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Effects of hydrolyzed and live yeasts on rumen microbial fermentation in a semicontinuous culture system (Rusitec)

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Abstract: The objective of this study was to investigate the effects of hydrolyzed and live forms of yeast products from *Saccharomyces cerevisiae* on in vitro fermentation of a 50:50 forage:concentrate substrate using the rumen simulation technique (Rusitec). The Rusitec system consisted of nine fermentation vessels: three of them received daily 0.5 g/L hydrolyzed yeast, three vessels received daily 0.5 g/L live yeast, and three vessels received no additives (control). After an adaptation period of 7 days, the main fermentation characteristics were evaluated for 7 consecutive days. Ruminal pH was decreased ($P < 0.05$) by hydrolyzed yeast, whereas no effects were observed after live yeast treatment. Both hydrolyzed and live yeasts tended to increase butyrate production ($P = 0.052$). Acetate to propionate ratio was significantly higher ($P < 0.05$) for live yeast. Both products increased NH_3-N concentration, and it was significantly higher ($P < 0.05$) in live yeast in comparison with that in hydrolyzed yeast. Digestibility of dry matter was not significantly affected by both yeast treatments. Overall, these results showed that different types of yeast products in terms of their viability status had significant actions in ruminal microbial metabolism in different ways.

Key words: Hydrolyzed yeast, live yeast, rumen, *Saccharomyces cerevisiae*

1. Introduction

Interest in the use of natural products as feed additives for ruminant livestock has increased since the use of antibiotics as growth promoters was banned in the European Union in 2006 (1). Among these natural products, yeast cultures, mainly from strains of *Saccharomyces cerevisiae*, are widely used in ruminant husbandry to change ruminal fermentation parameters and to have beneficial effects on animal production. However, results with yeast addition have been highly variable and inconsistent. These inconsistencies may be due to differences in yeast strains, concentrations of added yeast, types of animals, and feed composition (2). On the other hand, Sullivan and Bradford (3) showed that the efficacy of live yeast products was related to cell viability, which was greatly diminished during production, storage, and delivery of yeast products at elevated temperatures.

In most in vitro and in vivo experiments, live yeast cultures of *Saccharomyces cerevisiae* have been studied. Yeast culture is defined as the dried product composed of yeast and the medium it was grown on to preserve the fermenting activity of the yeast (4). Recently, attention has been directed at inactive yeast products and there has been a growing interest in research comparing the effects of live products to inactivated products on rumen microbial fermentation. There is no report on comparative effects of hydrolyzed and live yeast products originating from the same microorganism on ruminal metabolism within the same trial. This study was designed, therefore, to investigate and compare the effects of two yeast products containing only hydrolyzed or live cells of *Saccharomyces cerevisiae* (no culture medium) on ruminal fermentation of a 50:50 forage:concentrate diet using Rusitec.

2. Materials and methods

2.1. Incubation technique

The study was carried out using a semicontinuous culture system (5). The fermentation equipment included nine fermentation vessels with a capacity of 750 mL each. The inoculum was obtained from a freshly slaughtered beef bull (450 kg mean body weight) at a commercial slaughter facility and transferred in warm (39 °C) insulated flasks to the in vitro system within 30 min. The animal had been fed about 2 kg of barley straw and 9 kg of a commercial

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mineral- and vitamin-supplemented concentrate for beef cattle. The same diet was also used for in vitro incubation trial. The commercial concentrate had the following chemical composition: 91.56% dry matter and, on a dry matter basis, 13.91% crude protein, 2.61% crude lipids, 15.92% crude fibers, 50.95% nitrogen-free extract, and 8.17% total ash. At the beginning of the study, each fermentation vessel was filled with 750 mL of ruminal fluid, which was filtered through four layers of cheesecloth and two nylon bags (80 × 120 mm; 150 µm pore size), one containing 80 g of solid digesta and the other containing 10 g of feed (5 g of barley straw and 5 g of concentrate on feed basis). After 24 h, the solid digesta bag was replaced by a fresh feed bag. One feed bag was replaced daily, so that the retention time of each feed bag was 48 h. When the bag was being changed, the vessels were flushed with CO_2 to maintain anaerobic conditions. The liquid flow through the vessels was maintained by continuous infusion of a buffer solution with pH 7.4 and 293 mosm/L at a rate of 750 mL/day. The chemical composition of the buffer solution is given in Table 1 (6).

