

FIBROLYTIC ENZYMES AND LIVE YEAST CULTURES IN RATIONS FOR DAIRY COWS – EFFECT ON RUMEN DEGRADABILITY AND FERMENTATION*

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Abstract

The aim of the study was to determine if the addition of exogenous fibrolytic enzymes and/or live yeast cultures to rations for dairy cows will increase rumen degradability and rumen fermentation. In the first stage of the experiment with 3 dry cows fitted with rumen fistulas, ruminal degradation of NDF of roughages (meadow hay), starch of concentrates (barley) and protein of high-protein feed (soybean meal) was determined using the nylon bag technique. The degradability was determined after 21-day periods of feeding either unsupplemented ration (group C), or the ration with enzyme preparation (Fibrozyme™) containing active cellulase and xylanase (group E), or yeast preparation (Yea Sacc¹⁰²⁶) containing *Saccharomyces cerevisiae*¹⁰²⁶ yeast culture (group D), or both (group ED). The incubation times were 2, 4, 8, 16, 24, 48, 72 h for meadow hay; 2, 4, 8, 16, 24, 48 h for barley grain; and 4, 16, 24 h for soybean meal. In the second stage of the experiment, 24 cows being in the first period of lactation were assigned to 4 groups (with 6 animals per group) receiving TMR diets either unsupplemented (control group C) or supplemented with the same preparations as in experiment 1 (groups E, D and ED, respectively). The parameters determined were pH, NH₃-N content, total volatile fatty acids (VFA), and content of individual fatty acids in the samples of ruminal fluid, which were collected by stomach tube 3 h before the morning feeding and 3 h after feeding. The addition of exogenous fibrolytic enzymes and/or live yeast cultures to the standard ration increased significantly degradability in the rumen of meadow hay DM and NDF, barley grain DM and starch, and soybean meal DM and CP. Moreover, supplementation of diets with the enzyme and yeast preparations increased total VFA content and individual fatty acids content in total VFA. The preparations had no effect on pH and NH₃-N content in rumen fluid pre- and postprandially.

Key words: dairy cows, fibrolytic enzymes, yeast cultures, rumen, fermentation, degradation

The high production potential of modern Holstein-Friesian (HF) dairy cows caused conventional feeding and housing systems to become less effective (Strze-

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telski et al., 2009). Although modern dairy cows are genetically adapted to increased feed intake, reduced feed intake capacity and energy deficit are observed at some stages of the production cycle, especially in early lactation (Drackley, 1999). Feeding rations high in starchy concentrates during this period with inadequate supply of structural feed (roughages) may excessively lower the pH in the rumen, change the proportions and type of VFA in the rumen and influence the activity of ruminal microorganisms (Rabelo et al., 2003). In addition, high amounts of concentrates in the ration may lead to metabolic disorders (fatty degeneration of the liver, rumen acidosis, displacement of the abomasum), laminitis (Nocek, 1997), and impaired reproductive function (Staples et al., 1990). Rumination and gastrointestinal motility disorders and inhibition of rumen fermentation were also observed (Nocek, 1997; Rabelo et al., 2003). A considerable role in preventing these disorders is played by the stabilization of the digestive processes in the rumen, by increasing the digestibility of structural carbohydrates from roughages and by stimulation of appetite and feed utilization (Staples et al., 1990). Increasing attention has been given to the potential role of new generation feed additives, including live yeast cultures (especially the *Saccharomyces cerevisiae* strain) or their metabolites (Ghorbani et al., 2002; Denev et al., 2007) and exogenous fibrolytic enzymes (Beauchemin et al., 2003; Bowman et al., 2002; Nowak et al., 2003). In recent years attempts have also been made to feed dairy cows with a composite of several types of probiotic preparations (Erasmus et al., 2005; Mwenya et al., 2005; Lehloenyana et al., 2008), assuming their synergistic effect on the productivity and health of animals. However, only few comparative studies were conducted on the effect of different bacterial or yeast probiotic preparations on dairy cow health and productivity (Mwenya et al., 2005). Therefore, the present study was aimed to determine if the addition of live yeast cultures and/or exogenous fibrolytic enzymes to TMR diets for dairy cows will provide optimal conditions for rumen fermentation and nutrient degradability.

