



Effects of Subacute Ruminal Acidosis (SARA) on Epidemiological and Clinicopathological Parameters of Dairy Cattle

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ABSTRACT

Background: Subacute ruminal acidosis (SARA) is one of the most important metabolic disease of dairy cattle which decrease milk production, dry matter intake, productivity and increase culling rate and even cause death.

Methods: In this study the epidemiological status of SARA namely age, breed, stage of lactation, lactation number, general health condition, body condition score, daily milk production, milk fat content and biochemical alteration were investigated.

Result: Out of 100 cows examined 20 were found to be positive for SARA and the mean age of dairy cows suffering from SARA was found to be 6.53 $\pm$ 0.68 years. The prevalence of SARA was 70 and 30 per cent in Jersey and HF breed of cows respectively and was high in mid lactation and the lactation number ranged from 2 to 6. 65 per cent. SARA affected cows were in poor body condition score, milk production and milk fat content was significantly low ($P \leq 0.05$). The mean GGT (Gamma Glutamyl Transferase) and AST (Aspartate aminotransferase) values were significantly increased in SARA affected dairy cows. SARA seems to be a common problem in dairy cattle and it has to be considered when dealing with herd level problems like low milk fat, low milk production, poor body condition and appropriate management measures should be implemented to prevent it.

Key words: Dairy cattle, Rumenocentesis, Rumen-fluid pH, Subacute ruminal acidosis.

INTRODUCTION

Subacute ruminal acidosis is one of the most important metabolic disease in dairy cattle since it can negatively affect the dairy industry by decreasing dry matter intake, milk production, profitability and increase culling rate and even cause death of the animals (Enemark, 2008, McCann *et al.*, 2016). Subacute ruminal acidosis is defined as a period of moderately depressed ruminal pH, from about 5.5 to 5.0 and is more common in dairy farms (Krause and Otzel, 2006).

SARA affects fermentation in rumen, productivity and farm profit. It is estimated that in the United States the economic loss associated with SARA is US \$ 500 million to US \$ 1 billion annually. Garret *et al.* (1997) reported a prevalence of SARA in 19% of early lactating cows and 26% in mid lactation cows and more than 40 per cent of the total number of cows were found to have SARA. Similarly high prevalence (32.5-37.7%) of SARA was reported by Attia (2016).

The complex etiology of SARA necessitates routine monitoring and the clinical signs of SARA are subtle and can easily be overlooked. The clinical signs include decreased dry matter intake, laminitis, (Kleen *et al.*, 2003) and low fertility (Britt, 1995).

SARA has been linked to loss of body condition, milk fat depression and lameness (Garrett 1996, Oetzel 2000 and Livesey *et al.*, 2003). It has been pointed out recently

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that the pathogenic mechanism of SARA might consist of activation of systemic inflammatory responses due to lysis of gram-negative bacteria (Gozho *et al.*, 2006 and Gozho *et al.*, 2007). SARA cannot be diagnosed based on clinical signs alone, therefore studying rumen fluid pH is the only

method recommended for diagnosis of SARA in dairy cows (Tajik and Nazifi, 2011). Though estimation of lipopolysaccharide in rumen and rumen temperature are tested by earlier workers (Khafipour *et al.*, 2009a), Rumenocentesis remains the most reliable means of diagnosing of SARA (Abdela, 2016).

MATERIALS AND METHODS

This study was conducted in the Department of Veterinary Medicine Veterinary College, Hebbal, Bangalore 560024. from April 2018 to June 2019.

Animals

Dairy cows at Instructional Livestock Farm Complex (ILFC), Veterinary College, Hebbal, Bangalore and other organized dairy farms under the same feeding protocol in and around Bangalore served as experimental animals in this study. A total of 100 dairy cows were selected randomly for this study. Rumen fluid was collected from dairy cows by rumenocentesis as explained by Garrett *et al.* (1999). Rumen fluid was collected 4 hours after feeding the dairy cows.

Rumen fluid analysis

In the present study adequate rumen fluid was collected from individual dairy cows by rumenocentesis (Plate 1) four hours after feeding as per the method described by Duffield *et al.* (2004). A portable pH meter was utilized in the study to measure rumen fluid pH and it is considered suitable to measure pH of rumen fluid as it yields very similar pH reading as did a standard meter with a pH probe (Garrett *et al.*, 1999).