2.2. Experimental procedures

The incubation trial consisted of a 7-day adaptation period (to achieve steady state conditions) followed by a 7-day collection period. At the start of the collection period, hydrolyzed (Progut Rumen, Finland) or live yeasts (Yea-K, Alltech, Turkey) were added to the respective fermentation vessels. During the collection period, the 9 vessels were divided into 3 groups: three of them received daily 0.5 g/L of hydrolyzed yeast, three vessels received daily 0.5 g/L of live yeast, and three vessels received no additives (control). Progut is a patented commercial product from Suomen Rehu (Espoo, Finland). The product is derived from inactivated whole brewer's yeast (*Saccharomyces cerevisiae*). Unlike cell wall products, it also contains the extract components of the yeast. Yea-K is a probiotic yeast product consisting of only live cells of *Saccharomyces cerevisiae*. According to the manufacturer, Yea-K contains 2×10^9 live cells per gram. Both yeast products had similar dry matter content (approximately 94%), so the same dose as the supplementation rate for yeasts was used.

Table 1. Chemical composition of the buffer solution (mmol/L).

Ingredients	
NaCl	28.00
KCl	7.69
$CaCl_2.2H_2O$	0.22
$MgCl_2.6H_2O$	0.63
NH_4Cl	5.00
$Na_2HPO_4.12H_2O$	10.00
$NaH_2PO_4.H_2O$	10.00
$NaHCO_3$	97.90

2.3. Samplings and analytical procedures

The pH values were measured daily in each fermentation vessel at the time of feeding using an epoxy body pH electrode (WD-35801-00, Oakton, USA) connected to a pH-meter (Ion 6, Acorn series, Oakton). Liquid effluent was collected daily and samples were taken and frozen at –20 °C for volatile fatty acid (VFA) and NH_3-N determination. VFAs were quantified by the method of Oeztuerk et al. (7) using HPLC (Shimadzu LC-20AT, Japan) with a Rezex ROA-organic acid column (7.8 × 300 mm) at 60 °C, isocratic elution with 0.005 M H_2SO_4, and UV detection at 210 nm. NH_3-N concentration was determined using a colorimetric technique as described by Bhandari et al. (8). Digestibility of dry matter was detected by drying at 65 °C for 48 h. It was calculated as original dry matter sample weight minus dry matter residue weight divided by the original sample weight. This value was then multiplied by 100 to derive the digestibility of dry matter percentage.

2.4. Statistical analysis

Results are presented as means ± standard error of means (SEM). Statistical analysis was performed by a one-way analysis of variance (ANOVA) for repeated measures using the SigmaStat 3.1 software (Systat Software, Erkrath, Germany). In case of a significant ANOVA result, post-hoc Duncan tests were performed to evaluate the statistical differences between the groups. $P < 0.05$ was considered significant.

3. Results

The effects of hydrolyzed and live yeast products on in vitro ruminal fermentation are given in Table 2. Compared with the controls, live yeast supplementation significantly ($P < 0.05$) decreased ruminal pH, but hydrolyzed yeast did not. Both yeast treatments had no significant effects on the daily production of total VFA, acetate, and propionate. However, butyrate production tended to increase with yeast administrations ($P = 0.052$). On the other hand, the ratio of acetate to propionate was significantly increased ($P < 0.05$) by the addition of live yeast when compared with unsupplemented controls. Both yeast treatments increased ($P < 0.05$) ruminal NH_3-N concentrations, and the increase was significantly higher ($P < 0.05$) in live yeast than in hydrolyzed yeast. Digestibility of dry matter was not significantly influenced by both yeast treatments.

Table 2. Effects of hydrolyzed and live yeast products on ruminal fermentation in the Rusitec system.

Variable	Treatments			SEM	P-value
	Control	Hydrolyzed yeast	Live yeast		
pH	6.73[a]	6.71[b]	6.72[ab]	0.003	0.007
Total VFA (mmol/day)	26.53	27.86	27.82	0.036	0.205
Acetate	16.19	17.24	17.21	0.237	0.106
Propionate	6.68	6.78	6.60	0.098	0.725
Butyrate	3.65	3.84	4.02	0.066	0.052
C_2:C_3	2.44[bc]	2.56[ab]	2.62[a]	0.033	0.018
NH_3-N (mmol/L)	6.20[c]	6.99[b]	7.56[a]	0.101	<0.001
Dry matter digestibility (%)	57.09	56.89	56.51	0.486	0.910

Means within the same row with different letters (a, b, c) differ ($P < 0.05$). C_2:C_3 is the acetate to propionate ratio.

