



## Effect of supplement some feed additives to mitigate the risk of subacute ruminal acidosis in fattening calves on performance, rumen fermentation activity, and some blood parameters

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### Abstract

This study aimed to evaluate the effects of adding feed additives such as sodium bicarbonate (SB), magnesium oxide (MgO), calcareous marine algae (CMA), or seaweed to calves' rations on performance, rumen fermentation, blood parameters, and the economic efficiency of Holstein's calves. Twelve healthy Holstein calves aged nine months with an average body weight of  $272.7 \pm 3$  kg were randomly allocated into four dietary treatment groups. The animals in all groups received a basal diet consisting of a concentrate feed mixture (CFM) and wheat straw. The calves in the 1<sup>st</sup> group (T1) were fed CFM supplemented with 1% SB, the 2<sup>nd</sup> calves' group (T2) were fed CFM supplemented with 1% SB and 0.3% MgO, the 3<sup>rd</sup> calves' group (T3) were fed CFM supplemented with 1% CMA, and the 4<sup>th</sup> calves' group (T4) were fed CFM supplemented with 1% CMA and 0.3% MgO. The results revealed that the average daily gain, DMI, and feed conversion ratio were not significantly affected by dietary treatment. The calves in T3 had a higher ( $P < 0.05$ ) plasma albumin and A/G ratio and a lower ( $P < 0.05$ ) globulin compared with the other groups. The value of serum glucose was higher ( $P < 0.05$ ) in T1 than the other groups, with no significant differences observed among the T2, T3, and T4 groups. The values of ALT were increased in T2 and T3 compared with the T1 and T4 groups. Supplementation of antacids to calves' diets did not have a significant effect on ruminal pH or ammonia nitrogen concentration. The ruminal TVFAs increased ( $P < 0.05$ ) with the T2 diet compared with T1 and T4. In conclusion, dietary sodium bicarbonate and seaweed supplementation in calves' diets improve some blood parameters and rumen fermentation, particularly when mixed with MgO.

**Keywords:** sodium bicarbonate, calcareous marine algae, calves' performance, blood parameters, rumen fermentation.

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## 1. Introduction

Acidosis is a major health disorder in feedlots worldwide, and in Egypt, it has a direct impact on cattle productivity and health. Acute and subacute rumen acidosis frequently affects intensively reared beef cattle, which are fed high-energy diets rich in highly fermentable carbohydrates. Acidosis reduces feed intake and growth performance, damages the gastrointestinal tissue, leads to inflammation due to the release of lipopolysaccharides from the death of gram-negative bacteria, and predisposes cattle to diseases such as liver abscess and laminitis (Sgoifo Rossi and Compiani, 2016). Treatment of lactic acidosis is difficult, and its recovery depends on the severity of the condition (Karapinar *et al.*, 2008). Focusing mainly on correcting rumen acidosis and acid base imbalance, inhibiting further lactic acid production, and restoring a normal rumen microenvironment (Anderson and Rings, 2008; Koondhar *et al.*, 2020). Agents capable of neutralizing acids can be used to treat lactic acidosis. Numerous studies have summarized the effectiveness of sodium bicarbonate in increasing rumen pH (Erdman, 1988; Hu and Murphy, 2005; Udainiya *et al.*, 2020), but due to its rapid solubility, it is short lived in the rumen (Van Soest, 1994). Additionally, magnesium oxide (MgO) increases the rumen pH, but the effect develops slowly and is only relevant after 24 h of treatment (Calsamiglia *et al.*, 2012), and the optimal way to apply MgO in the diet is one part of MgO with two to three parts sodium bicarbonate (Hutjens, 2003). Calcareous marine algae are a natural product produced from the calcified seaweed

“*Lithothamnion calcareum*”, which contains high levels of calcium, magnesium, and essential trace elements, has a positive effect on rumen pH, and has the potential to prevent the onset of lactic acidosis (Cruywagen *et al.*, 2015). Sykes (2009) reported that seaweed is a natural multimineral supplement that contains all the minerals and trace elements an animal requires for a normal healthy life. Stokes *et al.* (1986) reported that adding sodium bicarbonate (NaHCO<sub>3</sub>) with magnesium oxide (MgO) increased dry matter intake when corn silage was the sole or major source of forage in the diet rumen pH by supplementing an increasing dose of CMA, from 0.125 to 1.2% dietary DM. The objective of this study was to evaluate the effect of supplementation with different feed additives, such as sodium bicarbonate, magnesium oxide, and calcareous marine algae (CMA) on animal performance, rumen fermentation and blood parameters in fattening Holstein calves.

