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EVALUATION OF THE EFFICACY OF DIFFERENT DIETARY RUMEN BUFFERS ON PREVENTION OF RUMINAL ACIDOSIS IN GOATS

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Abstract: This study was realized to evaluate the efficacy of dietary supplementation of sodium bicarbonate plus magnesium oxide "MgO", calcareous marine algae (AcidBuf) and essential oils (Actifor®Boost) on the animal performance and prevention of ruminal acidosis in goats moreover, evaluation the diagnostic accuracy of some biochemical parameters including blood pH, total antioxidant capacity (TAC), bicarbonate (HCO_3^-), partial pressure of carbon dioxide (pCO_2), beta-hydroxybutyric acid (βHBA) and plasma $_L$-lactate versus ruminal pH in diagnosis of ruminal acidosis. A total of 30 goats were divided into five equal groups (G1-5) according to the type of dietary treatment. G1 served as a control group and fed the concentrate diet only, G2 fed the concentrate diet supplemented with 10 g Na bicarbonate plus 4g MgO, G3 supplemented with 4g AcidBuf, G4 supplemented with 10 g Na bicarbonate plus 4g MgO plus 4g AcidBuf and G5 supplemented with 1g Actifor® boost/head/day for five days. Tachycardia, tachypnea and decreased ruminal motility were recorded in G1 only. The ruminal pH tended to return to its toward normal for G3 and G4 with a mean of (6.3±0.03 and 6.3±0.01, respectively) which significantly higher than those of G1 and G2 (6.19±0.01 and 6.17±0.02 respectively). A significant increase in plasma $_L$-lactate and a significant decrease in blood pH, HCO_3^-, pCO_2, TAC, and βHBA were recorded in G1 when compared with other groups. Calcareous marine algae with or without adding other compounds found to be a promising rumen buffer agent, moreover TAC and plasma $_L$-lactate showed better diagnostic performance versus rumen pH in the diagnosis of ruminal acidosis.

Key words: rumen buffers; ruminal acidosis; goats; diagnostic accuracy

Introduction

Today, both small and large producers look to goat farming as a source of income, thus their feed management has been modified to promote efficient growth and achieve quick weight gain through the use of highly fermentable carbohydrates, which puts the animals at risk for developing ruminal acidosis (RA) (1).

The microbial fermentation of these carbohydrates by the amylolytic bacteria present in the rumen produces volatile fatty acids and lactic acid leading to dropping of ruminal pH below the physiological levels, death of many gram -ve bacteria and favours the growth of other gram +ve bacteria, particularly lactate-producing bacteria like *Streptococcus bovis* and *Lactobacilli spp*. This has detrimental effects on animal health (2).

When the microbial production of lactic acid exceeds its utilization, lactic acid absorbed into the blood circulation and causing systemic changes that can be assessed by measuring some hematobiochemical parameters that give useful information in the diagnosis, prognosis, and treatment of RA (3, 4).

Dietary buffers play an important role in preventing ruminal pH from falling below optimal limits. There are a variety of them which could be used to control the rumen fermentation and

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improve the animal productivity and health including sodium bicarbonate (SB), sodium bentonite, magnesium oxide, calcium carbonate and calcified seaweed (5)

Sodium bicarbonate is the most widely used mineral buffer in the animal industry for its rumen buffering capacity and its inclusion in animal diet has become a common practice in most parts of the world (6), several reviews highlight the efficiency of SB in raising the ruminal pH, and there are practical recommendations for adding SB at 7–10 g/kg (DM) (7). MgO is a slow-release neutralizing agent that has been used to raise the ruminal pH; however, due to its low water solubility, its effect developed slowly and occurred only after 24 h of treatment (8). Two to three parts of SB to one part of MgO is the proper ratio to use it in the diet (9). Recently, calcareous marine algae (CMA), natural product made from calcified seaweed "Lithothamnion calcareum" and have been used recently to stabilize the rumen pH and prevent development of ruminal acidosis (10). Essential oils could be considered a natural alternative to modify the rumen microbial fermentation (11).

