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Effects of hydrolysed yeasts on ruminal fermentation in the rumen simulation technique (Rusitec)

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ABSTRACT: The objective of the present study was to investigate the effects of three different hydrolysed yeast products derived from *Saccharomyces cerevisiae* [hydrolysed whole yeast (HWY), less hydrolysed whole yeast (LHWY), and yeast cell wall (YCW)] on microbial fermentation characteristics using the rumen simulation technique (Rusitec) with three consecutive experiments. The Rusitec system consisted of six fermentation vessels. Each vessel received 5 g chopped meadow hay and 4 g concentrate (as-fed basis) daily for up to 22 days. Yeast products were added to the fermentation vessels at a concentration of 0.25 or 0.75 g/day. In most cases, ruminal microbial activity was stimulated by HWY and YCW, particularly at the 0.75 g/day level. HWY resulted in a decrease ($P < 0.05$) in ruminal pH and an increase ($P < 0.05$) in total short-chain fatty acid (SCFA), acetate, propionate and methane productions, and an increase in NH_3-N concentration when compared with the control values. Ruminal pH was not altered, but total SCFA, acetate, propionate, butyrate, and methane productions as well as NH_3-N concentration increased ($P < 0.05$) in response to YCW treatment. Digestibility of organic matter was not significantly affected by either HWY or YCW. The effects of LHWY on ruminal fermentation characteristics were negligible. These results indicate that degree of hydrolysation (low or high) and composition of yeasts (whole cell or cell wall) have remarkable effects on ruminal microbial activity in the Rusitec system.

Keywords: hydrolysed yeast; fermentation, rumen; *Saccharomyces cerevisiae*

Antibiotic feed additives have been successfully used in ruminant rations to improve the efficiency of nutrient use and thereby maximise animal production and lower product cost. However, the risk of residues in animal products as well as the concern about the appearance of resistant strains of bacteria led to the prohibition of antibiotic use in animal feeds in the European Union in January 2006 (OJEU 2003). This has shifted the focus of researchers to the study of natural alternatives to manipulate ruminal fermentation in order to improve ruminant productivity.

Yeast products based on *Saccharomyces cerevisiae* have been used to improve productivity in ruminants and represent an attractive alternative to antibiotic feed additives. Certain studies have indicated that supplementation of yeast to the diet may improve feed intake (Williams et al. 1991), weight gain (Tripathi and Karim 2011), digestion (Jouany et al. 1998), numbers of anaerobic and cellulolytic bacteria (Newbold et al. 1995), ruminal pH value (Bach et al. 2007) and alter the patterns of SCFA (Marden et al. 2008). However, animal responses to yeast supplementation have not been consistent. These inconsistencies may have arisen from differences in diet composition, the dose, type and quality of yeast used, and physiological stage of animal tested.

In most of the published literatures, live strains of *Saccharomyces cerevisiae* have been studied. Limited data are available on the effects of inactivated yeasts on rumen microbial fermentation. In a previous study, we demonstrated that live and autoclaved probiotic yeast *Saccharomyces boulardii* (synonym *Saccharomyces cerevisiae* Hansen CBS 5926) stimulated rumen microbial metabolism without major differences occurring between treatments, and that *Saccharomyces boulardii* func-

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tions as a prebiotic rather than as a probiotic agent (Oeztuerk et al. 2005). The objective of the current study was to evaluate the effects of two different doses of three different hydrolysed yeast products from *Saccharomyces cerevisiae* on *in vitro* ruminal fermentation of medium concentrate diet.