Material and methods

Yea-Sacc¹⁰²⁶ (a viable yeast culture based on the *Saccharomyces cerevisiae* yeast strain 1026 – Alltech Inc., USA), the enzyme preparation FibrozymeTM (powder preparation containing xylanase and cellulose from *Aspergillus niger* and *Trichoderma longibrachiatum* fungi fermentation extract with a cellulase and xylanase activity of 31.0 and 43.4 UI – Alltech Inc., USA), or a mixture of both preparations were used in both stages of experiment. The effect of the preparations, used alone or in combination, on the degradability of NDF roughages (meadow hay), starch from concentrate (barley grain) and protein from high-protein feed (soybean meal) was investigated in stage 1 of experiment. On the other hand in stage 2 the effect of these preparations on rumen fermentation in early lactation Polish Holstein-Friesian cows (PHF) was determined. The research was carried out in the years 2006–2008 at the Rudawa Experimental Station of the National Research Institute of Animal Production (stage 2), experimental farm in Aleksandrowice (stage 1).

Stage 1 was carried out with three dry cows fitted with permanent rumen fistulas (ANKOM Products, Fairport, NY, USA), 12.5 cm in diameter. Animals were kept in non-litter tie-up stalls equipped with automatic drinkers and trough partitions. Cows were fed individually twice daily (at approx. 0800 and 1500 h) with a standard diet composed of 6 kg meadow hay and 2.2 kg concentrate (0.98 UFL, 127 g PDIN and 115 g PDIE in DM). The weight ratio of roughage to concentrate dry matter in the ration was 70:30. The diet met maintenance requirements of cows (IZ-INRA, 2001). The diet was then supplemented with the enzyme preparation (15 g/animal/day), yeast preparation (10 g/animal/day) or both preparations (25 g/animal/day), which were added to the concentrate during morning feeding.

Rumen degradability was determined by *in sacco* method (Michalet-Doreau et al., 1987). Experimental feeds (meadow hay, barley grain, soybean meal) intended for incubation were dried in a 50°C forced-air drying oven to obtain permanent DM (air-dry samples). Then the samples were pulverized in a Fritsch mill (Germany) fitted with 1.5-mm mesh screens. Ankom polyester bags (internal dimensions 9 × 10 cm) were dried at 80°C, filled with 3 g of feed, sealed with a heat sealer (AVC, Super-Magnet-Sealer, The Netherlands) and then placed into a weighted large-mesh bag (35 cm × 60 cm, pore size 5 mm) and inserted into the ventral sac of the rumen. Degradability runs were performed four times, when the cows were fed diet without the preparations (control C) and when the diet fed contained one or both preparations – enzyme preparation (Fibrozyme™) containing active cellulase and xylanase (group E), or yeast preparation (Yea Sacc¹⁰²⁶) containing *Saccharomyces cerevisiae*¹⁰²⁶ yeast culture (group D), or both (group ED). Incubation time varied according to incubated feed (h): 2, 4, 8, 16, 24, 48 and 72 for hay; 2, 4, 8, 16, 24 and 48 for barley grain; 4, 16 and 24 for soybean meal. Before and after incubation, feed samples were analysed for NDF (Van Soest et al., 1966), starch (Faisant et al., 1995), DM and crude protein (AOAC, 1995).

