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# EFFECT OF DIFFERENT LEVEL OF CONCENTRATE FEEDING ON HEMATOBIOCHEMICAL PARAMETERS IN AN EXPERIMENTALLY INDUCED SUB ACUTE RUMINAL ACIDOSIS (SARA) IN SHEEP AND ITS MANAGEMENT WITH DIFFERENT CONCENTRATION OF ANTACIDS

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## ABSTRACT

The study was designed to detect the effect of SARA on hematobiochemical parameters and efficacy of some allopathic and herbal antacid drugs against SARA. A total of 16 sheep of the same age and weight were divided randomly into four groups. Group A was placed as a control group and fed on routine diet while Group B, C and D were fed on Barseem with 250 gm, 500 gm and 750 gm ground wheat flour, respectively to induce SARA. Blood samples were collected three times per day for five consecutive days and analyzed for blood parameters using calibrated blood gas analyzer. A significant increase was recorded in WBC count, whereas, non significant differences were observed in all other blood parameters. During therapeutic trials different concentration of antacids were used and their efficacy was analyzed. The 12 animals in which SARA were induced were selected and divided into 3 groups. The Group 1 and Group 2 were treated with different concentration of sodium bicarbonate, whereas, Group 3 was fed with monensin and SAFI. The ruminal fluid showed marked decrease in pH and increase in serum haptoglobin and WBC counts, but no change in other blood parameters was observed in any of the three groups.

**Key words:** SARA, prevalence, herbal buffer SAFI, ruminal fluid pH.

## INTRODUCTION

Detection of changes in hematological and biochemical parameters act as identification markers for SARA in sheep (González *et al*. 2010). As showed by Gianesella *et al*. (2010), there is a strong relationship between the rumen pH and blood pH. Acidosis is defined by a blood pH lower than the average range (Rose, 1995). In newborn calves, a decreased maintenance of colostrum because of hypercapnea is a clinical sign of acidosis (Bleul *et al*. 2007). In adult mammals (especially in ruminants), blood pH depends upon a variety of factors, like accumulation of different salts in the blood and mainly depends on the bicarbonate concentration (Owens *et al*. 1998). Direct pH testing of rumen fluid is considered to be the gold standard in SARA diagnosis, Measuring $CO_2$ levels in blood has been positively correlated to rumen pH, and retrieval of blood samples is less laborious than extracting rumen fluid samples (Morgante *et al*. 2009). In order to keep acidosis in check, nutritional management is crucial, particularly with respect to feed composition. As SARA is considered to be an economically significant factor in the bovine and ovine agronomy, the present study aimed to determine if acid neutralizing substances added to feed could help alleviate artificially induced SARA in sheep, and if increases in rumen pH (SARA mitigation) were accompanied by, and correlated to, concomitant increases of blood pH.

## MATERIALS AND METHODS

**Ethics Statements:** All the ruminal, blood and fecal samples were collected observing all the ethical formalities and the pain management of the animals was done effectively.

**Animal sampling:** The present investigation enlisted 16 sheep examined in two regions (Lahore and Okara) of the Punjab territory, Pakistan. 16 sheep 1-3 years old and measuring 28-35 kg was purchased from a nearby livestock market in the Lahore and Okara areas. The sheep were housed in individual boxes. Each sheep was given berseem twice a day i.e. at 9 am and 5 pm and water in adequate amount. The animals were adopted for this meal over a period of 20 days. These animals were then isolated into four groups- A, B, C and D. The four animals in group A was kept as control and was fed on the same diet throughout the study period, while the Berseem allotments were decreased to 90 % of optimal levels for next 3 days to stimulate hunger in 12 of the sheep groups B, C and D. SARA was then induced by administering 250 g of ground wheat flour to animals in test group B, 500 g to group C, and 750 gm to group D,

all thrice daily. The amount of berseem was cut in half. SARA was confirmed to have been induced in the test animals via routine clinical signs and monitoring of ruminal pH.

**Blood profile of experimentally induced SARA in sheep:** Blood tests from these sheep (n=12) and control group (n=4) were collected from jugular vein in vacuum tubes containing EDTA (BIO-VAC). Three ml samples from all sheep were obtained and tested for the following parameters; Hemoglobin (Hb), RBCs count, WBCs numbering and PLTs. The specimens were processed in an aligned blood gas analyzer (EasyStat, Medica, USA). The analyzer was set at the body temperature of the sheep before investigation. Ruminal pH was measured with portable pH meter.

