

Protozoal motility/activity

A drop of fresh rumen liquor was placed on a clean glass slide, covered with cover glass and examined under low power magnification of microscope. More is the number of protozoa more is the active rumen liquor. Depending upon protozoal activity graded as vigorous (+++), moderate (++) and sluggish (+).

Examination of rumen liquor for bacteria

Ten drops of rumen liquor was taken and evenly spread on a clean glass slide and heat fixed. The smear was stained with Gram's staining solution and observed under oil immersion of the microscope. Proliferation of gram positive cocci and rods at the expense of gram negative bacteria was specifically recorded.

The data obtained were statistically analyzed for ANOVA (Snedecor and Cochran 1994) [15]. Means showing significant differences were compared by Duncan's New Multiple Range Test (Duncan, 1955) [5]. Statistical significance was accepted at $p \leq 0.05$.

Results and Discussion

The work was conducted at the Department of Veterinary Medicine in collaboration with Veterinary Clinical Complex (VCC), Department of Veterinary Physiology and Biochemistry, Post Graduate Institute Veterinary Education and Research (PGIVER) Jaipur Rajasthan. Clinical cases of rumen indigestion in goats brought to the VCC, PGIVER were selected for detection of ruminal acidosis on basis of history of ingestion of large quantity of highly fermentable carbohydrate rich diet. The diet varied between goat to goat which mostly included barley, wheat, amla leaves, groundnut fodder, stale chapattis or other leftover cooked human food. The clinical examination was carried out and rumen liquor samples were collected from such cases for immediate estimation of pH. Those 50 cases having rumen pH below 5.5 were included in this study. Ten healthy goats were selected as control animals from nearby area of PGIVER Jaipur.

Rumen liquor evaluation

In the present study, rumen liquor was evaluated for pH, physical properties (colour, odour, consistency), protozoal activity and gram's staining.

Physical examination of rumen liquor

Ruminal Fluid pH

The pH of rumen liquor in all the ruminal acidotic cases was significantly ($p<0.05$) lower than the normal range of healthy goats (6.9±0.03). The mean values of rumen liquor pH were obtained 4.7 ± 0.3, 5.10 ± 0.16, 4.85 ± 0.16, 4.5±0.29 and 5.04±0.84 for group 1,2,3,4 and 5 respectively. It was observed that the rumen liquor pH in all the ruminal acidotic cases was significantly ($p<0.05$) lower than the normal range of healthy goats.

Ingestion of large amount of carbohydrates rich diet leads to change in the microbial population in the rumen. The changes in the microbial species inhabiting the rumen normal fermentation were accompanied by a change in the type of fermentation, becoming a lactic fermentation. This had an impact on the pH values of rumen liquor, which decreased in proportion to increased amount of lactate reported by (Nikolov,1998) [12]. The similar finding were documented by Valmik *et al*. (2017) [18], Taylor (2017) [16], Chavelikar *et al*. (2018) [3], Kaliappan *et al*. (2018) [8], Bhujange *et al*. (2019) [2], Kumar *et al*. (2019) [10], Koondhar *et al*. (2020) [9] and Zaki *et al*. (2020) [19].

Colour

The color of rumen liquor was noticed in affected and healthy control goats by visualization. The colour of rumen liquor in ruminal acidosis affected goats was milky grey as compared to healthy goats, it was greenish.

These findings are in accordance with Valmik *et al*. (2017) [18], Taylor (2017) [16], Chavelikar *et al*. (2018) [3], Kaliappan *et al*. (2018) [8], Bhujange *et al*. (2019) [2], Kumar *et al*. (2019) [10], Koondhar *et al*. (2020) [9] and Zaki *et al*. (2020) [19].

Consistency

The consistency of rumen liquor in acidotic goats was watery, slightly watery to semisolid when compared with healthy group was thick to viscous.

The consistency could be attributable to increased lactic acid concentration and osmolality of rumen liquor resulting in withdrawal of extra-cellular fluid in to rumen as recorded by (Slyter, 1976).

The change in consistency of rumen liquor due to acidosis in goats to semiliquid or watery as observed in the present study was comparable to the reports of Petrovski (2016) [13], Chavelikar *et al*. (2018) [3], Kaliappan *et al*. (2018) [8], Al-Azazi *et al*. (2018) [1], Bhujange *et al*. (2019) [2], Kumar *et al*. (2019) [10],Koondhar *et al*. (2020) [9] and Zaki *et al*. (2020)

[19]in goats.

Odour

The odour of the rumen liquor in acidotic goats was sour and in healthy group, it was aromatic.

The change of odour of rumen liquor might be due to excess putrifaction / fermentation of carbohydrates rich diet by proliferated gram positiveorganisms, which was correlated with the earlier observations recorded by Petrovski (2016) [13], Chavelikar *et al*. (2018) [3], Kaliappan *et al.* (2018) [8], Al-Azazi *et al.* (2018) [1], Bhujange *et al*. (2019) [2], Kumar *et al*. (2019) [10], Koondhar *et al*. (2020) [9] and Zaki *et al*. (2020) [19].

Activity of rumen protozoa

The protozoal motility of rumen liquor in affected goats was +/0 (sluggish/nil motility), because of complete absence of protozoa as compared to healthy group which had +++ (rapid vigorous motility)

It was observed that acidic pH of rumen liquor adversely affected the concentration and motility of rumen protozoa. Similar findings were reported by (Radostits *et al.*, 2007) [14].

These findings are in agreement with the observations of Valmik *et al.* (2017) [18], Kaliappan *et al.* (2018) [8] and Zaki *et al*. (2020) [19].

Bacterial Staining Method (Gram's Staining)

In goats affected with ruminal acidosis, there was predominance of Gram positive cocci and rod-shaped bacteria as compared to healthy goats which had Gram negative cocco-bacilli.

The findings are accordance with Petrovski (2016) [13], Al-Azazi *et al.* (2018) [1] and Bhujange *et al*. (2019) [2].

Table 1: Mean values of rumen fluid parameters in acidotic and healthy goats

Groups	Healthy Group (N=10)	Group-1 (N=10)	Group-2 (N=10)	Group-3 (N=10)	Group-4 (N=10)	Group-5 (N=10)
pH	6.9 ± 0.03^{aC}	4.7 ± 0.3	5.1 ± 0.16^{a}	4.85 ± 0.16^{a}	4.5 ± 0.29	5.04 ± 0.84^{aB}
Color	Greenish	Milky grey	Milky grey	Milky grey	Milky grey	Milky grey
Consistency	Viscous and thick	Watery	Watery	Watery	Watery	Watery
Odour	Aromatic	Sour	Sour	Sour	Sour	Sour
Protozoal motility	Vigorous +++	Nil 0	Sluggish +	Nil 0	Sluggish +	Nil 0
Gram staining	Gram -ve dominance	Gram +ve dominance	Gram +ve Dominance	Gram +ve dominance	Gram +ve dominance	Gram +ve dominance

Mean bearing same superscript donot differ significantly.

Conclusions

Fifty goats were presented with the history of dietary abnormalities, excessive ingestion of carbohydrate-rich diet. The cases were showing the symptoms of inappetence to anorexia, suspended rumination and clinical manifestations of distended rumen and diarrhoea and acidic pH of rumen liquor were selected and screened for ruminal acidosis basing on ruminal fluid pH which was less than normal as compared with ten healthy goats. It was concluded that the goats were confirmed as suffering from ruminal acidosis and rumen liquor analysis was undertaken.

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Department of Veterinary Medicine, and Department of Physiology and Biochemistry, Post Graduate Institute of Veterinary Education and Research (PGIVER), Jaipur Rajasthan.

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