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Effect of Ruminal Juice, Vitamins, and Minerals Mixture Supplementation on Calves Affected with Ruminal Acidosis

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Abstract

This study was planned to evaluate the efficacy of ruminal juice, vitamins, and minerals mixture supplementation on the improvement of ruminal acidosis in calves in a comparison with the group treated with the anti-acid only. In this direction, a total of 30 calves were used in this study; 10 were clinically healthy and assigned as a control (G1), and 20 were affected with ruminal acidosis. The latter 20 calves were further equally grouped into 2 subgroups (G2, and G3). Group 2 was treated with a ruminal anti-acid, while G3 was treated by the administration of a ruminal juice supplemented with a vitamins and minerals mixture beside the anti-acid. The result showed a significant decrease in WBCs, Hb, PCV, ALT, AST, and serum lactate. Furthermore, significant improvement in TAC, BHBA, ruminal protozoal count, and ruminal pH in calves treated with ruminal juice supplemented with vitamins and minerals more than in the group treated with the anti-acid only. It is concluded that the use of ruminal juice supplemented with vitamins, and minerals besides the anti-acid had a better impact on clinically affected calves with acute ruminal acidosis.

KEYWORDS
Ruminal acidosis, Ruminal juice, Vitamins, Minerals

INTRODUCTION

Ruminal disorders are considered the most common issue of ruminants that are fed on high-quality grains. Moreover, it is of particular importance because of the high morbidity, loss of productivity, and profitability of farms (Galbet, 2020).

Indigestion is the most common rumen dysfunction among other disorders which mainly describes reticulorumen dysfunction (Constable *et al*., 2017). Overfeeding, sudden change of diet from forage to a highly concentrated diet, and excess indigestible roughages are the most common etiological factors leading to indigestion (Hynd, 2019). There are different types of ruminal microbial fermentative illnesses such as acute ruminal acidosis, which can sometimes be fatal within less than 24 hours (Gentile *et al*., 2004).

Ruminant simple indigestion can be successfully treated with rumen transfaunation, a common procedure that is widely approved. Furthermore, this procedure provides the rumen microbial population with nutrients and energy (DePeters and George, 2014).

The productive and reproductive health of animals is correlated with minerals and vitamin supplementation (Khan *et al*., 2014). Immune system function has more requirements than manufacturing or reproduction needs (Xin *et al*., 1991; Weiss, 1998). To the best of the authors' knowledge, there is limited data related to adding ruminal juice with other mixtures in the treatment of ruminal acidosis. Therefore, this work aimed to evaluate the effects of adding ruminal juice with vitamins and mineral mixtures to the anti-acids in the treatment of rumen acidosis in calves.

MATERIALS AND METHODS

Animals and the study protocol

Thirty Holstein calves weighed 250 kg body weight located at a private farm in Fayum governorate, Egypt. Ten of these calves were healthy and were assigned as a control group (G1), 10 calves suffered from ruminal acidosis and were treated with oral anti-acid only (100 g sodium bicarbonate, and 50 g magnesium oxide for 3 successive days) represented the second group (G2), and 10 calves suffered from ruminal acidosis and were treated with oral anti-acid in addition to ruminal juice with vitamins and minerals mixture (SUMMIT PHARMA VET. S. B. V) were kept as the third group (G3). All calves were parasitic-free. The excessive corn in the diet may be the cause of ruminal acidosis. The diagnosis of acidosis was based on clinical manifestation and ruminal juice pH.

Clinical examination

Thorough clinical examination including body temperature, heart rate evaluation, mucus membrane, respiratory rate, and ruminal contraction was done for all calves (Edward, 2012). Specified clinical scores such as fecal scores were assessed once daily, and the findings were documented by fecal fluidity (0=normal, 1=soft, 2=runny, 3=watery) (Larson *et al*., 1977). Furthermore,

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respiratory and locomotor scores, which ranged from 0 to 3 depending on the severity of the illness, were recorded as previously described (Constable *et al*., 2017).

Samples

Jugular vein blood samples were obtained on clean, dry vacutainer tubes with EDTA as an anticoagulant. Another part of the blood samples was drawn into clean, dry centrifuge tubes, allowing the blood to flow freely and gently over the inside of the tube. The tubes were then cooled for 30 minutes at 4°C before being centrifuged for 15 minutes at 3000 rpm to separate the serum (Coles, 1986). The sera were put into Eppendorf tubes, labeled, and kept at -20 °C until further analysis. Samples from rumen juice were extracted directly from the rumen using a stomach tube and put in a clean, dry flask using a pump and suction during the collection. Ruminal juice samples were examined right away after being collected. Specimens were sieved and then divided into three parts. The first section was used to assess the ruminal juice's physical characteristics, including color, odor, consistency, and pH (Dirksen, 1969). The second section was inspected under a microscope to evaluate the microfauna's mobility (Misra and Singh, 1974). The protozoal account was evaluated in the third part of the samples.

