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Yeast cells as a feed supplement for cattle. 2. Effect of liquid yeast cultures on digestive processes in the rumen of bulls

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ABSTRACT

The experiment was carried out on 12 Black- and -White Lowland bulls with an average body weight of 400 ± 50 kg divided into 4 groups and fed concentrates and meadow hay according to the INRA system. After 2 months of adaptation, the level of metabolites in the rumen was determined and the diet for the respective groups was supplemented with yeast cultures: *Saccharomyces cerevisiae* 1026, *Saccharomyces carlsbergensis* brewery strains SK-1, BS-Bratislava or wine strain T-81. All yeast supplements caused changes in yeast cell density, protozoa number, pH, ammonia concentration and VFA proportion in the rumen of bulls. The greatest growth ability and largest effect on fermentation in the rumen was demonstrated by the brewery strains, *Saccharomyces carlsbergensis* and *Saccharomyces cerevisiae* 1026.

KEY WORDS: cattle, yeast, rumen, metabolism

INTRODUCTION

Many reports have recently dealt with the use of small amounts of viable yeast cultures as probiotics in ruminant rations to influence the rumen fermentation pattern; diet composition has been reported to play a role in modulating the effect of yeast (Wiedmeier et al., 1987; Williams, 1989; Huhtanen, 1991; Carro et al., 1992; Kumar et al., 1994; Olson et al., 1994; Plata et al., 1994; Rouzbehan et al., 1994; Zeleňák et al., 1994). In our previous study on calves, we demonstrated

that supplementing diets with viable *Saccharomyces carlsbergensis* yeast cells influenced calf growth and digesta enzymatic activity (Strzetelski et al., 1995). It is therefore reasonable to expect that different strains of yeast could alter the pattern of nutrient metabolism in the rumen. The aim of this study was to verify this suggestion by assaying nutrient metabolites, yeast cell and protozoa density in the rumen of bulls.

MATERIAL AND METHODS

Animals and experimental design

The experiment was carried out on 12 Black- and -White Lowland bulls of about 400±50 kg body weight fed individually with an average of 6.5 kg concentrate and 1 kg meadow hay daily in two equal portions. The concentrate mixture consisted of (in %): ground barley, 60; ground wheat, 10; soyabean oilmeal, 15; wheat bran, 13 and mineral mixture[1], 2. Daily rations were established according to the INRA system (1988), assuming a body weight gain of 1.2 kg/day. The animals had free access to water and were kept tied in single stalls with a rubber floor.

The period of adaptation to the diet lasted 2 months. In the second week of this period about 350 ml of rumen liquor were sampled every other day from each animal, mixed together and given back to the animals to equalize the microbial population in their rumens. At the end of this period, samples of rumen liquor were taken from all animals for control analysis (group C).

After the adaptation period the animals were divided into 4 groups of 3 animals. Ten grams of respective culture of viable yeast cells, obtained as described in a previous paper (Strzetelski et al., 1995), were given with each meal for 3 weeks:

- *Saccharomyces cerevisiae* 1026 (group Yp)
- *Saccharomyces carlsbergensis* brewery strain SK-1 (group S)
- *Saccharomyces carlsbergensis* brewery strain BS-Bratislava (group B)
- *Saccharomyces carlsbergensis* wine strain yeast T-81 (group T).

Each of the yeast strains was tested on 3 bulls by sampling rumen liquor on days 7, 14 and 21 after starting yeast administration. Samples were taken before the morning feeding (sample 0) and 1, 2, 3, 4, 5 and 7 h after feeding. Rumen liquor samples (about 250 ml) were filtered through 4 layers of cheese cloth and

[1] Composition, % – common salt 25, $CaHPO_4$ from Bonarka (Poland) 425, and industrial mineral mixture MM 50. One kg mixture contained, g: 199 Ca; 86 P; 212 Cl; 2 Mg; 0.32 Fe; 0.11 Cu; 0.10 Mn; 0.02 Co and 0.07 J

divided into two portions, one of them was treated with a formalin-glycerol solution.

Analysis

Determination of density and purity of yeast cultures administered to bulls, chemical analysis and nutritive value of feed were carried out as described in the previous study on calves (Strzetelski et al., 1995).