## 2. Materials and methods

The current study was carried out at the animal production farm of the New Sonta Feed Company in Alexandria, Egypt. Twenty Holstein calves aged 9 months with an average body weight of  $272.7 \pm 3$  kg were randomly assigned to four groups of five calves each, according to their average live body weight. The average initial weight was similar in all groups. The animals in all groups received a basal diet consisting of concentrate feed mixture (CFM) and wheat straw. The CFM was supplemented with 1% sodium

bicarbonate in the first group (T1), 1% sodium bicarbonate and 0.3% magnesium oxide in the second group (T2), 1% calcareous marine algae (CMA) in the third group (T3), and 1% calcareous marine algae and 0.3% magnesium oxide in the last group (T4). The experiment began from August to December 2021 and lasted for 120 days. The animals were weighed biweekly before morning feeding. The calves of each group were kept in one pin and fed individually. All animal groups were fed 80% of their requirements as a concentrate feed mixture, while the other requirement was covered with wheat straw as roughage. The quantity of concentrate mixture was

adjusted every month according to changes in body weight (NRC, 1996). Animals were subjected to veterinary quarantine for 40 days, and the necessary vaccinations were given. Cubes of vitamins, minerals, and freshwater were available free of choice. The concentrate feed mixture was offered twice daily at 8 am and 8 pm, while wheat straw was offered once daily at 10 am. Feed sample chemical analyses were conducted using AOAC (2005) methods. Acid-insoluble ash (AIA) was calculated according to Van Keulen and Young (1977). Chemical analysis and nutritional composition of experimental diets (% based on DM) are shown in Table (1).

Table (1): Chemical analysis and nutritional composition of experimental diets (% based on DM).

Items	Treatments				Wheat straw
	T1	T2	T3	T4	
Dry matter (DM)	89.7	90	90.1	89.8	92.6
Organic matter (OM)	81.4	82.4	81.6	82	83.0
Crude protein (CP)	15	14.7	14.6	14.7	3.5
Crude fiber (CF)	12.8	12.5	13	12.8	30.9
Ether extract (EE)	2.1	2.3	2.2	2.3	0.19
Nitrogen free extract (NFE)	61.8	62.9	61.7	62.4	56.31
Ash	8.3	7.6	8.5	7.8	9.1
*AIA	1.7	1.8	1.6	1.5	3.6
**Gross energy Mj/kg DM	1.76	1.56	1.76	1.78	0.69

T1: calves received 1% sodium bicarbonate, T2: calve received 1% sodium bicarbonate plus 0.3% magnesium oxide, T3: calves received 1% seaweed, T4: received 1% seaweed plus 0.3% magnesium Oxide. \*AIA = acid insoluble ash.  
 \*\* GE MJ/Kg DM = 0.0226 × % CP + 0.0407 × % EE + 0.0192 × % CF + 0.0177 × % NFE.

### 2.1 Blood sampling

Blood samples were collected at the end of the experimental period before the Morning feeding from four calves in all groups via the jugular vein in glass tubes heparinized with anticoagulant (EDTA). Blood samples were immediately centrifuged at 4000 rpm for 15 min. and

the plasma was stored at -20 °C until chemical analysis. Plasma was used to determine total protein (g/dl), albumin (g/dl), glucose (g/dl), cholesterol (mg/dl), urea (mg/dl), aspartate transaminase (AST) (U/l) and alanine transaminase (ALT) (U/l) levels using commercial kits and spectrophotometer methods. Globulin values were determined by subtracting

albumin value from total protein value.

