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Effect of sodium bicarbonate, Neem and Yeast on different ruminal fluid parameters in sub-acute ruminal acidosis

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Abstract
Present study was done to know the effect of different treatment regimens on ruminal fluid in Sub-acute ruminal acidosis in cattle. Eighteen SARA positive animals with ruminal fluid ranging from 5.2to 6.0 were divided equally into 3 treatment group. After sodium bicarbonate treatment animals showed changes in various ruminal fluid parameters with increased protozoal count. After treatment with *Azadirachta indica* alteration in ruminal motility and SAT was seen. After treatment with *Saccharomyces cervisiae*, improvement in ruminal motility was observed.

Keywords: Ruminal fluid, sodium bicarbonate, Neem, yeast

Introduction
Sub-acute ruminal acidosis (SARA) is a digestive disorder of ruminants occurring due to feeding of excess of rapidly fermentable carbohydrates and inadequate fibers leading to decrease in milk production. The present study was taken to know the various changes in ruminal fluid parameters before and after treatment with different drugs.

Materials and Methods
Eighteen SARA positive animals were divided into 3 different treatment groups containing 6 animals in each group. In group I, powder sodium bicarbonate 50 gm was given orally once daily, group II animals treated with dried leaves powder of *Azadirachta indica* mixed with 100gm jaggary orally once. Third group was treated with *Saccharomyces cervisiae* (1 X 10^{10} CFU/gm) @ 5gm orally daily once for 5 days.
The treatment was given for 5 days and the ruminal fluid was evaluated on day "0" (before treatment) and 7th and 14th day of treatment.

Results and Discussion
Rumen pH

Table 1: Mean of ruminal pH in Group I, Group II, Group III and Group IV (Healthy control) in SARA affected cattle.

	Day "0"	Day 7th	Day 14th	Pooled mean
Group I	5.65^{b}±0.08	6.23^{a}±0.15	6.25^{a}±0.11	6.04±0.16
Group II	5.73±0.09	5.93±0.09	5.93±0.07	5.87±0.09
Group III	5.68±0.08	5.82±0.08	7.05±0.10	5.84±0.11
Group IV	7.12±0.09	6.03±0.11	7.07±0.08	7.08±0.09
Pooled mean	6.04±0.27	6.26±0.22	6.32 ±0.21	

Group I CD 1% value is 0.474 and CD 5% value is 0.343
Similar superscripts are non-significant.

The mean ruminal pH in sub-acute ruminal acidosis affected cattle of group I was 5.65±0.08, group II was 5.73±0.09 and group III was 5.68±0.08. This was lower when compared with the control group IV (7.12±0.09). Similar observation was noted by Li *et al*., (2012) [10], Bipin *et al.*, (2016a) [2] and Nasr *et al*., (2017) [15].
After treatment mean ruminal pH of group I significantly increased on day 7th (6.23±0.15) and day 14th (6.25±0.11). Similar finding was observed by Bipin *et al.*, (2016b) [3] who noted increased ruminal pH after the treatment with sodium bicarbonate. After treatment mean ruminal pH of group II increased on day 7th and day 14th (5.93±0.09) as against day 0. After

treatment mean ruminal pH of group III increased on day 7th (5.82±0.08) and day 14th (7.05±0.10).
Sodium bicarbonate is having strong capacity to neutralize protons. It stabilizes ruminal pH. (Ruyet and Tucker, 1992) [18]. Buffers like bicarbonate help in preventing growth of lactobacilli (Garry 2002) [8].
Saccharomyces cerevisiae increases lactate utilization. It leads to improved ruminal function and ruminal pH (Nocek 1997) [17]. *Saccharomyces cerevisiae* stimulate growth of bacteria *Selenomonas ruminantiun* and *M. elsdenii* which help in lactate utilization (AlZahal *et al.*, 2014) [1]. Live yeast enhances fiber digestion and help in further accumulation of lactate (Marden *et al*., 2008) [12].

Colour
In SARA affected cattle of group I, II and III the colour of rumen fluid was slightly milky brown to yellow brown on day "0" before treatment and in group IV (control) it was greenish. Similar finding was noted by Nasr *et al*., (2017) [15]. After treatment on day 7th and day 14th animals of group I, II and III showed changed ruminal fluid colour due to changed ruminal pH. It was found to be turning to greenish in all treatment groups after treatment.