Hemato-Biochemical Analysis

Red blood cell count (RBCs $x10^{12}$ /l) and total white blood cell count (TWBCs $x10^{9}$/l) were measured using the hemocytometer method (Coles, 1986). Hemoglobin concentration (g/dl) was measured using the Acid Hematin method (Kelly, 1984). Alanine aminotransferase (ALT, U/L), Aspartate aminotransferase (AST, U/L), beta-hydroxybutyric acid (βHBA), total antioxidant capacity (TAC), and lactate (mg/dl) levels were determined according to the method described before (Kelly, 1984). All assay kits were based on the colorimetric analysis and were purchased from Merck KGaA, Darmstadt, Germany.

Ruminal juice analysis

Physical characteristics of ruminal juice samples as odor, color, and consistency were evaluated (Abdel-Salam, 1981). Ruminal microfauna was evaluated by microscopic examination (Fouda, 1995). A full count of ruminal protozoa was completed by using the described method (Ogimata and Imai, 1981).

Statistical analysis

Results were reported as Mean±SE. To assess the influence of the treatment groups on the different biochemical parameters, Tukey's Honestly Significant Difference (HSD) test was used as a post-hoc analysis after two-way repeated measures. Statistical significance was represented by the value of $P< 0.05$. The Statistical Package for Social Sciences version 24.0 (SPSS, IBM Corp., Armonk, NY) was used for all analyses, and Graph Pad Prism 8.0.2 (GraphPad Software, Inc) was used for charts.

RESULTS

Clinical findings

The different groups were nearly similar in different physical parameters (temperature, heart rate, and respiration rate) with no biological differences, while ruminal contraction was normal (2-3 moves/2 minute) in the first control group; but ruminal contraction in the second and third groups was absent or weak (0-1 move/2minute) and started to increase in G2 after the second dose of treatment (2-3 move/2 minutes) with a significant improvement until the end of the study. At the first dose of the experiment, the improvement in G3 (2–3 moves/2 minutes) became apparent. Likely, after treatment, the fecal, respiratory, and locomotor scores of G2 and G3 were significantly improved when compared to the control group (Table 1).

Hemato-biochemical analysis

Table 2 and Figure 1 showed the hemato-biochemical alternations. There was a relevant increase ($P < 0.05$) in Hb concentration, WBCs, and PCV% in G2 and G3 compared to G1. Furthermore, there was a significant increase ($P < 0.05$) in ALT, AST, and serum lactate while TAC and βHBA showed a significant decrease ($P < 0.05$) in G2 and G3 compared to G1. These haemato-biochemical changes returned toward the normal range on the 3rd-day post-treatment.

Ruminal juice analysis

In G2 and G3, ruminal juice experienced changes in its ap-

Table 1. Clinical scoring of different groups of fattening calves under investigation.

Parameters	G1 Healthy control calves	G2		G3	
		Before treatment	After treatment	Before treatment	After treatment
Temperature (°C)	38.5±0.6	37.7	38.36	37.77	38.46
Respiratory rate (per min)	23.95±0.85^{ab}	24.4±0.58^{ab}	23.65±0.45^{ab}	24.15±0.73^{ab}	23.65±0.45^{ab}
Heart rate (per min)	72.66±2.96^{a}	72.0 ±3.6^{a}	72.33±1.45^{a}	73.0 ±1.52^{a}	72.33 ±3.8^{a}
Mucous membrane	Rosy red	Slightly pale	Rosy red	Slightly pale	Rosy red
Ruminal contractions (per 2 min.)	3-Feb	0-1	3-Feb	0-1	3-Feb
Fecal score	Normal (0.65±0.18)	Scanty- defecation	Normal	Scanty- defecation	Normal
Respiratory score	1.4±0.26^{a}	1.1 ±0.23^{a}	1.2 ±0.39^{a}	1.3 ±0.44^{a}	1.12 ±0.39^{a}
Locomotors score	Normal (0.35±0.09^{a})	Mild lame (1)	Normal (0.35±0.13^{a})	Mild lame (1)	Normal (0.4 ±0.16^{a})
Dehydration	-ve	Mild to moderate	-ve	Mild to moderate	-ve
Abdominal distension (sublumbar fossa-ruminal part)	-ve	Slight left flank distension	-ve	Slight left flank distension	-ve
Defecation	Normal	Scanty or No defecation	Normal	Scanty or No defecation	Normal

G1: Group 1 (control); G2: Group 2 (impacted treated with ruminal anti-acid); G3: Group 3 (impacted treated with ruminal anti-acid with rumen juice supplemented with minerals and vitamins).