**Therapeutic trials to find out efficacy of different concentrations of antacids:** Once the animals in all the three induction groups B, C and D were confirmed positive for SARA through rumenocentesis then they were further subjected to treatment trial. Hence, when animals of group B becomes positive for SARA they were treated with treatment regimen 1, animals of induction group C and D were given treatment regimen 2 and 3 respectively, so the effect of three treatments was compared between all the animals positive for SARA. Thus, the treatment was compared not on the basis of severity but on basis of disappearance of clinical signs and change in ruminal pH to normal range. The Group B was treated with treatment regimen 1, which was comprised of Berseem, Sodium Bicarbonate 5% (Soda Bicarb. Symans Pharmaceuticals Pvt. Ltd.) and monensin (Sinosin Ghazi Brothers, 20 mg/ animal /day for 10 days). Animals from group C were treated with treatment regimen 2 i,e Berseem , sodium bicarbonate 10% and monensin (Sinosin Ghazi Brothers, 20 mg/host/day for 10 days), while group C animals were given treatment regimen 3 that was comprised of Berseem, monensin (Sinosin Ghazi Brothers, 20 mg/host/day for 10 days) and SAFI (Hamdard Pakistan Limited; ingredients are: Cassia angustifoli, Smilax China, Sphaeranthus indicus, Nymphaelotus, Daldergia sissoo, Fumaria varbiflora, Dauhinia variegata, Melia azadirachta, Swertia chirata, Tephrosia purpurea, Canscora decussata, Chrozophora Plicata, Curcuma caesia, Cuscuta reflexa, Ipomoea turpethum, Lavandula stoechas, Ocimum canum, Pterocarpus santalinus, Rosa damascene, Terminalia chedula, and Tinospora cordifolia) at a dosage of 20 ml/d. Treatment impacts upon host animals were evaluated by monitoring the following hematobiochemical parameters: Blood pH, Ruminal fluid pH, Serum Amyloid A and Haptoglobin, hemoglobin (Hgb), WBC, RBC counts and PLT.

## RESULTS

Experimental animals were divided in to 4 groups. Group A was kept control to compare the values of blood pH (BpH), ruminal fluid pH (RpH), hematological parameters red blood cell count (RBCs), hemoglobin (Hb%), platelet count (PLT) and white blood cell count (WBCs). The groups B, C and D were subjected to induction of SARA with rations 1, 2 and 3. The data revealed slight decline in blood pH from7.3 to 7.2 in group B 7.1 to 7.3 in group C and 7.3 to 7.1 in group D. The major decline was noted in ruminal fluid pH. 6.92 to 5.63 pH decline was noted in induction group B, while in group C it declined from 7.10 to 5.58 and in group D it was observed from 7.24 to 5.51. As for as hematological parameters were concerned, no significant change was noticed in RBCs and Hb % among control and inducted groups. There was a significant increase ($p<0.05$) observed in the WBCs count after the induction of SARA in experimental groups C and D. Significant change ($p<0.05$) in Haptoglobin was also noted in all induced groups.

**Efficacy of different concentration of antacids on blood pH, different blood parameters and ruminal fluid pH:** Experimental animals were isolated into four groups. Group A was kept as a control to establish baseline marks for the physiological parameters monitored in this study. Groups B, C and D were the experimental treatment groups induced with SARA via the application of three different dietary regimens. Animals in group B experienced a reduction of blood pH from 7.2 to 7.1. This was accompanied by an increase in the ruminal fluid pH. A decrease of blood pH was noted in treatment group C from 7.3 to 7.2 with increase ruminal pH from 5.58 to 5.99. While Group D exhibit no change in blood pH but ruminal pH increased from 5.51 to 6.03 after treatment. With respect to hematological parameters slight increase in RBC was observed in all the groups after treatment. There was an increase in WBC counts in groups B, C and slight decrease was observed in group D. Noteworthy was the change ($P<0.05$) in Haptoglobin levels in treatment groups. The SARA induced groups B, C, and D all responded well upon application of treatments designed to alleviate symptoms of SARA.