The pH of rumen fluid was determined immediately after collecting the rumen fluid. Dairy cows with rumen pH ranging from 5.2 to 5.6 were selected and cases where rumen pH continues to be in the range of 5.2 to 5.6 for minimum of

3 hours were considered as positive for SARA (Gozho *et al.*, 2005). Dairy cows with ruminal pH above 5.6 were considered as negative for SARA and dairy cows with ruminal pH less than 5.2 were considered to be positive for acute ruminal acidosis.

Epidemiological study of SARA

Information regarding age, breed, lactation number and stage of lactation of animals were collected from dairy cows positive for SARA as per the ruminal pH, using data collection sheet (Appendix 1).



Plate 1: Collection of rumen fluid by rumenocentesis.

Appendix 1: Sub acute ruminal acidosis data collection sheet.

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Name of the dairy farm _____ Case no _____ Date: _____ Animal no _____ Breed _____
Age _____ Lactation no _____ Date of recent calving _____ Lactation stages (Early Mid Late) Milk production _____ /day

Body Condition Scoring ('0' Very poor, '1' Poor, '2' Moderate, '3' Good, '4' Fat, '5' Grossly fat)

General health condition -----of the animal is not / slightly / moderately / severely disturbed.

Ruminal pH _____ Type of acidosis (SARA CRA) Normal

Milk Sample

Milk fat content % _____

Blood Sample

GGT _____ SGOT _____

Studies on SARA affected dairy cows

Twenty dairy cows suffering from SARA and twenty dairy cows not suffering from SARA (normal) as a control group were included in this study. The general health condition of dairy cows were assessed based on the physical examination of animals for parameters such as pasture, behavior, appetite, physical condition, respiratory rate, pulse rate and rectal temperature and was classified as "not disturbed", "slightly disturbed", "moderately disturbed" and "severely disturbed" as explained by Rosenberger (1979). Body condition score of dairy cows was determined as explained by Mulvaney (1977) and Radostits *et al.* (2000) and was graded as very poor, poor, moderate, good and fat. Daily milk production from dairy cows were recorded twice a day and the average daily milk production was calculated. Milk sample collected from dairy cows was subjected for estimation of milk fat using electronic milk fat tester (Rajasthan electronic and instrument Ltd, Jaipur, India).

Blood sample and biochemical study

Five ml of blood was collected from individual dairy cows in EDTA and plasma was separated. Plasma samples from individual animals were stored separately at 4°C in disposable sterile serum collection tubes until subjected for biochemical analysis. Biochemical parameters namely gamma glutamyl transferase (GGT) and aspartate amino transferase (AST/SGOT) were estimated using ARTOS semiautomatic biochemical analyzer as per the procedure described in the manual by using reagent kits supplied by M/S Rashmi Diagnostics, Bangalore.

Statistical analysis

Statistical analysis was performed using the statistical software graph pad prism, version 5 for windows. The data were analyzed by Student's t-test (One-sample, paired and unpaired) to arrive at conclusion and *P* value of ≤ 0.05 was considered significant.

RESULTS AND DISCUSSION

Out of 100 dairy cows examined for SARA based on rumen fluid pH, it was noticed that 20 dairy cows had rumen fluid pH ranging from 5.2 to 5.6 for more than 180 minutes/day. Thus, the prevalence of SARA in this study was 20 per cent. Rumen fluid pH of cows in control group was 6.53 ± 0.68 (Table 1). Garret *et al.* (1999) and Oetzel *et al.* (1999) have reported prevalence of SARA to be 20 to 30 per cent. However, Bramley *et al.* (2008) and O'Grady *et al.* (2008) observed SARA in only 10 per cent of the dairy animals. This difference may be attributed to difference in geographical area, type of feed and ruminal environment in term of flora and ruminal mucosa as rightly reported by Kleen *et al.* (2003).

Twenty cows from 80 normal group and twenty suffering from SARA were compared. The age of dairy cows suffering from SARA based on pH of rumen fluid ranged from 3-12

years and the mean $\pm$ SE age of cows suffering from SARA was 6.53 ± 0.68 . Perusal of available literature failed to provide any information regarding the relationship between age and occurrence of SARA in dairy cows.

The prevalence of SARA was high (70%) in Jersey breed of dairy cows as against 30 percent in HF. However, available literature failed to provide any information on the influence of breed on prevalence of SARA in dairy cows and it may be related to the high number of the Jersey breed of cows in the dairy farms.

