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REGULAR ARTICLES

Comparison of the effect of *Saccharomyces cerevisiae–Megasphaera elsdenii* and buffer on growth performance, digestibility, ruminal histomorphometry, and carcass characteristics of fattening lambs in high concentrate diet

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Abstract

This study aimed to investigate the effect of rumen pH-adjusting additives in the high-concentrated diet on functional traits, nutrient digestion, some meat parameters, and histomorphometry, and rumen histopathology. Twenty-four Arabia male lambs with 3 to 4 months old and initial body weight of 23.9 ± 3.15 kg were used in a completely randomized design with three treatments and eight replicates. The study was 77 days, including 14 days of the adaptation period and 63 days of the record taking and sampling period. The experimental treatments consisted of a control diet, control diet + sodium bicarbonate buffer, control diet + *Megasphaera elsdenii*, and *Saccharomyces cerevisiae* (bacterial–yeast). Rumen fluid was taken by stomach tube at 3 h after morning feeding to measure pH. The lambs were weighed every 3 weeks during the period, and the body weight changes, average daily gain, and total weight gain were measured, and the feed conversion ratio was calculated. At the end of the experiment, the lambs were slaughtered, and the longissimus dorsi muscle was prepared to determine the meat parameters. For histological studies, the abdominal rumen sac was sampled. There were no differences among treatments in dry matter intake (DMI), daily weight gain (ADG), and feed conversion ratio ($P > 0.05$). Propionate concentration was higher in the bacteria–yeast treatment than other treatments ($P < 0.05$). Protein digestibility was higher in control and bacteria–yeast treatments than buffer treatment ($P < 0.05$). The percentage of meat protein, carcass weight, and dressing percentage in bacterial–yeast treatment was higher than other treatments ($P < 0.05$). Rumen wall thickness in the buffer and bacterial–yeast receiving treatments was greater than the control treatment and was significant in the buffer treatment compared to the control treatment ($P < 0.05$). The thickness of rumen epithelial tissue in the buffer and bacterial–yeast recipient treatments was less than the control treatment ($P < 0.05$). Rumen papillae thickness was higher in the control treatment than other treatments ($P < 0.05$). Hydropic degeneration and parakeratosis were less in pH-regulating treatments than in control. The results showed that the use of *Megasphaera elsdenii* could be an effective way to modulate the ruminal fermentation conditions of lambs fed with high concentrate diets. In addition, to increaseing dressing percentage and meat protein, it can also reduce tissue damage and improve ruminal tissue structure.

Keywords Acidosis · Meat · Papillae · *Saccharomyces cerevisiae* · VFA

Introduction

Subacute acidosis is characterized by reduced feed intake and livestock performance, which leads to significant economic losses. Therefore, preventive measures to prevent acidosis and improve starch digestion, such as antibiotics, probiotics, and buffers, have been considered. New evidence suggests that some yeast products may be an effective and economical alternative to traditional mineral buffers to modulate ruminal pH (Der Bedrosian 2009).

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Reports indicate that the consumption of live *Saccharomyces cerevisiae* yeast cells leads to the removal and consumption of oxygen in the rumen environment as well as the release of some essential enzymes, vitamins, other nutrients, and growth factors that these factors could significantly help to the suitable life and activity of microorganisms in the rumen environment (Ding et al. 2008; Calsamiglia et al. 2012).

It has been suggested that the *Saccharomyces cerevisiae* may develop the population of *Megasphaera elsdenii* and increase lactate use (khorasani et al. 2020; Calsamiglia et al. 2012). Therefore, their simultaneous use with bacterial species, including *M. elsdenii*, will enhance the action of these bacteria. On the other hand, the use of yeast and bioactive compounds in comparison with chemicals can effectively reduce inflammation caused by acidosis (Aschenbach et al. 2019).

M. elsdenii and *Selenomonas ruminantium* are the dominant strains that consume lactic acid in the rumen, and among these two strains, *M. elsdenii* consumes 65 to 95% of the lactate in the rumen. Therefore, by consuming lactic acid, *M. elsdenii* prevents the drastic reduction of rumen pH as a result of lactic acid accumulation (Prabhu et al 2012). Considering the diverse ability of different species of *M. elsdenii* to produce volatile fatty acids (VFA) in acidosis conditions, further research can help to find new species that used high lactate (Sedigh and Alipour 2019). Therefore, considering their compatibility with the rumen environment and their naturalness, and the possibility of enhancing their effect with the yeast of *Saccharomyces cerevisiae* compared to chemical buffers (Der Bedrosian 2009), their use should be given more attention.

