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The effect of *Saccharomyces cerevisiae* on in vitro growth and fermentation of *Selenomonas ruminantium* and *Megasphaera elsdenii**

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Stimulation of lactate utilization by *Selenomonas ruminantium* and *Megasphaera elsdenii* may help in reducing problems associated with rumen acidosis. The objective of this study was to determine the effect of a *Saccharomyces cerevisiae* live culture and *Saccharomyces cerevisiae* fermentation products on in vitro growth and fermentation of lactate-utilizing ruminal bacteria, *S. ruminantium* (ATCC 19205) and *M. elsdenii* (ATCC 25940). The cultures were run for 0, 6, 12, 24 and 48 h under anaerobic conditions on a growth medium supplemented with a yeast live culture (SC) or with yeast fermentation products (SCFP) and, as reference, on the same medium without supplementation (CON). Neither SC nor SCFP had a significant effect on the growth of *S. ruminantium* after 6, 12 and 24 h of incubation, but the live yeast culture significantly (P≤0.05) improved the growth of these bacteria after 48 h of incubation. The yeast fermentation products significantly (P≤0.05) decreased pH and increased lactate synthesis by *S. ruminantium.* The *Saccharomyces cerevisiae* live culture significantly improved the growth of *M. elsdenii* after 12 and 24 h of incubation, and the *S. cerevisiae* fermentation products increased its growth after 48 h. The After 24 and 48 h of incubation the *Saccharomyces cerevisiae* live culture reduced the concentration of total volatile fatty acids (VFA), while caproate was the main product of in vitro fermentation of *M. elsdenii* (P≤0.05). *Saccharomyces cerevisiae* live cultures may improve microbial fibre fermentation in the rumen by maintaining optimal pH conditions.

KEY WORDS: *Saccharomyces cerevisiae* / live yeast culture / *Megasphaera elsdenii* / *Selenomonas ruminantium* / bacterial growth / fermentation / in vitro

Research results indicate that *Saccharomyces cerevisiae* yeast exert a beneficial effect on changes occurring in the rumen and nutrient digestion [5, 15], control ruminal pH [4], and reduce the risk of metabolic disease or fertility disorders [10]. Some in vitro

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studies have indicated that *S. cerevisiae* stimulates the growth of lactate-utilizing bacteria such as *Megasphaera elsdenii* and *Selenomonas ruminantium*, thereby reducing lactate concentrations [7]. Pinloche et al. [31] reported increased concentrations of propionate and butyrate in the rumen fluid in response to administration of yeast, and other studies have shown that yeasts increase the proportions of propionate [13], acetate [1] and butyrate [36]. Lynch and Martin [21], in an in vitro study of microbes from various ruminants, found that active cultures of *Saccharomyces cerevisiae* (SC) decreased lactate concentrations. *M. elsdenii* and *S. ruminantium* are common anaeraobic ruminal bacteria which may increase ruminal pH by fermenting lactate and reducing problems associated with rumen acidosis [25]. *M. elsdenii* is a Gram-negative, obligate anaerobic coccus that ferments soluble sugars and lactate [30]. According to Counotte et al. [9], *M. elsdenii* is a species that predominantly utilizes lactate and is capable of fermenting up to 97% of rumen lactate. In in vitro cultures, lactate utilization by *M. elsdenii* was greater than in the case of *S. ruminantium* [3]. *Saccharomyces cerevisiae* fermentation products (SCFP) have been shown to improve *M. elsdenii* and *S. ruminantium* growth and fermentation, but their mode of action is not fully understood [6, 18]. Live yeast cultures can survive in the rumen for a short time by utilizing traces of dissolved oxygen, creating a beneficial anaerobic environment for bacterial growth and contributing to breakdown of fibre [2]. The feed market offers several yeast products, differing slightly in their manufacturing process, but few studies have been conducted to compare yeast fermentation products under identical experimental conditions [26]. Recent years have seen increasing interest in comparing the effects of SC products and SCFP on ruminal fermentation.