Out of 20 SARA affected dairy cows, 5 dairy cows (25%) were in early lactation, 9 (45%) in mid lactation and 6 (30%) in late lactation (Table 2). The incidence of SARA was high during mid lactation followed by cows in late and early lactation. This observation agrees with the finding of Garrett *et al.* (1997), Oetzel *et al.* (1999) and Kleen (2004). The development of SARA in mid lactation is linked to the managerial factors like feeding frequency, processing of feed, housing and similar influences as indicated by Oetzel (2000). Further, change from dry-period diet to lactation diet puts cows at high risk for developing SARA. Similar opinion was expressed by Brand and Warner (1996) and Nocek (1997). The lactation number in SARA affected dairy cows ranged from 2 to 6 with mean $\pm$ SE of 3.00 ± 0.41 . This indicated that SARA can be observed in dairy cows irrespective of the lactation number.

Out of 20 cows suffering from SARA 11 (55%) had disturbed health condition but 19/20 (95%) had no disturbance on the general health condition. This observation agrees with the observations made by Nocek (1997). SARA

Table 1: Rumen fluid pH of normal and SARA affected dairy cows.

S/No.	Normal	SARA
1	6.5	5.5
2	6.3	5.5
3	6.4	5.4
4	6.7	5.2
5	6.5	5.4
6	6.4	5.4
7	6.5	5.5
8	6.5	5.5
9	6.6	5.6
10	6.3	5.6
11	6.6	5.6
12	6.6	5.6
13	6.4	5.6
14	6.6	5.6
15	6.5	5.5
16	6.5	5.5
17	6.4	5.4
18	6.4	5.4
19	6.4	5.4
20	6.5	5.5
Mean $\pm$ SE	$6.49^a \pm 0.02$	5.48 ± 0.01^b

Note: Mean bearing different superscript differ significantly ($P \leq 0.05$).

results in chronic inflammation, decreased dry matter intake (Webel *et al.*, 1997 and Oetzel, 2000), diarrhea (Nocek, 1997, Kleen *et al.*, 2003 and Oetzel, 2003), reduced fiber digestibility (Nordlund, 2004) and generalized abscessation. In addition, ruminal papillae adhere together to form bundles in SARA affected cows and thus decreasing ability of rumen wall to absorb volatile fatty acids (Nocek, 1997). These factors, either individually or jointly might have caused disturbance in the general health condition in SARA affected dairy cows.

Table 2: Epidemiological data of SARA in dairy cows.

Sr. no.	Age/ year	Breed	Lactation number	Stage of lactation
1	3.0	HF	2	Early
2	8.0	Jersey	5	Mid
3	10	Jersey	2	Late
4	4.0	Jersey	2	Late
5	3.5	HF	2	Early
6	5.0	HF	3	Early
7	12	Jersey	6	Mid
8	4.0	HF	3	Mid
9	7.0	Jersey	2	Late
10	4.5	HF	3	Early
11	4.0	HF	2	Mid
12	3.0	Jersey	2	Mid
13	4.0	Jersey	2	Late
14	6.0	Jersey	4	Mid
15	12	Jersey	3	Mid
16	10.5	Jersey	4	Late
17	8.0	Jersey	4	Late
18	5.0	Jersey	2	Early
19	7.0	Jersey	2	Mid
20	10	Jersey	5	Mid
Mean $\pm$ SE	6.53 $\pm$ 0.68		3.00 $\pm$ 0.41	

HF: Holstein Friesian.

It was observed that 65 per cent of the animals suffering from SARA were in poor and 35 per cent in moderate body condition score (Table 3), indicating that body condition was highly affected in SARA affected dairy cows. This observation agrees with the finding of Nordlund *et al.* (1995), Webel *et al.* (1997), Oetzel (2000) and Kleen *et al.* (2003). The poor body condition observed in SARA affected cows can be attributed to chronic inflammation or decrease dry matter intake. Similar opinion was also expressed by Webel *et al.* (1997) and Oetzel (2000). Chronic inflammation observed in cases of SARA antagonize growth by releasing cytokines which opposes anabolism leading to poor body condition (Kleen *et al.*, 2003). Further, it can be stated that the body condition score cannot be used as a sole parameter to differentiate between SARA affected and healthy dairy herd (Kleen, 2004 and Tajik *et al.*, 2009). Hence it may be concluded that body condition score can be only one of the parameters for diagnosis of SARA in dairy cows.