Stage 2 was conducted in a production facility with 24 early-lactation PHF cows with daily milk yield of 30–35 kg. Animals were kept in tie-up stalls bedded with straw and equipped with automatic drinkers and trough partitions. The 35-day experiment consisted of a 14-day preliminary period, in which animals were adapted to the analysed diet, and a 21-day trial period. Cows were assigned to four groups (with 6 animals per group) based on the analogue principle according to lactation number, milk yield during the previous 100-day lactation and expected calving date. In the control group (C) TMR contained no preparations, whereas the experimental cows received the same ration supplemented either with the enzyme preparation Fibrozyme™ (15 g/animal/day) in group E; the yeast preparation Yea-Sacc¹⁰²⁶ (10 g/ animal/day) in group D; or a mixture of both preparations (25 g/animal/day) in group ED. The preparations were added during the morning feeding to the concentrates. Cows were fed individually a complete diet (TMR) formulated according to the IZ-INRA system (2001). The TMR (containing in 1 kg DM: 0.88 UFL, 97 g PDIN and 93 g PDIE) consisted of (% DM): maize silage 27.1, wilted meadow grass silage 24.8, ensiled brewers' grains 5.3, meadow hay 3.7, concentrate mixture 39.1, containing (%) ground maize 21, ground wheat 13, ground barley 18, ground triticale 9, soybean meal 18, rapeseed meal 7.9, rapeseed cake 4.8, ground limestone

4, premix CJ 4, sodium bicarbonate 0.3. The TMR was offered twice daily. At day 21 of the trial ruminal fluid was collected from each cow using a stomach tube connected to a suction pump. Samples of ruminal fluid were collected twice, before the morning feeding and 3 h after feeding. The ruminal fluid was immediately analysed for pH using a Mera Tronik N517 pH meter. The other determinations in the samples of ruminal fluid (i.e. VFA and $\text{NH}_3\text{-N}$ content) were performed in a laboratory. For VFA determination the samples were stabilized by metaphosphoric acid whereas for $\text{NH}_3\text{-N}$ determination mercuric chloride was used. VFA analyses were performed by gas chromatography (Varian 3400CX, column DB-FFAP, 30 m \times 0.53 mm \times 1.0 micron, FID detection, 260°C, range 11, helium as carrier gas, 6 ml/min, injector temperature 200°C, analysis time 17 min, sample injection volume 1.0 μl), using 8200 CX autosampler. The $\text{NH}_3\text{-N}$ was determined using Conway's microdiffusion method (1962). All chemical analysis was performed at the Central Laboratory of the National Research Institute of Animal Production in Aleksandrowice, according to currents standards.

Statistical calculations of the results were performed by one-way analysis of variance (ANOVA) using SAS package (1996), and significance of differences between the groups was determined using Duncan's test.

Results

In sacco ruminal degradation of meadow hay DM and NDF, barley grain DM and starch, and soybean meal DM and CP is shown in Tables 1 to 3. The supplementation of the diet with the enzyme preparation (group E), the yeast preparation (group D) or a mixture of both (group ED) significantly ($P < 0.01$) increased the ruminal degradation of meadow hay DM and NDF between 4 and 72 h of incubation compared to group C (Table 1). The preparations had also a significant ($P < 0.01$ or $P < 0.05$) effect on increasing the degradation of barley grain DM and starch in the rumen (Table 2) and a significant ($P < 0.01$) effect on increasing the degradation of soybean meal DM and CP (Table 3).

The addition of the preparations (groups E, D and ED) increased ($P < 0.05$ or $P > 0.05$) total VFA content in the rumen fluid as well as the content of acids (acetic, propionic and butyric) in total VFA (Table 4). Regardless of the time when samples of ruminal fluid were collected (pre- and postprandially), the content of propionic and acetic acids was the highest in groups E and D, intermediate in group ED, and the lowest in the control group C. Compared to the other groups (C, E and ED), cows from group D had the highest percentage of propionic acid and animals from group E the lowest percentage of this acid in total VFA, with significant ($P < 0.05$) differences between groups E and D. Both before and after feeding, the lowest content of butyric acid was found in group C and the highest in group ED. Before feeding in group ED, the percentage of butyric acid in total VFA was significantly higher ($P < 0.01$) than in group D. Three hours postprandially, there were no significant differences between the groups in percentage of butyric acid in total VFA. The highest

acetic (C_2) to propionic acid (C_3) ratio was found in group E, intermediate in groups C and ED, and the lowest in group D, but the differences between the groups were not significant ($P>0.05$), both pre- and postprandially. The supplements had also no marked effect ($P>0.05$) on pH and concentration of ammonia (NH_3 -N) in the rumen fluid (Table 4).