The density of yeast cells in the rumen liquor samples withdrawn 0, 2, 4 and 7 h after feeding was determined by the dilution method, in two replicates, inoculating malt agar supplemented with chloromycitine (0.05 g/l) on Petri dishes. The number of yeast colonies was counted after 90 h of incubation at 25°C. Ammonia was estimated according to the Conway method (1962), VFA – on a Philips PU 4500 gas chromatograph, pH potentiometrically. Protozoa in the rumen liquor samples treated with formalin-glycerol solution were quantified in a Fusch Rosental chamber.

RESULTS

The average density of yeast cells was n x 10^6 cells/ml: 122, *Saccharomyces cerevisiae* 1026 and 109.5; 216.3; 168.7 for *Saccharomyces carlsbergensis* strains SK-1, BS-Bratislava and T-81, respectively. There were no bacterial cells in the liquid yeast cultures.

The chemical composition of feeds and nutritive value of concentrates and meadow hay are given in Table 1. Daily intake of feeds during the period of sampling rumen liquor was 3 to 4 kg of concentrates and 0.5 kg of hay. The animals ate about 80% of the hay and 50% of the concentrates during the first hour after offering feeds.

TABLE 1

Chemical composition (%) and nutritive value of feeds

Feed	Dry matter	Crude protein	Ether extract	Crude fibre	N-free extractives	Ash
Ground barley	86.46	12.09	1.37	4.38	66.01	2.57
Ground wheat	86.53	12.14	1.18	2.34	69.09	1.78
Soyabean oilmeal	88.03	42.62	1.55	5.70	30.76	7.40
Wheat bran	87.20	13.25	3.32	6.98	59.03	4.62
Concentrate mixture*	86.70	16.77	1.60	5.50	60.50	3.33
Meadow hay*	85.63	10.25	2.24	26.62	41.40	5.12

* according to INRA system (INRA, 1993) content in 1 kg DM of: concentrate mixture 124 g PDI (PDIN-PDIE = 8 g) and 1.11 UFL; meadow hay 86 g PDI (PDIN-PDIE = – 5 g) and 0.68 UFL

In all of the groups, the highest density of yeast cells in the rumen liquor was found 2 h after feeding (Figure 1). The density of the yeast cells was highest in groups Yp and S and the lowest in group T. After this time, yeast cell density decreased in all groups but more slowly in group Yp than in the remaining groups of animals receiving yeast supplements. The lowest yeast cell density was in the rumen liquor of control animals.

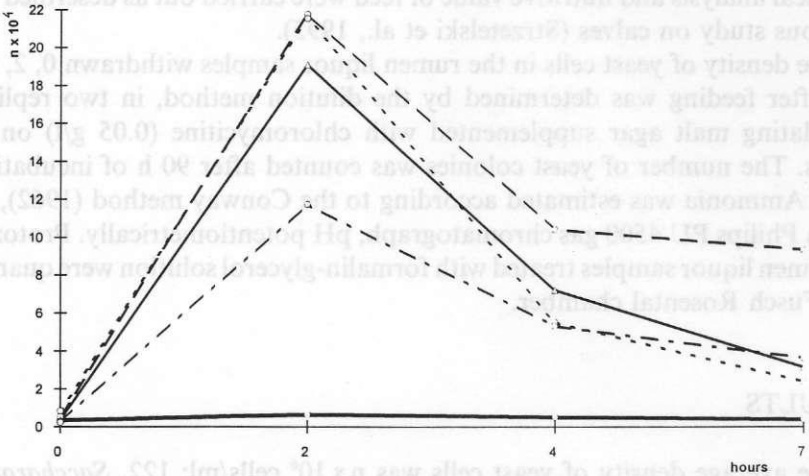

Figure 1. Daily pattern of different strains of yeast cultures density in the rumen liquor (n cells x 10^4/ml) counted over on 100 x 10^6 cells/ml of yeast cultures
groups: —o— C — o— Yp – –o– – S —△— B —o— T