MATERIAL AND METHODS

Incubation technique. The present study was carried out using the rumen simulation technique (Rusitec) described by Czerkawski and Breckenridge (1977). The complete unit consisted of six fermentation vessels with an effective volume of 750 ml each. Inocula for the fermentation vessels were obtained from a pooled sample from two mature ruminally cannulated sheep fed meadow hay and pelleted concentrate. The same diet was also used for *in vitro* incubation trials (Table 1). Fermentation inocula (solid and liquid) were collected through the rumen cannula immediately before the morning feeding and transferred to the Rusitec system. On the first day of the study, one nylon bag (150 µm pore size) was filled with 80 g of solid rumen contents to inoculate particle-associated microorganisms into the system and the other (150 µm pore size) with the daily diet, a mixture of 4 g of pelleted concentrate and 5 g of chopped meadow hay (approximately 0.5 cm pieces). The vessels were filled with rumen fluid to inoculate fluid-associated microorganisms. The nylon bag with solid rumen contents was replaced after 24 h of incubation with a bag containing the daily diet. The feed bag was changed after 48 h so that two bags were always present. This gave a retention time of 48 h for feed. When the bag was being changed, the vessels were flushed with nitrogen 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 was given in Table 2.

Table 1. Analysed composition of the experimental diet (g/kg as-fed basis)

Ingredient	Meadow hay	Pelleted concentrate
Dry matter	939	912
Crude protein	73	180
Crude lipids	9	39
Crude fibre	276	95
Total ash	61	72

Table 2. Chemical composition of the buffer solution (mmol/l)

Ingredients	
NaCl	28.00
KCl	7.69
$CaCl_2 \cdot 2H_2O$	0.22
$MgCl_2 \cdot 6H_2O$	0.63
NH_4Cl	5.00
$Na_2HPO_4 \cdot 12H_2O$	10.00
$NaH_2PO_4 \cdot H_2O$	10.00
NaH_2CO_3	97.90

Experimental procedures. Three incubation trials were carried out independently using six Rusitec fermentation vessels. Experimental procedures similar to those reported by Wallace et al. (1981), Durix et al. (1991) and Breves et al. (2000) were used in these trials. In **Experiment 1**, six vessels of the Rusitec system were run for a total period of 22 days. The first six days served as an adaptation period to allow for equilibration of microbial populations. The following six days were used to determine basic parameters of microbial fermentation under control conditions (control period). The last 10 days represented the treatment period during which the six fermentation vessels were divided into two groups with three vessels per group. Two doses, 0.25 or 0.75 g of hydrolysed whole yeast (HWY), were added daily to the first and second groups, respectively. The product used as HWY was Progut® Rumen (Suomen Rehu Oy, Helsinki, Finland), which is whole brewery yeast (*Saccharomyces cerevisiae*) hydrolysed according to a patented process (European patent no. 0946108). The process consists of heat treatment (70 °C) of the yeast for inactivation, storage below 10 °C in a storage tank and evaporation of water with an evaporator to raise the dry matter content of the yeast to up to 35–40%. After processing with acid-alkaline treatment, the product is spray-dried, cooled, sieved, and bagged. Unlike cell wall products, HWY also contains the extract components of the yeast. The composition of HWY as stated by the manufacturer was 34% crude protein, 1.8% crude fat, 25% crude ash and 5% moisture.

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Experiment 2 was performed under the same conditions as Experiment 1, except that in this experiment, less hydrolysed whole yeast (LHWY, Suomen Rehu, Espoo, Finland) was used. This product was made of the same raw material as HWY. The only difference was that this preparation was hydrolysed about three times less than the standard HWY because of the process in which lower amounts of acid and alkaline are used.

The experimental setup in **Experiment 3** was basically the same as in Experiments 1 and 2. However, in this experiment, control and treatment periods were five days each. During the treatment period, yeast cell wall product (YCW, Bio-Mos®, Alltech Inc, Nicholasville, KY, USA) was added directly to the fermentation vessels at levels of 0.25 or 0.75 g/day. Bio-Mos® is a yeast product derived from the outer cell wall of a specific strain of the yeast *Saccharomyces cerevisiae*.

The following considerations were the basis for the chosen yeast dosages. The Rusitec system is a dilute form of rumen fermentation, designed as such to give a final pH and SCFA concentrations similar to those found *in vivo*. Because of a lack of absorption of SCFA in the Rusitec system, their pH-depressing effects are decreased by dilution. As reported by Newbold et al. (1998), 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. Thus, in our experiments, the proportions of yeast products added to the Rusitec vessels were up to 8.3% of the feed whereas these amounts would be equivalent to 0.3% or less *in vivo*.

