Prevalence of Bovine Ruminal Acidosis in and Around Jammu and its Impact on Haemato-biochemical Profile

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ABSTRACT

Ruminal acidosis is a condition that follows ingestion of excessive amounts of readily fermented carbohydrate, is prominent production problem for ruminants fed diets rich in concentrate. The present study was planned to investigate the prevalence of bovine ruminal acidosis and its impact on haemato-biochemical profile. A total of 265 animals were screened from different areas of Jammu region including the cases that were presented in TVCC, F.V.Sc. & A.H. Forty three (43) animals were found positive for acidosis. The overall prevalence of ruminal acidosis including sub-acute form in Jammu region was 16.22%. The prevalence was highest (22.82%) in age group 4-7 years. The highest prevalence in early lactation period was found to be 21.87%. The incidence of laminitis was found to be 16.27%. There was significant changes in haemato-biochemical parameters were observed in acidotic animals.

Keywords: Lactic acidosis, Large ruminants, Probiotics, Treatment

Bovine ruminal acidosis is an important alimentary tract disease which results from feeding of highly fermentable feeds to increase productivity (Khafipour et al., 2009). When the ruminant abruptly ingests large amounts of readily fermentable carbohydrates (RFCs) or when the period of adaptation to RFCs is insufficient, RFCs are suddenly fermentated and volatile fatty acids (VFAs) are accumulated, resulting in the rumen pH to drop below 5.5 for a prolonged period and creating the condition of fermentative acidosis (Ding and Xu, 2003). In this condition, lactic acid-producing bacteria, such as Streptococcus bovis and Lactobacillus spp., are proliferated, leading to the accumulation of lactic acid, which is known as lactic acidosis (Gozho et al., 2007). It is a common Alimentary tract disorder causing severe economic losses to the dairy industry due to significant reduction in milk yield with high mortality rate in acute cases (Patil et al., 2008). Ruminants, regardless of age, breed, and sex are susceptible to overeating with grains and carbohydrates (Radostitis et al., 2007). The resulting production of large quantities of volatile fatty acids (VFA) and lactic acid decreases rumen pH to non-physiological levels, simultaneously weakening the buffering capacity of the rumen, and reduces the efficiency of rumen flora and fermentation. Lactic acidosis can cause ruminitis, metabolic acidosis, lameness, hepatic abscessation, pneumonia and death (Lean et al., 2007). Ruminal acidosis occurs when there is a sudden excess intake of highly fermentable carbohydrates, primarily starches and sugars. This normally manifests when feedlot cattle without proper adaptation are rapidly transitioned from roughage to high concentrate diets, or when dairy cow intake during transition pre- and post-partum is erratic (Beauchemin and Penner, 2009). It is also a function of the total load of readily fermentable carbohydrates in the rumen (Oetzel, 2003), since dairy cows often experience sub acute ruminal acidosis at the time of maximum carbohydrate consumption (Penner et al., 2007). The clinical signs of acidosis usually depends on the severity of the disease and can be either acute, posing a life-
threatening situation, or sub acute (chronic), resulting in reduced feed consumption and weight gain. Repeated bouts of sub acute ruminal acidosis can damage the surface of the rumen wall (Krause and Oetzel, 2006). Once the rumen wall is damaged, bacteria and toxins produced by bacteria can enter the portal circulation, causing liver abscesses and an inflammatory response (Gozho et al., 2007). A series of physiological effects may happen in acidic animals and the effects may relate to many factors. Decreased dry matter intake (DMI) is an obvious cause for acidosis and has been used as a clinical sign to diagnose sub acute ruminal acidosis (Kleen et al., 2009).

Therefore, keeping in view importance of ruminal acidosis in bovines, the present study was designed to study the prevalence of bovine ruminal acidosis and its impact on haemato-biochemical profile.

MATERIALS AND METHODS

The ruminal acidosis in dairy animals of Jammu region that included organized as well as unorganized dairy farms.

Source and number of animals

A total of 265 animals were screened. Screening of animals was done from different areas of Jammu region such as TVCC of F.V .Sc. and A.H, R.S. Pura, Areas in and around R.S. Pura (Kirpind, Simbal, Dablehar etc.), Govt. Sub-district Veterinary Hospital, R.S. Pura, Dairy Unit of ILFC, F.V .Sc. & A.H, R.S Pura Campus and Dairy farms in Jammu district as well as farmer houses.