So, this study was planned to evaluate the efficacy of dietary supplementation of SB plus MgO, AcidBuf and Actifor®Boost on prevention of ruminal acidosis in goats fed on acidogenic diet moreover, evaluation the diagnostic accuracy of some biochemical parameters versus the ruminal pH in the diagnosis of RA.

Materials and Methods

All the procedures used in this experiment were reviewed and approved by the Zagazig University Animal Research Ethics Committee (ZU- IACUC, approval number: ZU- IACUC/2/F/30/2022).

Animals, diet and experimental design

A total of 30 clinically healthy goats of both sexes weighed (20-25 kg) and aged (2–3 years) were admitted to the isolation section at Faculty of Veterinary Medicine, Zagazig University, Egypt.

Experimental design

Adaptation period

Upon arrival, the goats underwent to a thorough clinical examination and dewormed through subcutaneous injection of Ivomec® plus (Ivermectin/ Clorsulon, Merial) at a dose of 1ml/50kg body weight. This period lasted for 20 days during them the goats were fed on a forage diet and water ad libitum and kept on wheat straw beds in natural light and room temperature conditions.

Feeding period

This period began after the 20 days of the adaptation period and lasted for five days during them all goats received basal concentrate diet, crushed corn at 50 g/kg body weight per day. According to the type of dietary treatment, the goats were randomly divided into five equal groups, each with six goats. The dietary treatments were; G1 fed the basal concentrate diet only and served as an unbuffered control group, G2 supplemented with 10 g SB plus 4g MgO, G3 supplemented with 4g AcidBuf, G4 supplemented with 10 g SB plus 4g of MgO plus 4g of AcidBuf and G5 supplemented with 1g of Actifor ® boost / head / day for five days.

Clinical examination

All experimental goats underwent to thorough clinical examination according to Smith and Sherman (12), focusing on the evaluation of vital signs (heart rate, respiration rate, body temperature and rumen motility).

Sampling

Blood and ruminal fluid samples were collected before morning feeding at zero day (last day of adaptation period) then at interval of 24, 48, 72 and 96 h post feeding the concentrate diet.

Blood samples

Three blood samples were collected from each goat through jugular vein puncture: one on vacuum-heparinized tubes containing freeze-dried lithium heparin for hemogasometry analysis; measurements of blood pH, pCO_2 and HCO_3^-, were made within half an hour after collection, using blood gas analyzer (RAPIDlab 348EX SIEMENS blood gas system), the second sample was collected in clean and dry tubes without anticoagulant to obtain serum for the determination of βHBA, using commercial spectrophotometric kits (Pointe Scientific, Inc. USA) according to Koch and Fledbruegge (13) and TAC, using a commercial test kit (Sigma-aldrich, USA) according to Miller and

Evans (14); and the third sample in tubes with sodium fluoride for plasma $_{L}$-lactate determination using kits produced by spin react according to Burtis (15).

Ruminal fluid samples

Ten mL ruminal fluid was obtained via stomach tube to determine the pH. A digital pH meter (Adwa, AD11, ROMANIA) was used, calibrated by standard pH buffer according to Constable et al. (16).

Statistical analysis

Data were analyzed using R 4.1.0 (R Foundation for Statistical Computing, Vienna, Austria). Descriptive statistics for the different clinical and biochemical parameters in ruminal acidotic +ve and -ve cases were presented. The two-way analysis of variance (ANOVA) was used to test the effect of groups and different sampling times on the ruminal pH, while the one-way ANOVA was used to investigate the ruminal pH within each group in the different sampling times.