Samplings and analytical procedures. 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 SCFA and NH_3-N. The overflow flasks were placed into ice to stop microbial activity and preserve fermentation products. An aliquot of effluent was centrifuged at 40 000g for 20 min at 4 °C. The resulting supernatant was acidified with 0.1 ml of 98% formic acid and then centrifuged at 4000g for 10 min at 4 °C. The supernatant was analysed for SCFA by gas chromatography (model 5890 II, Hewlett Packard, Boblingen, 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 SCFA were estimated by multiplying the respective concentration by the volume of effluent collected. The concentration of NH_3-N in rumen fluid was measured using a colorimetric method (Weatherburn 1967). Methane production was calculated using the equations proposed by Abdl-Rahman (2010), based on the stoichiometry of Wolin (1960), as following:

$$\text{Fermentative } CO_2 = A/2 + P/4 + 1.5\ B$$
$$\text{Fermentative } CH_4 = (A + 2\ B) - CO_2$$

were:
A = mole of acetate
P = mole of propionate
B = mole of butyrate

Chemical compositions of experimental diets were analysed according to AOAC (2000). Analyses were conducted for moisture (AOAC method 925.09), crude protein (AOAC method 979.09), crude fat (AOAC method 4.5.01), crude fibre (AOAC method 962.09) and total ash (AOAC method 923.03). Organic matter content was determined based on the value of dry matter content minus ash content. The digestibility of organic matter was calculated from the organic matter contents present in the nylon bags before and after 48 h of incubation.

Statistical analysis. The constancy of equilibrium conditions during the un-supplemented control period was tested by one-way ANOVA with Statistica 5.0 (Statsoft, Inc., Tulsa, OK) within a repeated measures design (fermentation vessels in different incubation times). None of the parameters were significantly affected during this period. Therefore, mean values of the control measurements in the respective fermentation vessels served as control values to determine treatment effects of hydrolysed yeast products by one-way ANOVA. In the case of a significant result in the post-test, Dunnett's procedure for pair-wise multiple comparisons was used. Two-way ANOVA was performed in order to test the statistical significance of the two fixed effects, "treatment" and "time course", and the interaction of "treatment" with "time course" within a repeated-measures design. *P*-values of < 0.05 were considered significant.

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RESULTS

The effects of HWY on ruminal fermentation in the Rusitec system were examined in experiment 1 (Table 3). Compared with the control period, the culture pH in fermentation vessels was decreased by the addition of HWY, but these decreases were only statistically significant ($P < 0.05$) on Day 15 and Day 16 after the inclusion of 0.25 or 0.75 g, respectively.

A daily dose of 0.75 mg HWY increased the production rate of total SCFA from 22.19 ± 0.28 during the six-day control period to 25.55 ± 0.35 mmol/day. This increase was statistically significant ($P < 0.05$) from Days 9 to 15 (Figure 1). On the other hand, the results of two-way ANOVA showed no significant dose effect or treatment by time (day) interaction. In general, increased SCFA production with 0.75 mg HWY was mediated by respective changes in the production rates of acetate and propionate. Acetate and propionate production were increased ($P < 0.05$) by 15 and 24%, respectively. The addition of 0.25 g HWY resulted in significant increases ($P < 0.05$) in the production rates of acetate and propionate only on Day 11 and 16, respectively. Compared with the control period, 0.75 g HWY had no significant effect on butyrate production. However, a transient reduction ($P < 0.05$) in butyrate production (with a minimum on Day 2 after starting treatment) was recorded in response to 0.25 g HWY. At the same time, butyrate production was significantly higher ($P < 0.05$) in 0.75 g HWY compared with 0.25 g HWY.