Animals were categorized according to their age and on the basis of rumen liquor pH value ranges and animals that were showing rumen pH value in the range (5.1-6). Prevalence was determined by calculating percentage of acidic animals on the basis of history, important clinical signs/behavioral changes (anorexia, tympany, diarrhea, oliguria, excessive salivation, dyspnea, dullness, restlessness, and depression etc.) and finally after examining pH of rumen liquor (using pH paper strips), to decide whether animal was positive for acidosis. Screening of animals data related to lactation number, milk yield, lactation stage, feeding management and incidence of lameness was also recorded on designed farm questionnaire.

Biological sample analysis

Sampling was done on 0 day (pretreatment), 7th day and 14th day (postreatment). Three groups, each of (6 animals) were studied as pretreatment group, post treatment group on 7th and 14th day and parameters of rumen and hematobiochemicals were compared between the groups and with healthy control group (6 animals).

Haematological analysis

Blood was collected aseptically from jugular vein in EDTA vacutainers and was analyzed for some of the hematological parameters like (Hb, PCV, TLC, TEC, DLC and ESR). Hemoglobin was determined calorimetrically at 540 nm by cyanomethaemoglobin method using Drabkin’s reagent and expressed as g/dl. Pack cell volume (PCV) was determined by micro-haematocrit method by centrifuging at a speed of 12000 rpm for 5 min and expressed as volume percentage. Total leucocyte count (TLC) and Total erythrocyte count (TEC) was determined by using improved Neubauer chamber (Haemocytometer) and were expressed × 10^3/cu.mm and × 10^6/cu.mm respectively. Differential leucocyte count (DLC) was undertaken by using Giemsa staining method and expressed as percentage (%). Erythrocyte sedimentation rate (ESR) was determined within two hours after collection of blood in wintrobe hematocrit tube and was expressed in mm.

Biochemical analysis

Serum was aseptically separated by the method of (Gupta,1995), with few modifications and kept under refrigeration (4°C) for the analysis of some of the biochemical parameters (total protein, BUN, creatinine, glucose, total bilirubin, Ca and P) and enzymes (AST and ALT) in biochemical analyzer using commercial ERBA diagnostic kits (Transasia Biomedical Ltd. Mumbai, India). Serum lactate was quantified by D-lactate estimation kit of product code K-DATE (Megazyme International Ireland) on UV spectrophotometer using wavelength of 340nm. The Principle of quantification of D-lactic acid is based on two enzymatic reactions, first D-lactic acid is oxidized to pyruvate in presence (NAD^+) catalysed by D-lactate dehydrogenase, further the conversion of pyruvate to D-alanine and 2-oxoglutarate catalysed by enzyme D-glutamate pyruvate transaminase in the presence of
excess of D-glutamate. The amount of NADH formed is stoichiometric with the amount of D-lactic acid, thus amount of NADH formed was measured by increase in absorbance at 340 nm. Evaluation of impact of ruminal acidosis on liver and kidney functions was done on the basis of assessing alterations in the biochemical parameters and enzymatic activity.

RESULTS AND DISCUSSION

Out of 265 animals screened from different areas of Jammu region, forty three (43) animals were found positive for ruminal acidosis. The overall prevalence of ruminal acidosis including sub-acute form in Jammu region was 16.22% (Table 1).

Table 1: Prevalence percentage (%) of ruminal acidosis

| Sl. No | Source(s) | Total no. of animal screened | Total no. of positive cases | Prevalence of acidosis cases (including sub acute cases) |
|--------|------------|------------------------------|-----------------------------|---------------------------------------------------------|
| 1      | TVCC F.V.Sc & A.H, R.S. Pura | 27                            | 07                           | 25.92%                                                   |
| 2      | Areas in and around R.S. Pura (Kirpind, Simbal, Dablehar etc.) | 70                            | 13                           | 18.57%                                                   |
| 3      | Govt. Sub-district Veterinary Hospital, R.S. Pura. | 25                            | 06                           | 24%                                                      |
| 4      | Dairy unit ILFC, F.V.Sc & A.H., R.S Pura Campus. | 28                            | 03                           | 10.17%                                                   |
| 5      | Dairy animals in different farms of Jammu district as well as at farmers houses | 115                           | 14                           | 12.17%                                                   |
| Total  | 265                    | 43                           | 16.22%                       |

The animals screened in TVCC, F.V.Sc & A.H R.S. Pura showed maximum prevalence of 25.92% followed by Govt. Sub-district Veterinary Hospital, R.S. Pura with 24% prevalence whereas animals in Dairy unit ILFC, F.V.Sc & AH, R.S Pura Campus had lowest prevalence of 10.17% of ruminal acidosis. Prevalence was highest in age the group 4-7 years i.e.22.82% and lowest in age group 1-2 years i.e.11.29% among the animals screened for ruminal acidosis.