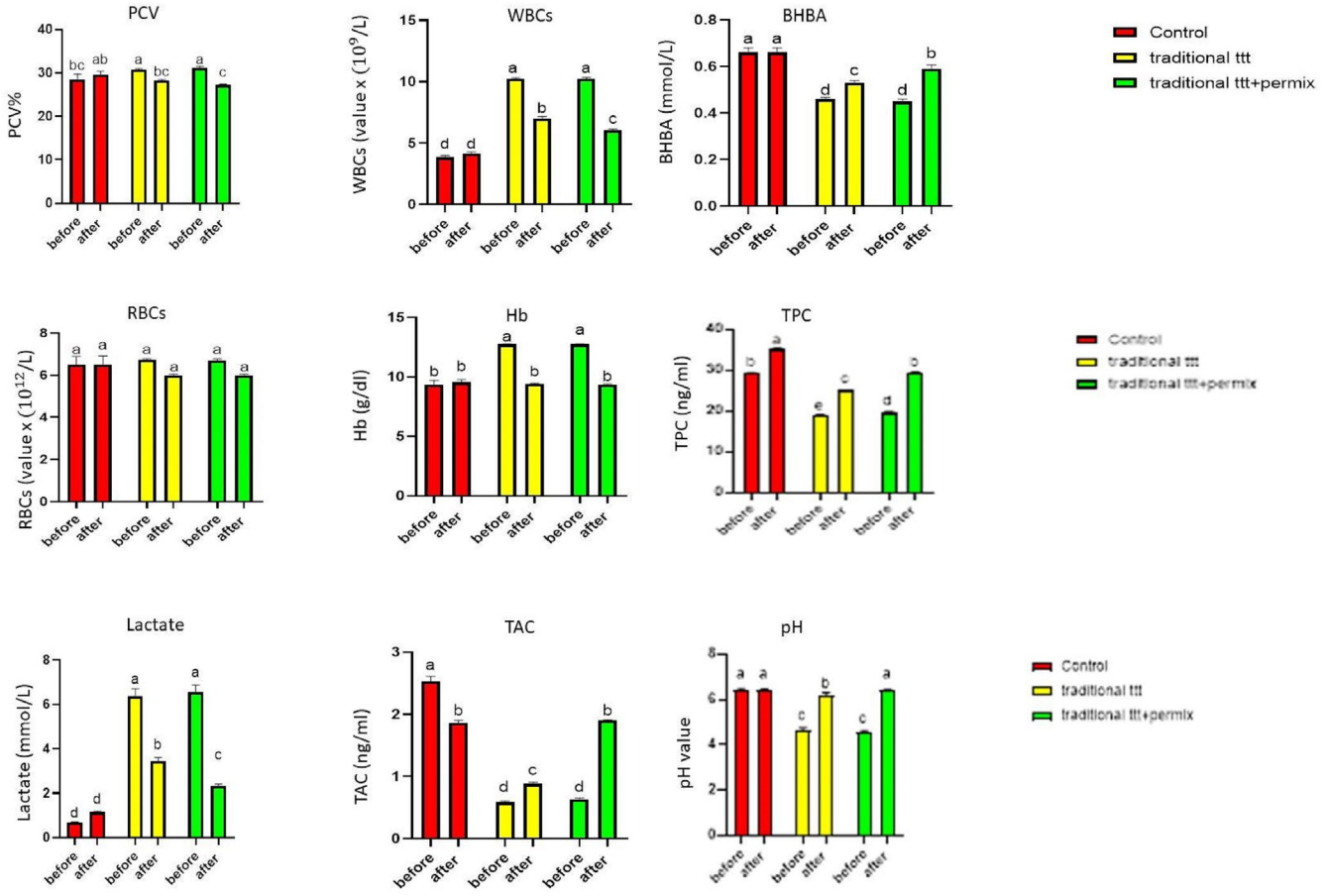

Fig. 1. Effect of ruminal juice, vitamins, and minerals mixture supplementation on calves affected with ruminal acidosis. Columns carrying different letter are significantly different at P< 0.05.

Table 2. Haemato-biochemical alternations in calves suffering from ruminal acidosis.

