

# Effects of live and autoclaved yeast cultures on ruminal fermentation *in vitro*

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## ABSTRACT

The objective of this study is to determine whether a yeast culture of *Saccharomyces cerevisiae* has an effect on parameters of ruminal fermentation and whether the potential positive effects of yeast culture are associated with the yeast's viability. For this purpose, live and autoclaved yeast cultures were tested simultaneously in the rumen simulation technique (Rusitec). Each fermentation vessel received daily 9 g of a basal diet consisting 5 g meadow hay and 4 g pelleted concentrate. Yeast preparations (1.5 g/day) were added with the feed. Compared to the control vessels, the pH of ruminal fluid was significantly ( $P<0.05$ ) lower in the vessels supplemented with autoclaved yeast culture. Live yeast culture had no effect on the pH. Both yeast preparations resulted in a significant increase ( $P<0.05$ ) in  $\text{NH}_3\text{-N}$  concentration, total and individual short-chain fatty acid (SCFA) production except valerate. The increase in  $\text{NH}_3\text{-N}$  concentration and propionate production was significantly higher ( $P<0.05$ ) in the vessels supplemented with live yeast culture than that with autoclaved yeast culture. Also, the ratio of acetate to propionate was significantly lower ( $P<0.05$ ) by live yeast culture, when compared with the control vessels. Organic matter digestibility was not significantly affected by both yeast preparations. In conclusion, these results indicated that the addition of live and autoclaved yeast cultures stimulated ruminal fermentation but this effect was more pronounced with live yeast culture.

**KEY WORDS:** live yeast culture, autoclaved yeast culture, *Saccharomyces cerevisiae*, rumen, *in vitro*

## INTRODUCTION

Dietary supplements of yeast cultures based on *Saccharomyces cerevisiae* have been reported to improve health and productivity of ruminants. In comparison

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with antimicrobial agents, yeast cultures offer a natural alternative to manipulate animal performance. Some data indicate that supplementation of live yeast culture into the feed ration may improve feed intake (Williams et al., 1991), weight gain (Lesmeister et al., 2004), digestion (Wiedmeier et al., 1987), increase of anaerobic and cellulolytic bacteria (Wiedmeier et al., 1987; Koul et al., 1998), increase the ruminal pH (Williams et al., 1991; Koul et al., 1998) and alter the patterns of SCFA production (Wiedmeier et al., 1987). However, yeast cultures have not been found to alter ruminal fermentation or improve animal performance in all cases (Chademana and Offer, 1990). These inconsistencies might be due to strain-dependent, or specific differences between commercial additives, or differences in dietary compositions (Oeztuerk et al., 2005).

The mode of action of yeast cultures on rumen metabolism remains unclear. Preliminary studies showed that the viability of yeasts was essential for their stimulatory effect in the rumen. Koul et al. (1998) examined the effects of live, autoclaved and  $\gamma$ -irradiated yeast culture of *Saccharomyces cerevisiae* (5 g/d, Yea-Sacc 1026) on rumen microbial population and fermentation pattern in buffalo calves fed a diet consisting of 56% roughage and 44% concentrate. They found that autoclaving of yeast culture destroyed almost all and  $\gamma$ -irradiation of yeast culture retained about 50% of stimulatory activity of live yeast culture. However, Oeztuerk et al. (2005), using the rumen simulating technique (Rusitec), reported that addition of live and autoclaved *Saccharomyces boulardii* (0.5 to 1.5 g/d) stimulated *in vitro* ruminal microbial metabolism, with only small differences between treatments, and concluded that *Saccharomyces boulardii* was functioning as a prebiotic rather than as a probiotic agent. Most of the studies reported have been conducted *in vivo* on animals and little is known about the longer-term effects of yeast's viability on *in vitro* ruminal fermentation. The rumen simulation technique (Rusitec) makes it possible to examine the direct effects of yeast cultures independent of the host ruminant. The aim of the present study was therefore to investigate the long-term effects of yeast's viability (live vs autoclaved yeast culture) on *in vitro* ruminal fermentation in the Rusitec fermenters.

## MATERIAL AND METHODS

### *Incubation technique*

Nine incubation vessels (1 l volume) were used simultaneously in this experiment. The nominal volume in each vessel was 750 ml. Each vessel was loaded with 2 nylon bags. The nylon bags (70×120 mm) had a pore size of 150  $\mu\text{m}$  which is usually used in *in vitro* rumen fermentation studies (Öztürk, 2003). At the start of the trial, one bag was filled with 80 g of solid rumen contents

to inoculate particle-associated microorganisms into the system and the other with the daily diet, a mixture of 4 g of pelleted concentrate and 5 g of meadow hay cut into 1 cm lengths. The energy value of the pelleted concentrate was 10.2 MJ ME/kg DM and it was based on wheat, maize gluten feed, lucerne meal, barley, sunflower extraction meal, soyabean extraction meal, sugar beet molasses, and mineral/vitamin premix. The chemical composition of the experimental diet is presented in Table 1. The incubation vessel was filled with

