Journal of Animal and Feed Sciences, 21, 2012, 276–284

The effect of *Saccharomyces cerevisiae* on ruminal fermentation in sheep fed high- or low-NDF rations

S. Grochowska[1,3], W. Nowak, R. Mikuła and M. Kasprowicz-Potocka

Poznan University of Life Sciences, Department of Animal Nutrition and Feed Management
Wołyńska 33, 60-637 Poznań, Poland

(Received 6 July 2011; revised version 10 May 2012; accepted 18 June 2012)

ABSTRACT

The aim of the study was to determine the effect of live yeast culture (LYC) and dehydrated yeast culture (YC) containing *Saccharomyces cerevisiae* on fermentation parameters and degradability in the rumen of sheep fed a high- (H-NDF; 56% DM) or low- (L-NDF; 35% DM) NDF diet. Three Polish Merino sheep fitted with ruminal cannulas were used. The effective degradability of maize grain and wheat straw was measured by an *in situ* procedure. Ruminal pH, ammonia, total volatile fatty acid concentrations, and molar percentages of acetate, propionate and butyrate were not modified by the addition of LYC or YC, irrespective of the applied diet. Compared with control (CON) and LYC supplementation of the L-NDF diet significantly ($P<0.05$) increased the caproate concentration, but in the H-NDF diet, it increased the lactate concentration ($P<0.05$). *In situ* rumen NDF degradability of maize grain and wheat straw was not affected by the treatments. When the sheep were fed the H-NDF diet, however, degradability of dry matter maize grain was significantly higher ($P<0.05$) than in the CON group. The results of this study did not confirm the hypothesis that NDF in the diet is the main reason for the considerable inconsistencies in the results of experiments with *Saccharomyces cerevisiae* products.

KEY WORDS: *Saccharomyces cerevisiae*, rumen fermentation, degradability, sheep

INTRODUCTION

One of the potential alternatives for antibiotics is fungal supplementation with *Saccharomyces cerevisiae*. The beneficial effects are associated with increased milk production and improved performance of ruminants (Abd-El-Ghani, 2004; Moharrery and Asadi, 2009). Several hypotheses concerning the mode of action of yeast supplementation in ruminant nutrition have been proposed, but most

[3] Corresponding author: e-mail: s.grochowska@wp-piast.pl

of them emphasize positive effects achieved by modifying rumen fermentation. Yeast cultures can stimulate utilization of hydrogen by acetogenic bacteria, lactate uptake by *Selenomonas ruminatium,* and enhance the growth of cellulolytic bacteria (Martin and Nisbet, 1992; Chaucheyras et al., 1995). Live yeast cultures increased the proportion of propionate, decreased the lactate concentration, and increased the numbers of total viable bacteria and cellulolytic bacteria (Newbold et al., 1990; Mutsvangwa et al., 1992; Lila et al., 2004). Corona et al. (1999) emphasised considerable inconsistencies between the quoted research results. This results mainly from the type of added yeast, their quantity, as well as diet composition (Wallace, 1994; Mendoza et al., 1995). Recently published studies focused on the effects of live yeast culture or metabolites on rumen fermentation. Comparative studies with the use of both yeast products supplementing high- and low-fibre diets of sheep could test the hypothesis that the level of NDF in the diet can limit the effectiveness of *Saccharomyces cerevisiae* products.

The aim of the experiment was to compare the effect of live yeast culture and dehydrated yeast culture on rumen fermentation and ruminal degradation of dry matter and NDF of maize grain and wheat straw, depending on the level of NDF in the diet.

MATERIAL AND METHODS

Experimental design

Live yeast culture, LYC, commercially available as Biosaf SC 47 (Lesaffre, $8x10^9$CFU) and yeast culture (dehydrated), YC, commercially available as Diamond V XP (Diamond V) containing *Saccharomyces cerevisiae*, were used in the study. Two experiments were carried out in a Latin square design on three cannulated Polish Merino sheep (average body weight of 65 kg; rumen cannula diameter, 30 mm). The protocol for the experiment was reviewed and approved by the Institutional Animal Care and Use Committee at the Poznan University of Life Sciences.

In experiment I, sheep were fed a low-NDF diet (35% DM; L-NDF) consisting of meadow hay (50%) and ground barley grain (50%) and in experiment II, only meadow hay (56% DM, H-NDF). They were fed *ab libitum*, twice a day as TMR. Feed compositions are given in Table 1. Additives were added intra-ruminally in the amount of 2 g/day of LYC or 15 g/day of YC, respectively. The experimental period lasted for 21 days, which was divided into a 14-day adaptation period and a 7-day experimental period. Ruminal samples were collected at 0, 1, 3 and 6 h after feeding according to the method described by Enjalbert et al. (1999). Ruminal pH was measured immediately after sampling and then 0.7 ml of 46 mM mercury chloride were added to 6.3 ml of the rumen liquid and stored in a freezer for further analysis.