The objective of the study was to determine the effect of *S. cerevisiae* live cultures and *S. cerevisiae* fermentation products on in vitro growth and fermentation of lactate-utilizing ruminal bacteria of the species *Megasphaera elsdenii* and *Selenomonas ruminantium.*

Materials and methods

Bacterial strains and growth conditions. Two strains of bacteria isolated from the rumen, *Selenomonas ruminantium* (ATCC 19205) and *Megasphaera elsdenii* (ATCC 25940), were used in the experiment. According to ATCC recommendations, *M. elsdenii* was cultured on Oxoid CM 149 medium and *S. ruminantium* on ATCC 602 E. The media were sterilized following removal of oxygen through the introduction of CO_2. Lyophilized bacteria were revived and then multiplied by repeated inoculation on specific media. Prior to the experiments, the growth media were inoculated with a specific bacterial strain and then CO_2 was introduced again in sterile conditions. The cultures were incubated in anaerobic conditions for 48 h in an atmosphere of 20% CO_2 and 75% N_2 at 37°C in a HEPA CLASS 100 Thermo Electron incubator.

Culture and tests. The inoculum of the microorganisms was prepared using growth media specific for the bacterial strains. The bacteria were cultured for 48 h at 37°C. The inoculum constituted 10% (v/v) of the culture medium. Two preparations were used in

the experiment – Biosaf SC 47, consisting of live cultures of *Saccharomyces cerevisiae* (5^9 log CFU/g), and Diamond V XP Mills, containing the media on which yeast were grown, the metabolites produced by yeast during the manufacturer's fermentation process, and dead yeast cells. The feed additives, i.e. 0.13 g of live yeast cultures (Biosaf SC 47) and 1 g of yeast metabolites (Diamond V XP Mills), were dissolved in 50 cm^3 of medium. After 10 min CO_2 was introduced and then 10 cm^3 of the solution was applied to the culture of each bacterial strain. The amount of the feed additives used was calculated based on the dosages recommended for dairy cows, taking into account average rumen capacity. *S. ruminantium* (ATCC 19205) and *M. elsdenii* (ATCC 25940) were cultured separately in 500 cm^3 Erlenmeyer flasks containing 130 cm^3 of the respective growth media with the addition of Biosaf SC 47 (SC group) and Diamond V XP Mills (SCFP group). A control culture without feed additives was conducted as well (CON group). To ensure anaerobic conditions, the flasks were plugged with cotton wool moistened with 10% pyrogallol solution and saturated sodium bicarbonate and sealed with parafilm, in two replicates per group. The cultures were grown at 37ºC for 48 h in a Mytron WT 120 incubator.

After 6, 12, 24 and 48 h of incubation, the supernatant was collected and the number of live bacterial cells, pH, and concentrations of volatile fatty acids (VFA), lactic acid and ammonia were determined in it. After each measurement, the same procedure as at the start of the culture was followed to ensure anaerobic conditions in the culture. The number of live yeast cultures during fermentation was determined by the plate count agar method. ATCC-recommended growth media with the addition of 1.5% agar were used. The plates were incubated in anaerobic conditions in a HEPA CLASS 100 Thermo Electron incubator for 48 h.

Analytical methods. Changes in the pH of the culture environment were measured using a CP 411 Elmetron electronic pH-meter with temperature compensation. VFA and lactic acid concentrations were measured by gas chromatography (Varian CP380) according to Jensen et al. [17]. Prior to separation in the gas chromatograph, 50 µL of the sample was incubated at 80°C for 30 min in a Thermolyne Dri Bath (Thermolyne, UK) in MTBSTFA (Fluka 19918) and with 2-ethylbutyric acid (Fluka 03190) as the internal standard. Ammonia-N was determined by the microdiffusion method [8].

The results presented are the means of the observations. Statistical analysis of the results was performed by one-way analysis of variance and the MEANS and GLM procedures of the SAS software package [34], using Duncan's test and significance levels of $P \leq 0.01$ and $P \leq 0.05$.