Table 2: Age wise prevalence of ruminal acidosis

| Sources                                           | Total          | Age group (in years) |
|---------------------------------------------------|----------------|----------------------|
|                                                   |                | 1-2 years | 2-4 years | 4-7 years |
| TVCC                                              | (43)           | (11)      | (15)      | (43)      |
| F.V.Sc & A.H, R.S. Pura                          | (27)           | (7)       | (15)      | (43)      |
| Areas in and around R.S. Pura (Kirpind, Simbal, Dablehar etc.) | (70)           | (13)      | (21)      | (43)      |
| Govt. Sub-district Veterinary Hospital, R.S. Pura. | (25)           | (6)       | (21)      | (43)      |
| Dairy unit ILFC, F.V.Sc & A.H., R.S Pura Campus.  | (28)           | (8)       | (21)      | (43)      |
| Dairy animals in different farms of Jammu district as well as at farmers houses | (115)          | (43)      | (43)      | (43)      |
| Total no. of animals examined.                    | (265)          | (92)      | (92)      | (265)     |
| Total no. of positive cases for acidosis.         | (43)           | (15)      | (21)      | (43)      |
| Prevalence of acidosis                            | 11.29%         | 13.51%    | 22.82%    | 16.22%    |
| (Age Wise)                                        |                |           |           |           |

Figures in parenthesis shows animals suffering with ruminal acidosis.

Among the animals screened prevalence of ruminal acidosis in non-lactating animals was 13.98% and that of lactating animals was 18.85% with the highest prevalence in early lactation period i.e. 21.87% (Table 3)
Table 3: Lactation wise prevalence of ruminal acidosis

| Items                                      | Early lactation period | Mid lactation period | Total no. of lactating animals and prevalence of acidosis |
|--------------------------------------------|------------------------|----------------------|----------------------------------------------------------|
| No. of animals examined                    | 64                     | 58                   | 122                                                       |
| Acidosis positive cases                    | 14                     | 09                   | 23                                                        |
| Prevalence of acidosis                    | 21.87%                 | 15.51%               | 18.85%                                                   |

In total forty three (43) animals suffering with ruminal acidosis (i.e. acidicotic animals), seven (7) animals showed signs of lameness and therefore incidence of lameness was 16.27%. The minimum pH values of rumen liquor was found 5.1, whereas maximum pH was 7.0. Eight animals showed a ruminal pH value of ≤ 5, seventeen showed pH 5-5.5, eighteen showed pH 5.5-6.2 and two hundred twenty two animals showed pH>6.2. The mean value of ruminal pH of all acidicotic animal was found 5.11 ±0.06.

High prevalence of acidosis in lactation period could probably be due to rapid increase in feed intake (grains) to meet the energy demand of high producing dairy cows and secondly concentrates are preferred over forage by lactating cows particularly in early lactation period. Tajik et al. (2009) has also concluded higher prevalence rate of sub acute ruminal acidosis in dairy cows with overall prevalence of 27.6%, with prevalence in early lactation of 29.3% and 26.4% in mid lactation period. The prevalence by (Kitkas et al., 2013) was found to be 16% which was at par with present study. Our results were also in accordance with the results of some field studies (Kleen, 2004; Tajik et al. 2009) who revealed the presence of sub acute ruminal acidosis in 11-29.3% of the early lactation cows and 18-26.4% of the mid-lactation cows.

Haematological profile

The mean value with standard error of haematological parameters of healthy, pre-treatment and post-treatment group was analyzed at 0 day (pre-treatment group) and 7th and 14th day (post-treatment group) and were compared with healthy group as depicted in Table 4.