Table 1. Meadow hay and barley grain composition, %

Nutrient	Meadow	Barley
Dry matter	91.5	89.2
NDF, % DM	55.6	14.8
ADF, % DM	32.0	3.9
Crude protein, % DM	10.5	9.2

Analysis

In situ disappearance of dry matter and NDF was measured by incubating 1 g of maize grain or wheat straw in polyester bags (45 x 50 mm, 46 micrometer pore size). Quadruplicate bags were incubated for 6, 12, 24, and 48 h starting on the second day of the measurement period. The composition of feeds incubated in the rumen is presented in Table 2. Based on dry matter and NDF digestibility in particular periods of incubation, the constant rate was calculated using a mathematical model elaborated by Ørskov and McDonald (1979). Samples of feeds were chemically analysed using standard methods (AOAC, 2005). Ammonia concentrations were determined according to Nessler's method. Volatile fatty acid (VFA) and lactic acid concentrations were measured by gas chromatography (Varian CP380) according to the method described by Jensen et al. (1995) with 2-ethyl-n-butyric acid as the internal standard.

Table 2. Composition of feeds incubated in rumen, %

Nutrient	Maize grain	Wheat straw
Dry matter	89.2	93.9
Crude ash, % DM	1.2	2.9
Crude protein, % DM	9.2	3.0
Crude fibre, % DM	2.9	48.3
ADF, % DM	3.9	53.6
NDF, % DM	14.8	80.5

Statistical analysis

Degradation data were fitted to a non-linear regression equation: $P = a + b (1 - e^{-ct})$ where: 'a' - the rapidly soluble fraction, 'b' - the less rapidly degradable fraction that disappears at a constant rate'c' per unit of time. The three constants were then used to calculate effective ruminal degradability (ERD) by the equation $ERD = a + ((b \times c) / (c + k))$ assuming a fractional outflow rate (k) of 0.06 h^{-1}. Degradation constants a, b, c were calculated using SAS 9.1. software (2004).

Data were analysed for a Latin square design using the general linear model procedure (PROC GLM) of SAS (2004). Probability values of $P<0.05$ were considered significant.

The model used was:

$$Y_{ijk} = \mu + C_i + P_j + T_k + E_{ijk}$$

where: Y_{ijk} - dependent variable, μ - overall treatment mean for parameter Y_{ijk}, C_i - effect of the sheep, P_j - effect of the period, T_k - effect of the diet, E_{ijk} - experimental error.

RESULTS

The addition of LYC and YC to L-NDF or H-NDF diets did not affect (P>0.05) ruminal pH or ammonia concentration (Tables 3 and 4). A trend for decreased ammonia by LYC and YC was observed when sheep were fed the

Table 3. The effect of LYC and YC on ruminal pH, VFA, lactate and ammonia concentrations (experiment I - L-NDF diet)

Parameter	CON	LYC	YC	SEM
Ruminal pH	6.3	6.3	6.3	0.1
mmol/l				
ammonia	15.4	14.9	14.9	0.5
total VFA	45.8	50.4	45.8	1.4
acetate	28.6	30.9	27.4	0.8
propionate	10.8	12.3	10.6	0.5
butyrate	4.7	5.5	6.0	0.3
valerate	0.6	0.8	0.6	0.0
caproate	0.5[b]	0.5[b]	0.6[a]	0.0
isobutyrate	0.4	0.4	0.4	0.0
isovalerate	0.2	0.2	0.2	0.0
acetate:propionate ratio	2.85	2.56	2.66	0.07
lactate	0.68	0.64	0.63	0.04

[a,b] means denoted with different letters are significantly different at P<0.05; CON - control group; LYC - live yeast culture; YC - dehydrated yeast culture; SEM - standard error of mean

Table 4. The effect LYC and YC on ruminal pH, VFA, lactate and ammonia concentrations (experiment II - H-NDF diet)

Parameter	CON[1]	LYC[1]	YC[1]	SEM[1]
Ruminal pH	6.7	6.8	6.7	0.0
mmol/l				
ammonia	13.9	13.0	13.2	0.3
total VFA	42.9	41.0	40.7	0.9
acetate	29.3	27.8	27.3	0.6
propionate	8.3	7.9	8.5	0.2
butyrate	3.6	3.7	3.4	0.1
valerate	0.4	0.4	0.4	0.0
caproate	0.5	0.5	0.5	0.0
isobutyrate	0.4	0.4	0.3	0.0
isovalerate	0.4	0.4	0.2	0.1
acetate:propionate ratio	3.5	3.5	3.2	0.1
lactate	0.9[b]	0.7[b]	1.1 [a]	0.1

