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The effect of live *Saccharomyces cerevisiae* yeast in the diet of rams on the digestibility of nutrients, nitrogen and mineral retention, and blood serum biochemical parameters

Barbara KOWALIK*, Jacek SKOMIAŁ, Renata MILTKO, Małgorzata MAJEWSKA
The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jabłonna, Poland

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Abstract: The effect of live *Saccharomyces cerevisiae* yeast added to the diet of rams on the digestibility of carbohydrates and protein in the total tract, retention of nitrogen and mineral elements, and concentration of biochemical parameters in blood serum was examined. The experiment was carried out on 12 Corriedale rams allocated to 2 groups of 6 animals each. The control group was fed meadow hay (45.4%), concentrate (53.2%), and a vitamin–mineral mixture (1.4%), while the second group was fed the same diet supplemented with 2 g day^{-1} live *S. cerevisiae*. Adaptation to feeding lasted 21 days; the sampling period lasted 7 days. The digestibility of carbohydrates, protein, nitrogen, macroelements (P, Na, Mg, K), and microelements (Cu, Mn, Zn) and their retention, as well as total protein, total cholesterol, triacylglycerol, HDL, LDL, and VLDL in blood serum, were determined. Supplementation with live yeast caused a tendency towards decreasing (P = 0.066) the digestibility of N-free extract compared with animals fed the control diet. Magnesium retention showed a tendency to increase (P = 0.053) when rams were fed a diet supplemented with live yeast cells. Total cholesterol, triacylglycerol, and LDL concentrations decreased significantly when yeast was added compared with animals fed the control diet.

Key words: Yeast, digestibility, minerals, nitrogen, blood, rams

1. Introduction

The use of live *Saccharomyces cerevisiae* yeast as a feed additive for domestic ruminants has increased in recent years. A small amount of live cell yeast supplemented to the diet for ruminants influences animal health and milk production, as well as daily gain (1). This effect has been associated with the ability of yeast to influence ruminal fermentation. Addition of *S. cerevisiae* to the diet stimulates cellulolytic bacteria, e.g., *Ruminococcus albus* and *Fibrobacter succinogenes* (2), and improves the digestibility of structural carbohydrates in the rumen (3). The increased bacteria count is probably the result of the improved anaerobic status of the rumen. Newbold et al. (4) found a correlation between the ability of different strains of *S. cerevisiae* to stimulate oxygen uptake by ruminal fluid and the ability of live yeast to stimulate the growth of ruminal bacteria. Moreover, Chaucheyras et al. (5) found that live yeast stimulates the ruminal fungi *Neocallimastix frontalis*. It cannot be precluded that ruminal fungi are able to digest structural carbohydrates. Marden et al. (6) observed increased total tract digestibility of NDF and ADF when dairy cows were fed a diet with live yeast. In contrast to the cited authors, Tripathi et al. (7) showed that neither NDF nor ADF were influenced when the diet was supplemented with live *S. cerevisiae*. The yeast additive can reduce lactate accumulation in the rumen (6) by increasing the count of lactate-utilizing bacteria, particularly *Selenomonas ruminantium*, and also by decreasing the number of the lactate-producing bacteria, *Streptococcus bovis* (3).

In addition, Marden et al. (6) and Kowalik et al. (8) have shown that addition of live yeast cells to the diet results in changing the concentration of volatile fatty acids (VFAs) in the rumen of cows. Increased VFA concentrations can slow down cholesterol and triacylglycerol synthesis in the liver and may alter the blood lipid profile. On the other hand, Pysera and Opałka (9) showed that the lipid profile in blood serum can be modified according to the age of the animals and by the amount and kind of fat or feed additives in the diet. Galip (10) and Grochowska et al. (11) showed that live *S. cerevisiae* did not modify VFA in the rumen of sheep.

All animals require mineral elements for normal life processes; these minerals have many differing physiological functions. The effect of live yeast on retention of macro- and microminerals by ruminants is little known. Petersen et al. (12) observed a positive effect of live yeast on mineral metabolism in sheep, with increased retention

* Correspondence: b.kowalik@ifzz.pl

of total minerals when the animals were fed a diet with yeast. Strusińska et al. (13) showed that a live yeast supplement increased the zinc and iron concentrations in milk but decreased that of copper. Iwańska et al. (14) did not observe any changes in the serum levels of calcium, phosphorus, magnesium, or iron, but an increase in the level of these minerals was found in milk.

We hypothesize that live *S. cerevisiae* can influence the digestibility of nutrients, particularly carbohydrates and protein, as well as the absorption of minerals and the lipid profile in the blood serum of rams.