By direct consumption of bacteria in the concentrated of beef calves (Elam et al. 2003), by voluntarily feeding *Aspergillus* to African goats (Belewu and Jimoh 2005), and by feeding the bacteria directly to heifers in high-concentrated diets (Huck et al. 2000), improvements in carcass weight have been reported. In most studies that have used microbial additives, the focus has been on rumen fermentation parameters (Geng et al. 2018), and its relationship to meat quality has not been investigated. One acceptable theory is that *M. elsdenii* causes more glucose to flow to cells in skeletal tissue in a high concentrate diet (DeClerck et al. 2020) and can affect meat properties.

Although many studies have been done to investigate of the effect of yeast on the quality of ruminant products, this subject has been more current in dairy cows, and according to these reports, yeast has a significant effect on milk fat and protein content (Poppy et al. 2012). In addition, it has been shown that the *Saccharomyces cerevisiae* can affect fat and protein metabolism in ruminants and have the most significant impact on product quality (Geng et al. 2016).

Consumption of starch and its fermentation in the rumen by producing VFA can cause the growth and development of ruminal tissue structure (Krause and Oetzel 2006). Although more production of VFA increases their uptake from ruminal villi, the sudden accumulation of VFA in the rumen leads to a decrease in ruminal pH and tissue changes (Zitnan et al. 2003; Garcia Diaz et al. 2018). In general, uptake from the rumen depends on its morphological structure and changes in morphology lead to dysfunction (Zitnan et al. 2003).

The use of sodium bicarbonate in livestock diets eliminates or reduces the damage caused by acute and subacute ruminal acidosis in various ways (Erdman et al. 1980). Therefore, using an available substance such as chemical buffers (e.g., sodium bicarbonate) or biological materials (e.g., bacteria that consume acid), while maintaining a healthy rumen, could provide a suitable environment for the activity of rumen microorganisms. In addition, weight gain costs and feed conversion ratio are improved and, on the other hand, prevent further problems such as lameness or problems that may not be identified until slaughter (Aschenbach et al. 2019).

Therefore, this study aimed to compare the effect of sodium bicarbonate and bacteria consuming lactic acid (*M.* elsdenii) + *Saccharomyces cerevisiae* on growth performance, nutrient digestibility, ruminal histomorphometry, and carcass characteristics in high concentrate diets, which were performed on Arabia lambs.

Materials and methods

Animal management

Twenty-four Arabi males (4 ± 1 months old and 23.9 ± 3.15 kg initial body weight) were used. The study was 77 days, including 14 days of the adaptation period and 63 days of the record taking and sampling period. Before starting the study, all lambs were vaccinated against external parasites (1 mL of Azantol 10% per 7 L of water, as spraying method; Bayer, Germany) and internal parasites (triclabendazole + levamisole 12 mL per each lamb; Darou-Pakhsh Co, Iran) and vaccinated against enterotoxaemia (3 mL per each lamb, Razi Vaccine and Serum Research Institute, Iran).

Lambs were maintained in individual feeding pens (1.4 × 1.2 m). Livestock was randomly assigned to one of three treatments, including (1) control diet (no additives), (2) control diet + sodium bicarbonate (1% daily diet in two meals), and (3) control diet + *M. elsdenii* and *Saccharomyces cerevisiae* (yeast – bacteria).

Experimental design

M. elsdenii 3 mg per animal (4.5×10^8 CFU/ml) plus 2 g of *Saccharomyces cerevisiae* was fed to the animals every morning, as direct-fed microbes (DFM) (Miles and Bootwalla 1991; Sedigh and Alipour 2019). The feeding and breeding conditions of the selected lambs were the same before the experiment. The constituent nutrients and chemical composition of the experimental diets are presented in Table 1. Lambs were fed ad libitum a total mixed ration (TMR) feed with a forage:concentrate (F:C) ratio of 30:70 twice per day in the morning (08:00) and afternoon (16:00) with free access to water. The diet was balanced using nutrient requirements of small ruminant standard tables (NRC 2007).

Sodium bicarbonate used in the present study was prepared from Kimia Sepahan Company (Isfahan-Iran), and *Saccharomyces cerevisiae* yeast (7×10^9 CFU/g) from Khuzestan Yeast Dough Company (Dezful-Iran). *M. elsdenii* bacteria (1.5×10^8 CFU/ml) were isolated from Najdi goat at Agricultural Sciences and Natural Resources University of Khuzestan (Malasani-Ahvaz-Iran).