[a,b] means denoted with different letters are significantly different at P<0.05; [1] see Table 3

H-NDF diet. The differences CON vs LYC and CON vs YC were 0.85 and 0.63 mmol/l, respectively. The concentration of VFA was low and did not exceed 50.4 mmol/l. In sheep fed the L-NDF diet, a tendency to incease the total VFA concentration by LYC in comparison with the other groups was found. The YC additive increased ($P<0.05$) caproate and lactate concentrations in rumen fluid of sheep fed the L-NDF and H-NDF diets, respectively, in comparison with other groups. Neither additive affected the other analysed organic acids and total VFA concentration, although a trend for higher VFA production by LYC was observed. This resulted primarily from increased synthesis of acetic and propionic acids on the L-NDF diet. There were no differences ($P>0.05$) in the acetate:propionate ratio between the additives used in both experiments. No impact of LYC or YC on effective dry matter and NDF degradability in maize grain and wheat straw were observed, except reduction of dry matter degradability by YC in the H-NDF diet ($P<0.05$) compared with the other groups (Tables 5 and 6).

Table 5. The effect of LYC and YC on effective degradability (%) of dry matter and NDF of maize grain and wheat straw (experiment I - L-NDF diet)

Diet	CON[1]	LYC[1]	YC[1]	SEM[1]
Maize grain				
DM	55.6	56.4	54.5	0.5
NDF	39.3	41.6	39.8	0.5
Wheat straw				
DM	20.8	20.1	19.7	0.5
NDF	16.7	16.6	16.1	1.9

[a,b] means denoted with different letters are significantly different at $P<0.05$; [1] see Table 3

Table 6. The effect of LYC and YC on effective degradability (%) of dry matter and NDF of maize grain and wheat straw (experiment II - H-NDF diet)

Diet	CON[1]	LYC[1]	YC[1]	SEM[1]
Maize grain				
DM	54.1[a]	55.4[a]	51.1[b]	0.7
NDF	40.2	41.2	40.2	0.6
Wheat straw				
DM	23.7	25.2	24.0	0.4
NDF	21.0	23.1	21.3	0.5

[a,b] means denoted with different letters are significantly different at $P<0.05$; [1] see Table 3

DISCUSSION

Irrespective of the NDF concentration in the diet, supplementation of diets by live yeast culture and dehydrated yeast culture did not affect rumen pH as reported by other authors (Plata et al., 1994). According to Michalet-Doreau and

Morand (1996), a reduction in ruminal pH by adding *Saccharomyces cerevisiae* to diets rich in only non-structural carbohydrates was observed. Previous experiments in sheep also showed that yeast culture supplementation of a diet with 50% barley resulted in decreasing the pH to below 6.0 (Mathieu et al., 1996). Directly fed microbial products containing *Saccharomyces cerevisiae* are known to increase ruminal pH by reducing the lactic acid concentration in rumen fluid. Martin and Nisbet (1992) suggested that YC enhanced the utilization of lactate by an increased presence of lactate-utilizing bacteria, thereby maintaining a constant pH. Lynch and Martin (2002) reported that live yeast culture reduced, while yeast culture increased, the lactate concentration, which was also found in the current study with the high-NDF diet.

In the present study, the rumen ammonia concentration was not affected by the yeast addition, as reported by others (Lila et al., 2004; Mwenya et al., 2005), although slight decreases in the ammonia concentration by LYC and YC for the L-NDF diet were observed. Decreased ammonia concentrations in the rumen caused by *Saccharomyces cerevisiae* were reported by Abd-El-Ghani (2004) and may indicate lower protein decomposition as well as faster flow of undegraded protein to the duodenum. Newbold et al. (1995) suggested that *Saccharomyces cerevisiae* improved the conditions for synthesis of microbial protein, resulting from increased availability of energy for its synthesis.