Results and discussion

Neither the active yeast (SC) nor the yeast fermentation products (SCFP) significantly affected ($P > 0.05$) the growth of *S. ruminantium* after 6, 12 or 24 h of incubation (Table 1, Fig. 1). After 48 h a statistically significant ($P \leq 0.05$) increase in the bacterial count was

Table 1

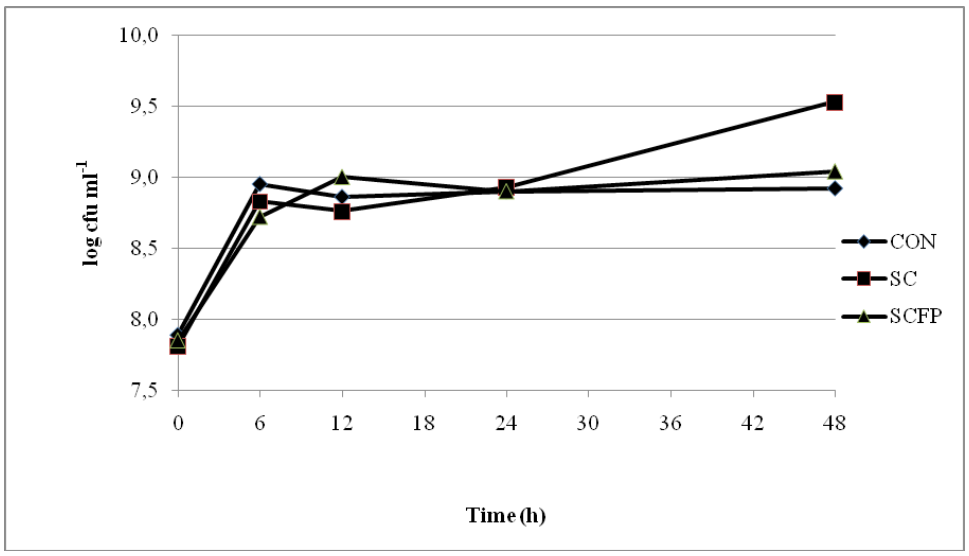
Effects of *Saccharomyces cerevisiae* live yeast culture (SC) and *Saccharomyces cerevisiae* fermentation products (SCFP) on in vitro growth and fermentation of *Selenomonas ruminantium*

Items	Incubation time (h)																			
	0			SEM	6			SEM	12			SEM	24			SEM	48			SEM
	CON	SC	SCFP		CON	SC	SCFP		CON	SC	SCFP		CON	SC	SCFP		CON	SC	SCFP	
1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**
Bacteria (log cfu ml^{-1})	7.89	7.81	7.85	0.02	8.95	8.83	8.72	0.09	8.86	8.76	9.00	0.12	8.90	8.93	8.90	0.02	8.92^{A}	9.53^{B}	9.04^{A}	0.11
pH	6.88	6.94	6.89	0.02	5.91^{A}	5.84^{B}	5.71^{C}	0.04	5.89^{A}	5.84^{B}	5.55^{C}	0.07	5.98^{A}	5.87^{B}	5.59^{C}	0.07	5.99^{A}	5.94^{B}	5.61^{A}	0.08
Ammonia (mmol l^{-1})	9.74	9.79	10.23	0.32	10.59	10.24	9.75	0.30	10.37	10.33	11.14	0.24	10.67	10.96	10.19	0.29	10.11	10.22	10.20	0.21
Total VFA (mmol l^{-1})	15.22	14.90	15.60	0.33	15.38	15.89	15.83	0.15	15.02^{A}	16.90^{B}	16.90^{B}	0.31	18.20	16.25^{A}	18.79^{B}	0.46	19.36	20.70	18.61	0.49
Acetate; A (mmol l^{-1})	9.70	9.78	9.94	0.23	10.40	10.91	10.25	0.15	9.55^{A}	11.11^{B}	11.06^{B}	0.25	12.56^{A}	10.55^{B}	12.63^{A}	0.40	13.28	14.28	12.69	0.38
Propionate; P (mmol l^{-1})	2.36	2.09	2.39	0.06	2.70^{A}	2.77^{A}	2.42^{B}	0.05	2.23^{A}	2.61^{B}	2.52^{B}	0.06	2.61^{A}	2.35^{B}	2.57^{A}	0.05	2.66	2.89	2.50	0.08
Butyrate (mmol l^{-1})	1.03	0.94	1.06	0.02	1.03	1.00	1.00	0.02	1.01	0.91	1.05	0.07	1.08	1.11	1.18	0.02	1.10	1.20	1.16	0.03
Valerate (mmol l^{-1})	0.33	0.32	0.34	0.01	0.36	0.36	0.34	0.01	0.40	0.46	0.41	0.02	0.43	0.38	0.49	0.02	0.39	0.42	0.39	0.01
Caproate (mmol l^{-1})	1.48	1.47	1.50	0.01	1.41	1.40	1.48	0.03	1.48	1.49	1.49	0.00	1.14	1.50	1.50	0.01	1.52	1.50	1.51	0.01
Isobutyrate (mmol l^{-1})	0.18	0.16	0.20	0.01	0.21	0.25	0.19	0.02	0.19	0.16	0.21	0.01	0.21	0.20	0.23	0.01	0.25	0.23	0.20	0.01
Isovalerate (mmol l^{-1})	0.09	0.09	0.12	0.01	0.11	0.10	0.10	0.00	0.09	0.08	0.10	0.00	0.10	0.10	0.11	0.00	0.10	0.11	0.10	0.00
Isocaproate (mmol l^{-1})	0.05	0.05	0.05	0.00	0.06	0.06	0.05	0.00	0.07	0.08	0.06	0.00	0.07	0.06^{A}	0.08^{B}	0.00	0.06	0.07	0.06	0.00