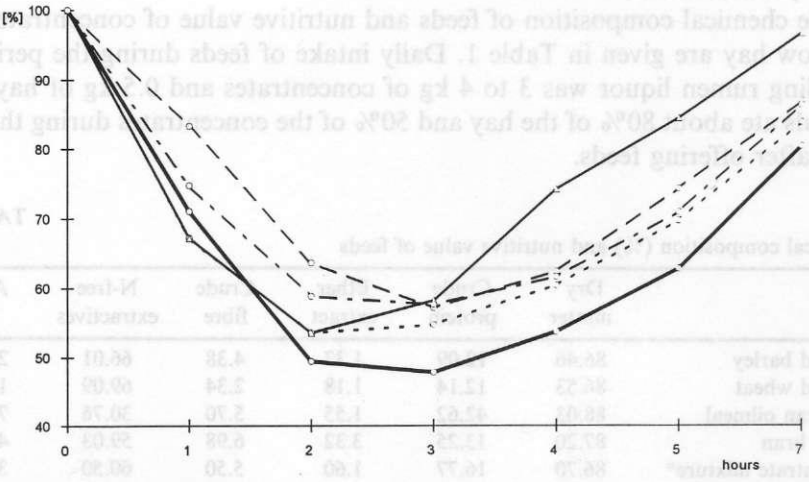

Figure 2. Daily pattern of protozoa density in the rumen liquor of bulls receiving different yeast strains, expressed as percent of initial value at 0 (100%)
groups: —o— C — o— Yp – –o– – S —△— B —o— T

The protozoa content in the rumen liquor of all of the animals decreased until the third hour after feeding, afterwards it rose (Figure 2). The lowest number of protozoa in the rumen, as the average from 1 to 7 h after feeding, was found in the control animals (60.8% of the number found at time 0). Supplementing feeds with yeast increased protozoa density in the rumen as compared to control animals at 0 h: 64.9%, group S; 68.2%, T; 71.3%, Yp and 74.4%, B.

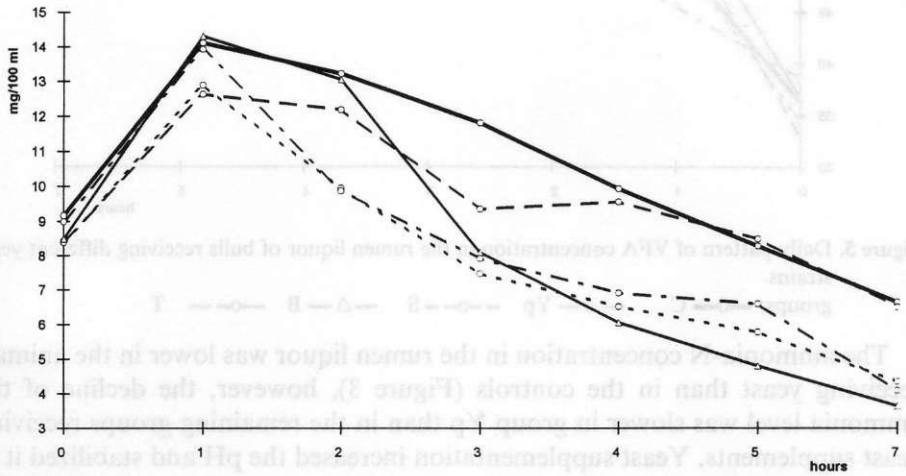

Figure 3. Daily pattern of ammoniaN concentration in the rumen liquor of bulls receiving different yeast strains
groups: —o— C —o— Yp - -o- - S —△— B —o— T