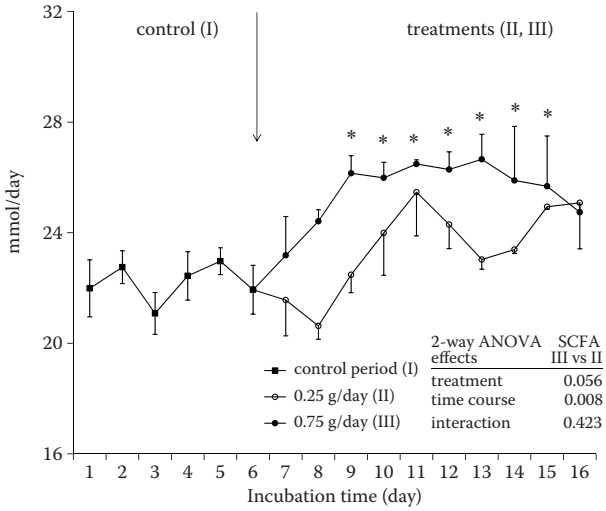

Figure 1. Effect of HWY on total SCFA production in Rusitec (Experiment 1). Vertical bars denote the SEM of vessels at each sampling time. Observation number is six for control period and three for treatments. Dunnett's method was used for multiple comparisons

*significantly different from control period within each treatment dosage in the same horizontal line ($P < 0.05$)

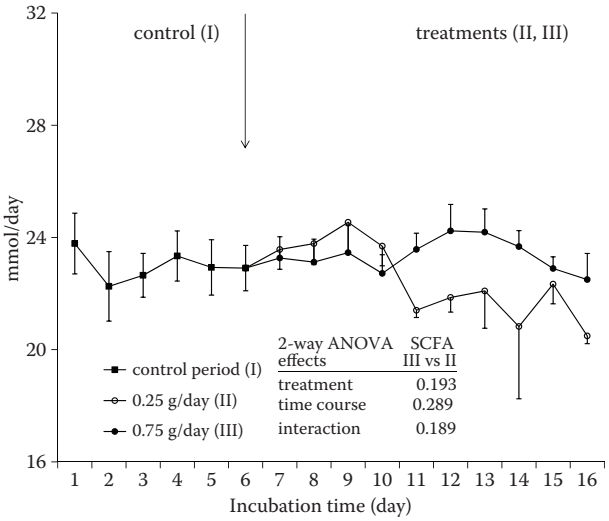

Figure 2. Effect of LHWY on total SCFA production in Rusitec (Experiment 2). Vertical bars denote the SEM of vessels at each sampling time. Observation number is six for control period and three for treatments

Methane production and NH_3-N concentration were significantly increased ($P < 0.05$) by the addition of 0.75 g HWY when compared with the control period. However, 0.25 g HWY had no significant effects on the same parameters.

Digestibility of organic matter was not significantly affected by HWY addition and ranged between 49.25 ± 0.63% during the control period and 47.70 ± 1.20% and 46.93 ± 0.93% in the presence of 0.25 g/day and 0.75 g/d HWY, respectively.

Experiment 2 was performed under the same experimental conditions as those used in experiment 1 except that LHWY was used instead of HWY (Table 4). In general, LHWY had no significant effects on most of the measured parameters as compared with the un-supplemented control period. Ruminal pH and the production of total SCFA (Figure 2), acetate and methane were not significantly affected by LHWY. However, a significant treatment × time interaction ($P = 0.019$) was recorded for propionate production. Propionate productions were greater for 0.75 g LHWY compared with 0.25 g LHWY from Days 12 to 14. Butyrate productions in the fermentation vessels were not significantly altered by either levels of LHWY addition. The production rates of butyrate, however,

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Table 3. Effects of HWY on ruminal fermentation in Rusitec (Experiment 1)