4. Discussion

The present study was designed to investigate and compare the effects of hydrolyzed and live yeast products on in vitro rumen microbial fermentation of a diet composed of 50% barley straw and 50% concentrate. In order to compare the results, two yeast products that were prepared only from whole cells of *Saccharomyces cerevisiae* (hydrolyzed whole yeast or live whole yeast) were chosen for the study. Throughout the whole experiment, the pH values of ruminal fluid ranged between 6 and 7 and were thus within the physiological range of rumen pH. When compared with unsupplemented control vessels, hydrolyzed yeast product decreased the ruminal pH, whereas no decrease was observed in vessels receiving live yeast product. One of the most consistent results in previous studies with live yeast products is a stabilization of rumen pH. This pH-stabilizing effect of live yeasts has been attributed to promoting the use of lactic acid by lactate-utilizing bacteria such as *Selenomonas ruminantium* and *Megasphaera elsdenii* (9,10) and competing for rapidly fermentable carbohydrates with lactate-producing bacteria such as *Streptococcus bovis* (11). This causes a reduction in lactate concentration, giving rise to higher ruminal pH. Brossard et al. (12) also showed that live yeast was efficient at stabilizing rumen pH by stimulating ciliate entodiniomorphid protozoa, which are known to rapidly engulf starch granules. In the current study, the lower ruminal pH observed with hydrolyzed yeast treatment may be associated with the absence of the above mentioned mechanism.

The addition of hydrolyzed or live yeast to the Rusitec vessels resulted in a slight, nonsignificant increase in the production of total and individual VFA (except propionate for live yeast treatment). The above described pH decline could also be a consequence of this increased VFA production. The profile of VFA was influenced by yeast treatments. Acetate to propionate ratio was increased by live yeast treatment. VFAs are of paramount importance for ruminants since they contribute approximately 70% of the ruminant's energy supply (13). Propionate is the major gluconeogenic substrate for ruminants, whereas acetate is a main precursor for de novo lipogenesis. Increased acetate production is associated with elevated milk fat yield in vivo (14). In this study, the results for the VFA response to yeast treatments are in agreement with previous studies (2,15,16). On the other hand, there are contradictory data in the literature concerning the effects of yeast products on VFA production and profile (17). These contradictory results may be related to ration composition (2,18), characteristics of the strain used (17,19), and differences among commercial additives (20).

The main significant finding from this study is the effect of yeast products on ruminal NH_3-N concentration. Both hydrolyzed and live yeast products increased the NH_3-N concentration, but the increase was higher for live yeasts. These findings are consistent with previous in vitro (2,6) and in vivo studies (21). The higher ruminal NH_3-N concentration in the current study can be associated with the microbial degradation of yeasts because of their high protein content. Pacheco et al. (22) reported that yeast biomass contains about half of its dry weight as proteins. In a recent study, Molist et al. (23) reported that hydrolyzed whole yeast (Progut) contains about 38% protein. But what caused the difference in NH_3-N concentrations between hydrolyzed whole yeast and live whole yeast used in this study? This difference could be caused by the different protein contents of the two products. Another possibility is that the hydrolyzation procedure of yeast could lead to more resistant proteins to microbial degradation, as the by-pass protein that escapes ruminal digestion.

Digestibility of dry matter was not influenced by both yeast treatments. A similar response in diet digestibility was observed by Opsi et al. (2) when inactivated and live yeasts were added, and by Carro et al. (18) with the addition of a live yeast culture on a medium of concentrate diet.

In conclusion, both yeast products tested in this study increased ruminal NH_3-N concentrations and tended to increase the production rate of butyrate. The live yeast product stimulated the production of acetate at the expense of propionate and possessed the capacity to stabilize ruminal pH. Hydrolyzed and live yeast products had no other effects on ruminal fermentation or on nutrient digestibility in the semicontinuous rumen simulation technique (Rusitec). These effects may be the consequences of changes in rumen microbial ecology arising from the yeast product treatments. However, further in vitro and in vivo studies are required to investigate the importance of yeast viability in rumen fermentation.

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