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Evaluation of serum haptoglobin and rumen liquor in goats affected with sub-acute ruminal acidosis (SARA)

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Abstract
Reports on sub-acute ruminal acidosis in goats are scarce as per available literature because the clinical appearance is subtle and usually goes under diagnosed. Study was conducted in various goat farms in Thrissur district of Kerala. Twenty four SARA affected goats were selected and compared with values obtained from 6 apparently healthy goats from a SARA negative herd. Rumen liquor showed a predominance of Gram positive bacterial population during SARA episodes. Total protozoal count was reduced. Volatile fatty acid profile showed an increase in TVFA, propionate and butyrate level with reduced acetate level. Rumen lactate concentration did not show any significant variation. Serum haptoglobin concentration was significantly higher in SARA affected group.

Keywords: TVFA, Acetate, propionate, butyrate, haptoglobin, SARA

1. Introduction
Sub-acute ruminal acidosis is defined as low ruminal pH, ranging from 5.0 to 5.8, caused by excessive accumulation of volatile fatty acids without persistent lactic acid accumulation which was later restored to normal pH by animal's own physiologic responses. It is considered as the most important nutritional disease which predisposes to various other disease conditions in dairy cattle (Tajik *et al*, 2010) [10].
Even though it is well recognized in dairy cattle herds, researches in goats are scarce. In the context of increased popularity of goat farming across the country, scientific information regarding the herd based diagnosis of SARA in goats is utmost important. Determination of rumen liquor pH alone might not be adequate for diagnosis in many circumstances. Estimation of volatile fatty acids in rumen liquor and serum acute phase protein like haptoglobin gives more value to the diagnosis.

2. Materials and Methods
The study was conducted across various goat farms in Thrissur district of Kerala from December 2015 to January 2017. Lactating female goats of more than one year of age were selected for the study. Rumen liquor for pH estimation was collected by rumenoentesis with the consent of owner. Goats with rumen liquor pH less than or equal to 5.8 and persisting for more than three hours were selected as SARA affected animals. Thereby, twenty four SARA affected goats were selected and recognized as group I. Another 6 goats from a SARA negative herd were selected as group II which was not maintaining low pH for more than 3 hours and not showing any clinical illness. Selected animals were subjected to further evaluation.
An adequate quantity of rumen liquor was collected by passing stomach tube. A dried smear of rumen fluid was stained with Gram's Method to understand the predominant type of bacteria. An adequate quantity of the strained rumen fluid was taken and the volume was made up to 25 ml using 10 per cent formol saline (10 per cent formaldehyde v/v in 0.85 per cent sodium chloride), 10 ml was taken from the above and stained by adding ten drops of 2 per cent Eosin, which stained protozoa only. A drop of this fluid was charged in a haemocytometer with improved Neubauer ruling. Protozoa were counted in the leukocyte counting chamber and counting was done in eight square milliliters and its average was multiplied by 50,000. Result was expressed as total number of protozoa per milliliter (n x 10^5).
Five ml of rumen liquor was sieved through double layer muslin cloth and acidified with 25% metaphosphoric acid

(0.2 ml of 25 per cent metaphosphoric acid (w/v) to 0.8 ml of strained rumen liquor) and stored at -80ºC for VFA analysis. Volatile fatty acid composition (acetic, propionic and butyric acid) of the innoculum was found out using 7890A GC System gas chromatograph, (Agilent Technologies, USA) as per standard procedure described by Filípek and Dvorak (2009) [3].

Enzymatic determination of lactate in rumen liquor was done by spectrophotometry using Megazyme K-DLATE Lactic acid assay kit (Gawehn, 1988) [4]. Serum haptoglobin level was determined by enzyme linked immunosorbent assay using Fine Test goat haptoglobin sandwitch ELISA kit.

The data obtained were statistically analyzed using independent sample 't' test using IBM SPSS Statistics, Version 24.0 software. Results were expressed as mean ± standard error.