Daily milk production in SARA affected dairy cows ranged from 6-13 liters with mean $\pm$ SE 8.92 $\pm$ 0.51 and the same in normal dairy cows ranged from 9-15 liters with Mean $\pm$ SE 12.32 $\pm$ 0.42. It was also noticed that SARA resulted in statistically significant ($P \leq 0.05$) reduction in daily milk production (Fig 1) Similar observations have been made by Stone (1999), Khafipour *et al.* (2009a) and George (2005). The rumen pH is the major determinant of the type of digestion that occurs in the rumen. A decrease in rumen pH causes the decrement of cellulolytic bacteria (Nagaraja *et al.*, 1978 and Goad *et al.*, 1998). Further SARA also affects fiber digestion (Nagaraja *et al.*, 1978). SARA also depresses dry matter intake in dairy cows (Kleen *et al.*, 2003). These factors either individually or jointly might have resulted in significant reduction in daily milk production in SARA affected dairy cows.

Milk fat content in SARA affected dairy cows and normal cows was 3.20 $\pm$ 0.18 and 4.81 $\pm$ 0.15 per cent respectively (Fig 1) and it was noticed that milk fat content was statistically ($P \leq 0.05$) low in dairy cows suffering from SARA as compared

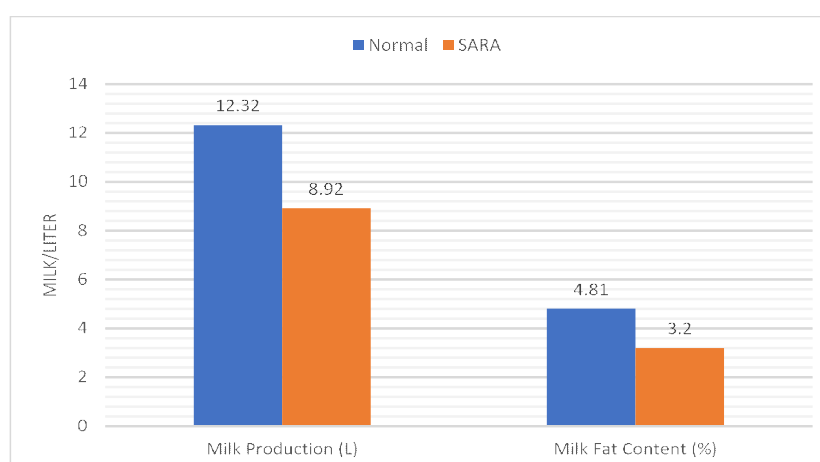


Fig 1: Daily milk production (L) and milk fat content (%) in normal and SARA affected dairy cows.

to normal cows. This observation agrees with the finding of Dirksen (1985), Nordlund *et al.* (1995), Allen (1997), Oetzel (2000) and George (2005). The alteration in the ruminal fermentation pattern in SARA affected dairy cows may be one of the factors for reduced milk fat content in SARA affected cows and similar opinion was expressed by Dirksen (1985). Low milk fat in cases of SARA can be related to the ratio of Acetate and Butyrate to Propionate in the rumen. Readily available fermentable carbohydrates in the diet favors a shift in rumen fermentation towards Propionate. This leads to low intra ruminal production of Vitamin B-12 which causes reduction in the conversion of Propionate to

Succinyl-co A, resulting in low fat synthesis in various tissues including the mammary gland resulting in low milk fat percentage in cases of SARA (Suton *et al.*, 1986 and Van Soest, 1994). As per the biohydrogenation theory, ruminal biohydrogenation will be affected in SARA affected dairy cows and intermediates resulting from altered biohydrogenation act on the mammary gland, thus inhibiting synthesis of fatty acids and decrease in milk fat content in these cows (Bauman and Griinari, 2001 and 2003). On the other hand, low milk fat content was not observed during some of the experimental induction of SARA (Oetzel, 2005 and Tajik *et al.*, 2009). This inconsistent response in milk fat in experimentally induced SARA may be related to the duration of the bouts of SARA. Milk fat content will not get affected if SARA observed in the dairy cow is of short duration (Oetzel, 2005).

The Mean $\pm$ SE GGT values in SARA affected dairy cows was 23.09 ± 1.44 IU/L and the same in normal cows was 7.10 ± 0.30 and there was statistically significant ($P \leq 0.05$) difference between the mean values. The mean GGT value was statistically elevated in SARA affected dairy cows (Fig 2). Perusal of available literature failed to provide information regarding influence of SARA on GGT activity in dairy cows. However, SARA is associated with inflammations of different organs (Nordlund *et al.*, 1995). In cases of SARA the function of ruminal mucosa as a barrier between ruminal environment and blood stream is impaired, thus enabling bacteria to translocate via the ruminal mucosa into the portal blood flow, colonizing in the liver tissue and from there spreading to other tissues in the body (Nocek, 1997). Hence it may be safe to conclude that inflammatory changes in the liver caused due to translocation of bacteria to the liver tissue might have resulted in statistically significant increase in the mean GGT values in SARA affected dairy cows.