For culture and isolation of *M. elsdenii*, ruminal fluid obtained from Najdi goats was filtered with four layers of cheesecloth. Rumen fluid was diluted with anaerobic dilution solution (ADS) to a dilution of 10^{-9} and added to the culture medium inside the hangite tubes, and then the tubes were incubated at 39 °C until bacterial growth. After observing the colony of bacteria, they were transferred to solid culture medium containing agar, and after rolling on ice and uniformity, the tubes were transferred to incubator again at 39 °C; after the growth of colonies, which usually occurs after 3 to 7 days, individual colonies were transferred to the liquid culture medium. Finally, the purified isolates were transferred to an anaerobic and sterilized glycerol solution for storage and stored in a − 20 °C freezer (Mohammadabadi et al. 2018).

Table 1 Feed ingredients and chemical composition of the experimental basal diet fed to lambs

Ingredients	Amounts (g/kg DM)
Alfalfa hay	201
Wheat straw	99
Barley grain	300
Corn grain	210
Soybeans meal	123.5
Wheat bran	55
Calcium carbonate	4
Salt	2.5
Vitamin and mineral supplements[a]	5
Chemical composition	
Dry matter	891
Ash	51.7
Crude protein	161
Ether extract	27
NDFom[b]	290
ADFom[c]	165
Non-fiber carbohydrates[d]	472

[a]Premix contained (per kg): vitamin A, 500,000 IU/mg; vitamin D_3, 100,000 IU/mg; vitamin E, 100 mg/kg; Ca, 180 g/kg; P, 60,000 mg/kg; Na, 60,000 mg/kg; Mg, 19,000 mg/kg; Zn, 3000 mg/kg; Fe, 3000 mg/kg; Mn, 19,000 mg/kg; Cu, 300 mg/kg; Co, 100 mg/kg; Se, 1 mg/kg; I, 100 mg/kg; antioxidant, 400 mg/kg; carrier, up to 1000 g

[b]NDFom, ash-free neutral detergent fiber

[c]ADFom, ash-free acid detergent fiber

[d]Calculated as NFC = 1000 − (NDFom g/kg + crude protein g/kg + ether extract g/kg + ash g/kg)

Growth performance

During the experimental period, the daily feed consumption and orts of feeds were recorded. The lambs were weighed every 3 weeks during the period, before feeding at least after 14 h of starvation. The body weight changes, average daily gain (ADG), and total weight gain were measured, and the feed conversion ratio was calculated.

Nutrient digestibility

In the last 7 days of the experiment (on days 57 to 63), fecal and the orts of feed of lambs were collected, weighed, and 10% of it sampled. At the end of this course (on day 63), fecal and the orts of feed of each lamb were pooled, and one representative sample was taken and stored in a cold room (− 20 °C) for analyzing the chemical composition. The apparent digestibility of nutrients was calculated by the difference between the amounts of nutrients consumed and excreted.

Chemical analysis

To measure the chemical composition of the feeds, sorts, and feces, they were dried at 60 °C for 48 h and grounded using a mill with a sieve of 1 mm. All samples were analyzed according to the standard analytical procedures for dry matter (DM) (method 967.03), ash (method 923.03), crude protein (CP) (Kjeldahl N × 6.25, method 2001.11), ether extract (EE) (method 920.85), and acid detergent fiber by ash removal (method 973.18) (ADFom) (AOAC 2016). Neutral detergent fiber (NDFom) was analyzed without alpha-amylase (Van Soest et al. 1991).

Rumen pH measurement

On day 42 of the experiment, rumen fluid was collected from each lamb via a stomach tube 3 h after the morning feeding, and pH was measured immediately using a glass electrode pH-meter (Model 3110, WTW, Germany); the samples were filtered with four layers of cheesecloth.

Volatile fatty acid concentrations in rumen fluid

On the last day of the experiment, ruminal fluid was taken to measure the concentration of VFA. About 5 ml of ruminal fluid was acidified with 2 ml of meta-phosphoric acid 25% (w/v), and then preserved at −20°C until analysis. The concentration of VFAs was measured by gas chromatography (GC device, model YL6100, manufactured by Young Lin, South Korea) using silicone capillary column (CP-Wax Chrompack Capillary Column; Varian, Palo Alto, CA, USA), helium gas as carriers, and the crotonic acid as an internal standard (Malekkhahi et al. 2015; Khorasani et al. 2020).