2. Materials and methods

2.1. Animals and management

The experiment involved 12 Corriedale rams of 40 kg (±2.7) body weight and similar body conditions, allocated to 2 groups of 6 animals in each. Adaptation feeding lasted 21 days and the sampling period lasted 7 days. During adaptation, all animals were housed in individual pens provided with separate facilities for feeding and watering. The control group was fed meadow hay (45.4%), concentrates (53.2%), and a vitamin–mineral mixture (1.4%), while the second group was fed the same diet supplemented with 2 g day^{-1} live *S. cerevisiae* yeast (Levucell SC, *S. cerevisiae* CNCM I-1077). The yeast was given in the morning, mixed with the concentrate. The Levucell yeast preparation contains select viable lyophilized *S. cerevisiae* cells. The daily dry matter intake was 1.4 kg per ram. The composition of the diet is presented in Table 1. The daily ration was divided into 2 equal parts. Drinking water was available ad libitum. Because animals were fed with limited amounts of feed, no orts were observed.

All procedures were performed with the consent of the Local Ethics Committee for Animal Experimentation.

2.2. Sampling procedures and analytical methods

During the sampling period, rams were maintained in individual metabolic cages for 7 days. Feces and urine were collected every morning. Feces samples were weighed and 10% of the material was frozen for analysis. Urine collection containers were acidified (100 mL of 6 N HCl) daily. Ten percent of the total daily urine output was collected and frozen for future analysis. Blood samples were taken from the jugular vein into heparinized centrifuge tubes 2 h after morning feeding for 2 consecutive days. Serum was separated by centrifugation at 2500 × *g* for 15 min and stored at –20 °C until analyzed. Serum samples were analyzed for total protein, total cholesterol (Chol), triacylglycerol (TG), and HDL (high density lipoprotein). LDL (low density lipoprotein) was calculated according to the following formula: Chol – HDL – TG/2.2, and VLDL (very low density lipoprotein) as TG/2.2.

Feces samples were dried at 60 °C for 48 h and ground through a 1-mm screen. Dry matter (934.01), crude fiber (978.10), ADF (973.18), NDF (2002.04), ash (930.05), and crude fat (920.39) in feed and feces were analyzed according to AOAC methods (15). Nitrogen in feed, feces, and urine samples was analyzed by the Kjeldahl method (954.01) (15). Organic matter, N-free extract, and crude protein were calculated. The concentration of minerals (Na, Mg, K, Cu, Mn, Zn) in feed, feces, and urine was analyzed by atomic absorption spectrophotometry (AA-6300 Shimadzu), except for phosphorus, which was estimated photometrically. Biochemical indicators of blood serum were determined using a VITROS DT 60 II

Table 1. Composition of diets (%) and feeding value of ration (% DM).

Ingredients		Chemical composition	
Meadow hay	45.4	Organic matter	94.3
Crushed barley	27.4	Crude protein	15.8
Rapeseed oil meal	14.1	Crude fiber	17.9
Potato starch	11.7	Ether extract	2.3
Vitamin–mineral premix*	1.4	Ash	4.0
		NDF	43.7
		ADF	22.7
		N-free extract	58.6
		UFV kg^{-1}	1.2

*Premix contents per kilogram: iron, 500 mg; zinc, 2500 mg; calcium, 250 g; magnesium, 65 g; manganese, 3000 mg; sodium, 60 g; phosphorus, 125 g; cobalt, 15 mg; selenium, 3 mg; vitamin A, 300,000 UI; vitamin D_3, 30,000 UI; vitamin E, 1500 mg. DM: Dry matter; ADF: acid detergent fiber; NDF: neutral detergent fiber; UFV: feed unit of maintenance and meat production.

system and diagnostic kits from ICN Instruments Poland Ltd.

2.3. Statistical analysis

Data were expressed as means and pooled standard error of mean values. The data were processed by variance analysis in accordance with the linear model:

$$Y_{ij} = \mu + A_i + T_i + e_{ij}$$

where: Y_{ij}, observation mean; μ, overall mean; A_i, animals; T_j, the treatment effect; e_{ij}, random error.

The significance of differences between the effect of dietary treatment and the control diet was calculated by Tukey's test. Statements of statistical significance were based on $P < 0.01$ and trends were discussed at $P < 0.10$. The Statistica 10.0 software package was used for statistical analysis.

3. Results

The addition of live yeast to the diet of the rams did not affect the total tract digestibility of organic matter, crude protein, crude fiber, ADF, or NDF (Table 2). A tendency towards decreased N-free extract digestibility ($P = 0.066$) was observed when sheep were fed live yeast cells in comparison with the control diet (72% vs. 75%, respectively).

Nitrogen intake, N-excreted in feces and urine, and N retained did not change significantly when live yeast was supplemented (Table 3). However, N retention showed a numerical decrease following supplementation with live yeast.