Parameter	G1		G2		G3	
	Before	After	Before treatment	After treatment	Before treatment	After treatment
RBCs (10^{12}/L)	6.50±0.407^{a}	6.52±0.403^{a}	6.74±0.051^{a}	6.00±0.063^{a}	6.72±0.066^{a}	6.00±0.063^{a}
WBCs (10^{9}/L)	3.84±0.157^{d}	4.14±0.142^{d}	10.26±0.049^{a}	6.99±0.177^{b}	10.23±0.113^{a}	6.03±0.103^{c}
Hb (g/dl)	9.38±0.340^{b}	9.54±0.254^{b}	12.76±0.05^{a}	9.44±0.051^{b}	12.76±0.051^{a}	9.36±0.087^{b}
PCV (%)	28.56±1.184^{bc}	29.68±0.741^{ab}	30.78±0.203^{a}	28.20±0.272^{bc}	31.10±0.447^{a}	27.20±0.200^{c}
ALT (U/L)	13.72±0.433^{e}	30.25±0.707^{d}	43.96±0.488^{a}	40.36±0.051^{b}	43.86±0.474^{a}	37.50±0.176^{c}
AST (U/L)	43.13±0.816^{d}	26.70±0.649^{e}	97.22±0.549^{a}	72.60±0.540^{b}	97.52±0.597^{a}	65.96±0.464^{c}
Lactate (mmol/L)	0.68±0.015^{d}	1.15±0.048^{d}	6.37±0.341^{a}	3.44±0.176^{b}	6.57±0.298^{a}	2.34±0.067^{c}
BHBA (mmol/L)	0.66±0.020^{a}	0.66±0.020^{a}	0.46±0.008^{d}	0.53±0.009^{c}	0.45±0.011^{d}	0.59±0.017^{b}
TAC (ng/ml)	2.53±0.082^{a}	1.86±0.044^{b}	0.59±0.015^{d}	0.88±0.029^{c}	0.63±0.026^{d}	1.90±0.013^{b}

Data are expressed as Mean±SE.
Mean values with different superscripts within rows are significantly different (P < 0.05). G1: Group 1 (control); G2: Group 2 (impacted treated with ruminal anti-acid); G3: Group 3 (impacted treated with ruminal anti-acid with rumen juice supplemented with minerals and vitamins).

pearance, flavor, and consistency. In comparison to G1, the sedimentation activity time in minutes increased significantly (P < 0.05) in G2 and G3. When ruminal juice was examined under a microscope, it revealed a highly significant decrease (P < 0.05) in the number of live protozoa in G2 and G3 compared to G1. Ruminal pH decreased significantly (P < 0.05) in G2 and G3 compared to G1 in both groups (Table 3 and Figure 1). On the third day after treatment, these changes returned to the normal level.

DISCUSSION

Ruminal acidosis is characterized by a rumen fermentation problem. Moreover, it has a lower pH than usual, indicating an imbalance in the microbial population, microbial utilization, and ruminal absorption of volatile fatty acid (VFA) (Castillo *et al.*, 2012). Most of the clinical and systemic abnormalities seen in the affected calves were caused by a drop in the ruminal pH (Plaizier *et al.*, 2008; Afshin *et al.*, 2012; Constable *et al.*, 2017).

The observed clinical alternations were similar to those reported before (Commun *et al.*, 2009; Afshin *et al.*, 2012; Zain El-Din, 2013; Mohamed, 2014; Garrett and Oetzel, 2015; El-Nady *et al.*, 2019). Such alterations confirmed the severity of lactic acidosis. Depending on the degree of the pH value of the ruminal contents, the indications changed. Anorexia, dullness, depression, ruminal atony or complete stasis with light tympany, and congested mucous membrane were among the signs that were

Table 3. Ruminal juice Parameters in calves suffering from ruminal acidosis.

Parameter	G1		G2		G3	
	Before	After	Before treatment	After treatment	Before treatment	After treatment
Ruminal pH	6.44±0.051[a]	6.44±0.024[a]	4.68±0.080[c]	6.22±0.080[b]	4.58±0.058[c]	6.42±0.037[a]
Color	Olive green	Olive green	Milky white	Olive green	Milky white	Olive green
Odor	Aromatic	Aromatic	Sour odor	Aromatic	Sour odor	Aromatic
Consistency	Slightly viscid	Slightly viscid	Watery rumen contents	Slightly viscid	Watery rumen contents	Slightly viscid
Protozoal count (x10^5)	29.28±0.040[b]	35.23±0.161[a]	18.99±0.096[e]	25.06±0.057[c]	19.60±0.212[d]	29.28±0.245[b]
Protozoal motility and population	Moderate active with moderate number	Moderate active with moderate number	Inactive with low number	Moderate active with moderate number	Inactive with low number	Highly active with moderate number