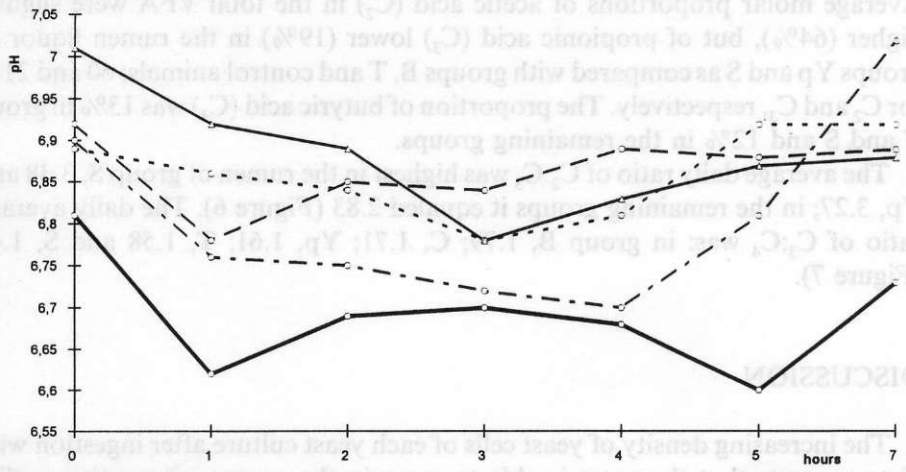

Figure 4. Daily pattern of pH of rumen liquor of bulls receiving different yeast strains
groups: —o— C —o— Yp - -o- - S —△— B —o— T

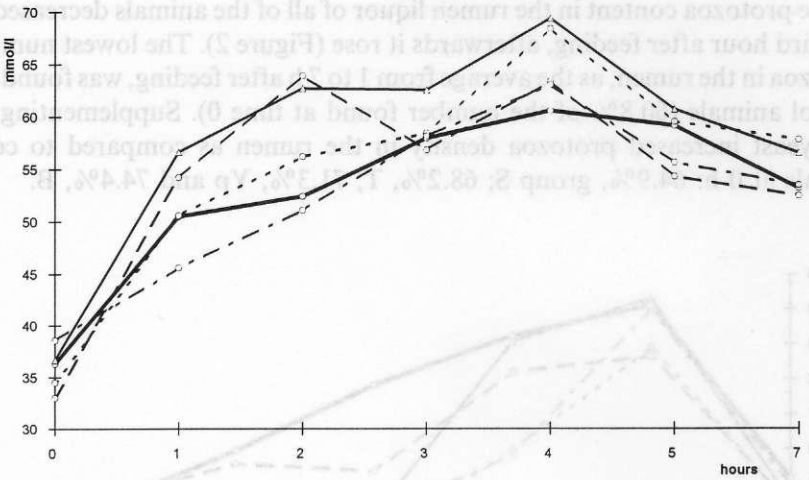

Figure 5. Daily pattern of VFA concentration in the rumen liquor of bulls receiving different yeast strains
groups: —o— C — o— Yp – –o– – S —△— B —o— T

The ammonia-N concentration in the rumen liquor was lower in the animals receiving yeast than in the controls (Figure 3), however, the decline of the ammonia level was slower in group Yp than in the remaining groups receiving yeast supplements. Yeast supplementation increased the pH and stabilized it in time as compared to control animals, especially in groups Yp and S (Figure 4).

The total volatile fatty acid (VFA) level in the rumen liquor was similar in the animals of all groups being, however, slightly higher in group B (Figure 5). Average molar proportions of acetic acid (C_2) in the total VFA were slightly higher (64%), but of propionic acid (C_3) lower (19%) in the rumen liquor of groups Yp and S as compared with groups B, T and control animals: 60 and 21% for C_2 and C_3, respectively. The proportion of butyric acid (C_4) was 13% in group T and S and 12% in the remaining groups.

The average daily ratio of C_2:C_3 was highest in the rumen of group S, 3.38 and Yp, 3.27; in the remaining groups it equaled 2.83 (Figure 6). The daily average ratio of C_3:C_4 was: in group B, 1.79; C, 1.71; Yp, 1.61; T, 1.58 and S, 1.48 (Figure 7).

DISCUSSION

The increasing density of yeast cells of each yeast culture after ingestion with diets suggests that the yeast is able to grow in the rumen, supporting earlier results of experiments on sheep (Ryś et al., 1962) and results obtained on