Variable	Control period (day) 1–6	Treatments (g/day)	Experimental period (day)									SEM	Two-way ANOVA, *P*-values		
			7	8	9	10	11	12	13	14	15	16		treatment	time	interaction
pH	6.80	0.25	6.81	6.82	6.80	6.79	6.76	6.76	6.76	6.74	6.73*	6.77	0.004	0.297	0.001	0.733
		0.75	6.78	6.77	6.80	6.79	6.76	6.77	6.75	6.75	6.74	6.74*				
Acetate (mmol/day)	12.96	0.25	12.98	12.32	13.34	13.88	15.10*	14.24	13.36	13.66	14.66	14.90	0.142	0.048	0.017	0.393
		0.75	13.51	14.23	15.26*	15.04*	15.34*	15.46*	15.52*	15.11*	15.25*	14.45				
Propionate (mmol/day)	4.36	0.25	4.34	4.20	4.45	5.05	5.06	5.12	5.00	4.99	5.38	5.40*	0.073	0.145	0.026	0.782
		0.75	4.68	5.05	5.50*	5.42*	5.56*	5.58*	5.85*	5.62*	5.41*	5.46*				
Butyrate (mmol/day)	4.87	0.25	4.25	4.12*	4.69	5.06	5.31	4.94	4.67	4.74	4.90	4.78	0.048	0.050	0.001	0.255
		0.75	4.99	5.13	5.39	5.52	5.58	5.25	5.29	5.16	5.02	4.82				
Methane (mmol/day)	7.82	0.25	7.53	7.17	7.90	8.21	8.94	8.31	7.76	7.95	8.43	8.49	0.075	0.042	0.008	0.307
		0.75	8.08	8.42	8.95*	8.93	9.07*	8.96*	8.94	8.73	8.78	8.27				
NH_3-N (mmol/l)	10.53	0.25	10.22	10.67	11.08	10.87	10.74	10.19	10.37	10.31	10.09	10.29	0.070	0.005	0.001	0.594
		0.75	10.36	11.57*	11.81*	12.17*	11.79*	11.45	10.95	11.01	11.10	11.38				
OMD (%)	49.25	0.25	49.91	40.78	42.77	46.69	50.33	49.83	50.01	44.49	50.09	52.11	0.577	0.581	0.533	0.516
		0.75	40.55	45.85	49.70	44.54	49.71	50.00	48.68	47.14	45.67	47.48				

Observation number is six for control period and three for treatments. Dunnett's method was used for multiple comparisons
HWY = hydrolysed whole yeast, SEM = standard error of mean, OMD = organic matter digestibility
*significantly different from control period within each treatment dosage in the same horizontal row ($P < 0.05$)

were significantly higher ($P = 0.006$) on Days 8 and 16 for the fermentation vessels supplemented with 0.75 g LHWY than for the vessels supplemented with 0.25 g LHWY.

The addition of 0.75 g/day LHWY resulted in a significant increase ($P < 0.05$) in ruminal NH_3-N concentration from days 10 to 16 when compared with the un-supplemented control period, while 0.25 g/day of LHWY had no significant effect.

The digestibility of organic matter was significantly decreased ($P < 0.05$) in response to both levels of LHWY addition. Furthermore, a significant treatment by time interaction ($P = 0.032$) was detected; the digestibility of organic matter was significantly lower for 0.75 g LHWY on Days 10 and 15 than for 0.25 g LHWY.

In experiment 3, the effects of daily addition of YCW on rumen microbial fermentation were evaluated (Table 5). The pH values of rumen fluid ranged between 6.79 and 6.84 and were neither affected by 0.25 g nor by 0.75 g YCW addition.

The daily addition of 0.75 g YCW resulted in a significant increase ($P < 0.05$) in the production of total SCFA (Figure 3), acetate, propionate, butyrate, and methane, when compared with the control period. At the same time, a dose-related increase ($P = 0.050$) in propionate production was observed in response to increasing doses of YCW.

Significant increases in NH_3-N concentrations were recorded from Days 8 to 10 after the addition of 0.75 g YCW. *In vitro* dry matter digestibility was not significantly affected by the addition of YCW.