Haemoglobin of animals suffering with ruminal acidosis showed significant increase (p<0.05) as compared to healthy group. The value of haemoglobin in post-treatment group reached to normal (10.95±0.29) at 14th day. Pack cell volume in animals suffering with ruminal acidosis was also significantly increased (p<0.05) as compared to healthy group but was restored to normal (31.43±0.50) following post probiotics treatment at 14th day. Total erythrocyte count was significantly increased (p<0.05) in animals suffering with ruminal acidosis as compared to healthy group and the value was restored to normal (6.28±0.09) following post probiotics treatment at 14th day.

Table 4: Haematological profile in different trial groups at 0, 7th and 14th days

| Parameters                              | Healthy Group (n=6) | Pre-treatment Group (n=6) | Post-treatment Group (n=6) |
|-----------------------------------------|---------------------|---------------------------|---------------------------|
| Haemoglobin (g/dl)                      | 10.75±0.25a         | 15.11±0.09b               | 13.40±0.47b               |
| Packed Cell Volume (%)                  | 29.00±1.41a         | 41.08±1.20b               | 37.57±0.56b               |
| Total Erythrocyte Count (10⁶/µL)        | 6.83±0.60a          | 10.33±0.31c               | 8.85±0.17b               |
| Erythrocyte Sedimentation Rate (mm/24hr)| 12.41±0.26a         | 21.48±0.22c               | 19.03±0.48b               |
| Total Leukocyte Count (10³/µL)          | 7.16±0.54a          | 19.16±0.51d               | 14.47±0.72c               |
| Neutrophils (%)                         | 24.50±0.76a         | 47.87±1.17d               | 44.50±0.43c               |
| Lymphocytes (%)                         | 59.17±1.01a         | 41.16±0.92b               | 47.17±0.70b               |
| Monocytes (%)                           | 3.50±0.43b          | 1.85±0.15a                | 2.07±0.10a                |
| Eosinophils (%)                         | 1.50±0.18a          | 1.38±0.05a                | 1.20±0.08a                |
| Basophils (%)                           | 0.50±0.07a          | 0.56±0.02b                | 0.30±0.06a                |

Mean±SE bearing different superscript differ significantly (P<0.05).
ESR value in animals suffering with ruminal acidosis was significantly increased (p<0.05) as compared to healthy group and the value was restored within normal range (10.28±0.33) following post probiotics treatment at 14th day. Total leukocyte count was significantly increased (p<0.05) in animals suffering with ruminal acidosis as compared to healthy group and the value was restored within normal range (54.33±1.56) following post probiotics treatment at 14th day. Neutrophilic count was significantly increased (p<0.05) in animals suffering with ruminal acidosis as compared to healthy group and the value was restored within normal range (37.75±0.63) following post probiotics treatment at 14th day. Lymphocytic count was significantly decreased (p<0.05) in animals suffering with ruminal acidosis as compared to healthy group and the value was increased to normal level (54.33±1.56) following post probiotics treatment at 14th day. Similarly, the monocyte, eosinophil and basophil count in percentage for healthy group was recorded as: 3.50±0.43, 1.50±0.18 and 0.50±0.07; for pre-treatment group as 1.85±0.15, 0.53±0.05 and 0.13±0.02 and for post-treatment group at 14th day as 3.15±0.12, 1.67±0.08 and 0.20±0.04 respectively. Non significant change was observed in the mean values of monocyte, eosinophil and basophil count. The increase in hemoglobin and PCV values might be due to haemo-concentration and systemic dehydration caused by drawing of fluid from circulation to rumen when the osmolality in rumen is increased in acidosis. Our results were comparatively similar to that of (Mousa et al., 2013) who revealed hemoglobin and PCV values as 16.10 and 43.72 respectively in cattle with acidosis. But these results are in contrast to the result of (Shukla et al., 2004) who reported non-significant increase in hemoglobin value in experimental acidotic calves. The erythrocyte count was significantly (p<0.05) increased in acidotic animals in this study that could be also probably due to haemo-concentration caused by dehydration which was evident from clinical signs and elevated haematocrit. Our results were substantiated by the findings of (Shihabudheen, 2003). The erythrocyte sedimentation rate in affected animals was significantly (p<0.05) increased i.e. 0.90 (mm/hr) that could probably due to acute localized infection of serosal membrane or tissue destruction and the same parameter with increased value was reported by (Shihabudheen et al. 2003) in acidotic goat. There was significant (p<0.05) leukocytosis, neutrophilia and lymphopenia in present study.