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
A/P ratio	4.11	4.20	4.16	0.02	3.85^{A}	3.94^{B}	4.24^{C}	0.06	4.28	4.26	4.39	0.05	4.81	4.49	4.91	0.11	4.99	4.94	5.08	0.09
Lactate (mmol l^{-1})	3.24	3.60	3.58	0.19	5.02^{A}	5.10^{A}	11.84^{B}	1.41	17.53^{A}	21.66^{B}	23.99^{B}	0.90	18.43^{A}	16.80^{A}	26.11^{B}	1.38	10.26	11.43	14.93	0.90
Molar proportion (mol/100 mol)																				
Acetic acid	74.1	74.3	74.2	0.06	73.6	74.3	75.0	0.27	74.7	75.9	75.6	0.44	77.3	75.3	77.1	0.40	77.9	77.8	77.6	0.28
Propionic acid	18.0	17.7	17.9	0.08	19.1^{A}	18.9^{A}	17.7^{B}	0.22	17.4	17.9	17.2	0.18	16.1	16.8	15.7	0.27	15.6	15.7	15.3	0.22
Butyric acid	7.9	8.0	7.9	0.04	7.3	6.8	7.3	0.17	7.9	6.2	7.2	0.47	6.6^{A}	7.9^{B}	7.2^{C}	0.19	6.5	6.5	7.1	0.17

CON – without yeast supplement; SC – live yeast culture; SCFP – yeast fermentation products
SEM – standard error of the mean
A/P ratio – acetate to propionate ratio
Means denoted with different letters are significantly different: a, b, c at $P \leq 0.05$; A, B, C at $P \leq 0.01$

observed following the addition of live yeast (SC). Compared to the control and the live yeast cultures, the yeast fermentation products (SCFP) significantly increased the lactate level and decreased pH after 6, 12, 24 and 48 h of incubation. The total VFA concentration after 12 h was statistically significantly higher (P≤0.05) in the SC and SCFP groups than in the control, mainly due to the higher concentrations of acetate and propionate, while the acetate to propionate ratio (A:P) was not affected. The highest propionate concentration was observed in the SC group and the lowest in the SCFP group (P≤0.01), which resulted in the highest A:P ratio. Significantly (P≤0.05) lower total VFA and lactate, acetate and propionate concentrations as compared to the control were observed after 24 h of incubation with SC. Also after 24 h of incubation, the isocaproate concentration was lower in the SC group than in the SCFP group (P≤0.01). Neither the *S. cerevisiae* fermentation products nor the live yeast cultures had a significant (P>0.05) effect on the ammonia concentration.