DISCUSSION

The present study was designed to evaluate the effects of two different doses of three different hydrolysed yeast supplements on *in vitro* ruminal fermentation of medium concentrate diet. Overall, the hydrolysed yeast products tested in this study had only slight effects on the culture pH in the Rusitec vessels; the pH value was lower for only one day at the end of the study with HWY compared with the un-supplemented control period. All the measured values, however, always remained

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Table 4. Effects of LHWY on ruminal fermentation in Rusitec (Experiment 2)

Variable	Control period (day) 1–6	Treatments (g/day)	Experimental period (day) 7	8	9	10	11	12	13	14	15	16	SEM	Two-way ANOVA, *P*-values treatment	time	interaction[1]
pH	6.80	0.25	6.81	6.81	6.79	6.84	6.84	6.83	6.82	6.79	6.85	6.83	0.003	1.000	0.263	0.221
		0.75	6.83	6.83	6.84	6.83	6.82	6.79	6.81	6.81	6.85	6.81				
Acetate (mmol/day)	13.83	0.25	14.20	14.38	14.96	14.44	12.94	13.20	13.36	12.67	13.84	12.79	0.119	0.366	0.685	0.131
		0.75	13.91	13.62	13.96	13.47	14.04	14.46	14.57	14.21	14.04	13.93				
Propionate (mmol/day)	4.72	0.25	4.91	4.90	5.01	4.76	4.16	4.23	4.27	4.13	4.38	3.94	0.055	0.099	0.171	0.019
		0.75	4.68	4.56	4.84	4.50	4.82	5.09	5.11	5.01	4.58	4.48				
Butyrate (mmol/day)	4.42	0.25	4.45	4.50	4.57	4.49	4.31	4.42	4.47	4.02	4.11	3.76	0.048	0.345	0.006	0.981
		0.75	4.67	4.94	4.66	4.75	4.72	4.68	4.51	4.45	4.27	4.09				
Methane (mmol/day)	7.95	0.25	8.10	8.22	8.52	8.28	7.59	7.75	7.85	7.31	7.88	7.29	0.063	0.350	0.442	0.551
		0.75	8.12	8.14	8.10	7.98	8.17	8.30	8.26	8.08	8.01	7.89				
NH_3-N (mmol/l)	11.45	0.25	10.94	10.84	11.09	11.02	11.42	11.55	11.74	12.26	12.14	11.75	0.113	0.038	0.000	0.611
		0.75	11.74	11.91	12.56	13.11*	13.14*	13.33*	13.02*	13.85*	13.97*	14.04*				
OMD (%)	50.41	0.25	49.25	50.19	48.52	50.05	39.47*	42.23	42.11	44.53	49.87	40.27*	0.632	0.076	0.056	0.032
		0.75	47.21	43.50	41.70	40.06*	41.98	45.03	47.69	45.57	40.01*	38.58*				

Observation number is six for control period and three for each treatment. Dunnett's method was used for multiple comparisons
LHWY = less hydrolysed whole yeast, SEM = standard error of mean, OMD = organic matter digestibility
[1]multiple comparisons regarding significant interactions were given in the text
*significantly different from control period within each treatment dosage in the same horizontal row ($P < 0.05$)

within a normal physiological range (6–7). This decrease in pH with HWY is difficult to explain and is perhaps of little biological significance. Published reports on the effect of yeasts on ruminal pH are variable. In an *in vitro* study, Newbold et al. (1995) reported that Yea-Sacc 1026 Strain (0.59 g/l) did not modify ruminal pH. Similarly, Lila et al. (2004) observed no effect of a twin-strain of *Saccharomyces cerevisiae* live cells (0.33 to 1.32 g/l) on pH. In contrast, Bach et al. (2007) demonstrated that yeast supplementation (*Saccharomyces cerevisiae* Strain CNCM I-1077) to dairy cattle (5 g/day) contributes to the rumen environment by enhancing the ruminal pH. On the other hand, Mutsvangwa et al. (1992) showed that ruminal pH was depressed by the addition of yeast culture (Yea-Sacc) in bulls (8 to 10 g/day). Nevertheless, one of the main effects of yeasts is to stabilise the rumen pH. But what causes this stabilisation in the rumen pH is not clear. Among different hypotheses explaining the effects of yeasts on ruminal pH, the ability of yeasts to stimulate the uptake and growth of L-lactate-utilising bacteria was proposed by Nisbet and Martin (1991) and Williams et al. (1991). This action would result in a reduction of lactic acid and, thus, a stabilisation in the ruminal pH. The mechanism by which the hydrolysed yeast products induced the stabilisation in culture pH could not be clarified.