Table 1. Ruminal degradation (%) of meadow hay DM and NDF at different incubation times

Ingredients	Incubation time (h)	Group				SEM	P
		C	E	D	ED		
DM	2	30.46	31.29	31.40	31.03	0.25	0.59
	4	32.70 B	36.50 A	33.47 B	33.25 B	0.38	0.001
	8	35.59 C	39.46 A	35.81 C	36.92 B	0.38	0.001
	16	40.97 B	42.96 A	39.39 B	39.45 B	0.39	0.001
	24	43.21 C	52.60 A	48.33 B	49.56 B	0.78	0.001
	48	63.66 C	69.25 A	66.07 B	67.85 AB	0.53	0.001
	72	73.73 C	81.74 A	75.00 C	78.62 B	0.76	0.001
NDF	2	1.02	1.63	2.07	1.93	0.16	0.09
	4	3.43 C	10.47 A	5.88 B	10.34 A	0.64	0.001
	8	6.96 D	12.31 A	8.49 C	10.15 B	0.46	0.001
	16	13.05 B	17.26 A	13.69 B	15.69 A	0.42	0.001
	24	20.72 C	31.57 A	24.42 B	30.32 A	0.98	0.001
	48	49.47 C	56.54 A	51.67 BC	53.43 AB	0.67	0.001
	72	63.88 C	76.65 A	66.27 C	71.68 B	1.08	0.001

A, B, C – values in rows with different letters differ highly significantly ($P<0.01$).

Table 2. Ruminal degradation (%) of barley grain DM and starch at different incubation times

Ingredients	Incubation time (h)	Group				SEM	P
		C	E	D	ED		
DM	2	60.96 B	65.59 A	64.54 AB	64.29 AB	0.56	0.01
	4	63.46 B	74.03 A	72.60 A	73.85 A	1.04	0.001
	8	74.70 B	80.85 A	77.05 B	81.03 A	0.69	0.001
	16	80.28 B	83.69 A	82.17 A	83.34 A	0.33	0.001
	24	84.96	86.70	83.63	85.83	0.45	0.04
	48	87.59 B	89.80 A	88.89 AB	88.44 AB	0.26	0.02
Starch	2	72.7 b	78.68 a	76.21 a	77.11 a	0.89	0.01
	4	75.17 b	82.68 a	81.46 a	82.24 a	1.61	0.01
	8	78.67 B	87.99 A	84.44 A	88.07 A	1.71	0.001
	16	84.13 b	90.52 a	89.81 a	89.02 a	1.55	0.05
	24	85.38 b	91.66 a	90.49 a	91.56 a	1.28	0.05
	48	85.63 C	91.79 B	92.57 AB	97.50 A	1.22	0.001

a, b, c – values in rows with different letters differ significantly ($P<0.05$).

A, B, C – values in rows with different letters differ highly significantly ($P<0.01$).

Table 3. Ruminal degradation (%) of soybean meal DM and CP at different incubation times

Ingredients	Incubation time (h)	Group				SEM	P
		C	E	D	ED		
DM	4	41.10 C	55.28 A	48.39 B	47.85 B	1.11	0.001
	16	77.06 B	79.91 A	84.12 B	80.03 B	0.72	0.001
	24	88.34 B	90.51 B	96.61 A	94.44 A	0.80	0.001
CP	4	26.17 C	43.30 A	38.37 B	37.50 B	1.34	0.001
	16	68.97 B	76.12 A	76.80 A	76.53 A	0.82	0.001
	24	83.77 C	92.25 B	96.79 A	93.03 B	0.95	0.001

A, B, C – values in rows with different letters differ highly significantly ($P < 0.01$).