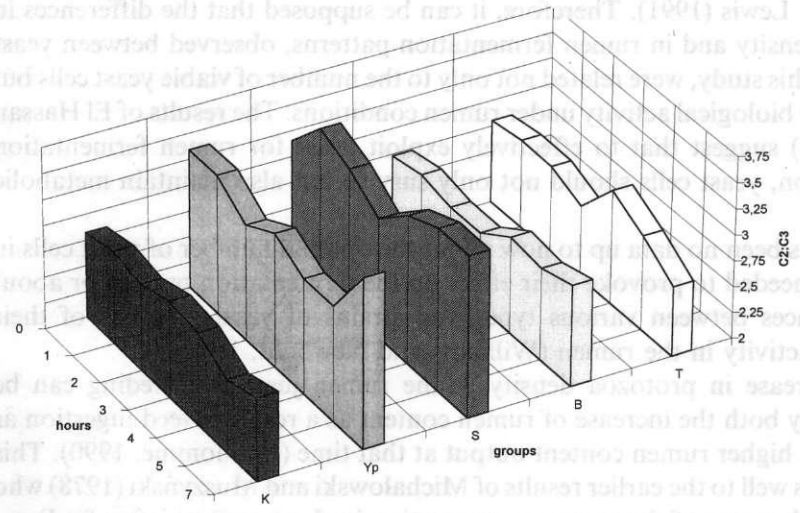

Figure 6. Ratio of C_2:C_3 in the rumen liquor of bulls receiving different yeast strains

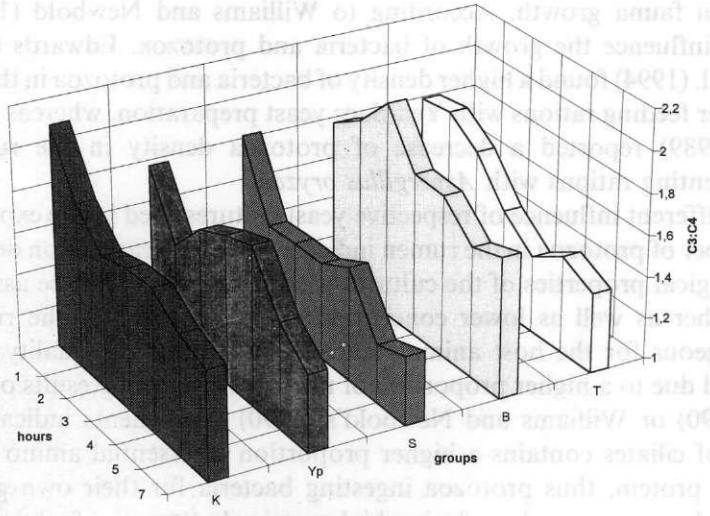

Figure 7. Ratio of C_3:C_4 in the rumen liquor of bulls receiving different yeast strains

simulated rumen with *Saccharomyces cerevisiae* 1026 (Dawson, 1987). On the other hand, the quick decline of yeast cell concentration in the rumen liquor after reaching the peak value leads to the supposition that yeast growth in the rumen can be limited (Newbold et al., 1990, 1993) but this does not exclude its metabolic activity in the rumen (Williams and Newbold, 1990). Decreasing density of yeast

populations in relation to fermentation intensity with brewery yeast was also reported by Lewis (1991). Therefore, it can be supposed that the differences in yeast cell density and in rumen fermentation patterns, observed between yeast cultures in this study, were related not only to the number of viable yeast cells but also to their biological activity under rumen conditions. The results of El Hassan et al. (1993) suggest that to effectively exploit yeast for rumen fermentation manipulation, yeast cells should not only survive but also maintain metabolic activity.

There has been no data up to now about the optimal number of yeast cells in the rumen needed to provoke their effect on the fermentation pattern or about the differences between various types and strains of yeast in terms of their metabolic activity in the rumen (Williams and Newbold, 1990).

The decrease in protozoa density in the rumen just after feeding can be explained by both the increase of rumen content as a result of feed ingestion as well as by a higher rumen content output at that time (Bonhomme, 1990). This corresponds well to the earlier results of Michałowski and Muszyński (1978) who reported a decrease of the protozoa proportion in sheep rumens after feeding.

A higher protozoa density in the rumen of all animals given feeds supplemented with yeast compared to control animals suggests a stimulating effect of yeast on rumen fauna growth. According to Williams and Newbold (1990) fungi cultures influence the growth of bacteria and protozoa. Edwards (1991) and Plata et al. (1994) found a higher density of bacteria and protozoa in the rumen of bulls after feeding rations with Yea-Sacc yeast preparation, whereas Frumholtz et al. (1989) reported a decrease of protozoa density in the rumen after supplementing rations with *Aspergillus oryzae*.