3. Result and Discussion

In the present study, rumen pH was significantly low ($p \leq 0.01$) in SARA affected goats. A predominance of Gram positive bacterial population was observed in stained smear of rumen fluid collected from goats suffering from SARA while SARA negative group showed Gram negative bacterial predominance (Fig. 1). Total protozoal count of SARA affected goats were significantly reduced and a significant positive correlation was observed between rumen pH and total protozoal count as well (Table: 1 and 2).

Rumen volatile fatty acid profile showed an increase in the concentration of TVFA together with propionate and butyrate levels while, acetate level and acetate: propionate ratio was reduced upon lowering the pH of rumen (Table: 1 and Fig. 2). Increased propionate concentrations together with reduction in aceate caused lowering of acetate to propionate ratio (Gozho *et. al.*, 2006; Fairfield *et al.,* 2007; Khafipoor *et al.,* 2007; Plaizier *et. al*., 2008) [5, 6, 7, 8].

No statistically significant difference in rumen lactate concentration were recorded between SARA affected and SARA negative group (Table: 1). Stone (2004) [9] and Fernando *et al*. (2010) [2] reported that lactate content found to be very negligible or absent in rumen fluid of cattle during the occurrence of SARA, since lactate-utilizing bacteria such as *Megasphaera elsdenii*, Prevotella spp. and *Streptococcus ruminantium* immediately convert lactate to propionate, butyrate and valerate. Therefore, in the present study, reduction in ruminal pH in SARA affected goats could be associated with raise in concentration of total volatile fatty acids within rumen rather than of lactic acid (Allen, 1997) [1].

Serum haptoglobin concentration was significantly higher ($p \leq 0.01$) in SARA positive animals compared to SARA negative and a significant negative correlation was observed between rumen pH and serum haptoglobin as well (Table: 1 and 2; Fig. 3). Haptoglobin concentration could increase due to inflammation within the rumen resulted as a sequelae of sub-acute ruminal acidosis. This might also indicate that SARA affected goats were under stress.

Table 1: Rumen liquor parameters and serum haptoglobin level of SARA positive and negative groups

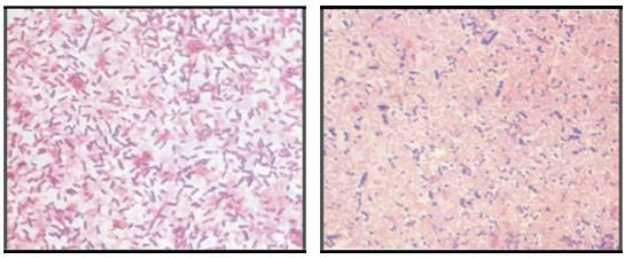
Parameter	Group I	Group II	p-value
pH	5.64±0.03	6.67±0.07	< 0.001**
Total Protozoal Count (nx10^5/ml)	1.13±0.06	3.09±0.09	< 0.001**
Lactate (mmol/L)	1.10±0.09	0.76±0.13	0.083 ^{ns}
TVFA (mmol/L)	63.91±0.89	54.57±0.60	< 0.001**
Acetate (mmol/L)	29.22±0.52	36.96±0.83	< 0.001**
Propionate (mmol/L)	22.24±0.57	12.44±0.35	< 0.001**
Butyrate (mmol/L)	12.45±0.53	5.17±0.31	< 0.001**
A/P ratio	1.34±0.05	2.99±0.13	< 0.001**
Haptoglobin (mg/L)	297.10±9.96	63.79±9.23	< 0.001**

** Means differ significantly at at p≤0.01
^{ns} Non-significant

Table 2: Correlation matrix of rumen pH with other parameters in SARA affected goats

Parameter	pH
Total Protozoal Count	0.485**
Lactate	-0.250
TVFA	-0.321
Acetate	0.329
Propionate	-0.275
Butyrate	-0.282
A/P ratio	0.253
Haptoglobin	-0.558**

** Highly significant correlation at p≤0.01
Negative value indicated negative correlation



Gram +ve predominance in SARA positive **Gram -ve predominance in SARA negative**

Fig 1: Microbial composition of rumen liquor (100x)

