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A Study on Occurrence and Haemato-biochemical Alterations in SARA in Cattle Treated with Different Therapeutic Regimens

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ABSTRACT

Background: Subacute ruminal acidosis is one of the most important nutritional diseases in cattle. The consequence of feeding excessive amounts of rapidly fermentable carbohydrates in conjunction with inadequate fiber to ruminants leads to subacute ruminal acidosis. Cattles are at a high risk of developing SARA. The present research work was undertaken to study haemato-biochemical alterations in SARA affected cattle treated with different treatment regimens.

Methods: Present work was done to study the efficacy of sodium bicarbonate powder, *Azadirachta indica* (Neem) dried leaves powder and *Saccharomyces cervisiae* (Yeast) in sub-acute ruminal acidosis (SARA) in cattle. Out of 148 cattle screened, 24 (16.22%) were diagnosed as SARA, 13 (56.52%) animals were in mid stage of lactation followed by early and late lactation (5 cases, 21.74% each).

Result: After sodium bicarbonate treatment, animals showed changes in various haemato-biochemical parameters. However decreased neutrophils and ALT was also observed. After treatment of *Azadirachta indica* reduction in lymphocyte and eosinophil count was seen.

Key words: *Azadirachta indica* (Neem), SARA, Sodium bicarbonate, Yeast.

INTRODUCTION

Subacute ruminal acidosis is considered subacute when the low ruminal pH is caused by excessive accumulation of volatile fatty acids without persistent lactic acid accumulation, later restored to normal pH by animal's own physiologic responses. It is an economically important condition seen even in well managed herds which results in subsequent deterioration of health and loss of production (Prasanth and Ajithkumar 2016).

The present research work was undertaken to study the occurrence and haemato-biochemical alterations in SARA affected cattle treated with different treatment groups.

MATERIALS AND METHODS

The present research work was conducted from February to July 2019, in and around Parbhani district of Maharashtra at department of veterinary clinical medicine, college of veterinary and animal sciences Parbhani. Cattle presented with complain of suboptimal appetite, reduced body condition in spite of adequate energy intake, intermittent diarrhoea and suboptimal milk production were subjected to further clinical evaluation and rumen fluid analysis. Animals having indigestion and ruminal pH ranging from 5.2 to 6 were selected for further study. Eighteen SARA positive animals were randomly divided into 3 treatment groups (n=6). Group I was treated with powder sodium bicarbonate 50 gm orally once, group II was given 100 gm of dried leaves powder of *Azadirachta indica* with jaggary (to reduced bitter effect of dried neem leaves taste) orally once daily, while group III was treated with yeast (*Saccharomyces cerevisiae,* 1 X 10^{10} CFU/gm) @ 5 gm orally daily once. Treatment for all groups was continued for 5 days and observations taken on day 0 (before treatment) day 7 and day 14 after treatment. A group of healthy cattle (Group IV) was used as control group.

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RESULTS AND DISCUSSION

Occurrence

Occurrence of SARA in cattle was studied at Parbhani and surrounding area during February to July 2019. Total 148 animals were screened. Out of these 24 (16.22%) animals were diagnosed as sub-acute ruminal acidosis. Similar findings were observed by Kleen *et al*., (2009) who found 13.8% SARA cases, while Petrujkic *et al*., (2010) found 20% occurrence of this condition in cattle.

Kleen *et al*., (2003) mentioned that due to different geographical region, difference of feed and ruminal environment change in occurrence was observed.

Sex wise, 23 females (95.83%) and only one male (4.17%) was found to be suffering from SARA. Kleen *et al*.,

(2009) mentioned that it is common problem of dairy farm. Alzahal *et al*., (2014) opined that dairy cows fed on fermentable carbohydrates are commonly encountered with SARA. The age of cattle suffering from SARA was ranging from 3 to 9 years. Breed wise, SARA was observed in 19 (79.16%) HF, 2 (8.33%) non-descript, 1 (4.16%) Kankrej, 1 (4.16%) Jersey x HF cross and 1 (4.16%) Red Kandhari animal. Nawid (2012) observed 70% incidence of SARA in Jersey and 30% in HF cow.

Table of Breed wise occurrence of SARA in cattle.

Breed of animal (no. of animals)	Per cent occurrence (%)
Holstein Friesian (19)	79.16
Non-descript (2)	8.33
Kankrej (1)	4.16
Jersey x HF cross (1)	4.16
Red Kandhari (1)	4.16

Age wise study revealed that SARA was observed at the mean age of 4.63±0.68 years. Nawid (2012) found that cattle affected with SARA were of 3-12 years age.