## 2.2 Rumen liquor parameters

At the end of the experimental trial, samples of rumen liquor were collected from each four calves using a stomach tube. Rumen liquor samples were collected after morning feeding. Rumen liquor samples were filtrated through four layers of cheesecloth. The filtrated portion was used immediately for the measurement of pH using a digital pH meter (Beckman, model 45, USA). The  $\text{NH}_4\text{-N}$  concentration in the rumen liquor was determined according to the atomic absorption spectrophotometer methods described by Van Anken and Scphorst (1974). Strained rumen liquor samples were acidified with 0.1 N hydrochloric acid and 2-3 drops of formalin or formaldehyde to stop the microbial activity before its storage for analysis, and then the samples were kept frozen at  $-20^\circ\text{C}$  for determination of total volatile fatty acids (VFA) and total bacterial count. The concentration of TVFAs was determined by the steam distillation method (Wanner, 1964) using a Markham microdistillation apparatus.

## 2.3 Economic evaluation

The economic efficiency in the present study was calculated according to marketing prices during December 2022. The prices of animal feedstuffs during this period for one Kg (DM basis) of concentrate mixture, wheat straw,

calculated considering the price of one Kg (DM basis) of concentrate mixture, wheat straw, sodium bicarbonate, magnesium oxide, and seaweed were 7, 1.2, 7, 7.5 and 10 LE, respectively. Also, the price of one Kg of fattening calves live body weight was 62 LE. Entomic analysis of data was done using the technique of Perrin *et al.* (1979).

## 2.4 Statistical analysis

The data were statistically analyzed using the General Linear Model (GLM) procedure of the SAS (2001) program, version 8.2. Differences between groups in blood parameters, and rumen liquid parameters were evaluated by one-way ANOVA. The significant differences between treatments means were tested by Duncan's Multiple Range Test (Steel and Torrie, 1980). The data are presented as means  $\pm$  SE. Probability values of less than 0.05 ( $P \leq 0.05$ ) were significant. The following model was used:

$$Y_{ij} = \mu + T_i + E_{ij}$$

Where  $Y_{ij}$  = experimental observation,  $\mu$  = general mean,  $T_i$  = the effect of treatment,  $E_{ij}$  = the errors related to individual observation.

## 3. Results and Discussion

### 3.1 Calves' performance

The results in Table (2) indicated that the average daily gain was not significantly affected by dietary treatment. However,

the average daily gain tended to be lower by about 16.34 14.09 and 7.91% for calves that received 1% sodium bicarbonate plus 0.3% magnesium oxide when compared with T4, T1 and T3 groups respectively. for calves that received 1% sodium bicarbonate plus 0.3% magnesium oxide when compared with other groups. Also, the total DM intake and feed conversion ratio were not affected by dietary treatments. While the total DMI tended to decrease by about 4-8% of T2 when compared with other groups. The results

of the present study were similar to the previous studies that reported the voluntary feed intake of the animals was not affected by supplementation of sodium bicarbonate (Fellner *et al.*, 2000; Mess *et al.*, 1985). Similarly, Santra *et al.* (2003) reported that daily dry matter intake was not affected by increasing the level of sodium bicarbonate in rations from 0.75 to 2.25%. Furthermore, Wu *et al.* (2015) found that cows feeding calcareous marine algae (CMA) prepartum does not affect dry matter intake (DMI) or serum metabolites.

Table (2): Effect of antacids supplement to calves’ rations on performance of fattening calves.

Items	Treatments				Sig.
	T1	T2	T3	T4	
Initial weight (Kg)	272.70 ±4.918	273±2.444	275.8 ± 4.244	271.6± 3.176	NS
Daily gain (kg)	1.49±0.133	1.28 ± 0.150	1.39±0.078	1.53± 0.032	NS
Total DMI (kg/ day)	9.36± 0.44	8.94± 0.47	9.73± 0.42	9.86± 0.30	NS
Feed conversion (kg/kg gain)	6.29 ± 0.277	6.98 ± 0.950	7.05 ± 0.237	6.44± 0.242	NS

<sup>a, bc</sup> Means of the same row in each item with different superscripts are significantly different (P<0.05). T1: calves received 1% sodium bicarbonate, T2: calve received 1% sodium bicarbonate plus 0.3 magnesium oxide, T3: calves received 1% seaweed, T4: received 1% seaweed plus 0.3% magnesium oxide. Sig. = Significant, NS = not significant.