Supplementation of live *S. cerevisiae* to the ration did not affect the retention of minerals (Table 4). In sheep fed the diet containing live yeast, a tendency towards increased ($P = 0.053$) retention of magnesium (1.1 g day^{-1}) in comparison with control animals (0.8 g day^{-1}) was found. Furthermore, retention of microelements was numerically decreased when rams were fed the yeast-supplemented diet.

The total protein content and lipid profiles of blood serum in the experimental rams are presented in Table 5. Total triacylglycerol and cholesterol, as well as the LDL concentration, decreased significantly when live cell yeast (0.2 and 1.4, as well as 0.6 Mmol L^{-1}) was added to the diet compared with animals fed the control diet (0.3, 1.7, 0.8 Mmol L^{-1}, respectively). No effect of yeast in the diet on the serum level of total protein, HDL, and VLDL was found.

4. Discussion

Supplementation with live yeast caused a decreasing tendency ($P = 0.066$) in total tract digestibility of N-free extract when compared with control rams. Moharrery and Asadi (16) demonstrated that addition of yeast to the diet increased amylase activity (19%) in the rumen compared with control lambs. This effect suggests increased degradation of starch in the rumen and reduced starch flow to the duodenum. In the current study, the total tract digestibility of other nutrients did not increase when yeast was added to the diet. Most literature data show no improvement in digestibility of dry matter and organic matter when live yeast is added to the ration (7,16,17). In several trials, higher dry and organic matter digestibility was reported (6,18) when live *S. cerevisiae* cells were supplemented, due to the increased structural carbohydrate digestibility. These differences cannot be clearly attributed to the species and physiological state of animals or to diet composition. Our results suggest that live *S. cerevisiae* did not affect digestion in the rumen or that its effect was masked by greater hindgut fermentation in control rams as compared with animals fed live yeast (19). On the other hand, Moharrery and Asadi (16) showed that cellulase activity in the rumen was higher in lambs fed live yeast compared with animals on a control diet. These results show that yeast can stimulate ruminal degradation of nutrients, but digestion in the postruminal tract counteracted this effect. Moreover, it cannot be ignored that the dose of live yeast in our study was rather small.

In the present study, the nitrogen excreted in feces and urine, as well as N retained, was not modified by addition of live *S. cerevisiae*. Tripathi et al. (7) showed a

Table 2. Digestibility coefficient in gastrointestinal tract of rams (%).

Nutrient	Control	Live yeast	SEM	P-value
Organic matter	73.7	71.6	0.72	0.150
Crude protein	90.8	90.0	0.41	0.372
Crude fiber	59.1	57.5	1.36	0.586
ADF	54.3	53.8	1.19	0.831
NDF	62.7	62.8	0.78	0.967
N-free extract	75.1	72.1	0.83	0.066

Explanations given at the bottom of Table 1. SEM: Standard error of the mean.

Table 3. Retention of minerals in rams.

Specification	Control	Live yeast	SEM	P-value
Total mineral (g day^{-1})*	6.53	6.79	0.524	0.535
Macroelements (g day^{-1})				
Phosphorus	2.84	2.52	0.131	0.262
Sodium	0.77	0.82	0.142	0.892
Magnesium	0.84	1.11	0.072	0.053
Potassium	2.00	2.28	0.240	0.605
Microelements (mg day^{-1})				
Copper	1.72	0.99	0.267	0.133
Manganese	43.60	33.39	3.313	0.250
Zinc	34.80	30.58	2.095	0.351

*Total mineral: macroelements + microelements. SEM: Standard error of mean.

Table 4. Nitrogen utilization in rams.

Specification	Control	Live yeast	SEM	P-value
N utilization (g day^{-1})				
N intake	35.56	35.56		
N excreted				
Feces	10.21	10.83	0.352	0.409
Urine	11.14	11.28	0.358	0.853
Total N excreted	21.35	22.11	0.450	0.427
N retained	14.21	13.45	0.450	0.427

SEM: Standard error of mean.

Table 5. Total protein concentration (g L^{-1}) and some lipid parameters (Mmol L^{-1}) in the blood serum of rams.