Biochemical parameters

The mean value with standard error of biochemical parameters of healthy, pre-treatment and post treatment group was analyzed and were recorded at 0 day (pre-treatment group) and 7th and 14th day (post-treatment group) and compared with healthy group as presented in Table 5.

It was observed that serum lactate level was significantly increased (p<0.05) in animals suffering with ruminal acidosis following grain engorgement i.e. 24.10±0.37 as compared to healthy group 9.25±0.55.

Table 5: Biochemical profile in different trial groups at 0, 7th and 14th days

| Parameters                     | Healthy Group (n=6) | Pre-treatment Group (n=6) | Post-treatment Group (n=6) |
|-------------------------------|--------------------|--------------------------|---------------------------|
| D-lactate (mg/dl)             | 9.25±0.55<sup>a</sup> | 24.10±0.37<sup>c</sup>  | 19.87±0.30<sup>b</sup>    |
| Blood Urea Nitrogen (mg/dl)   | 11.17±0.82<sup>a</sup> | 31.81±0.27<sup>b</sup>  | 24.97±0.55<sup>c</sup>    |
| Total Protein (g/dl)          | 6.93±0.05<sup>c</sup>  | 6.63±0.06<sup>a</sup>   | 6.65±0.06<sup>b</sup>     |
| Creatinine (mg/dl)            | 1.16±0.09<sup>a</sup>  | 2.90±0.09<sup>c</sup>   | 2.23±0.07<sup>b</sup>     |
| Glucose (mg/dl)               | 62.92±0.08<sup>b</sup> | 79.42±1.03<sup>d</sup>  | 66.93±0.70<sup>c</sup>    |
| Total bilirubin (mg/dl)       | 0.46±0.01<sup>a</sup>  | 0.90±0.01<sup>c</sup>   | 0.83±0.08<sup>b</sup>     |
| Aspartate amino transferase (U/L) | 53.92±1.24<sup>a</sup> | 131.50±2.11<sup>d</sup>| 112.00±1.46<sup>c</sup>  |
| Alanine amino transferase (U/L) | 28.50±0.92<sup>a</sup> | 50.75±0.89<sup>b</sup>  | 32.70±0.68<sup>c</sup>    |
| Calcium (mg/dl)               | 9.70±0.21<sup>c</sup>  | 6.98±0.15<sup>a</sup>   | 7.58±0.41<sup>b</sup>     |
| Phosphorus (mg/dl)            | 4.70±0.12<sup>a</sup>  | 8.70±0.09<sup>d</sup>   | 7.53±0.11<sup>c</sup>     |

Mean±SE bearing different superscript differ significantly (P<0.05).
level was restored to normal value i.e.10.08 ±0.29 on 14th day following post probiotics treatment. Blood urea nitrogen and Creatinine level in animals suffering with ruminal acidosis were significantly increased (p<0.05) and were 31.81±0.27 and 2.90±0.09, respectively. These values were restored to the normal range i.e.19.92±0.89 and 1.43±0.09, respectively following post probiotics treatment at 14th day. Total protein level in animals suffering with ruminal acidosis (i.e. acidotic animals) was 6.63±0.06 as compared to healthy group i.e. 6.93±0.05. The value was restored in the normal range i.e. 7.00±0.06 following post probiotics treatment at 14th day. Total bilirubin level in animals suffering with ruminal acidosis (i.e. acidotic animals) was 0.83±0.08 as compared to healthy group i.e. 0.46±0.01. The value was restored in the normal range i.e.0.53±0.02 following post probiotics treatment at 14th day. The values of AST and ALT parameters in animals suffering with ruminal acidosis (i.e. acidotic animals) were significantly (p<0.05) increased i.e. 131±2.11 and 50.75±0.89 respectively. These values were restored in the normal range i.e.75.50±1.73 and 29.03±0.45 respectively following post probiotics treatment at 14th day. Blood glucose level in animals suffering with ruminal acidosis (i.e. acidotic animals) remained 79.42±1.03 as compared to healthy group i.e. 62.92±0.88 but the glucose level reached in the normal range i.e. 54.10±0.58 following post probiotics treatment at 14th day. There was significant (p<0.05) increase in the inorganic phosphorous level in animals suffering with ruminal acidosis (i.e. acidotic animals) 8.70±0.09. The value reached in the normal range i.e. 6.20±0.12 following post probiotics treatment at 14th day. There was non-significant (p>0.05) decrease in calcium level in the acidotic animals.