**Table 1. Experimental Induction of SARA in Sheep and Treatment.**

| | Group | BpH | RpH | RBCs | Hb% | WBCs | Hp (mg/l) | SAA (mg/l) |
|---|---|---|---|---|---|---|---|---|
| Pre-induction Day-0 | A Control | 7.2 | 7.21 | 8.55±1.21[a] | 12.99±1.10[a] | 6.88±0.25[a] | 81 ±98[a] | 3.66 ±4.8[a] |
| | B | 7.3 | 6.92 | 8.13±2.20[a] | 11.98±0.78[a] | 5.66±1.31[a] | 78±101[a] | 2.95 ±3.1[a] |
| | C | 7.1 | 7.10 | 8.63±0.22[a] | 12.85±0.40[a] | 7.14±2.03[a] | 89±107[a] | 2.78 ±5.7[a] |
| | D | 7.3 | 7.24 | 7.89±1.43[a] | 12.68±1.04[a] | 7.35±0.18[a] | 85±90[a] | 3.44 ±4.6[a] |
| Induced Day 1-10 | B | 7.2 | 5.63 | 8.12±2.07[a] | 11.45±0.50[a] | 8.11±2.01[a] | 179±226[b] | 3.1 ±4.7[a] |
| | C | 7.3 | 5.58 | 8.65±0.75[a] | 12.76±1.32[a] | 9.45±1.56[b] | 233±414[b] | 3.5 ±2.3[a] |
| | D | 7.1 | 5.51 | 8.12±1.84[a] | 12.56±0.76[a] | 10.99±2.1[b] | 289±315[b] | 2.7 ±1.6[a] |
| Treatment Day 11-20 | B | 7.1 | 6.32 | 8.55±1.61[a] | 11.98±1.55[a] | 10.07±1.81[b] | 99±156[a] | 4.7 ±3.2[a] |
| | C | 7.2 | 5.99 | 7.94±1.09[a] | 10.88±2.09[a] | 9.88±0.74[b] | 81±223[a] | 3.4 ±2.9[a] |
| | D | 7.1 | 6.03 | 9.35±2.73[a] | 11.53±0.67[a] | 9.57±1.67[b] | 78±105[a] | 3.5 ±2.6[a] |

## DISCUSSION

**Experimental induction of SARA in sheep and its effect on different blood parameters:** SARA is a condition occurring in ruminants as a result of excessive intake of fermentable carbohydrates. SARA clinical signs are not always obvious; symptoms often overlap those of a variety of other conditions, making differential diagnoses challenging. The present investigation focused on identifying hematological/physiological parameters that might serve as markers to detect SARA in sheep, provided that parameter changes have been positively correlated to SARA-induced acidification of rumen fluid. As the standard technique for diagnosing SARA is rumen fluid extraction and pH testing is an arduous process stressful to the animal, validation of blood test results as a diagnostic tool to detect SARA would be very useful to the ruminant veterinary community. A previous study by Gonzal *et al.* (2010) outlined a feed regimen developed to induce SARA experimentally. In their report SARA was induced in goats with 60% mixed ration, 40% hay fed to over a 5 day period. The dietary regimen resulted in a reduction of rumen pH to significantly less than 5.5. Haptoglobin levels increased during this period, while SAA did not change. As indicated in the result of present study SARA is associated with low ruminal pH, blood pH and fecal pH which are in agreement with the findings of Anne Mette Danscher *et al*. (2015). Ruminal acidosis is caused by heavy intake of fermentable starch. The fermentation process induces an increase of lactic acid concentrations, lowering pH, boosting the population of fermentative bacteria while suppressing other species of rumen commensals (Oetzel, 2000). Increasing levels of lactic acid in the rumen is accompanied by a buildup of unsaturated fatty acids, which can damage the rumen lining. In most cases of SARA, the ruminal pH drops under 5.0 (Brossard *et al*. 2003; Brown *et al*. 1999). Diagnosis of SARA is more difficult than acute acidosis, in light of the fact that the plasma pH may stay in the physiological range in animals afflicted with SARA (Enemark *et al*. 2004). Normally SARA is caused by ingestion of excess amounts of easily fermentable carbohydrates (Kezar and Church, 1979). While some researchers define SARA as a rumen pH drop from 6.25 to 5.5 (Kezar and Church, 1979), others consider SARA occur only when rumen pH levels drop lower than 5.5 (Oetzel GR, 2000). The lack of consensus in a precise definition for SARA, coupled with the lack a quick, convenient method of testing for the disorder makes diagnosing SARA a stiff challenge for veterinarians (Enemark *et al*. 2004). Acute acidosis is much more easily diagnosed; animals with this condition for the most part display rumen pH levels lower than 5.0, and generally have rumen fluid lactic acid concentrations up to 0.1 M, as well as high concentrations of fatty acids. Acute rumen acidosis is usually accompanied by a significant drop in blood pH. (Nour, 1998). Minimal lactic acid concentrations indicative of SARA are thought to be much lower-in some cases, above 2 mM level (Owens, 1998) and animals with SARA often show no significant changes in hematological parameters, including blood pH. (Goad *et al*. 1998).