Ruminal acidosis case definition was based on the ruminal pH value of ≤ 5.5 as described previously (17), the receiver operating characteristic curve (ROC) was executed for some biochemical parameters to test their diagnostic accuracy versus the ruminal pH, in-between such parameters the blood pH, TAC, HCO3-, pCO2, βHBA and L-lactate. The optimal cut-off values plus sensitivities (Se) and specificities (Sp) were presented for each of the tested parameters, such Se and Sp were calculated from the following formulas:

$$Se = TP / (TP + FN)$$
$$Sp = TN / (FP + TN), \text{ where}$$

TP: true positive: Rumen pH ≤ 5.5 and positive biochemical test (value > cut off point)
FP: false positive: Ruminal pH >5.5 and positive biochemical test (value > cut off point)
FN: false negative: Rumen pH ≤ 5.5 and negative biochemical test (value < cut off point)
TN: true negative: Ruminal pH >5.5 and negative biochemical test (value < cut off point)

Results

Clinical examination

The results of clinical findings of all groups were summarized in Table 1. The vital parameters (temperature, heart rate, respiratory rate, and rumen contraction) showed non-significant changes between the different buffered groups (G2-5), while the unbuffered control group G1 recorded significant increase in the mean of respiratory and heart rate per minute. Non-significant changes in the mean of body temperature between all groups, while the ruminal contraction per 2 minutes was significantly lower in G1 only (P-values between G1 and other groups were < 0.001).

Hemogasometry and biochemical parameters

Mean blood pH was significantly lower in G1 compared to other groups (7.37±0.07) and was significantly higher in G2 group compared to other groups (7.48±0.01) and mean TAC and βHBA were significantly lower in G1 in comparison to other groups (6.39±5.38, 0.49±0.18) respectively. The mean plasma L-lactate level was significantly higher in G1 compared to other groups (26.4±15.5), all such comparisons and others are summarized in Table 1.

Efect of dietary treatments on the ruminal pH

Results of the two-way ANOVA for the effect of groups, times of sampling, and their interaction were presented in Table 2. There was a significant effect of the groups, times, and their interaction on the level of the ruminal pH (P-value for all was <0.001). When the post-hoc test was implemented, the mean ruminal pH for G3 and G4 groups at the five sampling times was (6.3±0.03 and 6.3±0.01, respectively) that was significantly higher than those of G1, G2 and G5 (5.34±0.07, 6.19±0.01 and 6.17±0.02, respectively) as shown in Table 3. Figure 1 is showing the different ruminal pH values across groups and times.

Table 4 is showing the rumen pH in the different sampling times was compared within each of the treated groups. Interestingly, in groups G2-G5, there was a significant difference between rumen pH at zero h and 96h, but for all group the rumen

pH values were not less than 5.5, this indicating the efficiency of all buffers in preventing the occurrence of ruminal acidosis.

Diagnostic accuracy for some biochemical parameters versus ruminal pH

Based on the selected cut-off point to define the +ve RA, the ruminal pH for animals in group C was 22 times equal to or below this selected cut-off point (≤ 5.5), indicating the presence of RA. Blood pH, TAC, HCO3-, pCO2, βHBA, and plasma L-lactate accuracy were tested versus the accuracy of the ruminal pH in RA diagnosis (Figure 2). The more the curve moved toward the upper left corner of the ROC space is an indicator of higher test accuracy. TAC has the best diagnostic accuracy among other parameters (Se 97.7%, Sp 81.8%, and AUC 0.92) while HCO3- showed the lowest accuracy (Se 77.3%, Sp86.4%, and AUC 0.88).

Table 1: The effect of dietary treatments on the physical and biochemical parameters in the different groups through the five sampling times

	G1	G2	G3	G4	G5
Respiratory rate/ min	29.7±4.95^{a}	22.6±1.19^{c}	24.2±1.12^{bc}	24.1±1.25^{bc}	24.5±1.38^{b}
Heart rate/min	87.2±8.3^{a}	77.1±1.4^{b}	77.6±2.2^{b}	77±1.8^{b}	76.4±1.14^{b}
Temperature °C	39.5±0.08 ^{a}	39.5±0.06 ^{a}	39.6±0.05^{a}	39.5±0.07 ^{a}	39.5±0.08 ^{a}
Blood pH	7.37±0.07^{d}	7.48±0.01^{a}	7.44±0.03^{b}	7.44±0.01^{b}	7.4±0.02^{c}
TAC (ng/ml)	6.39±5.38^{b}	9.33±3.85^{a}	11.5±3.01^{a}	9.77±2.21^{a}	9.07±3.23^{a}
Lactate (mg/dl)	26.4±15.5^{a}	16.1±9.05^{b}	12±4.81^{b}	11.2±4.5^{b}	15.1±6.11^{b}
βHBA (mmol/L)	0.49±0.18 ^{b}	0.64±0.09^{a}	0.66±0.08^{a}	0.61±0.04 ^{a}	0.61±0.11 ^{a}
HCO_3 (mmol/L)	20.4±4.9^{d}	29.5±3.02^{a}	26.4±1.03^{b}	23.6±1.5^{c}	24.2±2.6^{c}
pCO_2 (mmHg)	29.06±3.8^{d}	37.7±4.4^{b}	38.9±3.7^{b}	33.4±4.5^{c}	42.3±2.3^{a}