In the present study, the total VFA concentration was low in comparison with results reported by Kowalik et al. (2011) for goats fed diets containing 523 g NDF per kg of dry matter. Molar percentages of acetate, propionate and butyrate, as well as the acetate-to-propionate ratio, were not significantly affected by the addition of yeast products. Using live yeast cultures, Corona et al. (1999) demonstrated a tendency towards increased quantities of total VFAs, propionate and acetate, as well as decreased butyrate concentrations, which was demonstrated in part in the present study ($P>0.05$) for the L-NDF diet. Piva et al. (1993) observed a reduction of the acetate:propionate ratio when YC was fed to lactating cows, while total VFA was not affected. Mwenya et al. (2005) reported increasing VFA concentrations, especially those of acetate and isoacids, in the rumen fluid and this may have indicated increased activity of cellulolytic bacteria. This effect was not observed in the present study. Lila et al. (2004) found in their *in vitro* study that yeast increased total VFAs and elevated levels of propionate during batch culture incubations, which was confirmed in the case of the LYC additive for the L-NDF diet. Nonetheless, in the experiments conducted by Newbold et al. (1995), LYC did not affect VFA concentrations. The results of other researchers are also inconsistent. Some investigators reported increased proportions of acetate (Mutsvangwa et al., 1992), while others found increased proportions of propionate (Plata et al., 1994). The hypothesis that considerable discrepancies are caused

mainly by the ratio of structural to non-structural carbohydrates was not confirmed in our study using high- and low-fibre diets.

In the current study, no significant effects of LYC and YC were observed on effective degradation of NDF from maize grain and wheat straw, similarly as in other experiments (Mutsvangwa et al., 1992; Enjalbert et al., 1999). Live yeast culture in the low-NDF diet increased the degradation of NDF from wheat straw ($P<0.05$) after 6 h incubation (data not shown), but the rate of degradation and effective degradability were not modified. Similarly, Doreau and Jouany (1998) showed a positive effect of adding yeast culture. In *in vitro* studies Martin and Nisbet (1990) confirmed the effect of yeast on the degradation parameters of feeds incubated for a short period in the rumen. In the present study, yeast culture supplementing a high-fibre NDF diet reduced ruminal maize grain dry matter effective degradability. Plata et al. (1994) demonstrated an improvement of *in situ* degradation of feed dry matter and NDF following their supplementation with *Saccharomyces cerevisiae*, while other researchers detected an improvement only at certain incubation times (Mendoza et al., 1995). Wallace (1994) attributed these positive trends to increased activity of cellulolytic bacteria resulting from the capability of live yeast cultures to reduce oxygen. Martin et al. (2000) demonstrated a positive effect of LYC on ruminal NDF degradation of bulky feeds containing high levels of crude fibre, while Abd-El-Ghani (2004) demonstrated the impact of yeast on increasing ruminal degradation and total digestibility.

CONCLUSIONS

Very few positive effects of live yeast culture and dehydrated yeast culture on rumen fermentation and ruminal degradation were observed in this experiment. Our results did not confirm the hypothesis that the nature of diets (content of structural carbohydrates) is the main reason for considerable inconsistencies in the results of experiments with directly fed fungal microbials containing *Saccharomyces cerevisiae.*

REFERENCES

Abd-El-Ghani A.A., 2004. Influence of diet supplementation with yeast culture (*Saccharomyces cerevisiae*) on performance of Zaraibi goats. Small Ruminant Res. 52, 223-229

AOAC, 2005. Association of Official Analytical Chemists, Official Methods of Analysis. 18th Edition. Gaithersburg, MA

Chaucheyras F., Fonty G., Bertin G., Gouet P., 1995. *In vitro* h_2 utilization by a ruminal acetogenic bacterium cultivated alone or in association with an archea methanogen is stimulated by a probiotic strain of *Saccharomyces cerevisiae*. Appl. Environ. Microbiol. 61, 3466-3469

Corona L., Mendoza G.D., Castrejon F.A., Crosby M.M., Cobos M.A., 1999. Evaluation of two yeast cultures (*Saccharomyces cerevisiae*) on ruminal fermentation and digestion in sheep fed a corn stover diet. Small Ruminant Res. 31, 209-214

Doreau M., Jouany J.P., 1998. Effect of a *Saccharomyces cerevisiae* culture on nutrient digestion in lactating dairy cows. J. Dairy Sci. 81, 3214-3221

Enjalbert F., Garett J.E., Moncoulone R., Chicateau P., 1999. Effect of yeast culture (*Saccharomyces cerevisiae*) on ruminal digestion in non-lactating dairy cows. Anim. Feed Sci. Tech. 76, 195-206

Jensen M.T., Cox R.P., Jenses B.B., 1995. Microbial production of skatole in the hindgut of pigs given different diets and its relation to skatole deposition in backfat. J. Anim. Sci. 61, 293-304

Kowalik B., Michałowski T., Pająk J.J., Taciak M., Zalewska M., 2011. The effect of live yeast, *Saccharomyces cerevisiae*, and their metabolites on ciliate fauna, fibrolytic and amylolytic activity, carbohydrate digestion and fermentation in the rumen of goats. J. Anim. Feed Sci. 20, 526-536