Table of Distribution of SARA according to stage of lactation.

Stage of lactation	No. of animals	Per cent occurrence
Early (1-2 months)	5	21.74%
Mid (3-5 months)	13	56.52%
Late (5 months above)	5	21.74%

It was observed that out of 23 female animals, 13 (56.52%) were in mid lactation. SARA occurs due to high feed intake in mid lactating cows which are sensitive to sudden change in feed. Similar finding was observed by Enemark (2009). Nawid (2012) observed no impact of lactation number on SARA. Dietary changes in dry period to lactation diet is one of the reason of occurrence of SARA in mid and early lactation. (Garrett *et al*., 1999). It is common practice to feed large amount of concentrates to cows during early and mid lactation to full fill the requirement of nutrition in dairy cows. Similar finding was observed by Humer *et al*., (2017).

Kleen *et al*., (2009) mentioned that no significant difference was observed in body condition and ruminal pH in SARA. Li *et al*., (2012) observed that SARA did not affect health condition of the cows.

Haematology: **Hemoglobin** (**g %), Packed cell volume (%), Total erythrocyte count (x 10^6/µL).**

Hemoglobin (**g %)**

In SARA affected animals, mean Hb (g/dL) concentration on day "0" in Group I, II and III was 8.58±0.42, 8.75±0.42 and 8.40±0.58 as against the control group IV (8.02±0.67). SARA affected animals showed increased Hb concentration when compared with control group. Similar finding was recorded by Ceroni *et al*., (2012) and Marchesini *et al*., (2013). Bipin *et al*., (2016a) observed that in SARA affected animals there was increased TEC and Hb. Huber (1971) opined that increased haemoglobin concentration might be due to increased osmolarity in rumen content that caused withdrawal of fluid from intravascular compartment.

After treatment in Group I significantly increased mean Hb (g/dL) concentration was observed on 7th day (9.92±0.19 g/dL) and 14th day (10.07±0.22 g/dL) Table 1. Similar finding was observed by Bipin *et al*., (2016b) who found increased mean Hb concentration after sodium bicarbonate treatment in SARA affected cows. In Group II and III, there was non-significant increase in haemoglobin concentration 7th and 14th day of treatment.

The finding of present study is in corroboration with finding of Constable *et al.*, (2017) who observed that the hemoglobin (g/dL) concentration of SARA affected animals ranged within normal range *i.e.* 8.5-12.2 g/dL.

Packed cell volume (%)

Increased mean PCV was observed in SARA affected animals as compared to control group (Table 1). Similar finding was observed by Li *et al*., (2012), Marchesini *et al*., (2013), Shah *et al*., (2013) and Danscher *et al*., (2015). Huber (1971) opined that increased PCV concentration in SARA might be due to to increased osmolarity in rumen content that caused withdrawal of fluid from intravascular compartment.

After treatment in Group I, it was observed that PCV % has increased on day 7th (31.24±0.84) and on day 14th (31.35±0.75). These PCV values were non-significantly high. Similar finding has been noted by Bipin *et al*., (2016b) who observed increased mean PCV concentration after sodium bicarbonate treatment in SARA affected cows. In group II and III also non-significant changes in PCV percentage were seen on 7th (28.93±1.18) and (29.35±1.09) 14th day of treatment.

As per Constable *et al.,* (2017) PCV in SARA remain within normal range. However, in present study an increase in PCV values was observed in SARA affected animals.

Total erythrocyte count (x 10^6/µL)

Mean TEC (x 10^6/µL) in SARA affected group I, II and III was 6.01±0.32, 5.76±0.19 and 5.74±0.35 and in control group it was 5.66±0.37. All groups showed non-significant increase in TEC as compared to control group. Similar finding was observed by Li *et al*., (2012) and Bipin *et al*., (2016a)

After treatment in Group I, mean TEC (x 10^6/µL) on day 7th was 6.21±0.16 and on 14th day it was 6.40±0.11. This increase in TEC after treatment was non-significant (Table 1). Similar finding was noted by Bipin *et al*., (2016b) who observed increased mean TEC concentration after sodium bicarbonate treatment in SARA affected cows. In group II and group III, non-significant changes in TEC was seen on 7th and 14th day of treatment.

Total leucocyte count (x 10^3/µL)

TLC has been found to be increased in SARA affected animals as compared to control group (Table 1). Similar finding was noted by Bipin *et al*., (2016a) who mentioned

Table 1: Changes in haematological parameters with different treatment regimens at weekly interval.