Results are expressed as means ± SD
Different superscripts within the row indicate statistically significant differences ($p<0.05$).
TAC: total antioxidant capacity; βHBA: beta hydroxybutyric acid; HCO_3^-: bicarbonate concentration; pCO_2: carbon dioxide partial pressure. Treatments: G1 = control; G2 = sodium bicarbonate + magnesium oxide; G3= calcareous marine algae; G4= calcareous marine algae+ sodium bicarbonate + magnesium oxide; G5 = essential oil.

Table 2: Two-way analysis of variance (ANOVA) table for groups and times effect on the rumen pH

	Sum of squares	Df	Mean square	F-value	P-value
Groups	19.8	4	4.9	384.9	<0.001
Time	25.8	4	6.4	501.04	<0.001
Group*Time	8.2	16	0.5	39.85	<0.001

Df: degree of freedom

Table 3: The effect of dietary treatments on the rumen pH in the different groups

	G1	G2	G3	G4	G5
Rumen pH	5.34±0.07^{d}	6.19±0.01^{b}	6.3±0.03^{a}	6.3±0.01^{a}	6.17±0.02^{bc}

Results are expressed as mean ± SD (i.e., mean pH through the five sampling times).
Different superscripts within the row indicate statistically significant differences ($p<0.05$).
Treatments: G1 = control; G2 = sodium bicarbonate + magnesium oxide; G3= calcareous marine algae; G4= calcareous marine algae + sodium bicarbonate + magnesium oxide; G5 = essential oil.

Table 4: The effect of rumen buffers on the rumen pH along the time in the different groups

Time / Groups	Zero h	24h	48h	72h	96h
G 2	6.87±0.08^{a}	6.08±0.07^{b}	5.98±0.19^{b}	6.07±0.1^{b}	5.97±0.05^{b}
G 3	6.87±0.05^{a}	6.42±0.16^{b}	6.07±0.13^{c}	6±0.09^{c}	6.15±0.16^{c}
G 4	6.85±0.05^{a}	6.28±0.11^{b}	6.1±0.09^{c}	6.13±0.15^{bc}	6.17±0.05^{bc}
G 5	6.83±0.05^{a}	5.95±0.12^{b}	5.97±0.15^{b}	5.97±0.13^{b}	5.97±0.1^{b}

Results are expressed as mean ± SD.
Different superscripts within the row indicate statistically significant differences ($p<0.05$).
Treatments: G1 = control; G2 = sodium bicarbonate + magnesium oxide; G3= calcareous marine algae; G4= calcareous marine algae+ sodium bicarbonate + magnesium oxide; G5 = essential oil