Table 4. Content of ammonia ($\text{NH}_3\text{-N}$), volatile fatty acids (VFA), and pH of ruminal fluid

Item	Group				SEM	P
	C	E	D	ED		
Before morning feeding:						
pH	6.36	6.44	6.40	6.47	0.08	0.14
Ammonia (mg/dL)	51.6	54.0	48.1	58.7	1.21	0.14
Total VFA (mMol/L):	70.55	85.82	79.05	74.30	6.99	0.18
acetic (C2)	46.22	56.13	54.14	51.77	4.70	0.19
propionic (C3)	15.20 b	18.12 a	18.26 a	16.88 a	1.59	0.05
butyric	6.34	7.82	6.53	7.83	1.02	0.11
Proportion in total VFA (%):						
acetic (C2)	68.21	68.39	68.55	68.12	1.57	0.48
propionic (C3)	22.43 AB	22.08 B	23.12 A	22.21 B	1.85	0.01
butyric	9.35 AB	9.53 AB	7.88 B	10.63 A	1.05	0.01
C2:C3 acid ratio	3.04	3.10	2.96	3.07	0.33	0.06
3 h after feeding:						
pH	6.33	6.39	6.27	6.26	0.13	0.44
Ammonia (mg/dL)	115.3	117.9	114.8	118.3	1.27	0.21
Total VFA (mMol/L):	94.00 b	113.89 a	108.28 a	102.55 b	10.91	0.04
acetic (C2)	61.06 b	74.30 a	72.15 a	67.91 ab	5.59	0.03
propionic (C3)	20.11 c	23.92 b	26.09 a	22.58 bc	3.44	0.05
butyric	8.43 b	10.69 a	11.15 a	11.23 a	1.87	0.05
Proportion in total VFA (%):						
acetic (C2)	68.15	68.22	68.58	68.58	2.06	0.16
propionic (C3)	22.44 ab	21.96 b	24.80 a	22.80 ab	1.84	0.03
butyric	9.41	9.81	9.50	9.58	1.09	0.29
C2:C3 acid ratio	3.04	3.11	2.77	3.01	0.32	0.06

a, b, c – values in rows with different letters differ significantly ($P < 0.05$).

A, B, C – values in rows with different letters differ highly significantly ($P < 0.01$).

Discussion

The results of ruminal degradation experiment indicate that the feed additives used here had different effects on this process. Best results for degradation of meadow hay DM and NDF were obtained when the enzyme preparation was used. Similar findings for degradation of hay DM and NDF were also obtained by Yang et al. (1999) who supplemented exogenous fibrolytic enzymes in liquid form, by Giraldo et al. (2008) in an *in situ* experiment with sheep, and by Eun et al. (2007) in an *in vitro* experiment on degradation of hay and maize silage DM, NDF and ADF. In digestibility trials with fistulated cows, the degradation of TMR DM and NDF was higher when enzymes were used but only during the initial hours of incubation (Nowak et al., 2003) or as late as after 32 hours of incubation (Lewis et al., 1996). Nsereko et al. (2002) suggest that the exogenous fibrolytic enzymes may stimulate the proliferation of hemicellulose-degrading rumen bacteria, and thus improve the extent of roughage NDF degradation in the rumen and the digestibility of nutrients found under the cell wall of plants. This may be particularly important for feeding high-yielding dairy cows in early lactation, when decreased feed intake capacity could be compensated by better digestibility (Rode et al., 1999). Also adding the yeast preparation had a beneficial effect (although to a lesser degree than the enzyme preparation) on improving the ruminal degradation of meadow hay DM and NDF and barley grain starch, which can be attributed to the effect of yeast on increased activity of cellulolytic bacteria (Kumar et al., 1997). When the Yea-Sacc preparation was used, the digestibility of hay DM increased in an *in vitro* study (Lila et al., 2004), and the degradation of NDF and CP increased in an *in sacco* experiment with heifers and beef bulls (Olson et al., 1994). However, these results were not confirmed in experiment with dairy cows (Carro et al., 1992). The beneficial effect of the Yea Sacc¹⁰²⁶ yeast preparation on improving the ruminal degradation of soybean meal DM and CP at all incubation times could be due to the increased amount of proteolytic bacteria in the rumen as a result of using the yeast additive (Yoon and Stern, 1996). It is worth noting that there was no effect of mixture of both feed additives (enzymes and yeast) on the ruminal degradation of DM, CP, starch and NDF. Tang et al. (2008) in an *in vitro* study with a mixture of live yeast culture and exogenous fibrolytic enzymes, demonstrated that the best results for the degradation of DM and organic matter of incubated maize silage, wheat straw and rice straw were obtained for a mixture in which the ratio of yeast and enzyme is 5.0 and 7.5 g respectively in kg DM of the tested roughage.