Data are expressed as Mean±SE.
Values with different superscripts within rows are significantly different ($P < 0.05$). G1: Group 1 (control); G2: Group2 (impacted treated with ruminal antiacid); G3: Group 3 (impacted treated with ruminal antiacid with rumen juice supplemented with minerals and vitamins).

seen in the sick cases. The majority of sick calves had soft, yellowish-white feces or diarrhea. Grinding on teeth was a prominent clinical sign in most severely diseased cases. Diarrhea is caused by the conversion of lactic acid to sodium lactate, which then travels through the intestinal tract and creates an osmotic gradient and attracts water into the small intestine (Constable *et al*., 2017). The elevated locomotion score raises the possibility of a histamine relationship. Although it appears that there is little net histamine absorption from the rumen, low rumen pH and gut lesions may enhance absorption since it is either inactivated during or after being absorbed into the blood (Aschenbach and Gabel, 2000; Constable *et al*., 2017;). In our study, the significant improvement in treatment with both ruminal anti-acid & ruminal juice with vitamins and minerals mixture is attributed to improving feed utilization and growth rates. Stabilize the rumen ecology and thus nutrient utilization. The improvement of these animals could be noticed from the second day of treatment. This was reflected by the disappearance of the symptoms of indigestion and the improvement of appetite.

Haemato-biochemical alternation observed in the impacted calves in this study includes no significant change in RBCs (Nasr-ElDin *et al*., 2017), and a significant increase in Hb, WBCs, and PCV%. The same results were recorded before (Hussain and Uppal, 2012; Vieira *et al*., 2012; Soha, 2017) which verified the efflux of liquid from intra- and extracellular compartments to the rumen in case of ruminal acidosis to keep the inner rumen in balance, this caused an increase in hematocrit. On the other hand, as a result of the stress brought on by acidosis, which causes splenic contraction, the number of red blood cells released into the peripheral bloodstream and the subsequent rise in hematocrit may cause hemoconcentration. The leukogram findings coincided with previous reports (Stocker *et al*., 1999; Garry, 2002; Vieira *et al*., 2012; Zain El-Din, 2013; Soha, 2017) which described how ruminitis is connected to neutrophil function, which causes the entire ruminitis process and irritates the epithelium (inflammation of the rumen mucosa caused by the high concentration of lactic acid in the rumen fluid). Elevation of AST and ALT was also previously reported (Abd El-Samee and Abddou, 1997; Bionaz *et al*., 2007; Madan *et al*., 2013).

High ALT activity indicates hepatocellular damage, which may include sub-lethal necrosis or degeneration; AST activity is raised as a result of hepatocellular damage or released from degenerated skeletal muscle (Zain El-Din, 2013). In addition, Xu and Ding (2011) reported that ruminal acidosis altered the animal metabolism, environment, and pH of the enzymes, which caused some hepatic enzymes to change in activity. Acidotic calves' serum enzyme profiles showed increased hepatic enzyme activity. On contrary, Soha (2007) recorded that the enzymes AST and GGT show no significant variation observed in ruminal acidosis.

Significantly higher levels of serum lactate were observed in impaction cases, which may have been caused by increased lactate production brought on by impaction in the rumen. These outcomes were agreed with (Ghanem, 2010; Sudhakara *et al*., 2014). Beta hydroxybutyric acid (βHBA) and total antioxidant capacity (TAC) showed a significant decrease in the case of ruminal acidosis (Yongqing *et al*., 2013).

The obtained results exhibited that treatment of the impacted calves with ruminal juice transplantation supplemented with vitamins and minerals mixture in addition to ruminal anti-acid had a significant effect on hemato-biochemical parameters more than ruminal anti-acid alone. This is because of the direct effect of rumen juice supplemented with vitamins and minerals on providing intestinal microflora. Improvements in cellulolytic bacteria colonization result in improved digestion and nutritional utilization (Soren *et al*., 2013).

In this study, the characteristics of ruminal juice returned to an olive green color, aromatic odor, and slightly viscous consistency. This is because ruminal juice supplemented with vitamins and minerals helps relieve the changes in the characters of the ruminal fluid of the impacted calves, as improvement in ruminal protozoa induced marked changes in the concentration of the constituents of the rumen causing a marked increase in rumen pH (Ibrahim, 1993; El-Nady *et al*., 2019) improve digestibility and refaunation of ruminal protozoa (Galbet. 2007; El-Nady *et al*., 2019).

CONCLUSION

It is concluded that using ruminal juice supplemented with vitamins and minerals mix with ruminal anti-acid has a good impact on calves clinically affected with ruminal acidosis.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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