Slaughter and chemical composition of meat

At the end of the experiment, five lambs from each treatment closer to the mean weight were selected (for slaughter weight) after fasting with free access to water for 16 h and slaughtered, and carcasses were weighed (Eynipour et al. 2019; Karami 2018). To determine the chemical composition of meat, a sample of meat was kept in the oven (EMMERT-UF 450-Germany) at 60 °C for 24 h; and then the DM was calculated, and from the difference between the moisture content of the meat, also the CP (Kjeldahl N × 6.25, method 990.03; FOSS 2033—Sweden) was measured (AOAC 2006).

Histomorphometry and histology of rumen

To evaluate the rumen histomorphometry of slaughtered lambs, after digesta removal and rumen lavage with distilled water, a fragment of approximately 1cm^2 was collected from the ventral sac region of the rumen. Ruminal tissue samples were washed in phosphate buffer solution (0.1 M, pH 7.4) and fixed in 10% buffered formalin solution. Each sample was placed separately in sealed containers containing 10% formalin (Garcia Diaz et al. 2018). After 24 h, the formalin of the containers was replaced, and the samples were transferred to the Isfahan-Iran veterinary pathology center for histomorphometric and histological changes.

In the laboratory, each sample was embedded in paraffin wax and finally cut into transverse sections at 5 µm. The 5 mµ sections were made with the paraffin embedding method and stained with hematoxylin–eosin (Wang et al. 2009). Tissue changes were examined with a Nikon microscope (model NS 100 made in Japan). The studied indices were measured at different magnifications with calibrated lenses. In each group, three samples and from each sample and five tissue sections and in each tissue section, at least four microscopic fields were counted and measured. Micrometric studies including villi height, thickness, and depth and histomorphometric studies such as epithelial tissue thickness, muscle layer, and total ruminal wall thickness were performed on the samples.

Statistical analysis

Data were analyzed as a completely random design using SAS 9.4 software (SAS (Statistical Analysis Systems Institute Inc.) 2017). The statistical model used was $Yij = \mu + Ti + \varepsilon\ (i)j$, in which Yij = experimental response measured on the treatment i and repetition j; μ = population mean; Ti = treatment effect of treatment i; and $\varepsilon\ (i)j$ = residual error. Duncan's test at the 5% probability level was used for comparisons of means. Significant differences were accepted if $P \leq 0.05$.

Results

Growth performance

There was no significant difference between the experimental groups for dry matter intake (DMI), final body weight, average daily gain (ADG), and feed conversion ratio (Table 2).

Table 2 Comparison of performance characteristics in lambs fed with the experimental diets

Variables	Treatments				
	Control	Buffer	Me + Sc	SME	*P* value
Dry matter intake (g/d)	1020.01	1035.90	1043.25	16.77	0.58
Initial body weight, Kg	24.33	23.60	23.53	0.65	0.87
Final body weight, Kg	37.30	37.10	37.18	0.67	0.98
Body weight gain, kg	12.96	13.49	13.66	0.31	0.59
Average daily gain (g)	207.08	214.72	218.67	4.35	0.61
Feed conversion ratio	4.93	4.81	4.78	0.13	0.68

Control = without any supplementation, buffer = sodium bicarbonate supplementation, Me + Sc = *Megasphaera elsdenii* + *Saccharomyces cerevisiae*

Means with different superscripts in the same row differ significantly ($P < 0.05$). *SEM* standard error of means

Nutrient digestibility

The feed intake and apparent digestibility of CP, DM, NDF, and ADF were not affected by experimental treatments (Table 3). Crude protein digestibility was the highest in yeast–bacteria recipient treatment, but this difference was only significant compared to buffer treatment ($P < 0.05$).

Meat composition and carcass characteristics

The chemical composition of the *longissimus dorsi* muscle of lambs is shown in Table 4. The percentage of protein in the meat sample of yeast–bacteria treatment was higher than other treatments ($P < 0.05$). The effect of treatments on carcass characteristics of fattening lambs is shown in Table 5. Hot carcass weight and carcass yield in bacterial–yeast treatment were higher than other treatments, and carcass drop was less ($P < 0.05$).

Histomorphometry and histopathology of rumen

The effect of experimental treatments on the histomorphometric structure of the rumen is shown in Table 6 and Fig. 1. Rumen wall thickness was higher in the buffer and bacterial–yeast treatments than the control treatment and was significant in the buffer treatment compared to the control treatment ($P < 0.05$). The thickness of ruminal epithelial tissue in buffer and bacterial–yeast treatments was less than the control treatment ($P < 0.05$). Rumen villi thickness was higher in the control treatment than other treatments ($P < 0.05$). There was no significant difference between other rumen indices ($P > 0.05$). Rumen villi height was not affected by experimental treatments; but the height of ruminal villi in the control treatment was numerically higher than the buffer and bacterial–yeast treatments ($P < 0.05$).