Group	Haemoglobin (As on day)			PCV (As on day)			TEC (As on day)			TLC (As on day)		
	0	7	14	0	7	14	0	7	14	0	7	14
I	8.58[b]±0.42	9.92[a]±0.19	10.07[a]±0.22	29.35±1.66	31.24±0.84	31.35±0.75	6.01±0.32	6.21±0.16	6.40±0.11	11.58±0.27	10.74±0.27	10.57±0.34
II	8.75±0.42	8.97±0.41	8.98±0.35	28.90±1.44	28.93±1.18	29.35±1.09	5.76±0.19	5.88±0.15	5.77±0.17	11.82±0.21	11.70±0.16	11.52±0.32
III	8.40±0.58	8.58±0.34	8.60±0.42	28.30±1.53	27.52±1.47	28.10±1.08	5.74±0.35	6.18±0.15	5.98±0.22	11.03±0.41	10.98±0.28	11.28±0.29
IV	8.02±0.67	8.30±0.55	8.25±0.51	24.97±1.93	25.13±1.55	25.23±1.31	5.66±0.37	5.65±0.25	5.69±0.25	9.38±0.14	9.35±0.16	9.31±0.18

that during stress increased cortisol level leads to increase in TLC. However, Li *et al*., (2012) observed fall in TLC count in SARA when compared with control group.

After treatment in Group I, it was observed that mean TLC on day 7th was decreased to 10.74±0.27 and on day 14th it was 10.57±0.34. This decrease in TLC count was non-significant. Similar finding has been observed by Bipin *et al.*, (2016b) after the treatment with sodium bicarbonate. After treatment in Group II, TLC decreased on day 7th (11.70±0.16) and day 14th (11.52±0.32) but statistically it was non-significant fall. After treatment in Group III decreased TLC was seen on day 7th (10.98±0.28) and it increased on day 14th (11.28±0.29). But these changes were also statistically non-significant.

Differential leucocyte count (DLC) %: Neutrophil, Lymphocyte, Monocyte, Basophil and Eosinophil

Neutrophil (%)

SARA affected cattle showed increased netrophil (%) count when compared to control group. Similar finding was observed by Bipin *et al*., (2016a) and Rodriguez-lecopte *et al*., (2014). Increase in neutrophil may be due to increased WBC count (Cannizzo, 2009).

In all treatment groups, a non-significant reduction in neutrophil count was observed on 7th as well as 14th day of treatment (Table 2). Similar finding was observed by Bipin *et al*. (2016b) who observed decreased mean neutrophil (%) count after sodium bicarbonate treatment in SARA affected cows.

Lymphocyte (%)

In SARA affected cattle showed decreased lymphocyte (%) count when compared with the control group. Similar observation was observed by Gozho *et al*., (2007), Rodriguez-lecopte *et al*., (2014) and Bipin *et al*., (2016a).

After treatment with sodium bicarbonate in Group I, increased mean lymphocyte (%) was observed on 7th day (43.83±1.74) and on 14th day (51.83±1.72) (Table 2). Similar finding was observed by Bipin *et al*., (2016b) in sodium bicarbonate treatment group. After treatment with *Azadirachta indica* in Group II, decreased mean lymphocyte (%) was observed on day 7th (42.67±1.86) and significantly increased count was seen on day 14th (52.83±2.44). After treatment with *Saccharomyces cervisiae* in Group III, decreased mean lymphocyte was observed on day 7th (44.67±1.74) which further increased on day 14th (44.83±1.33). This increase in lymphocyte (%) value was non-significant.

Monocyte

The mean monocyte count in group I and II was decreased when compared with control group. Similar observation was noted by Gozho *et al*., (2007), Rodriguez-lecopte *et al*., (2014) and Bipin *et al*., (2016a).

After treatment with sodium bicarbonate in Group I, decreased mean monocyte count (%) was observed on 7th day (4.33±0.42) which increased on day 14th (4.83±0.55)

Table 2: Change in neutrophil, lymphocyte, monocyte, basophil and eosinophil with different treatment regimens at weekly interval.