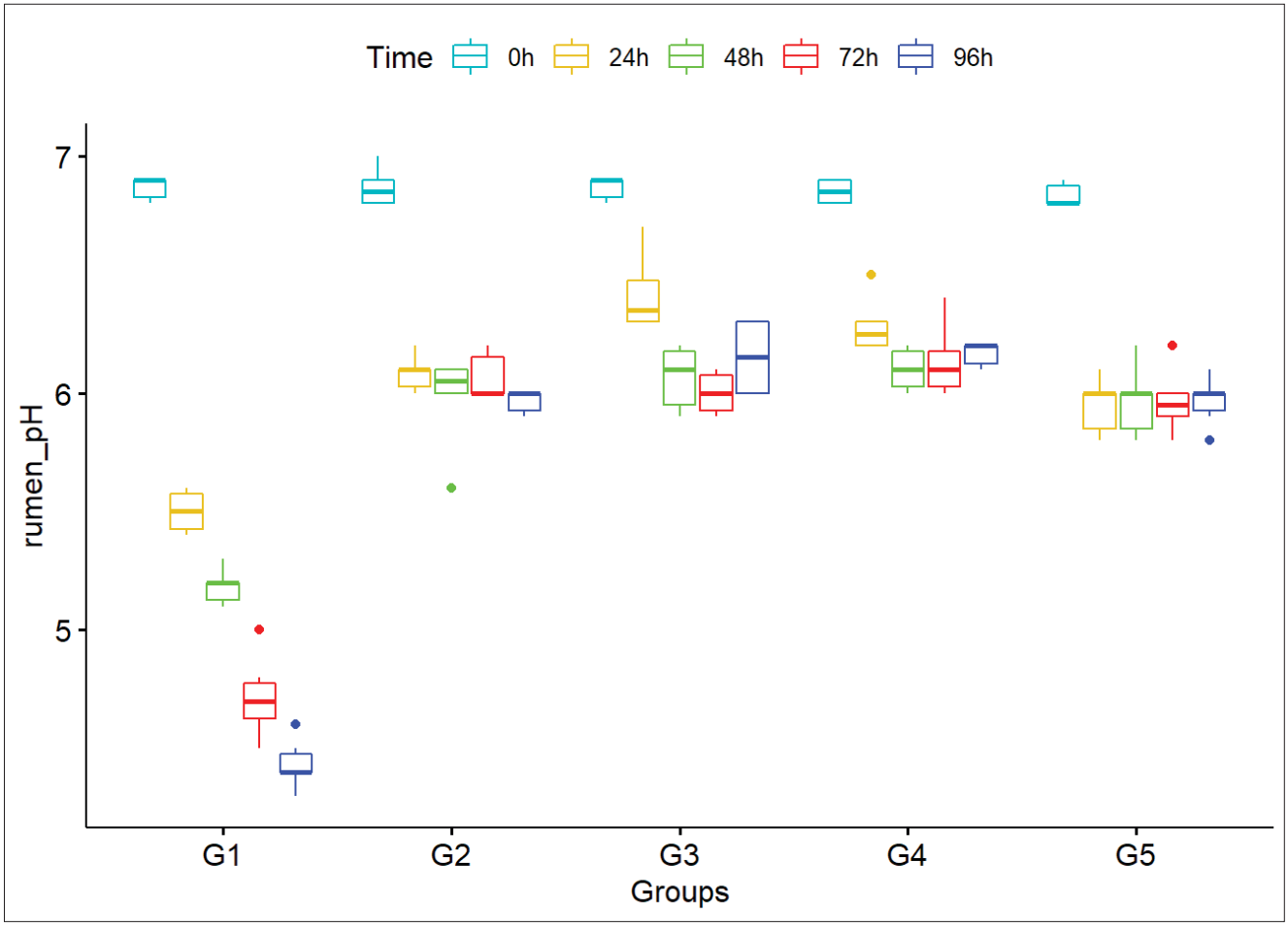

Figure 1: Box plot for the rumen pH values in different groups/times.