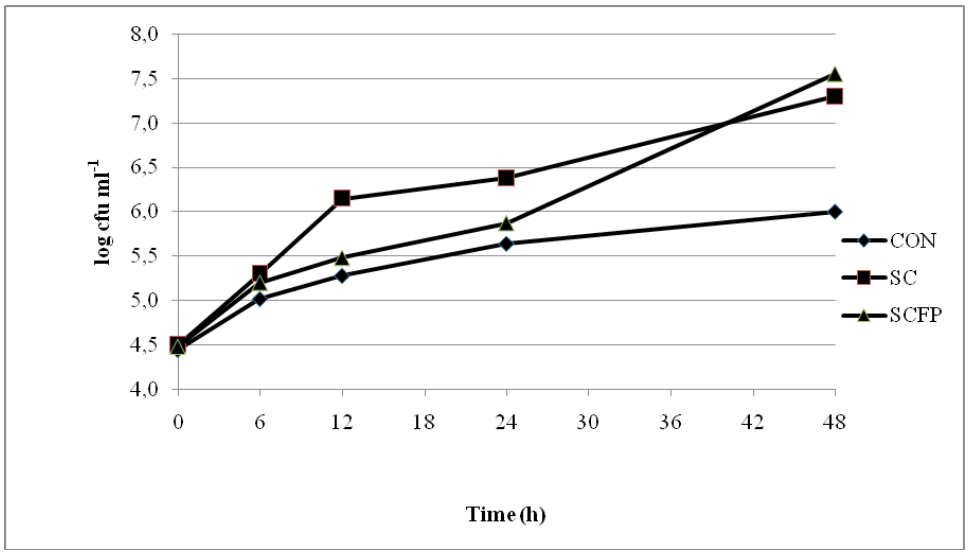


CON – without yeast supplement
SC – live yeast culture
SCFP – yeast fermentation products

Fig. 1. Effects of *Saccharomyces cerevisiae* live yeast culture (SC) and *Saccharomyces cerevisiae* fermentation products (SCFP) on in vitro growth of *Selenomonas ruminantium* (ATCC 19208)

Growth of *M. elsdenii* increased significantly when SC was added to the medium (Table 2, Figure 2). The greatest effect of the live yeast cultures was observed after 12 and 24 h of incubation. Both *S. cerevisiae* supplements significantly increased the *M. elsdenii* count

after 48 h of incubation as compared to the control. The main end products of *M. elsdenii* fermentation were caproate and butyrate. Compared with the untreated control (CON) and live yeast culture (SC), SCFP significantly increased total VFA concentration (P≤0.05) after 24 and 48 h of incubation, mainly due to increased levels of butyrate, valerate, caproate, isobutyrate and isovalerate. The acetate to propionate ratio also increased significantly (P≤0.05) after 48 h. SC significantly (P≤0.05) decreased the lactate level and increased pH after 48 h of incubation. The highest ammonia level was observed in the SCFP group after 12 and 48 h of incubation.



CON – without yeast supplement
SC – live yeast culture
SCFP – yeast fermentation products

Fig. 2. Effects of *Saccharomyces cerevisiae* live yeast culture (SC) and *Saccharomyces cerevisiae* fermentation products (SCFP) on in vitro growth of *Megasphaera elsdenii* (ATCC 19208)

Stimulation of lactate utilization by *S. ruminantium* and *M. elsdenii* may help to reduce problems associated with rumen acidosis [7]. Pinloche et al. [31] observed increased ruminal abundance of *M. elsdenii* and *S. elsdenii* when live yeast cultures were fed to cows. Callaway and Martin [6] suggest that identifying *S. cerevisiae* fermentation products that affect the growth and metabolism of key ruminal bacteria may lead to the development of microbial feed additives specific to the diet of individual ruminant species. In our study, neither SC nor SCFP added to the medium affected the growth of *S. ruminantium* after 6,

Table 2

Effects of *Saccharomyces cerevisiae* live yeast culture (SC) and *Saccharomyces cerevisiae* fermentation products (SCFP) on in vitro growth and fermentation of *Megasphaera elsdenii*