Gr.	Neutrophil (As on day)			Lymphocyte (As on day)			Monocyte (As on day)			Basophil (As on day)			Eosinophil (As on day)		
	0	7	14	0	7	14	0	7	14	0	7	14	0	7	14
I	51.83a ±1.38	50.00a ±1.79	41.67[b] ±1.63	0.33 ±0.21	0.50 ±0.22	0.33 ±0.21	0.33 ±0.21	0.50 ±0.22	0.50 ±0.22	0.33 ±0.21	0.50 ±0.22	0.50 ±0.22	0.83 ±0.17	1.17 ±0.31	1.00 ±0.26
II	50.83a ±1.96	49.83a ±1.30	39.33b ±2.14	0.33 ±0.21	0.33 ±0.21	0.33 ±0.21	0.33 ±0.21	0.33 ±0.21	0.33 ±0.21	0.33 ±0.21	0.33 ±0.21	0.50 ±0.22	1.00b ±0.26	1.00b ±0.26	2.00a ±0.34
III	49.33 ±1.28	49.17 ±1.25	48.67 ±1.38	0.33 ±0.21	0.17 ±0.17	0.33 ±0.21	0.33 ±0.21	0.17 ±0.17	0.17 ±0.17	0.33 ±0.21	0.17 ±0.17	0.33 ±0.21	0.83 ±0.31	0.67 ±0.21	0.67 ±0.21
IV	42.67 ±2.04	43.17 ±0.83	43.17 ±1.62	0.17 ±0.17	0.17 ±0.17	0.17 ±0.17	0.17 ±0.17	0.17 ±0.17	0.17 ±0.17	0.17 ±0.17	0.17 ±0.17	0.33 ±0.21	2.17 ±0.48	1.83 ±0.54	1.83 ±0.31

(Table 2). Similar finding was observed by Bipin *et al*., (2016b) in sodium bicarbonate treatment group. This change in monocyte count was non-significant. After treatment with *Azadirachta indica* in Group II non-significant increased mean monocyte (%) count was observed on day 7th (6.17±0.48) and on day 14th the count was 5.33±0.76. After treatment with *saccharomyces cervisiae* in Group III decreased mean monoocyte value was observed on day 7th (5.33±0.80) and on day 14th (5.50±0.56). Monocyte (%) count in all groups was found to be within the normal range (0-8%) (Kahn and Line, 2010).

Basophil

There was increase in mean basophil count (%) in SARA affected animals when compared with control group.

All the treatment groups showed non-significant changes in basohil count on 7th as well as 14th day of treatment (Table 2).

Basophil (%) count in all groups was found to be within the normal range (0-2%) (Kahn and Line, 2010).

Eosinophil

SARA affected animals showed decreased eosinophil (%) when compared with control Group IV.

After treatment mean eosinophil (%) in Group I increased on day 7th (1.17±0.31) and decreased on day 14th (1.00±0.26). This change was non-significant. After treatment in Group II significant increase on 14th day (2.00±0.337) was seen. After treatment in Group III decreased eosinophil (%) count was seen on 7th day and on 14th day it was 0.67±0.21.

This change was non-significant (Table 2). Easophil (%) count in all groups was found to be within the normal range (0-20%) (Kahn and Line, 2010).

Biochemical analysis: TSP (g/dL), Albumin (g/dL), Globulin (g/dL), Aspartate aminotransferase (AST) (IU/L) and Alanine aminotransferase (ALT) (units/L)

Total serum protein (g/dL)

Total serum protein in SARA positive cattle on day "0" in group I, II, III and control group was 7.76±0.33, 7.55±0.27, 7.75±0.29 and 7.48±0.31 respectively. SARA affected cattle have shown non-significant increase in total serum protein when compared to control group (Table 3). Similar finding was observed by Cannizzo *et al*., (2012) and Danscher *et al*., (2015) and contrast finding was observed by Rodriguez-Lecompte *et al*., (2014) and Trevisi *et al*., (2014), After treatment in all groups, total protein was found to be altered non-significantly on both observations days, Constable *et al*., 2017.

Albumin (g/dL)

Mean albumin in treatment groups was increased as compared to control group. Similar finding was observed by Cannizzo *et al*., (2012) Trevisi *et al*., (2014) and contrast finding was noted by Nasr *et al*., (2017).

After treatment all groups showed non-significant alteration in albumin concentration on both observation days (Table 3).

Albumin (g/dL) in all groups found within the normal range (2.1-3.6 gm/dL) (Constable *et al.* 2017).

Globulin (g/dL)

The mean Globulin on day "0" in group I, II, III and IV was 4.57± 0.30, 4.43± 0.05, 4.60± 0.19 and 4.41±0.12 respective lean globulin was found to be increased in treatment group I, II and III as compared to control group. However Rodriguez-Lecompte *et al*., (2014) has observed that the globulin remained within the reference range in SARA affected animals.