### 3.2 Blood parameters

The plasma parameters data are summarized in Table (3). The results indicated that supplementing seaweed to the diets of calves increased (P<0.05) albumin and A/G ratio and decreased (P<0.05) globulin in blood plasma when compared with other groups. However, no significant (P>0.05) effects were detected among the other treatment groups. The value of plasma total protein was not significantly affected by antacids supplementation to the ration of calves. Leanna (2002) found that the blood total protein was not affected by diet or free-choice options of sodium bicarbonate.

Also, Unni *et al.* (2022) found that no significant differences were observed in the values of serum biochemical parameters with cattle receiving oral rumen buffer compared with of the control. The value of serum glucose was higher (P<0.05) in T1 than in other treatment groups, with no significant differences observed among T2, T3, and T4 groups. The fixed value of glucose of T2, T3 and T4 may be explained by the adaptation of calves to antacids in the experimental diet (Rossi *et al.*, 2019). These results conflict with that reported by Karatzia *et al.* (2012) who stated that seaweed supplementation at 80 g/cow per day markedly increased blood glucose.

Table (3): Effect of supplements antacids to calves Holstein on some blood plasma constituents.

Items	Treatment				Sig.
	T1	T2	T3	T4	
Total protein (g/dl)	7.75 ± 0.06	7.75±0.04	7.62 ± 0.02	7.8 ± 0.04	NS
Albumin (g/dl)	2.80 <sup>b</sup> ± 0.04	2.92 <sup>b</sup> ± 0.63	3.15 <sup>a</sup> ± 0.029	2.92 <sup>b</sup> ± 0.06	**
Globulin (g/dl)	5.00 <sup>a</sup> ± 0.09	4.77 <sup>a</sup> ± 0.06	4.50 <sup>b</sup> ± 0.04	4.87 <sup>a</sup> ± 0.08	**
A/G	0.55 <sup>b</sup> ± 0.016	0.61 <sup>b</sup> ± 0.020	0.69 <sup>a</sup> ± 0.011	0.59 <sup>b</sup> ± 0.02	**
Glucose (g/dl)	91.00 <sup>a</sup> ± 5.656	76.75 <sup>b</sup> ± 2.898	76.00 <sup>b</sup> ± 1.78	73.00 <sup>b</sup> ± 1.10	**
Cholesterol (mg/dl)	98.75 ± 12.52	86.00 ± 8.68	80.5 ± 5.838	103.25 ± 4.45	NS
ALT (U/L)	17.80 <sup>c</sup> ± 0.08	18.87 <sup>a</sup> ± 0.13	18.52 <sup>ab</sup> ± 0.184	18.12 <sup>bc</sup> ± 0.17	**
AST (U/L)	21.65 ± 0.47	22.37 ± 0.29	22.02 ± 0.29	22.2 ± 0.43	NS

<sup>abc</sup> Means of the same row in each item with different superscripts are significantly different (P<0.05). T1: calves received 1% sodium bicarbonate, T2: calves received 1% sodium bicarbonate plus 0.3 magnesium oxide, T3: calves received 1% seaweed, T4: received 1% seaweed plus 0.3% magnesium oxide. Sig. = Significant, NS = not significant, \*\* = highly significant.