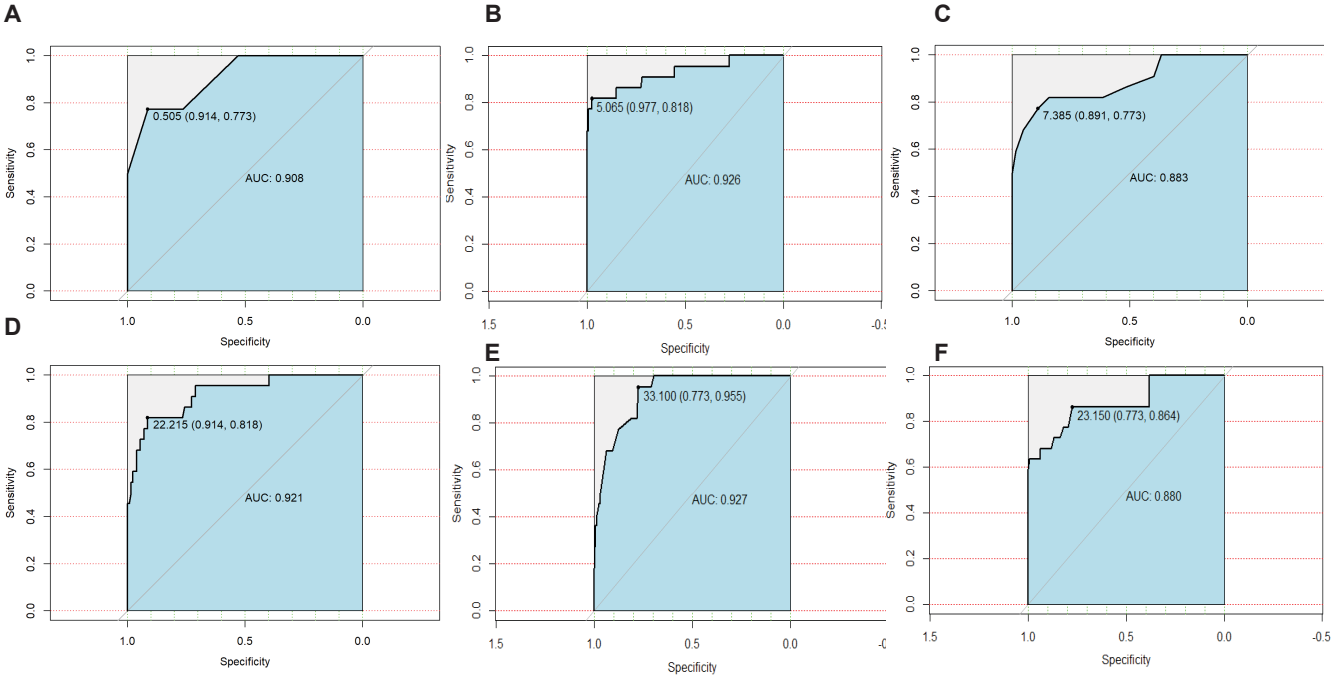

Figure 2: The receiver operating characteristic curves (ROC) for blood pH (A), TAC (B), bicarbonate (C), pCO2 (D), βHBA (E), and plasma lactate (F). The optimal cut-off points were shown for each parameter and the maximum sensitivity and specificity, also the area under curve (AUC)

Discussion

In order to improve animal performance and increase the weight gain, the highly and rapidly fermentable carbohydrates have been used widely in animal nutrition but this leads to some microbial disturbances and predispose the animal to the risk of RA (1) which considered an important and common clinical emergency of small ruminants and if not manipulated properly may lead to high economic losses due to the high morbidity and mortality, making detection of the disease in the field is very important (16).

On clinical examination, there were significant increase in heart and respiratory rate per minute found to be characteristic for the acidotic goats in the unbuffered control group (G1) compared to others. The recorded tachycardia in G1 coincided with the dropping of the ruminal pH to values < 5.5, increased ruminal osmolarity and consequently, mobilization of water from the circulation to the rumen causing hemoconcentration and increased heart rate. The increased respiratory rate may be attributed to stimulation of the respiratory center by the acidic blood pH that helps in removing the excess of CO_2 as a compensatory mechanism for correction of the metabolic acidosis occurred (18). Those results agree with previous studies (18-21).

The ruminal contraction/ 2minutes show significant decrease in G1 only which was similar to that was reported in previous studies (22-24) in which the ruminal contraction was significantly decreased with the ruminal acidosis that may be attributed to the low ruminal fluid pH and increased ruminal osmolarity (25).

Concerning ruminal pH and the buffer effect on it, the control diet used in this study caused ruminal pH to fall below 5.5 in the unbuffered control group only which had the lowest mean ruminal pH compared to other groups. This might be due to excess accumulation of lactate resulting from fermentation of the highly fermentable carbohydrates in the rumen (3).