Table 3 Nutrient digestibility in fattening lambs fed with the experimental diets

Variables	Treatments			SME	*P* value
	Control	Buffer	Me + Sc		
Total apparent digestibility, g/kg					
Dry matter	732.8	712.9	731.0	7.9	0.62
Crude protein	789.3^{a}	743.9^{b}	798.6^{a}	8.0	0.01
Neutral detergent fiber	541.7	468.9	515.8	15.1	0.24
Acide detergent fiber	512.2	501.9	518.9	17.4	0.91

Control = without any supplementation, buffer = sodium bicarbonate supplementation, Me + Sc = *Megasphaera elsdenii* + *Saccharomyces cerevisiae*

Means with different superscripts in the same row differ significantly ($P < 0.05$). *SEM* standard error of means

Table 4 Meat composition in fattening lambs fed with the experimental diets

Variables	Treatments			SME	*P* value
	Control	Buffer	Me + Sc		
Moisture (%)	70.501	69.325	71.998	1.211	0.6128
Dry matter (%)	29.021	29.998	28.100	1.154	0.6201
Protein (%)	16.7700^{b}	16.970^{b}	17.9200^{a}	0.188	0.0012

Control = without any supplementation, buffer = sodium bicarbonate supplementation, Me + Sc = *Megasphaera elsdenii* + *Saccharomyces cerevisiae*

Means with different superscripts in the same row differ significantly ($P < 0.05$). *SEM* standard error of means

Discussion

Growth performance

No significant difference in DMI was observed by fattening calves with consumption of *M. elsdenii* in the highly concentrated diet (Drouillard et al. 2012), which is consistent with the results of the present experiment. Some researchers have reported that consumption of *Saccharomyces cerevisiae* increases feeds intake (AlZahal et al. 2014), while reducing the daily feed of cows with consumption of this yeast has also been reported (Kung et al. 1997).

Some studies have shown that the addition of sodium bicarbonate increases DMI (Kawas et al. 2007), and others have reported no effect on DMI (Tripathi et al. 2004). No significant difference was observed between experimental treatments for daily weight gain and feed conversion ratio. In Baluchi lambs fed diets containing high concentrate, *Saccharomyces cerevisiae* had no significant effect on feed conversion ratio and daily weight gain (Malekkhahi et al.

Table 5 Effect of treatments on carcass characteristics in fattening male lambs fed with the experimental diets

Characteristic	Treatments			s.m.e	*P* value
	Control	Buffer	Me + Sc		
Initial body weight, kg	23.51	22.95	24.52	0.56	0.51
Final body weight, kg	35.85	36.53	37.97	0.516	0.22
Carcass weight (kg)	17.09^{b}	17.051^{b}	19.05^{a}	0.34	0.007
Dressing (%)	47.61^{b}	46.75^{b}	50.21^{a}	0.65	0.035
Drop carcass (%)	52.32^{a}	53.25^{b}	49.80^{b}	0.34	0.038

Control = without any supplementation, buffer = sodium bicarbonate supplementation, Me + Sc = *Megasphaera elsdenii* + *Saccharomyces cerevisiae*

Means with different superscripts in the same row differ significantly ($P < 0.05$). *SME* standard error of means

Table 6 Histomorphometric rumen in lambs fed with diets

Characteristic (μm)	Treatments			SME	*P* value
	Control	Buffer	Me + Sc		
Wall thickness	2069.8[b]	2314.5[a]	2247.2[ab]	48.38	0.11
Thichness of tunica muscularis	1627.4	1802.9	1541.2	62.34	0.21
Thickness epithelial tissue	186.2[a]	143.8[b]	114.9[b]	62.29	0.23
Papillae thickness	515.85[a]	419.61[b]	339.70[c]	23.12	0.0007
Papillae height	2745.3	2312.6	2298.8	122.35	0.21

Control = without any supplementation, Buffer = sodium bicarbonate supplementation, Me + Sc = *Megasphaera elsdenii* + *Saccharomyces cerevisiae*

Means with different superscripts in the same row differ significantly ($P < 0.05$). *SME* standard error of means