**Efficacy of different concentrations of antacids on blood pH, blood parameters and pH of ruminal fluids:** SARA is a serious concern even in well organized dairy farms and accurate diagnosis of SARA is crucial. Continuous monitoring of ruminal pH is possible with rumenocentasis. Different parameters measuring metabolic acidosis may be useful tools. The prevention of SARA requires establishment of feeding and management guidelines to minimize ruminal acidosis. Standard monitoring of animals for SARA may assist an early detection of the condition and thus can limit economic losses (Enemark, 2008). It was observed that during development of SARA there is marked decrease in dry matter intake (DMI) by the animals. Many studies documented a decreased feed intake during SARA (Krajcarski-Hunt *et al*. 2002). It was reported that when

animals were given a choice of feed, cattle change their diet selection in order to assuage SARA, and sodium bicarbonate was not a choice of the cattle under experiment (Keunen *et al*. 2002). SARA was associated with feeding conditions and the correction of nutrition or feeding management was important in resolving the condition. SARA is said to be associated with inflammations of different organs and tissues in dairy cows. SARA has long-term health and economic consequences, which include feed intake depression, fluctuations in feed intake, reduced diet digestibility, reduced milk yield, reduced milk fat percent, gastrointestinal damage, liver abscesses, and lameness (Nejash Abdela 2016). The introduction of fiber has proven to be particularly useful in treating SARA (Zebelli *et al*. 2006). In North America addition of chemical buffers has been a routine practice (Hutjens, 1991) and was documented to be beneficial in the prevention of acidosis in dairy cows (Garry, 2002). Buffers were suggested to be used in conditions where the fiber component in feed rations was less than optimal (Erdman, 1988). Many reports revealed that addition of 150 g of sodium bicarbonate to the diet of the lactating cattle per day had a positive effect on the performance (Downer and Cummings, 1985), milk fat percentage and feed intake (Erdman, 1988) of the cattle. Normally buffer is used as single treatment but combinations were reported to have a significant effect on dry matter intake, milk yield and fat percentage (Hutjens, 1991). Bicarbonate buffers prevented overgrowth of acid tolerant lactobacilli (Garry, 2002). Natural products have also been used such as Probimax Acid Buff (Engormix). This product in particular was found to have doubled the buffering capacity of sodium bicarbonate and contributed to an increase milk yield and feed conversion (Enemark, 2008). Yeast has also applied as a treatment for SARA (Williams *et al*. 1991). In the present study buffers were added to food rations of experimentally induced SARA in sheep. Two different concentrations of sodium bicarbonate and herbal buffer SAFI were added. The buffers were proved beneficial in increasing the ruminal pH of sheep in all groups. However, the effect was not significantly different ($P>0.05$). The study depicts the importance of buffers in feed rations for adjustment of the ruminal pH. Mould *et al*. (1983) in their study treated mild cases of acidosis through withholding concentrates and feeding hay. The authors emphasized on the supplementary therapy with oral antacids (magnesium hydroxide, magnesium oxide or sodium bicarbonate) at 1 g/Kg body weight. Similarly, oral solutions (electrolyte) containing sodium bicarbonate was also advised.

**Conclusions:** In the present study significant differences were recorded in WBC counts and in the haptoglobin levels in SARA afflicted and healthy sheep and non significant differences in the other blood parameters i.e. Hb estimation, RBCs count and PLT count. The highest WBC counts were recorded in animals suffering from SARA while lowest were recorded in control group. After administration of different concentrations of sodium bicarbonates and local herbal drugs a light sudden drop in blood pH (7.3 to 7.1) was recorded whereas a major decline was recorded in the pH of ruminal fluid i.e. 1.29, 1.52 and 1.73 in groups B, C and D, respectively. As a result of therapeutic trials with different antacids, it was concluded that there was non significant difference in efficacy of different concentrations of soda bicarbonates and local herbal drugs although pH levels were increased in three different groups of animals. We conclude that SARA may be diagnosed via the measurement of key hematobiochemical parameters.

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**Authors Contributions:** **A**SC, MAK, MSK and KA designed and plan the study. ASC and MHS collected the samples and data from the field, ASC executed the experimental work and wrote the initial draft of paper, TU, NUK and IA helped in analyzing the data and in writing of manuscript. All authors read and approved the manuscript.

**Conflict of interest statement:** All the authors declare that they have no conflict of interest.

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