Odour
Before treatment on day "0" odour of ruminal fluid in SARA affected cattle of group I, II and III was sour to aromatic. This occurred due to decreased protozoal activity in rumen and also due to increased Gram negative bacterial population (Driksen, 1990 and Nasr *et al.*, 2017) [15]. On day 7th and day 14th change in odour of ruminal fluid was found due to change in ruminal pH.

Consistency
Consistency of rumen fluid in SARA affected group I, II and III was watery to slightly viscous on day "0" before treatment and in control group rumen fluid was slightly viscous. After treatment on day 7th and 14th consistency of rumen fluid was changed to slightly viscous.
Decreased activity of rumen protozoa and increased Gram positive bacteria leads to change in consistency of rumen fluid. (Nasr *et al*., 2017) [15].

(SAT) Sedimentation activity time (minutes)

Table 2: Mean SAT (min) in Group I, Group II, Group III and Group IV (Healthy control)

	Day "0"	Day 7th	Day 14th	Pooled mean
Group I	2.67^{b}±0.28	4.25^{a}±0.51	4.00^{a}±0.13	3.64±0.44
Group II	2.58^{b}±0.24	3.42^{a}±0.20	3.50^{a}±0.22	3.17 ±0.27
Group III	2.75±0.25	2.75±0.11	2.75±0.11	2.75±0.16
Group IV	4.58±0.37	4.42±0.27	4.42±0.27	4.47±0.29
Pooled mean	3.14±0.44	3.71±0.40	3.67 ±0.32	

Group I CD 5 % value is 1.040
Group II CD 5 % value is 0.668
Similar superscripts are non-significant.

In SARA affected cattle sedimentation activity time (min) on day "0" in group I, II and III was 2.67±0.28, 2.58±0.24 and 2.75±0.25. It was found to be decreased when compared with control group IV (4.58±0.37 minutes). Similar finding was recorded by Nawid (2012) who observed that mean SAT time for normal animal was 5.84±0.32 and in SARA positive cases it was 2.50±0.13. However, Nasr *et al*., (2017) [15] observed mean SAT in SARA positive as 14.9±0.33 minutes, in SARA marginal cases as 6.4±0.47 minutes and in SARA negative cases as 5.4±20 minutes.
After treatment, sedimentation activity time (minutes) in Group I significantly increased on day 7th (4.25±0.51) and on day 14th (4.00±0.13) as compared with SAT before treatment. After treatment sedimentation activity time (minutes) in Group II significantly increased on day 7th (3.42±0.20) and on day 14th (3.50±0.22) when compared with day 0. This increase of SAT on day 7th and day 14th was statistically non-significant to each other. In Group III no change in SAT was seen before as well as after treatment.
Reduced dry matter intake leads to reduction in rumen fluid pH and SAT. (Enemark, 2002; Chako, 2014).

MBRT (minutes)

Table 3: Mean MBRT (min) in Group I, Group II, Group III and Group IV (Healthy control)

	Day "0"	Day 7th	Day 14th	Pooled mean
Group I	3.67±0.31	3.00±0.13	3.25±0.17	3.30±0.23
Group II	3.58±0.33	3.25±0.17	3.33±0.17	3.39±0.23
Group III	3.42±0.15	3.58±0.30	3.17±0.11	3.39±0.20
Group IV	3.17±0.11	3.08±0.08	3.08±0.08	3.11±0.09
Pooled mean	3.46±0.24	3.23±0.20	3.21±0.13	

Mean value of MBRT in SARA affected groups I, II, and III was 3.67±0.31, 3.58±0.33 and 3.42±0.15. These values were increased than control group IV (3.17±0.11). Similar finding was observed by Nawid (2012) and Nasr *et al*., (2017) [15].
After treatment mean MBRT (min) in Group I decreased on 7th day (3.00±0.13) and again increased on day 14th (3.25±0.17). But this change was statistically non-significant. Decreased MBRT (min) in sodium bicarbonate treated animals in SARA condition has been reported by Bipin *et al*., (2016b) [3]. After treatment mean MBRT (min) in Group II decreased on 7th day (3.25±0.17) and again increased on day 14th (3.33±0.17). But this change was also statistically not significant. After treatment mean MBRT (min) in Group III increased on 7th day (3.58±0.30) and again decreased on day 14th (3.17±0.11). This change was also statistically not significant.
Normal MBRT is < 3 min when rumen is healthy (Enemark *et al*., 2002).