There were no effects of the preparations on pH and NH₃-N content in the rumen fluid, which indicates that when cows have constant access to TMR diet throughout the day, these preparations have little effect on rumen fermentation. Similar results were also obtained in *in vivo* (Beauchemin et al., 2003; Giraldo et al., 2008) and *in vitro* studies (Wang et al., 2001). The increase in total VFA and in the content of individual acids in the ruminal fluid of cows receiving the enzyme or yeast preparation compared to group C may be an evidence of their beneficial impact on the degree of degradation of starch and NDF of feeding ration. The increased proportion of propionic acid in total VFA, especially in the group of cows supplemented

with live yeast culture may indicate increased ruminal degradation of concentrates and the higher utilization of acetic acid by yeast cells for growth compared to other feeding groups (Chu et al., 1981). A study by Miller-Webster et al. (2002) showed that the high proportion of propionic acid in the ruminal fluid of cows fed diets supplemented with live yeast cultures also had a favourable effect on improving protein metabolism in early-lactation cows. An increased concentration of propionic acid in the ruminal contents of cows receiving the yeast preparation Yea Sacc¹⁰²⁶ containing the *Saccharomyces cerevisiae*¹⁰²⁶ strain was also reported by Lynch and Martin (2002). A decrease in percentage of propionic acid in total VFA paralleled by the increased C2:C3 ratio in the ruminal contents, observed in cows supplemented with the enzyme preparation, may indicate the increased degradation of roughage fibre. Similar results to ours when feeding exogenous fibrolytic enzymes to early-lactation dairy cows were also reported by Beauchemin et al. (2000) when feeding exogenous fibrolytic enzymes to early-lactation dairy cows. However, these relationships were not confirmed by Giraldo et al. (2008) who fed enzyme preparations to sheep and by Krause et al. (1998) who used enzyme preparations to fatten young bulls. In accordance with the suggestion of Sutton et al. (2003), it can be assumed that the differences in the results obtained in different studies could be due to environmental factors, in particular the type of feed and the mode of enzyme application.

It is concluded that supplementation of the diet with exogenous fibrolytic enzymes in the form of FibrozymeTM preparation containing active xylanase and cellulase, and the yeast preparation Yea-Sacc¹⁰²⁶ containing live yeast culture of the *Saccharomyces cerevisiae* strain 1026, increased *in sacco* degradation of DM, NDF, starch and CP in the rumen. The addition of the enzymes and yeast to the TMR diet for early-lactation cows increased total VFA content and the contents of individual VFA. The feed additives used had no considerable effect on pH, NH₃-N concentration and the acetic (C2) to propionic acid (C3) ratio in the rumen. Our results failed to confirm the synergistic effect of using a mixture of both preparations on the parameters studied.