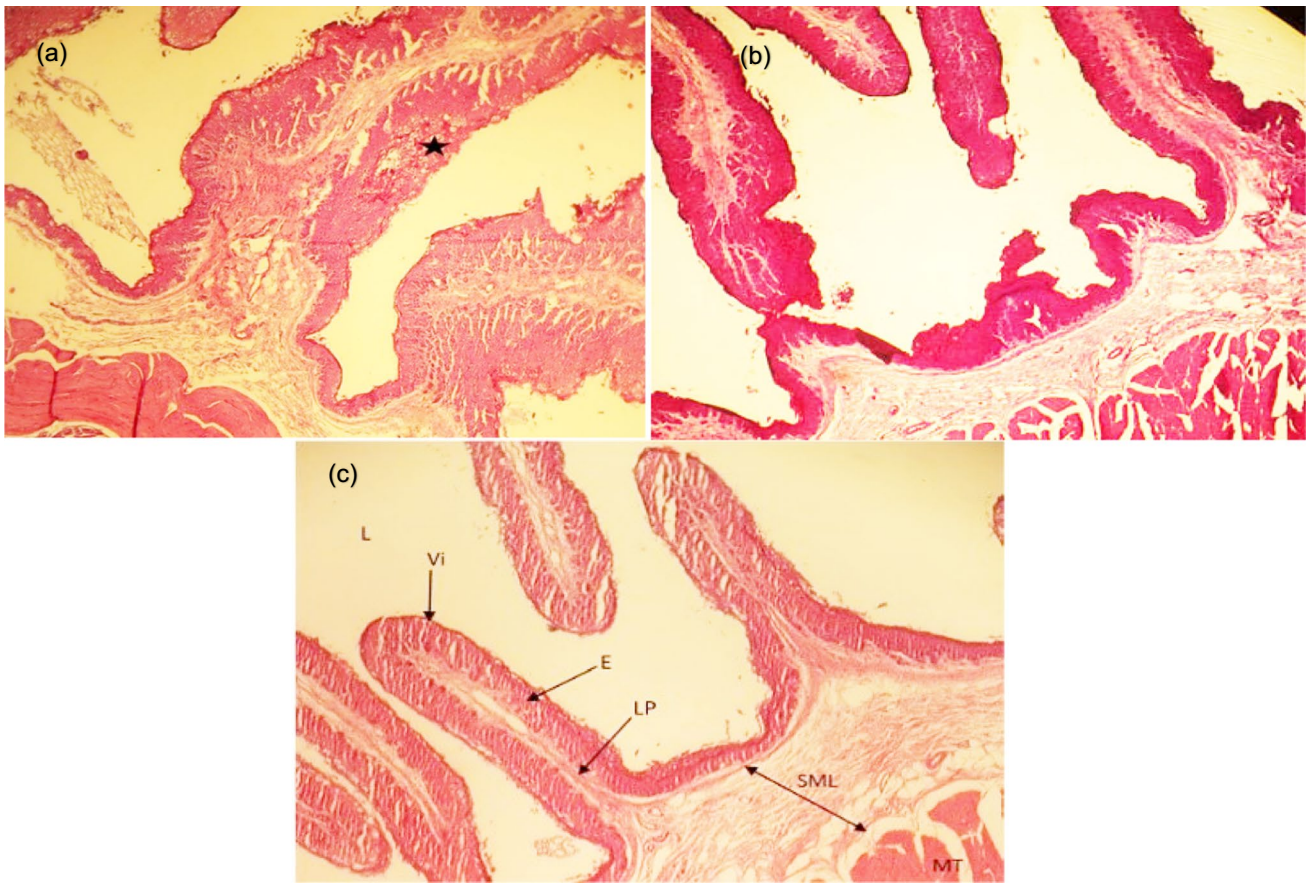

Fig. 1 Histopathology of rumen. **a** Control treatment, **b** buffer recipient treatment, **c** bacterial–yeast recipient treatment. Hydropic degeneration (star). Histological structure of the rumen (**c**): L, lumen; Vi, villi; E, epithelium; LP, lamina propria; SML, submucosal layer; MT, muscular tunica. Hematoxylin–eosin staining 100×

2015), in Awassi lambs and Shami goat kids. Similar results were also seen (Titi et al. 2008), which is consistent with the results of the present experiment. Agreed to the results of the present experiment, no significant difference was observed in feed conversion ratio and daily weight gain in fattening calves with consumption of *M. elsdenii* in high concentrate diets (Drouillard et al. 2012).