The serum lactate level was significantly (p<0.05) increased (24.10 mg/dl) in the acidotic animals might be due to grain engorgement and absorption of lactic acid produced in rumen through rumen wall that reached to general circulation. Similar results was reported by (Shukla et al., 2004) of increase in lactate level (25.75) in cattle following grain engorgement at 24 hr. Where as the mean serum lactate level reported by (Chehreh et al., 2014) in sub acute case of acidotic cattle was found to be 31.1. Blood urea nitrogen and Creatinine levels were significantly (p<0.05) increased in acidotic animals (31.80 and 2.90 respectively) which might probably due to renal damage, and of decreased glomerular filtration rate which is evident from clinical sign (oliguria). The increased level of BUN studied in the present study was comparatively similar as reported by (Mohan et al., 2015) who reported BUN level of (31.17) in acidotic buffalo. The increased level of Creatinine in this study was in concurrence with the result of (Mousa et al., 2013) who reported Creatinine level of (3.34) in acidotic cattle. The total serum protein was significantly (p<0.05) increased (6.63 g/dl) in the study group, which could probably due to hepatic damage in ruminal acidosis. Similar results were reported by (Chehreh et al., 2014) who reported total protein level of (6.30 g/dl) in acidotic cattle. Blood glucose level was significantly (p<0.05) increased (79.42 mg/dl) in acidotic animals which might have occurred due to decreased utilization of glucose by peripheral tissues coupled with hepatic glycogenolysis under the influence of corticosteroid released during stress in acidotic animals. Our results were in accordance with the results of (Shukla et al., 2004) who found glucose level of (84.80) in acidotic cattle. Total bilirubin of serum in cattle with acidosis was higher than healthy ones, but this increase was not enough large (1 mg/dl) to be considered as hyper-bilirubinemia. Therefore the jaundice was not seen in any acidotic animal in spite of increase of serum bilirubin. The increase in bilirubin could be probably due to liver abscess secondary to ruminal lactic acidosis as also reported by (Dore et al., 2007).

The activities of AST and ALT enzymes (IU/L) in serum was significantly (p<0.05) increased in acidotic animals might be due to hepatic necrosis or damage as a result of absorption of endotoxins and some harmful bacteria such as Fusobacterium necrophorum and Arcanobacter pyogens through ruminal wall to portal circulation. Our results were in concurrence with that of (Shukla et al., 2004) who reported the significant increase in AST and ALT level i.e. 123.20 and 50.60 in acidotic cattle. But our results were in contrast to the results of (Chehreh et al., 2014) who found AST and ALT level as 107.80 and 26.9 respectively in acidotic cattle. The inorganic phosphorous level was significantly (p<0.05) increased (8.70 mg/dl) whereas calcium level was non-significantly (P>0.05) decreased (6.98 mg/dl) in the acidotic animals. The increased value of inorganic phosphorous could be probably due to increase in buffering action so as to maintain the acid-base balance in blood. Our results were in accordance to that of (Mousa et al., 2013) who reported the significant increase...
in phosphorous level (8.22 mg/dl) in acidotic cattle. The non-significant (P>0.05) decrease in calcium level might be due to mal-absorption and increased serum inorganic phosphorous level in acidotic animals.

CONCLUSION
The overall prevalence of ruminal acidosis (acidotic) including sub-acute form during the study period in Jammu region was 16.22%. The prevalence was highest in age group 4-7 years i.e. 22.82%. The highest prevalence was in early lactation period i.e. 21.87% as compared to mid lactation period i.e. 15.51%. There was significant changes in haematological parameters viz., Haemoglobin, PCV, TEC, ESR and TLC were observed in acidotic animals. The altered haematological parameters (Haemoglobin, PCV, TEC, ESR and TLC) were returned to normal following fortnight probiotics treatment. The biochemical parameters viz. serum lactate level, BUN, creatinine, total bilirubin, ALT, AST, glucose and phosphorus was significantly increased in acidotic animals and was restored to normal value on 14th day following post probiotics treatment and confirm improved impact on liver and kidney functions.

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