After treatment, non-significant changes in globulin concentration were seen in all treatment groups on 7th as well as 14th day of treatment (Table 3).

Aspartate aminotransferase (AST) (IU/L)

The mean AST (IU/L) was increased non- significantly in SARA affected group I, II and III when compared with control group. Similar finding was observed by Prasanth and Ajithkumar (2016) and Nasr *et al*., (2017). Nawid (2012) and Rodriguez-Lecompte *et al*., (2014) opined that due to the transfer of bacteria from rumen to various organs inflammatory changes are observed in other organs. It may lead to increased AST (IU/L) value. Marchesini *et al*., (2013) mentioned that increased AST may be due to stress on liver due to various dietary changes.

After treatment non-significant changes in AST were observed in all treatment groups on 7th as well as 14th day of treatment (Table 3).

Aspartate aminotransferase in all groups was within the normal range *i.e*. 78-132 IU/L (Constable *et al*., 2017).

Alanine aminotransferase (ALT) (units/L)

In SARA affected cattle showed increased mean value of ALT when compared to control group. Similar finding was noted by Nasr *et al.,* (2017).

After treatment with sodium bicarbonate mean Alanine aminotransferase in Group I was significantly decreased. Mean ALT on 7th day was 31.75±1.68 and on 14th day it was 32.48±1.76. After treatment with *Azadirachta indica* mean ALT in Group II was decreased on 7th day (38.98±1.37) and increased on 14th day to 39.10±1.36. This change was non-significant. After treatment with *Saccharomyces cervisiae* mean value of ALT in Group III was decreased. Mean ALT on 7th day was 38.90±1.08 and on 14th day it was 38.85±0.96. This reduction in AST value was non-significant. (Table 3).

Glucose (mg/dL)

The mean glucose level was found to be increased in SARA affected group I, II and III when compared with control Group IV (Table 3). Similar finding was noted by Khafipour *et al*., (2009), Li *et al*., (2012) and Trevisi *et al*., (2014). Due to feeding of more fermentable carbohydrate diet concentration of glucose in blood plasma might have increased (Malekkhahi *et al*., 2015).

Table 3: Change in TSP, Albumin, Globulin, Glucose, AST and ALT with different treatment regimens at weekly interval.

Group	TSP (As on day)			Albumin (As on day)			Globulin (As on day)			Glucose (As on day)			AST (As on day)			ALT (As on day)		
	0	7	14	0	7	14	0	7	14	0	7	14	0	7	14	0	7	14
I	7.76 ±0.33	8.14 ±0.19	8.21 ±0.16	3.19 ±0.12	3.18 ±0.17	3.43 ±0.08	4.57 ±0.30	4.97 ±0.14	4.78 ±0.14	57.33 ±2.67	58.83 ±1.72	58.83 ±1.60	97.58 ±2.23	92.62 ±3.24	95.27 ±2.67	39.07a ±1.17	31.75b ±1.68	32.48b ±1.76
II	7.55 ±0.27	7.70 ±0.12	7.68 ±0.14	3.12 ±0.25	3.34 ±0.08	3.35 ±0.15	4.43 ±0.05	4.36 ±0.05	4.34 ±0.06	57.17 ±2.18	57.83 ±1.76	58.67 ±1.43	97.88 ±2.36	98.02 ±2.03	99.95 ±2.19	39.20 ±1.38	38.98 ±1.37	39.10 ±1.36
III	7.75 ±0.29	7.72 ±0.19	7.79 ±0.13	3.16 ±0.22	3.38 ±0.15	3.35 ±0.11	4.60 ±0.19	4.34 ±0.13	4.45 ±0.12	57.83 ±2.21	58.17 ±1.92	58.50 ±2.28	97.90 ±2.08	97.65 ±1.99	97.13 ±2.43	39.23 ±1.21	38.90 ±1.08	38.85 ±0.96
IV	7.48 ±0.31	7.54 ±0.28	7.64 ±0.29	3.08 ±0.24	3.10 ±0.21	3.16 ±0.16	4.41 ±0.12	4.43 ±0.20	4.48 ±0.21	56.13 ±2.98	57.63 ±0.95	57.17 ±1.99	89.77 ±1.93	89.98 ±1.48	89.90 ±1.55	31.47 ±0.45	31.65 ±0.47	31.73 ±0.45

CONCLUSION

SARA occurs mostly in cows of 4.63±0.68 years age in their mid-lactation. Treatment with soda-bicarbonate is found to be better as compared to treatment with *Azadirachta indica* (Neem) dried leaves powder or *Saccharomyces cerevisiae*.

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