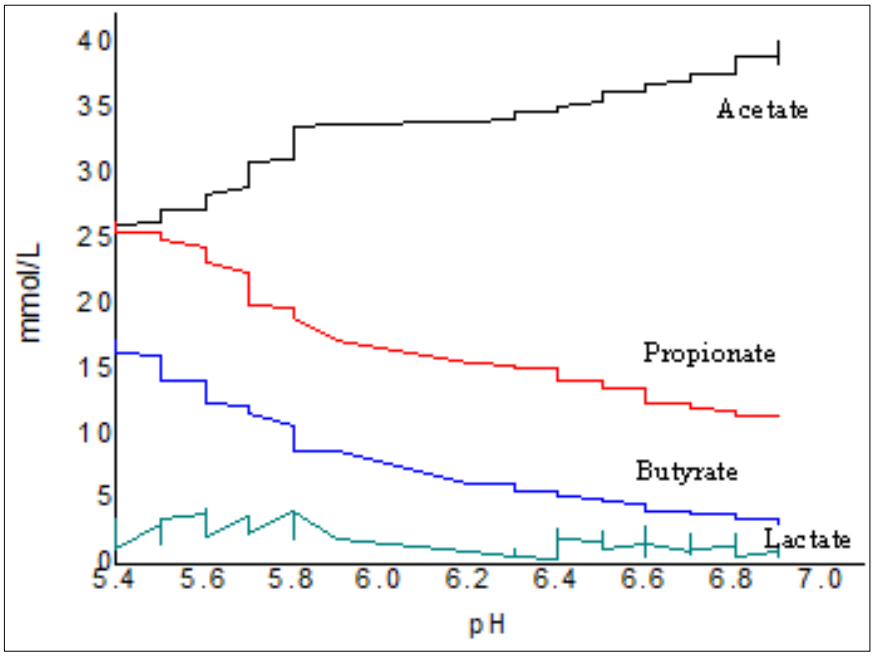


Fig 2: Relationship between individual volatile fatty acids and lactate upon pH changes in SARA affected goats

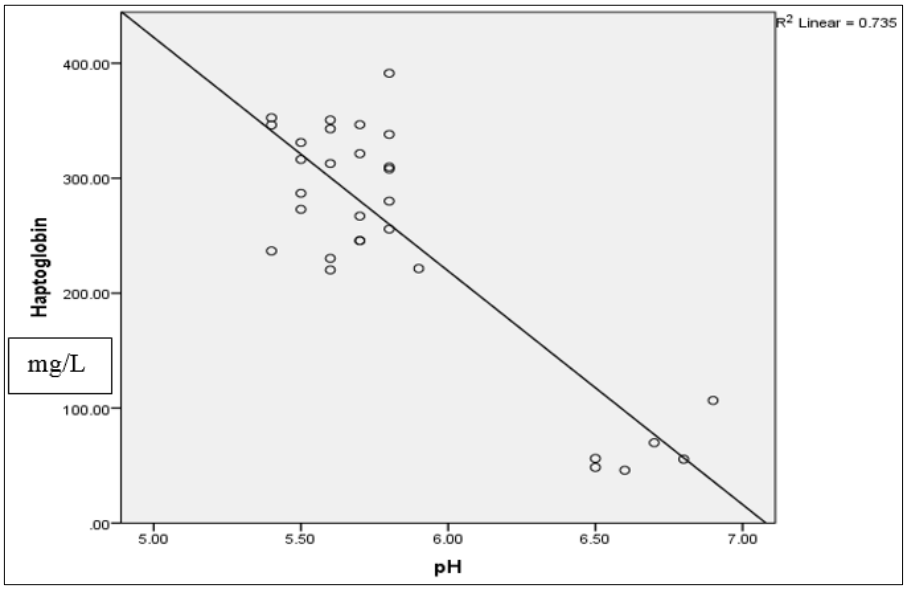


Fig 3: Regression graph showing relationship of rumen liquor pH with serum haptoglobin level

4. Conclusion

Estimation of serum haptoglobin, though a costly procedure, was identified as a useful parameter to monitor SARA in goat herds. Estimation of volatile fatty acid of rumen liquor would give more value to the diagnosis of SARA.

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