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Enzymy fibrolityczne i żywe kultury drożdżowe w dawkach pokarmowych dla krów mlecznych – wpływ na rozkład i fermentację w żwaczu

STRESZCZENIE

Celem badań było określenie, czy dodatek egzogennych enzymów fibrolitycznych i/lub żywych kultur drożdżowych do dawek pokarmowych dla krów mlecznych, w porównaniu z dawkami bez ich udziału, wpłynie na poprawę rozkładu w żwaczu podstawowych składników pokarmowych oraz aktywności fermentacyjnej żwacza. Pierwszy etap badań wykonano na 3 krowach zasuszonych z założonymi przetokami do żwacza. Określono rozkład w żwaczu komponentów paszowych: objętościowych – włóknistych (siano łąkowe), treściwych – skrobiowych (jęczmień) i treściwych – wysokobiałkowych (poekstrakcyjna śruta sojowa) metodą woreczków nylonowych *in sacco*. Badania strawności komponentów dawki pokarmowej przeprowadzono każdorazowo po 21-dniowym okresie jej skarmiania bez udziału (grupa K) lub z dodatkiem danego preparatu: enzymatycznego – FibrozymeTM, zawierającego aktywną celulazę i ksylanazę (grupa E), preparatu drożdżowego Yea Sacc¹⁰²⁶, zawierającego kulturę drożdży szczepu *Saccharomyces cerevisiae*¹⁰²⁶ (grupa D) lub mieszaninę obydwu preparatów (grupa ED). Stosowano określony dla poszczególnych komponentów czas inkubacji (godziny): 2, 4, 8, 16, 24, 48, 72 – w przypadku siana łąkowego; 2, 4, 8, 16, 24, 48 – w przypadku ziarna jęczmienia i 4, 16, 24 – w przypadku poekstrakcyjnej śruty sojowej. Po zakończeniu inkubacji wyliczono rozkład: SM i NDF siana łąkowego, SM i skrobi ziarna jęczmienia oraz SM i białka ogólnego poekstrakcyjnej śruty sojowej. Drugi etap

badania wykonano na 24 krowach w pierwszym okresie laktacji, przydzielonych do 4 grup (po 6 sztuk), żywionych dawką pełnoporcjową TMR; bez udziału (grupa kontrolna K) lub z dodatkiem badanych preparatów (grupy E, D i ED – analogicznie jak w doświadczeniu 1). Oznaczano pH, zawartości N-NH₃, sumę lotnych kwasów tłuszczowych (LKT) i zawartość poszczególnych kwasów tłuszczowych w próbkach treści żwacza, pobranych sondą żwaczową z pompą ssącą od krów 3 godz. przed i 3 godziny po karmieniu. Stwierdzono, że dodatek egzogennych enzymów fibrolitycznych i/lub żywych kultur drożdżowych do standardowej dawki pokarmowej wpłynął istotnie na zwiększenie tempa rozkładu *in sacco* SM i NDF siana łąkowego, SM i skrobi ziarna jęczmienia oraz SM i BO poekstrakcyjnej śruty sojowej w żwaczu. U krów otrzymujących dodatek preparatu enzymatycznego i drożdżowego wykazano również wzrost ($P < 0,05$ lub $P > 0,05$) sumy LKT oraz zawartości poszczególnych kwasów tłuszczowych w sumie LKT. Nie stwierdzono natomiast istotnego wpływu dodatków na pH i zawartość N-NH₃ w próbkach treści żwacza pobranych przed i po zadaniu paszy. U krów z grupy E odnotowano obniżenie w treści żwacza procentowego udziału kwasu propionowego w sumie LKT oraz podwyższenie stosunku kwasów C2:C3. W grupie D wykazano natomiast zwiększenie procentowego udziału kwasu propionowego w sumie LKT oraz obniżenie stosunku kwasów C2:C3.