In the current study, a daily dose of 0.75 g HWY and YCW generally increased the production of total and individual SCFA. This increased production is indicative of an increased fermentation taking place in the Rusitec vessels. The stimulation of microbial fermentation can be beneficial in providing more energy for microbial growth and for the maintenance and production activity of the ruminant. As is well known, SCFA are the most important by-products of fermentation and the main energy source for ruminants, contributing 50–75% of the total energy supply (Faverdin 1999). Although HWY and LHWY were made from the same raw material and the only difference was that LHWY was hydrolysed about three times less than HWY, LHWY had very small and generally non-significant effects on ruminal fermenta-

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Table 5. Effects of YCW on ruminal fermentation in Rusitec (Experiment 3)

Variable	Control period (day) 1–5	Treatments (g/day)	Experimental period (day)					SEM	Two-way ANOVA, *P*-values		
			6	7	8	9	10		treatment	time	interaction
pH	6.82	0.25	6.82	6.82	6.82	6.83	6.82	0.003	0.354	0.441	0.762
		0.75	6.79	6.82	6.80	6.83	6.80				
Acetate (mmol/day)	13.55	0.25	13.77	13.97	13.97	14.70	14.51	0.203	0.058	0.261	0.921
		0.75	15.63*	15.56	16.23*	16.71*	17.19*				
Propionate (mmol/day)	4.47	0.25	4.67	4.65	4.62	4.72	4.70	0.079	0.050	0.630	0.745
		0.75	5.26	5.32*	5.49*	5.58*	5.92*				
Butyrate (mmol/day)	4.01	0.25	3.87	3.93	3.96	4.17	4.18	0.072	0.132	0.557	0.562
		0.75	4.63	4.45	4.89*	4.12	5.11*				
Methane (mmol/day)	7.66	0.25	7.66	7.79	7.81	8.26	8.17	0.111	0.074	0.255	0.788
		0.75	8.82*	8.68	9.19*	9.02*	9.67*				
NH_3-N (mmol/l)	11.27	0.25	11.05	11.95	11.88	12.08	12.45	0.127	0.301	0.005	0.309
		0.75	11.80	11.59	12.90*	12.99*	13.47*				
OMD (%)	51.49	0.25	43.59	45.12	49.93	48.83	51.83	0.644	0.308	0.278	0.095
		0.75	47.77	52.34	46.00	50.40	48.92				

Observation number is six for control period and three for each treatment. Dunnett's method was used for multiple comparisons
YCW = yeast cell wall, SEM = standard error of mean, OMD = organic matter digestibility
*significantly different from control period within each treatment dosage in the same horizontal row ($P < 0.05$)

tion. This may be due to the fact that smaller particles of HWY can be more easily metabolised by rumen microorganisms. In a previous study, we investigated the effects of live and autoclaved probiotic yeast

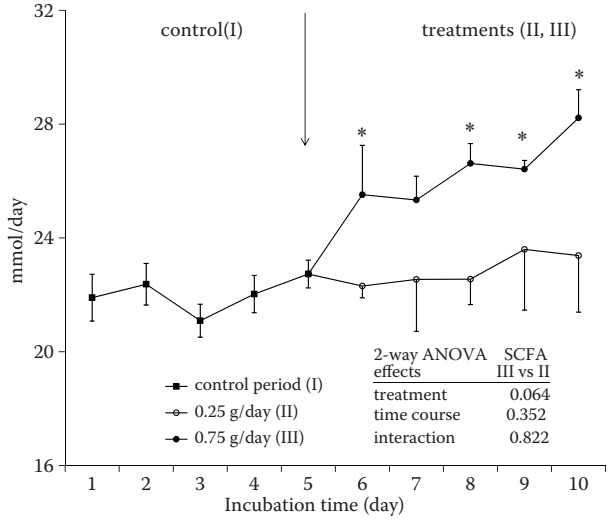

Figure 3. Effect of YCW on total SCFA production in Rusitec (Experiment 3). Vertical bars denote the SEM of vessels at each sampling time. Observation number is six for control period and three for treatments. Dunnett's method was used for multiple comparisons