Microscopic examination
Microscopic examination of ruminal fluid was done to study protozoal motility and density. Protozoal density was expressed as vigorous (++++) when more than 40 protozoa were present in one field of microscope. When 30-40 protozoa were observed in one field it was expressed as abundant (+++), while when 10 – 30 protozoa were present per field it was expressed as moderate (++) and less than 10 protozoa per field was expressed as few (+). Similar observation was noted for protozoal motility. When rumen pH was 5.4, at that time protozoal motility and density was (+) and when ruminal pH was more than 5.5, at that time protozoal motility and protozoal density was (++). Protozoal motility and density was decreased on day "0" when compaired with control group. Similar finding was observed by Mohan *et al*., (2015) who opined that due to lack of nutrients and optimum ruminal pH protozoal motility was sluggish. Protozoal motility and protozoal density was increased in group I when compared with other treatment group. Similar finding was observed by Nasr *et al*., (2017) [15].

Protozoal count (x 10^5/ml)

The Mean protozoal count in group I, II, III and IV (control) before treatment on day "0" and after treatment on day 7th and day 14th are presented in Table 4.

Table 4: Mean protozoal count (x 10^5/ml) in Group I, Group II, Group III and Group IV (Healthy control)

	Day "0"	Day 7th	Day 14th	Pooled mean
Group I	0.61b±0.10	1.15a±0.19	1.37a±0.18	1.04±0.20
Group II	0.62±0.10	0.92±0.10	0.90±0.10	0.82±0.11
Group III	0.66±0.08	0.83±0.10	1.02±0.16	0.84±0.13
Group IV	1.30±0.14	1.25±0.15	1.34±0.12	1.30±0.13
Pooled mean	0.80±0.16	1.04±0.15	1.16±0.16	

Group I CD 5% value is 0.483.
Similar superscripts are non-significant.

Protozoal count in SARA affected cattle of group I, II and III was 0.61±0.10, 0.62±0.10 and 0.66±0.08. This count was less as compared with control group IV (1.30±0.14). Similar finding was observed by Nawid (2012) [16], Mohan *et al*., (2015) [13] and Malekkhahi *et al*., (2015) [11].

pH of ruminal fluid is critical factor in growth of ciliated protozoa in rumen because ciliated protozoa are more sensitive than bacteria to changes of altering rumen pH (Granja-Salcedo *et al*., 2016) [9]. As per Nagaraja and Titgemeyer (2007) [14] reduced rumen ciliated population may be a sign of SARA.

After treatment mean protozoal count (x 10^5/ml) in group I significantly increased on day 7th (1.15±0.19) and day 14th (1.37±0.18) when compared with day 0. After treatment mean protozoal count (x 10^5/ml), in Group II increased on day 7th (0.92±0.10) and decreased on day 14th (0.90±0.10). But this change was statistically non significant. After treatment mean protozoal count, in Group III increased on day 7th (0.83±0.10) and on day 14th it was 1.02±0.16.

Bipin *et al*., (2016b) [3] observed increased protozoal count from 1.17±0.18 to 2.28±0.30 on day 3rd after treatment with sodium bicarbonate. *Saccharomyces cerevisiae* stimulates maturation of microbial ecosystem in rumen (Malekkhahi *et al*., 2015) [11]. Yeast helps to stabilize rumen microbiome which reduces effect of SARA (AlZahal *et al*., 2014) [1].

Conclusion

Rumen fluid examination of SARA affected animals showed rumen pH ranging from 5.2 to 6.0, change in colour i.e. green to slightly milky brown, change in consistency (watery to slightly viscous), reduced protozoal count and alteration in protozoal motility and density. Sedimentation activity time was reduced than the normal.

Use of powder sodium bicarbonate 50 gm orally once daily was found to be more effective than *Azadirecta indica* dried leaves powder along with jaggary and use of *Saccharomyces cerevisiae.*

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