The different influence of respective yeast cultures used in the experiment on the number of protozoa in the rumen indicates that the yeast action depended on the biological properties of the cultures used. However, it can be assumed that both higher as well as lower concentrations of protozoa in the rumen were advantageous for the host animal, since in both cases the quality of protein improved due to a higher proportion of microbial protein. Results of Bonhomme's (1990) or Williams and Newbold's (1990) experiments indicate that the protein of ciliates contains a higher proportion of essential amino acids than bacterial protein, thus protozoa ingesting bacteria for their own growth can improve the nutritive value of microbial protein. In the case of a low number or lack of protozoa in the rumen, bacterial and undegraded feed protein can avoid ingestion by protozoa increasing their outflow from the rumen (Veira et al,. 1984; Kayouli et al., 1986).

The ammonia-N concentration depended on the type of yeast culture used and was higher at higher protozoa density. Bonhomme (1990) reported that protozoa are characterized by considerable proteolytic activity and their

presence stimulates ammonia production. It can not be excluded that some strains of yeast might stimulate proteolysis in the rumen (Frumholtz et al., 1989; Fondevila et al., 1990; Williams and Newbold, 1990).

An increased pH value and its better stabilization in the rumen after feeding rations with yeast cultures suggests an advantageous influence on bacterial cellulolytic activity (Istasse and Ørskov, 1983; Williams, 1988).

Changes in VFA concentration and proportions suggest that various yeast cultures changed the rumen fermentation pattern, similarly as in the experiments of other authors (Gray and Ryan, 1988; Williams, 1989; Mutsvangwa et al., 1992; Wallace and Newbold, 1992; Williams and Newbold, 1992; Piva et al., 1993). It can be assumed that changes in the VFA pattern depended on the activity of the yeast culture used in the experiment, as the feeding and sampling regimen was similar for all animals. Williams (1989) and Williams and Newbold (1990) reported that differences between the strains *Saccharomyces cerevisiae* and *Aspergillus oryzae* in influence on the pattern of rumen fermentation are considerable.

The results of this experiment demonstrated that cultures of *Saccharomyces carsbergensis* brewery strains SK-1, BS-Bratislava and *Saccharomyces cerevisiae* 1026 can be used as a supplement to rations for bulls, because they demonstrate the ability grow and are metabolically active under rumen conditions. They exert a favourable, although differentiated among strains, influence on the pattern of fermentation.

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STRESZCZENIE

Zastosowanie drożdży jako dodatku paszowego w żywieniu bydła. 2. Wpływ dodatku płynnych żywych kultur drożdży na procesy trawienne w żwaczu buhajków

Wpływ różnych płynnych kultur drożdży na przemiany zachodzące w żwaczu badano w doświadczeniu na 12 buhajkach rasy o średniej masie ciała około 400 kg podzielonych na 4 grupy. Wszystkim zwierzętom podawano 6,5 kg mieszanki treściwej i 1 kg siana łąkowego dziennie. Po 2 miesiącach adaptacji badano poziom metabolitów w żwaczu zwierząt, a następnie do pasz dodawano przez 3 tygodnie, odpowiednio w grupach, kultury drożdży: *Saccharomyces cerevisiae* 1026; *Saccharomyces carlsbergensis* szczepów piwowarskich SK-1, BS-Bratysława lub szczepu winiarskiego T-81. Wszystkie szczepy drożdży powodowały zmiany liczby komórek drożdżowych i pierwotniaków oraz wartości pH, stężenia azotu amonowego oraz stężenia i proporcji lotnych kwasów tłuszczowych w treści żwacza. Największą zdolnością wzrostu i aktywnością w żwaczu odznaczały się drożdże *Saccharomyces carlsbergensis* szczepów piwowarskich SK-1 i BS-Bratysława oraz *Saccharomyces cerevisiae* 1026, natomiast drożdże szczepu winiarskiego T-81 miały mniejszy wpływ na przebieg fermentacji w żwaczu.