*significantly different from control period within each treatment dosage in the same horizontal line ($P < 0.05$)

(*Saccharomyces boulardii*) on rumen microbial metabolism. The living yeast cells were killed and lysed by the autoclaving process. The results showed that both live and autoclaved forms of yeast stimulated *in vitro* ruminal microbial metabolism, without major differences occurring between treatments, and we concluded that ruminal microbes digested yeast cells as a substrate (as prebiotic) rather than utilising it as a probiotic (Oeztuerk et al. 2005). Some authors also reported that yeasts provide various growth factors, pro-vitamins, and/or micronutrients that help stimulate microbial metabolism in the rumen (Miller-Webster et al. 2002).

Except for LHWY, ruminal methane production was increased by the addition of 0.75 g HWY and YCW. These results are consistent with the data of Opsi et al. (2012), who found that ruminal methane production was increased by inactivated cells of *Saccharomyces cerevisiae* (0.25 to 0.5 g/l) when total mixed rations with variable forage-to-concentrate ratios (ranging from 0.2 : 0.8 to 0.4 : 0.6) were fermented. Similar results in methane production have been also observed with the *in vitro* incubation of other *Saccharomyces cerevisiae* cultures (up to 1 g/l) with mixed rumen microflora (Martin et al. 1989). Despite increased methane production, there was a trend toward lower acetate-to-propi-

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onate ratio with HWY and YCW compared with the control incubations (2.97 vs. 2.76 for HWY and 3.03 vs. 2.94 for YCW). This means that these yeast products would be able to improve the utilisation of feed energy for production purposes. It is well known that propionate is the only major volatile fatty acid that contributes to hepatic gluconeogenesis (Young 1977). On the other hand, propionate is energetically more efficient because its production is in indirect competition with methanogens for metabolic hydrogen (Hegarty 1999).

The effects observed on NH_3-N with hydrolysed yeast products are consistent with previous *in vitro* and *in vivo* experiments using inactivated yeast products (Oeztuerk et al. 2005; Oeztuerk 2009; Vyas et al. 2014). The increase in NH_3-N concentration, especially at 0.75 g, can be associated with the microbial degradation of yeast products because of their high protein content. However, most of the early animal studies have shown either no effects (Thrune et al. 2009; Tripathi and Karim 2011) or reduced ruminal NH_3-N concentrations (Lascano and Heinrichs 2007; Hristov et al. 2010) in response to yeast supplementation. These differences among studies might have been due to differences in yeast preparations, type of diets and/or experimental conditions.

Paradoxically, in spite of increased fermentation rate, organic matter digestibility was not enhanced significantly by addition of HWY and YCW. This result suggests that rumen microorganisms digested the supplied yeasts as an additional substrate. A similar response in diet digestibility was observed by Opsi et al. (2012) when inactivated (Thepax 100 R) and live yeasts (Yea-Sacc) were added (up to 0.5 g/l), and by Carro et al. (1992) with the addition of yeast culture (0.17 g/l; Yea-Sacc) to a medium concentrate diet.

CONCLUSIONS

Our experiments demonstrated that the degree of hydrolysis (low or high) and composition of yeast (whole cell or cell wall) had remarkable effects on ruminal fermentation in the *in vitro* semi-continuous culture system (Rusitec). In most cases, rumen fermentation was stimulated by hydrolysed yeast products, especially those with a high degree of hydrolysis and at the 0.75 g/day level. If these effects would also be induced when hydrolysed yeasts are added to the diets of ruminants, beneficial responses could be expected.

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