Table 1. Chemical composition of the experimental diet, %

Nutrients	Meadow hay	Concentrate
Dry matter	93.94	90.48
Crude protein	7.27	15.96
Crude lipids	0.88	2.32
Crude fibre	27.59	10.49
N-free extractives	46.01	43.36
Total ash	6.13	8.83

rumen fluid to inoculate fluid-associated microorganisms. Rumen contents were taken from two rumen cannulated adult donor sheep that were maintained on a diet of meadow hay and concentrates. Animals had free access to hay, water, and a vitamin-enriched salt lick. The same dietary components were used for *in vitro* fermentation trials. The nylon bag with solid rumen contents was replaced after 24 h of incubation with a bag containing the diet. The feed bag was changed after 48 h so that 2 bags were always present. This gave a retention time of 48 h for feed. Bags were exchanged under anaerobic conditions using N<sub>2</sub> to flush the incubation vessels. To maintain conditions as close to those of the *in vivo* rumen as possible, the incubation temperature was 39°C and rumen fluid turnover was simulated by modified artificial saliva (pH 7.4, 293 mosmol/l; McDougall, 1948) at a rate of 750 ml/d. The chemical composition of modified artificial saliva is presented in Table 2. By moving the inner vessel up and down continuously rumen motility was simulated and exchange between the fluid and particle phases

Table 2. Chemical composition of modified McDougall's artificial saliva

Ingredient	mmol/l
NaCl	28.00
KCl	7.69
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.22
MgCl <sub>2</sub> ·6H <sub>2</sub> O	0.63
NH <sub>4</sub> Cl	5.00
Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O	10.00
NaH <sub>2</sub> PO <sub>4</sub> ·H <sub>2</sub> O	10.00
NaHCO <sub>3</sub>	97.90

was facilitated. Rumen gas was collected in gas-tight collecting sacs to ensure a closed system; the fluid outflow was collected in ice-cooled Erlenmeyer flasks.

### *Experimental procedure*

*In vitro* incubation trial (16 days) consisted of an adaptation period of 8 days (to achieve steady state conditions) followed by a collection period of 8 days. At the start of the collection period, live and autoclaved yeast culture (Yea-Sacc 1026, Alltech Biotechnology Centre, Nicholasville, KY) were added to the respective fermentation vessels. During the collection period the 9 vessels were divided into 3 groups. One group served as controls; 1.5 g/day of live and 1.5 g/day of autoclaved yeast culture were added to the second and third group, respectively. The following considerations were the basis for the chosen yeast dosage. Rusitec is a dilute form of rumen fermentation, designed as such to give a final pH and SCFA concentrations similar to those *in vivo*. Absorption of SCFA does not occur in Rusitec as it does *in vivo* and their pH-depressing effects are decreased by dilution. Therefore, when testing soluble additives, it is necessary to add amounts of the additive that are calculated to give the required final concentration in the liquid phase rather than as a proportion of the feed (Newbold et al., 1998). Thus the proportion of yeast cultures fed to the Rusitec was 16.6% whereas this amount would be equivalent to 0.7% or less *in vivo*.

Inactivation of yeast culture was achieved by autoclave standard operating procedures (213 kPa at 121°C for 20 min). To test if the inactivation was complete, 10 mg of autoclaved yeast culture was incubated at 30°C in yeast extract/peptone/dextrose liquid medium (Serva, Heidelberg, Germany) in a shaking incubator (GFL shaking inkubator 3031, Burgwedel, Germany) at 200 rpm for 16 h. Then, 100 µl of inoculate the liquid medium was plated onto yeast extract/peptone/dextrose agar, and incubated for 3 d at 30°C. No live yeasts were detected.

### *Analytical procedures and samplings*

The pH values were measured daily in each vessel at the time of feeding using a pH electrode (Typ 408, Mettler Toledo, Steinbach, Germany) connected to a Knick pH meter (digital pH meter 646, Knick, Berlin, Germany). Liquid effluent was collected daily and samples were taken for analyses of SCFAs and NH<sub>3</sub>-N. The overflow flasks were placed into ice to stop microbial activity and preserve fermentation products. An aliquot of effluent was centrifuged at 40.000 g for 20 min at 4°C. The resulting supernatant was acidified with 0.1 ml of 98% formic acid and then centrifuged at 4.000 g for 10 min at 4°C. The supernatant was analysed for SCFAs by gas chromatography (model 5890 II, Hewlett Packard, Böblingen, Germany) equipped with a 1.8 m × 2 mm glass column packed with