These findings agree with previous studies (21, 26). The results show that the four dietary buffer treatments have a positive effect on the mean ruminal pH and all the buffers used in this study have the ability to prevent lowering of ruminal pH below 5.5 compared with the control group. The buffer effect on rumen pH at all sampling times in the different groups was greater for G3 and G4 that had the highest mean ruminal pH. Also, G3 and G4 groups were most effective in maintaining high rumen pH with a greater daily mean rumen pH as shown in table 4. It has been claimed previously that CMA is more effective than SB, has twice the buffering capacity of it probably due its high content of calcium, magnesium, and essential trace elements (10) and give better results when mixed with the SB (27). These findings agreed with previous studies (28, 29) where the authors reported that CMA decreased the time spent under pH 5.5 compared to SB and, agreed with (30) who mentioned previously that the addition of 90 g/day CMA to high concentrate diets was more efficient than addition of 180 g/day SB in prevention of ruminal pH reductions.

Blood gas analysis offers a proper assessment of acidosis while being less invasive than ruminal pH so, it can be considered a valuable tool to diagnose RA (31). Blood pH relies on the relative amounts of bases, acids, and buffers (bicarbonate) (32) and there is a tight relationship between the ruminal pH and blood pH (31). There were a significant decrease in the mean values of blood pH, HCO_3^- and pCO_2 in G1 group in comparable to other groups. Lactate produced from the digestion of concentrates absorbed into the blood circulation and metabolized by the liver until the amount of lactate overwhelmed the liver's capacity, lactate accumulates, causing a reduction of blood pH and development of metabolic acidosis (1, 3). Bicarbonate reserves consumed through buffering of the accumulated acid and cause a reduction of blood pH (1). Low blood pH acts as stimuli for the respiratory center and increasing alveolar ventilation for elimination of extra CO_2 from the lungs decreasing pCO_2 concentration (3). Those findings have been agreed with previous studies (1, 3) in which the authors mentioned significant decrease in blood pH, $HCO_3^-$and pCO_2 in sheep and goats during induction of ruminal acidosis.

Concerning plasma $_{L-}$lactate concentration, there was a significant increase in the mean values of plasma $_{L-}$lactate in G1 group compared to other groups. Lactate is produced in large amount during feeding of concentrates and absorbed into the blood circulation increasing its level in the blood. Similar results were reported previously (1, 33).

TAC and βHBA serum concentration recorded a significant decrease in their mean values in G1 group in comparable to others. Ruminal acidosis was associated with excessive reactive oxygen

species (ROS) and oxidative stress leading to exhaustion of enzymatic and non-enzymatic antioxidants in their neutralization as it was obvious by decreased TAC (22). Those results agree with previous studies (23). The high concentration of blood glucose reduced the βHBA concentration (34) as mentioned in a previous work (35).

To the best of our knowledge, our study is the first to report the diagnostic accuracy of some biochemical parameters versus the ruminal pH in RA diagnosis. The precision and accuracy of a diagnostic test serve as two independent descriptors of the test performance, Se and Sp are usually measured to represent the accuracy of such diagnostic test (36). The receiver operating characteristic curves are graphical plots that illustrate the diagnostic ability of a test as its discrimination threshold is varied (37). Recently, such diagnostic accuracy measures are used extensively in the veterinary practice.

In this study, testing TAC and plasma $_{L-}$lactate versus the ruminal pH in diagnosis of ruminal acidosis showed the best Se (97.7%, 91.4%, respectively) and Sp (81.8% for both) rather than other biomarkers, with outstanding area under ROC curves for both parameters (nearly 0.92 for both). Ruminal pH was found to be decreased along with decreasing TAC and increase the plasma $_{L-}$lactate as reported in previous studies (23, 38, 39). Reasons for such correlation were described earlier in the discussion section.

The interpretation of our diagnostic accuracy results could be limited to the small sample size , only 22 times RA was diagnosed, further studies with larger sample size are recommended. The same recommendation is advised to verify the buffer results on a large scale and longer study duration. In conclusion, calcareous marine algae with or without adding other compounds found to be a promising rumen buffer agent. TAC and plasma lactate found to be good diagnostic biomarkers for RA when tested versus the ruminal pH as a gold standard.

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