The use of different levels of sodium bicarbonate (0, 0.75, 1.5, 2.5 percentage) in the high-concentrated diet of lambs, no significant difference was observed between the experimental groups in feed conversion ratio. Also, daily weight gain in the groups receiving 0.75 and 2.5% did not significantly differ from the control (Tripathi et al. 2004), which is consistent with the results of the present experiment.

Nutrient digestibility

The mode of action of yeast is primarily to alter ruminal fermentation and may not affect the digestibility of all components (Chung et al. 2011). On the other hand, compensatory digestion in the post-ruminal parts may obscure the digestive effects of yeast in the rumen. For example, ruminal digestion of organic matter and crude protein was reported to be improved by yeast, but apparent digestion of organic matter and crude protein was similar throughout the gastrointestinal tract (Yoon and Stern 1996). Dry matter digestibility was not affected by the addition of sodium bicarbonate and yeast to the feed lambs (Kawas et al. 2007), which is consistent with the findings of the present experiment.

Meat composition and carcass characteristics

Bacteria that consume lactic acid, such as *Megasphaera elsdenii*, reduce the lactic acid concentration in the rumen and produce higher concentrations of propionate in the rumen (Table 8). These propionates can be converted to glucose through the Krebs cycle. In addition, lactic acid-consuming bacteria can reduce methane production and increase feed efficiency, so direct feeding of livestock with microorganisms can increase feed intake and weight gain (Khorasani et al. 2020; Kalebich and Cardoso 2017) (Table 2). Studies have shown that cows fed DFM excrete fewer bacteria in the feces, which results in a better use of dietary nitrogen, greater efficiency, and less protein excretion in the feces (Kalebich and Cardoso 2017). Therefore, the increased concentration of meat protein in yeast–bacteria treatment in the present experiment can be considered the result of more microbial protein synthesis and more good use of ruminal ammonia nitrogen.

Research on the use of DFM and its effect on meat compositions is scarce, and most of them are related to their effect on milk composition. In an experiment on dairy cows using *Saccharomyces cerevisiae* as DFM, milk protein was significantly higher than the control treatment (Nocek et al. 2003). On the other hand, feeding the yeast of *Saccharomyces cerevisiae* to fattening cows (Geng et al. 2016) or sodium bicarbonate to fattening lambs (Bodas et al. 2007) fed a concentrated diet did not affect the chemical composition of the meat (DM and CP), except for protein, which was consistent with the results of the present experiment. The reason for this lack of difference in chemical composition may be the similarity in consumption of energy to protein ratio among treatments (Bodas et al. 2007; Geng et al. 2016).

Improving carcass weight and yield in bacterial–yeast treatment in the present experiment can be attributed to the presence of probiotics (yeast–bacteria in the present experiment) that inhibit harmful microbes and improve fiber digestion and, in turn, increase weight (Inyang and Udoh 2019).

Table 7 Rumen tissue changes in fattening lambs fed experimental diets

Characteristic	Treatments		
	Control	Buffer	Me + Sc
Hydropic degeneration	+ + +	+ +	+
Parakeratosis	+ + +	+ +	+

Do not see tissue changes (natural structure), + mild damage, + + moderate damage, + + + severe damage

Table 8 Rumen pH and concentration of volatile fatty acids in fattening lambs fed experimental diets

Characteristic	Treatments			SME	*P* value
	Control	Buffer	Me + Sc		
pH	6.13	6.24	6.11	0.039	0.87
Individual VFA, mmol					
Acetate (A)	51.24^{b}	57.98^{b}	68.95^{a}	3.25	0.01
Propionate (P)	29.04^{b}	27.15^{b}	56.18^{a}	5.19	0.0003
Butyrate (B)	20.05^{a}	15.98^{ab}	8.99^{b}	2.2	0.16

SME standard error of means; *VFA* volatile fatty acid; control = without any supplementation, buffer = sodium bicarbonate supplementation, Me + Sc = *Megasphaera elsdenii* + *Saccharomyces cerevisiae*

Means with different superscripts in the same row differ significantly ($P < 0.05$)

Numerically increased carcass weight was reported in fattening calves by feeding *Megasphaera elsdenii* bacteria in a high concentrate diet compared to the control (DeClerck et al. 2020). By adding some probiotic bacteria (*Lactobacillus* and *Propionibacterium*) directly to the diets of fattening calves (Elam et al. 2003), fattening heifers (Huck et al. 2000), or *Aspergillus* to the diets of African goats (Belewu and Jimoh 2005) in diets with high concentrate, weight improvement and carcass yield were reported. On the other hand, using a commercial probiotic in the high concentrate diet of goats did not cause any change in the weight and ratio of carcass components (Whitley et al. 2009).