### 3.3 Rumen fermentation

Supplementation of antacids to calves' diets did not have a significant effect on ruminal pH (Table 4). However, calves fed sodium bicarbonate supplement tended to lower rumen pH values than steers fed other treatments. Similar results were found by Cruywagen *et al.* (2007) and Calitz (2009) in dairy cows and Montañez-Valdez *et al.* (2012) in steers. The mechanism of CMA to increase rumen pH is probably the same as SB, which is an increase in water intake, dilution of acids, and reduced production of propionate in rumen (Melo and Moura 2009). In addition, Cruywagen *et al.* (2015) reported that CMA is sparingly soluble in water compared to SB which is highly soluble in water, and its effects are shorter than SB. This may have also contributed to the improved pH profile in the CMA and CMA with magnesium oxide treatments compared to the SB treatment. Moreover, Erdman *et al.* (1982) reported that MgO supplemented with the diet of lactating dairy cows at

0.8% DM was the most effective treatment for increasing rumen pH compared to the control. The addition of dietary antacids to calves' rations does not affect on ruminal Ammonia nitrogen. Similarly, Farghaly *et al.* (2019) stated that supplementation of sodium bicarbonate to rams diets do not effect on rumen ammonia concentration. Also, Harrison and McAllan (1980) reported that sodium bicarbonate improved the efficiency of the synthesis of microbial protein from ammonia and reduced the level of rumen ammonia through an increased rate of absorption. The ruminal TVFAs was increased (P<0.05) with the addition of calcareous marine algae at a level of 1 % of the concentrate diet (T3) as compared with T1 and T4. However, no significant effect between T3 and T2 or T2 and T4. The difference in total VFA concentration among treatments may be due to differences in rumen microbial populations (Neville, 2022). Erdman (1988) reported that rumen buffering decreases the existence of acidity produced by volatile fatty acids base

status. Cruywagen *et al.* (2015) found that pH, acetate production and total VFA CMA had a positive influence on rumen production in TMR-fed dairy cows.

Table (4): Effect of antacids on rumen liquid parameters of fattening calves.

Items	Treatment				Sig.
	T1	T2	T3	T4	
pH	5.90± 0.04	6.15± 0.05	6.25± 0.12	6.13 ± 0.19	NS
NH <sub>3</sub> (mg/100 ml R.L)	1905.25 ± 24.93	1920.50 ±20.32	1951.25 ± 12.28	1936.00 ± 37.13	NS
TVFAs (meq/100 ml/ R. L)	5.75 <sup>b</sup> ± 0.37	7.15 <sup>ab</sup> ± 0.30	8.59 <sup>a</sup> ± 0.75	6.41 <sup>b</sup> ± 0.06	*

<sup>a,b</sup> Means of the same row in each item with different superscripts are significantly different (P<0.05). T1: calves received 1% sodium bicarbonate, T2: calves received 1% sodium bicarbonate plus 0.3 magnesium oxide, T3: calves received 1% seaweed, T4: received 1% seaweed plus 0.3% magnesium oxide. Sig. = Significant, NS = not significant, \*\* = highly significant.

### 3.5 Economic efficiency

The results in Table (5) show that the supplements of sodium bicarbonate to calves’ rations tend to decrease the cost of feed consumption during the experiment as compared with other

groups diets. However, the net revenue was markedly improved for calves received T1 and T4 diets. The improved net revenue and economic efficiency of T1 and T4 may be due to the tendency to increase body weight gain for these groups.

Table (5): Effect of antacids on rumen liquid parameters of fattening calves.

Items	Treatment			
	T1	T2	T3	T4
No of animals	5	5	5	5
Duration (days)	120	120	120	120
Total gain (kg)	179.27	154.32	166.8	184.08
Price of 1kg gain	62	62	62	62
Total Feed intake (kg)	1131.44	1077.77	1172.78	1188.116
Price of ration, L.E/kg	7.22	6.9	6.91	6.94
Total feed cost, L. E	6326	5936.18	6401.97	6569.93
Price of total gain, L. E	11114.74	9567.84	10341.6	11412.96
Net revenue, L. E	4788.74	3631.66	3939.63	4843.03
Economic efficiency %	75.7	61.2	61.5	73.7

## 4. Conclusion

The supplement sodium bicarbonate and seaweed to calves’ diets improve some blood parameters and rumen fermentation particularly when mixed with MgO.

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