Chromosorb WAW (mesh 80/100) with 20% neopentyl glycol succinate and 2% orthophosphoric acid. Helium was used as a carrier gas with a flow rate of 25 ml/min. Injection port, detector, and oven temperatures were 220, 250 and 130°C, respectively. Daily production rates of SCFAs were estimated by multiplying the respective concentration by the volume of effluent collected. Ammonia N was measured using the steam distillation method of Kjeldahl (Stuck et al., 1995). Dry matter was determined by drying at 65°C for 48 h (Typ 600, Memmert, Schwabach, Germany). Ash concentration was determined after ignition at 600°C for 12 h in a muffle furnace (Typ M110 Heraeus, Hanau, Germany) and used to calculate organic matter. The digestibility of organic matter at 48 h was calculated as original organic matter sample weight minus organic matter residue weight divided by the original sample weight. This value was then multiplied by 100 to derive the digestibility of organic matter percentage.

### *Statistical analyses*

Statistical analyses were performed using SigmaStat 3.1 (Jandel Scientific, Erkrath, Germany). All the data were analysed by one-way ANOVA and the differences between the means were compared by the Duncan's multiple range test. Probability values of  $P < 0.05$  were considered as significant.

## RESULTS

Effects of live and autoclaved yeast culture on *in vitro* ruminal fermentation are shown in Table 3. Addition of autoclaved yeast culture in the fermentation vessels decreased the pH by 0.03 units ( $P < 0.05$ ) while live yeast culture had no effect on the pH compared to the control vessels. Both live and autoclaved yeast culture increased ( $P < 0.05$ ) the daily production of total and individual SCFA except valerate, which was not significantly altered by both treatments. The increase in propionate production was significantly ( $P < 0.05$ ) higher in the fermentation vessels receiving live yeast culture than in that receiving autoclaved yeast culture. As a result, the ratio of acetate to propionate in the vessels supplemented with live yeast culture was 4.3% smaller ( $P < 0.05$ ) than that in the control vessels. However, this ratio obtained after adding autoclaved yeast culture did not significantly differ from that in the control vessels. The production of total SCFA, acetate, propionate, butyrate and iso-valerate was 8, 6, 11, 7 and 64% higher in the vessels supplemented with live yeast culture, and 6, 5, 6, 4 and 53% in the vessels supplemented with autoclaved yeast culture, respectively, when compared with the control vessels. Addition of live and autoclaved yeast culture to the fermentation vessels increased ( $P < 0.05$ ) the

Table 3. Effects of live and autoclaved yeast culture on *in vitro* ruminal fermentation in Rusitec

Item	Control	Yeast culture	
		live	autoclaved
pH	6.81 ± 0.01 <sup>a</sup>	6.82 ± 0.01 <sup>a</sup>	6.78 ± 0.01 <sup>b</sup>
Total SCFAs, mmol/day	31.89 ± 0.44 <sup>a</sup>	34.44 ± 0.44 <sup>b</sup>	33.73 ± 0.36 <sup>b</sup>
acetate	17.86 ± 0.09 <sup>a</sup>	18.94 ± 0.13 <sup>b</sup>	18.72 ± 0.12 <sup>b</sup>
propionate	6.44 ± 0.06 <sup>a</sup>	7.16 ± 0.08 <sup>b</sup>	6.85 ± 0.09 <sup>c</sup>
butyrate	5.36 ± 0.12 <sup>a</sup>	5.75 ± 0.09 <sup>b</sup>	5.60 ± 0.07 <sup>b</sup>
iso-valerate	0.55 ± 0.02 <sup>a</sup>	0.90 ± 0.04 <sup>b</sup>	0.84 ± 0.03 <sup>b</sup>
valerate	1.68 ± 0.02	1.69 ± 0.03	1.71 ± 0.02
C <sub>2</sub> /C <sub>3</sub> <sup>1</sup>	2.77 ± 0.09 <sup>a</sup>	2.65 ± 0.07 <sup>b</sup>	2.73 ± 0.09 <sup>a</sup>
NH <sub>3</sub> -N, mmol/l	9.31 ± 0.05 <sup>a</sup>	10.72 ± 0.07 <sup>b</sup>	10.02 ± 0.09 <sup>c</sup>
OMD, %	60.67 ± 1.01	59.08 ± 0.85	59.33 ± 0.85

<sup>a-c</sup> different letters in the same row indicate statistical significance (P<0.05). Values are means ± SEM; n=24; OMD - organic matter digestibility; <sup>1</sup> ratio of acetate to propionate

concentration of NH<sub>3</sub>-N by 15 and 8%, respectively, when compared with the control vessels. Neither live nor autoclaved yeast culture significantly affected the digestibility of organic matter.