Very few studies have investigated the effect of yeast nutrition on carcass traits (Ran et al. 2018). *Saccharomyces cerevisiae* supplement and a plant mixture in the high-concentrated diet of beef cattle improved carcass yield (Mahyuddin and Winugroho 2010), which is consistent with the results of bacterial–yeast treatment in the present experiment. No effects on hot carcass weight, carcass yield, and other carcass components were reported when using *Saccharomyces cerevisiae* in calves fed with high concentrate diets (Ran et al. 2018).

Histomorphometry and histopathology of rumen

In the present experiment, ruminal epithelial thickness and villi thickness increased in the control treatment; excessive production of VFA in high concentrate diets leads to uncontrolled growth of epithelial cells of the epithelial tissue and ultimately to parakeratosis at the surface of the ruminal wall (Table 7 and Fig. 1). Parakeratosis causes a physical barrier against the dangers of acid, reducing the level of effective absorption, reducing epithelial blood flow, and reducing ruminal movements (Khan et al. 2016). Therefore, in the present experiment, the increase in the thickness of ruminal epithelial, and the villi in the control treatment, can be considered as the result of increasing the VFA and decreasing the pH (Table 8), and keratinization and stratum of ruminal epithelial tissue, which is consistent with the results of other experiments by researchers on high-concentrated diets (Steele et al. 2011).

However, in treatments receiving pH adjusters, especially bacterial–yeast treatment due to higher production of acetate (Table 8), which is the result of more activity of cellulolytic bacteria (Malekkhahi et al. 2016), also compared to the control treatment, a significant reduction of butyrate was observed (Table 8), which play a significant role in the longitudinal growth of villi in acidosis condition (Niwińska et al. 2017), so the villi thickness and the thickness of the epithelial tissue in the rumen were significantly reduced.

With the consumption of high concentrate in lambs, an increase in the height and density of ruminal villi was reported (Odongo et al. 2006). Butyrate and propionate both increase mitotic activity in villi, but the spread of these activities is more robust in butyrate (Niwińska et al. 2017). In general, the size, surface, and other properties of villi are mainly affected by VFA, especially propionic, and butyric, and pH (Shen et al. 2004).

Increasing the height and the level of the villi increases the absorption capacity and, in turn, protects the animal from the accumulation of VFA in the rumen of those animals consuming a large amount of dense material. Therefore, in these conditions, the ability of ruminal epithelium to absorb VFA faster helps stabilize ruminal pH. When VFA is produced beyond the absorption capacity of the rumen villi, VFA accumulates in the rumen, thereby lowering the rumen pH and causing ruminal acidosis (wang et al. 2009; Krause and Oetzel 2006). Therefore, considering that one of the reasons for increasing the villi height, thickness, or surface in acidosis conditions is to increase the absorption capacity of volatile fatty acids to maintain ruminal pH stability (Mashayekhi et al. 2020), reducing these indices in treatments with buffer and bacterial–yeast is the result of a sudden decrease in the accumulation of volatile fatty acids, significantly a decrease in the concentration of butyrate (Table 8), which plays a vital role in the development of acidosis.

In treatments containing pH regulators, in fact, due to the relative control of acidosis, this amount of increase is not required, and the decrease in the amount of butyrate and increased acetate secretion (Table 8) due to the higher activity of cellulolytic bacteria confirms this (Malekkhahi et al. 2016).

Conclusions

In general, the results of the present experiment showed that the use of *Megasphaera elsdenii* bacteria as a consumer of ruminal acid in comparison with the sodium bicarbonate chemical buffer could be an effective way to modulate the ruminal fermentation conditions of lambs fed with concentrates. In addition, to increasing dressing percentage and meat protein, it can also reduce tissue damage and improve ruminal tissue structure, which is helpful for health and increase the economic life of livestock.

Author contribution M and O conceived and designed the research. O and M conducted the experiments. O analyzed the data. O wrote the manuscript. M and F provided expertise and revised the manuscript. All authors read and approved the manuscript.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical approval The manuscript does not include clinical studies or patient data.

Consent to participate The manuscript did not involve human subjects or human transplantation studies, and no organs/tissues were obtained from the prisoners.

Consent for publication This manuscript did not contain any individual person's data in any form (including any individual details, images, or videos).

Conflict of interest The authors declare no competing interests.

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