Specification	Control	Live yeast	SEM	P-value
Total protein	77.7	76.2	5.63	0.565
Triacylglycerol	0.25^{A}	0.21^{B}	0.027	0.003
Total cholesterol	1.72^{A}	1.41^{B}	0.213	0.000
HDL	0.76	0.72	0.100	0.361
LDL	0.84^{A}	0.59^{B}	0.206	0.003
VLDL	0.11	0.10	0.012	0.110

HDL: High density lipoprotein; LDL: low density lipoprotein; VLDL: very low density lipoprotein; A, B: $P < 0.01$; SEM: standard error of mean.

nonsignificant decrease in N retention in lambs fed live cell yeast. Moreover, Mwenya et al. (20) reported that live yeast added to the ration for cows significantly increased the amount of nitrogen in urine and nonsignificantly decreased nitrogen excreted in feces and final nitrogen retention when compared with animals fed live yeast mixed with galacto-oligosaccharides. Our results are not in agreement with those of Paryad and Rashidi (18), who

reported a significant improvement in nitrogen retention in sheep fed 2 and 4 g day^{-1} live *S. cerevisiae* compared to the control group. Paryad and Rashidi (18) suggested that the increase in nitrogen retention in rams fed live yeast was probably caused by higher proteolytic activity of the rumen microorganisms. According to Mwenya et al. (20), differences in the results may be caused by the type of ration, animal species and age, and mode of production of strain of live yeast, i.e. mass production vs. laboratory preparation.

In the present study, the retention of minerals was not affected significantly by live yeast supplementation. Petersen et al. (12) reported nonsignificant increases in sodium and manganese retention when sheep were fed live cell yeast; our results are consistent with those of the cited authors. The same authors showed a significant increase in retention of total minerals, as well as potassium, copper, and zinc, when animals were fed a diet with live *S. cerevisiae* in comparison with the control ration. Moreover, Iwańska et al. (14) showed that a supplement of live *S. cerevisiae* or mineral bioplexes increases the availability of minerals for ruminal microorganisms and increases their absorption to the blood of the host.

Our results showed only a tendency to increase ($P = 0.053$) the retention of magnesium in rams fed the diet supplemented with live yeast. Petersen et al. (12) also failed to find a significant increase in magnesium retention in sheep supplemented with live *S. cerevisiae*. Magnesium absorption in ruminants depends on their age and on the feeding system. In ruminants, the majority of magnesium is absorbed in the reticulorumen part of the digestive tract; the absorption rate in adult ruminants is from 5% to 30% (21). Furthermore, McDowell (21) showed that availability values for magnesium as high as 70% have been reported in young milk-fed calves, which decreased to 30% to 50% in older calves. This may depend on the composition of the diet. According to Giduck and Fontenot (22), magnesium absorption was 15% in sheep fed hay alone and increased to 38% for animals supplemented with readily fermentable carbohydrates. Strusińska et al. (13) observed increased concentrations of magnesium in milk 60 days after calving in cows fed a ration containing live yeast.

In the current study, the retention of copper numerically decreased. On the other hand, Iwańska et al. (14) reported that the content of copper in milk was insignificantly decreased in cows fed a mixture of live yeast and vitamin–mineral premix compared with control animals. Hemken (23) suggests that low availability of copper depends on the degradation dynamics of copper bioplexes in the rumen and on the necessity to improve copper status, most probably on adequate copper accumulation in the liver. According to Du et al. (24), ruminal bacteria prefer peptides and amino acids from Cu-bioplex and release copper as a free ion that cannot be absorbed as effectively as copper bound with peptides or amino acids.

In the present experiment, a decrease was observed in total cholesterol and triacylglycerol levels in the blood serum of rams fed the diet with yeast. The effect of yeast on the triacylglycerol level found in our study was in accordance with the results described by Strusińska et al. (25) when live yeast was introduced into a dairy cow diet. Dobicki et al. (26) showed that yeast in the diet significantly decreased total cholesterol in the blood serum of calves. The decrease in triacylglycerol and total cholesterol could be caused by changes in rumen fermentation. The increase in the concentration of rumen short-chain fatty acids (propionate, butyrate, and valerate) is capable of reducing the synthesis of triacylglycerols and total cholesterol in the liver, and may change the lipid profile of blood. Furthermore, the cell wall of yeast is a rich source of β-glucans. These polysaccharides reduce serum total cholesterol (27). According to Bauchart (28), the total cholesterol concentration in blood depends on age, physiological state, and diet, particularly on the total lipid and long-chain fatty acid content in the ration. In contrast to the results of our study are the earlier results of Payandeh and Kafilzadeh (29), who observed a significant increase in the concentration of triacylglycerols and no significant enhancement of total cholesterol in blood serum of lambs. In contrast to the present study, Campanile et al. (30) observed no effect from adding yeast to the diet on the LDL level in buffalo cows. However, Pysera and Opałka (9) showed a significant increase in the LDL fraction in the blood of calves. According to these authors, application of live yeast may modify the lipid composition of the VLDL and LDL fractions in blood.

We conclude that live cell yeast added to the diet of rams slightly increased magnesium retention and decreased the concentrations of total cholesterol, triacylglycerols, and LDL in blood serum. These results may suggest that live yeast may influence ruminant product (meat, milk) quality and fertility. However, these results are not univocal, and further studies could explain the influence more precisely.

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