Items	Incubation time (h)																			
	0			SEM	6			SEM	12			SEM	24			SEM	48			SEM
	CON	SC	SCFP		CON	SC	SCFP		CON	SC	SCFP		CON	SC	SCFP		CON	SC	SCFP	
1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**7**	**18**	**19**	**20**	**21**
Bacteria (log cfu ml^{-1})	4.45	4.5	4.48	0.07	5.02	5.30	5.20	0.10	5.28^{Aa}	6.15^{B}	5.48^{Cb}	0.07	5.64	6.38	5.87	0.11	6.00^{a}	7.30	7.55^{b}	0.19
pH	6.01	6.03	6.01	0.03	6.02	5.99	6.05	0.04	5.99	5.92	6.12	0.04	5.96	6.11	6.05	0.03	6.01^{A}	6.31^{B}	6.02^{A}	0.06
Ammonia (mmol l^{-1})	8.35	8.35	8.41	0.12	8.62	9.50	10.35	0.15	9.30^{a}	10.43	11.86^{b}	0.52	14.65	14.28	13.99	0.14	13.39	14.90^{a}	13.05^{b}	0.35
Total VFA (mmol l^{-1})	18.84	19.21	18.40	0.45	21.29	20.13	20.56	0.56	22.09	21.27	20.52	0.59	25.85^{a}	26.49^{a}	33.10^{b}	1.31	34.40^{A}	27.93^{B}	39.39^{C}	1.42
Acetate; A (mmol l^{-1})	14.51	14.46	13.98	0.45	14.89	13.44	14.24	0.49	14.10	13.24	12.76	0.44	5.29	5.32	6.50	0.28	3.13^{A}	3.84^{B}	3.93^{B}	0.12
Propionate; P (mmol l^{-1})	0.20	0.22	0.18	0.01	0.38	0.38	0.35	0.01	0.43	0.42	0.39	0.01	0.39	0.43	0.44	0.01	0.49^{a}	0.52^{A}	0.39^{Bb}	0.02
Butyrate (mmol l^{-1})	0.99	1.02	0.95	0.05	2.24	2.41	2.24	0.03	3.16	3.46	3.28	0.10	9.37^{A}	9.66^{a}	12.46^{Bb}	0.54	9.42^{A}	9.53^{A}	10.54^{B}	0.16
Valerate (mmol l^{-1})	0.21	0.24	0.22	0.02	0.26	0.26	0.24	0.01	0.28	0.27	0.28	0.01	0.54^{a}	0.62	0.73^{b}	0.03	0.82^{A}	0.65^{B}	0.93^{C}	0.04
Caproate (mmol l^{-1})	2.58	2.86	2.71	0.04	2.80	2.90	2.76	0.03	3.14	2.89	2.83	0.04	6.93^{A}	7.12^{A}	9.04^{B}	0.34	17.32^{A}	9.77^{B}	20.27^{C}	1.33
Isobutyrate (mmol l^{-1})	0.12	0.14	0.12	0.01	0.21	0.22	0.21	0.00	0.26	0.26	0.26	0.01	1.13	1.14	1.20	0.09	1.23^{A}	1.42^{B}	1.28^{A}	0.03
Isovalerate (mmol l^{-1})	0.18	0.21	0.18	0.01	0.45	0.46	0.46	0.01	0.66	0.67	0.66	0.02	2.14	2.14^{a}	2.67^{b}	0.10	1.93^{A}	2.14^{B}	1.98^{A}	0.03
Isocaproate (mmol l^{-1})	0.05	0.06	0.06	0.01	0.06	0.06	0.06	0.00	0.06	0.06	0.06	0.00	0.06	0.06	0.06	0.00	0.06	0.06	0.07	0.00