Lila Z.A., Mohammed N., Yasui T., Kurokawa Y., Kanda S., Itabashi H., 2004. Effects of twin strain of *Saccharomyces cerevisiae* live cells on mixed ruminal microorganisms fermentation *in vitro*. J. Anim. Sci. 82, 1847-1854

Lynch H.A., Martin S.A., 2002. Effects of *Saccharomyces cerevisiae* culture and *Saccharomyces cerevisiae* live cells on *in vitro* mixed ruminal microorganism fermentation. J. Dairy Sci. 85, 2603-2608

Martin S.A., Nisbet D.J., 1990. Effect of *Aspergillus oryzae* fermentation extract on fermentation of amino acids, bermudagrass and starch by mixed ruminal microorganisms *in vitro*. J. Anim. Sci. 68, 2142-2149

Martin S.A., Nisbet D.J., 1992. Effect of direct fed microbials on rumen microbial fermentation. J. Dairy Sci. 75, 1736-1744

Martin A.S., Prado I.N., Zeoula L.M., Brnco A.F., Nascimento W.G., 2000. Apparent digestibility of diets containing corn or cassava hull as energy source and cottonseed meal or yeast as protein source, in heifers. Rev. Brasil. Zoot. 29, 269-277

Mathieu F., Jouany J.P., Senaud J., Bohatier J., Bertin G., Mercier S., 1996. The effect of *Saccharomyces cerevisiae* and *Aspergillus oryzae* on fermentations in the rumen of faunated and defaunated sheep; Protozoal and probiotic interactions. Reprod. Nutr. Develop. 36, 271-287

Mendoza M.G.D., Ricalde V.R., Esparza B.H., Velazquez T.L., 1995. Effect of two yeast cultures with *Saccharomyces cerevisiae* on ruminal neutral detergent fiber degradability. Invest. Agraria. Prod.Y San. Anim. 10, 33-38

Michalet-Doreau B., Morand D., 1996. Effect of yeast culture, *Saccharomyces cerevisiae* on ruminal fermentation during adaptation to high-concentrate feeding. Ann. Zootech. 45, Suppl. 1, 337 (Abstr.)

Moharrery A., Asadi E., 2009. Effects of supplementing malate and yeast culture (*Saccharomyces cerevisiae*) on the rumen enzyme profile and growth performance of lambs. J. Anim. Feed Sci. 18, 283-295

Mutsvangwa T., Edwards I.E., Topps J.H., Peterson G.F.M., 1992. The effect of dietary inclusion of yeast culture (Yea Sacc) on patterns of rumen fermentation, feed intake and growth of intensively fed bulls. Anim. Prod. 55, 35-41

Mwenya B., Santoso B., Sar C., Pen B., Morikawa R., Takaura K., Umetsu K., Kimkura K., Takahashi J., 2005. Effects of yeast culture and galacto-oligosaccharides on ruminal fermentation in holstein cows. J. Dairy Sci. 88, 1404-1412

Newbold C.J., Wallace R.J., Chen X.B., Mcintosh F.M., 1995. Different strains of *Saccharomyces cerevisiae* differ in their effects on ruminal bacterial numbers *in vitro* and in sheep. J. Anim. Sci. 73, 1811-1918

Newbold C.J., Williams P.E.V., Mckain N., Walker A., Wallace R.J., 1990. The effects of yeast culture on yeast numbers and fermentation in the rumen of sheep. Proc. Nutr. Soc. 49, 47A (Abstr.)

Ørskov E.R., McDonald I., 1979. The estimation of protein degradability in the rumen from incubation measurements weighed according to rate of passage. J. Agr. Sci. 93, 499-502

Piva G., Belladonna S., Fusconi G., Sicbaldi F., 1993. Effects of yeast on dairy cow performance, ruminal fermentation, blood components, and milk manufacturing properties. J. Dairy Sci. 76, 2717-2722

Plata P.F., Mendoza M.G.D., Barcena-Gama J.R., Gonzales M.S., 1994. Effect of a yeast culture (*Saccharomyces cerevisiae*) on neutral detergent fiber digestion in steers fed oat straw based diets. Anim. Feed Sci.Tech. 49, 203-210

SAS, 2004. SAS/STAT User's Guide. Version 9.1. SAS Institute Inc. Cary, NC

Wallace R.J., 1994. Ruminal microbiology, biotechnology, and ruminant nutrition: progress and problems. J. Anim. Sci. 72, 2992-3003