The AST values in SARA affected cows and unaffected normal cows were 130.5 ± 10.97 and 80.86 ± 2.11 IU/L respectively and were statistically ($P \leq 0.05$) higher in SARA affected dairy cows (Fig 2). SARA brings about inflammatory

Table 3: General health condition and body condition score of normal and SARA affected dairy cows.

S/ no.	General health condition		Body condition score	
	Normal	SARA	Normal	SARA
1	Not disturbed	Not disturbed	Moderate	Poor
2	Not disturbed	Not disturbed	Moderate	Poor
3	Not disturbed	Not disturbed	Moderate	Poor
4	Not disturbed	Not disturbed	Moderate	Poor
5	Not disturbed	Not disturbed	Moderate	Poor
6	Not disturbed	Not disturbed	Good	Poor
7	Not disturbed	Not disturbed	Good	Poor
8	Not disturbed	Not disturbed	Good	Poor
9	Not disturbed	Not disturbed	Good	Poor
10	Not disturbed	Slightly disturbed	Good	Poor
11	Not disturbed	Slightly disturbed	Good	Poor
12	Not disturbed	Slightly disturbed	Good	Poor
13	Not disturbed	Slightly disturbed	Good	Poor
14	Not disturbed	Slightly disturbed	Good	Moderate
15	Not disturbed	Slightly disturbed	Good	Moderate
16	Not disturbed	Slightly disturbed	Good	Moderate
17	Not disturbed	Slightly disturbed	Good	Moderate
18	Slightly disturbed	Slightly disturbed	Good	Moderate
19	Not disturbed	Slightly disturbed	Fat	Moderate
20	Not disturbed	Slightly disturbed	Fat	Moderate

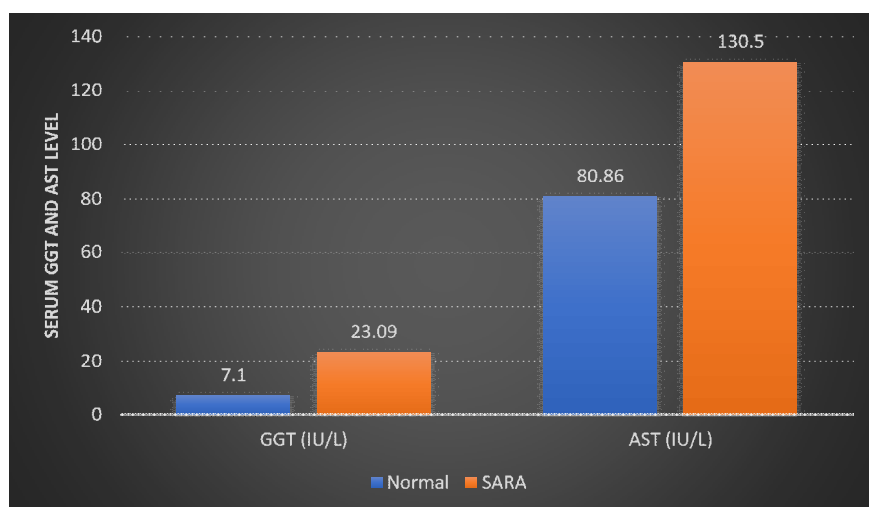


Fig 2: Mean $\pm$ SE GGT and AST in normal and SARA affected dairy cows.

changes in liver (Garry, 2002), kidney (Oetzel, 2000), lungs (Nordlund *et al.*, 1995), heart (Oetzel, 2000) and other tissue in the body (Nocek, 1997). Hence the elevation of mean AST values in SARA affected dairy cows observed in this study may be attributed to SARA associated inflammatory changes in various organs caused due to translocation of bacteria from rumen to various organs.

CONCLUSION

Rumenocentesis is a suitable method for collecting rumen fluid in dairy cattle and pH of rumen fluid can be measured using portable pH meter. Diagnosis of SARA based on rumen fluid pH examination can be applied in the field condition. The incidence of SARA was 20 per cent and was higher in Jersey breed of dairy cows compared to HF. SARA can be observed in dairy cows irrespective of the lactation number. Incidence of SARA was high during the mid-lactation and SARA disturbed the general health condition of dairy cows. For diagnosis of SARA body condition score can be used and SARA resulted insignificant reduction in daily milk production and altered milk composition. SARA caused inflammatory change in the liver and resulted in statistically significant increase in the mean GGT/AST values.

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