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
A/P ratio	72.55	65.73	77.67	1.52	39.18	35.37	40.69	2.13	32.79	31.52	32.72	0.89	13.56	12.37	14.77	0.34	6.39^{A}	7.38^{A}	10.08^{B}	0.51
Lactate (mmol l^{-1})	1.67	1.74	1.18	0.15	1.90	1.94	1.94	0.20	2.15	1.91	2.10	0.12	2.26	2.06	2.57	0.09	1.96^{a}	1.36^{b}	2.06^{a}	0.14
Molar proportion (mol/100 mol)																				
Acetic acid	92.4	92.1	92.5	0.15	85.0	82.8	84.6	0.11	79.7^{Aa}	76.6^{B}	78.1^{b}	0.47	35.1^{a}	34.5^{a}	33.5^{b}	0.38	24.0^{Aa}	27.6^{B}	26.5^{b}	0.55
Propionic acid	1.3	1.4	1.2	0.07	2.2	2.3	2.1	0.05	2.4	2.5	2.3	0.06	2.6^{a}	2.8^{a}	2.3^{b}	0.06	3.8^{A}	3.8^{A}	2.6^{B}	0.19
Butyric acid	6.3	6.5	6.3	0.09	12.8	14.9	13.3	0.10	17.9^{A}	20.9^{B}	19.6	0.44	62.3^{a}	62.7^{a}	64.2^{b}	0.41	72.2^{A}	68.6^{Ba}	70.9^{b}	0.55

CON – without yeast supplement; SC – live yeast culture; SCFP – yeast fermentation products
SEM – standard error of the mean
A/P ratio – acetate to propionate ratio
Means denoted with different letters are significantly different: a, b, c at $P \leq 0.05$; A, B, C at $P \leq 0.01$

12 and 24 h of incubation, but the live yeast cultures increased bacterial growth after 48 h. Both kinds of yeasts caused changes in the fermentation end products. This is consistent with other research [14] in which the end products of *S. ruminantium* fermentation were acetate and propionate. The yeast fermentation products additionally increased the lactate concentration and significantly decreased pH after 6, 12 and 24 h of incubation. SC and SCFP increased the total VFA level after of 12 h of incubation, mainly by stimulating acetate and propionate production. The acetate to propionate ratio did not change in the *S. ruminantium* culture. In contrast, Martin and Nisbet [23] observed a constant pH and enhanced utilization of lactic acid by *S. ruminantium* following the addition of yeast metabolites (Diamond V XP). They also showed that the addition of yeast fermentation products increased synthesis of acetate ($P \leq 0.05$) and total VFA during incubation of *S. ruminantium* H18 and observed an increasing trend in the propionate level [6].

S. ruminantium increased lactate utilization and decreased methane production [3]. Nisbet and Martin [28] reported a positive effect of *Aspergillus oryzae* on lactate utilization and growth of *S. ruminantium*. Martin and Streeter [24] suggest that the addition of fungi may be a source of dicarboxylic acids which improve utilization of lactate by *S. ruminantium*. Evans and Martin [14] reported that *S. ruminantium* produces malate, which stimulates lactate utilization and increases pH and propionate concentration, but unfortunately there is little information regarding the effect of malate lactate-utilizing bacteria.

In our study, live cultures of *S. cerevisiae* stimulated the growth of *M. elsdenii* mainly after 12 and 24 h of incubation, while the fermentation products improved the growth of this bacterium after 48 h. It is very likely that live yeast cells remain viable and metabolically active for 24 h. Kung et al. [19] reported that *S. cerevisiae* did not multiply in sterile rumen fluid, but they did survive and were metabolically active. In contrast, El Hassan et al. [11] found that the number of live yeast cells in the rumen of sheep decreased at a rate of 8.6% per hour. On the other hand, Kung et al. [19] observed that yeast cells were metabolically active throughout 48 h of incubation. Oeztuerk et al. [29] observed no additional benefit of using live *Saccharomyces boulardii* as compared to autoclaved bacteria, which suggests that yeasts act more as prebiotics than probiotics, although the reasons for this are not fully understood. In our study, SC significantly reduced ($P \leq 0.05$) lactate content and increased pH after 48 h of growth of *M. elsdenii*. Similarly, Lynch and Martin [21] reported that live cultures of *S. cerevisiae* reduced lactate concentration, while *S. cerevisiae* fermentation products increased lactate content and lowered pH. Lila et al. [20] observed that an increase in the concentration of *S. cerevisiae* live cultures linearly decreased lactate concentration. Rossi et al. [32], in a study on the effect of live yeast cultures (Yea-Sacc), also observed a linear improvement in lactate utilization and bacterial biomass production. Callaway and Martin [6] found no effect of a 1% culture filtrate of *S. cerevisiae* on the growth of *M. elsdenii*. In our study, the ammonia concentration during the *M. elsdenii* culture was not modified by the addition of SC or SCFP, which is in line with results obtained *in vitro* [20] and *in vivo* [27].