## DISCUSSION

The effect of yeast cultures on ruminal pH, perhaps the most easily measurable of the rumen variables, has been reported widely. Here autoclaved yeast culture decreased the pH values in rumen fluid, while live yeast culture had no effect on the pH. *In vitro* and *in vivo* effects of *Saccharomyces cerevisiae* on ruminal pH have been varied. Results of *in vitro* studies suggested that Yea-Sacc 1026 strain (0.5 g/d; Newbold et al., 1995) and Diamond V XP yeast culture (0.35 to 0.73 g/l; Lynch and Martin, 2002) did not modify mean pH. In contrast, Williams et al. (1991) reported that *Saccharomyces cerevisiae* NCYC 1026 (7.5g/d; Nutfield, Surrey, UK) increased ruminal pH in steers fed a hay plus barley diet. It has been suggested that the stabilizing effect of live yeasts on ruminal pH might be due to reduced lactate concentration in the rumen (Williams et al., 1991), through the increase of activity of lactate-utilizing bacteria such as *Selenomonas ruminantium* and *Megasphaera elsdenii* and/or the competition with *Streptococcus bovis*, the main lactic acid producer in the rumen, for soluble sugars uptake (Chaucheyras et al., 1996; Callaway and Martin, 1997).

Other major effects of live and autoclaved yeast culture on ruminal fermentation included increased concentrations of NH<sub>3</sub>-N. In an *in vitro* study, Newbold et al. (1998) and Oeztuerk et al. (2005) reported that live culture of *Saccharomyces cerevisiae* strain 47 (500 mg/d, Biosaf, Lesaffre Developments, Paris) and live

cells of *Saccharomyces boulardii* (1.5 g/d) increased ruminal  $\text{NH}_3\text{-N}$  concentration, respectively. In the current study, the levels of yeast culture additions were high (1.5 g for each yeast culture in 9 g of diet) and it is likely that the increases in the  $\text{NH}_3\text{-N}$  output represent a response to the microbial degradation of high amount of yeast cells because of their high protein content. This result was confirmed by the higher iso-valerate production in the vessels supplemented with both yeast cultures. The iso-SCFA (iso-butyrate and iso-valerate) are produced mainly from microbial degradation of branched-chain amino acids by rumen microorganisms (Richter and Flachowsky, 1989). Moreover, in the present study, the increase in  $\text{NH}_3\text{-N}$  concentration caused by live yeast culture was higher than that caused by autoclaved yeast culture. This difference between both yeast preparations might have been associated with a stimulation of proteolytic activity of rumen bacteria by live yeast culture. Further evidence supporting this notion was provided by the study of Yoon and Stern (1996), who reported that the addition of yeast supplement (57 g/d, Diamond V XP yeast culture) to the rumens of cows fed a basal diet increased numbers of proteolytic bacteria. It has been suggested that the increased bacterial population is central to the action of the yeast. However, the reason of increased bacterial count is not still clear. Popular theories suggest yeast culture provides various growth factors, pro-vitamins, and/or micronutrients and removes potentially harmful oxygen from the rumen environment. Thus, yeast culture stimulates the growth of the ruminal bacteria in the rumen (Newbold et al., 1996).

In the present study, both yeast preparations led to an increase in the SCFA productions except valerate. The significant increase in propionate production after supplementation live yeast culture was the reason of lower acetate to propionate ratio than that in the vessels supplemented with autoclaved yeast culture. Published reports of the effect of yeast culture on production rates of SCFA are variable. Chademana and Offer (1990) reported that Yea-Sacc 1026 had no effect on total SCFA or SCFA composition, but others found stimulation in the proportion of propionate at the expense of acetate (Plata et al., 1994) or even an increase in the proportion of acetate (Mutsvangwa et al., 1992). In an *in vitro* study, Miller-Webster et al. (2002) evaluated two yeast culture products (0.2 g/l, Diamond V XP yeast culture and 0.2 g/l, A-Max yeast culture) and both of them increased propionate concentration and reduced the ratio of acetate to propionate, similar to this study.

Organic matter digestion was not stimulated by both yeast preparations. In the present study, the SCFA production and the  $\text{NH}_3\text{-N}$  concentration were significantly increased by both yeast preparations, although digestibility of organic matter was not changed. This suggested that rumen microorganisms digested the supplied yeast cultures as additional substrates for their metabolic purposes.

## CONCLUSIONS

Despite ruminant nutritionists' increasing interest in the differences between live and autoclaved yeast cultures, few studies have been conducted to evaluate the effects of the yeast's viability on ruminal fermentation. Under the conditions of this study, the addition of live and autoclaved yeast cultures stimulated ruminal fermentation. These changes in the measured parameters of ruminal fermentation may be the consequences of altered rumen microbial population arising from yeast culture supplementation. The stimulatory effect observed in this study was more pronounced with live yeast culture and a part of the stimulatory effect was lost when yeast culture was autoclaved. These data suggest that the ability of yeast culture to influence ruminal fermentation could be associated with live yeast cells or some heat labile component(s) of the yeast cells.

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