Lactate utilization by *M. elsdenii* generally results in the production of propionate and acetate, but a few strains of *M. elsdenii* also produce valerate and butyrate. Both of these metabolic pathways enable the re-oxidation of reduced coenzymes [22, 33]. The main

end products of *M. elsdenii* fermentation were butyrate and valerate [37]. Cultures with *M. elsdenii* had greater amounts of isobutyrate, butyrate, isovalerate and valerate [18]. In our study, apart from butyrate, the main VFA was caproate, which is in agreement with *Bergey's Manual of Systematic Bacteriology* and Elsden et al. [12]. In contrast, Marounek et al. [22] concluded that the main product of glucose metabolism is butyrate; however, caproate content increased linearly with an increase in glucose in the medium. *M. elsdenii* is a sucrose-fermenting ruminal bacterium that can also break down amino acids, but its primary task is lactate utilization [16, 22]. Kung et al. [19] reported that live yeast cultures had no effect on the total concentration or molar percentages of VFA, with the exception of valerate production in the case of high doses of yeast. In our study, after 24 and 48 h incubation the *S. cerevisiae* fermentation product increased the total VFA concentration, mainly caproate, acetate and butyrate, and also improved the propionate ratio. In the SC group, the total VFA and caproate concentrations were reduced after 24 and 48 h of incubation. This result was different from that reported by Soto-Cruz et al. [35], who found that live Yea-Sacc cells stimulated butyrate production. Newbold et al. [27], in a review of the literature, reported that fungal extracts had no effect or tendency to increase the rumen acetate to propionate ratio, while live yeast cultures had no effect or decreased the A:P ratio. The addition of the hydrophilic peptides fraction from *S. cerevisiae* stimulated the growth of *M. elsdenii* and the production of butyrate (+100%) and valerate (+76.1%), as well as lactate metabolism [33]. Miller-Webster et al. [26] showed that supplementation with two different yeast fermentation products increased total VFA and propionic acid production and reduced the A:P ratio. The addition of both live and autoclaved *Saccharomyces boulardii* increased total VFA and the concentrations of butyrate, isovalerate and valerate with no significant effect on propionate [29]. *In vitro* experiments have also shown that the *S. cerevisiae* fermentation products had no significant effect on the production of VFA [21]. Treatment with yeast fermentation products had less effect on the production of acetate, propionate, butyrate and valerate by *M. elsdenii* B159 and T81 [6]. The addition of a 2.5% and 5% filtrate of a live yeast culture (Yea-Sacc) improved total VFA production and slightly decreased acetate concentrations [33].

Diets containing high concentrations of cereal grain reduce pH in the rumen. Neither SC nor SCFP had a significant effect on the growth of *S. ruminantium* after 6, 12 and 24 h incubation. SC was not found to decrease lactate concentration or to affect the end products of *Selenomonas ruminantium* fermentation, while the yeast fermentation products increased lactate concentration and decreased the pH of the rumen fluid. The addition of SC improved growth of *M. elsdenii* after 12 and 24 h of incubation, while SCFP significantly increased the growth of this bacterium after 48 h. Live cultures of *Saccharomyces cerevisiae* increased pH and reduced the content of lactate and caproate, which were the main products of *in vitro M. elsdenii* fermentation.

The study showed that *Saccharomyces cerevisiae* can be used to increase lactate utilization by *S. ruminantium* and *M. elsdenii* and to reduce problems associated with rumen acidosis. The *Saccharomyces cerevisiae* live cultures had more positive effects than the *Saccharomyces cerevisiae* fermentation products.

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