

Yeast cells as a feed supplement for cattle

1. Liquid viable yeast cultures for calves

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ABSTRACT

An experiment lasting 73 days was carried out on 40 Black-and-White Lowland 7-day-old calves divided into 5 groups of 8. The animals received 8 kg of whole milk daily for 5 weeks, 6 kg in the 6th and 3 kg in 7th week with free access to concentrate mixture and meadow hay. After 7 weeks of the experiment the calves were fed only concentrate to appetite and 0.3-0.4 kg of meadow hay. This basic control diet was supplemented with wort without yeast and in the experimental groups with liquid cultures of the yeast, *Saccharomyces cerevisiae* 1026 or *Saccharomyces carlsbergensis* of the brewery strain SK-1, BS-Bratislava or wine strain T-81. All strains of yeast significantly stimulated amylolytic and proteolytic activity of the digesta in the duodenum, small intestine and ileum but not in the abomasum. Body weight gains of calves fed diets supplemented with SK-1 and B-Bratislava yeast were higher than in control or in groups supplemented with the other two yeast strains.

KEY WORDS: calf performance, yeast, enzymatic activity, amino acid profile

INTRODUCTION

Natural fungal additives containing viable cells have become of interest in ruminant nutrition (Pusztai et al., 1990; Williams and Newbold, 1990). It was reported that such additives as fungal cultures based on *Saccharomyces cerevisiae* 1026 and *Aspergillus oryzae* had an advantageous effect on calf and

mature ruminant performance, protein and carbohydrate metabolism in the digestive tract and on animal product quality (Williams, 1989; Williams and Newbold, 1990; Edwards, 1991; Wallace and Newbold, 1992; Alonzo et al., 1993; Caton et al., 1993; Shievert and Shaver, 1993; Skórko-Sajko, 1993). However, information is lacking on fungal culture activity in the ruminant digestive tract, particularly beyond the rumen, and on the beneficial effect of new species and strains on digestion and utilization of nutrients.

The objective of this study was to compare different liquid cultures of *Saccharomyces carlsbergensis* and *Saccharomyces cerevisiae* 1026 on the performance, enzyme activity and amino acid profile of the intestinal digesta of calves.

MATERIAL AND METHODS

Animals and experimental design

At the age of 7 days, forty Black-and-White Lowland calves were divided into 5 groups of 8 (4 males and 4 females in each) allotted to the groups sequentially, according to calving order. The animals were kept tied in straw-bedded pens and fed individually at controlled intake and permanent access to water.

New-born calves were fed 2 kg of colostrum on the first day between 2 and 6 h of life, increasing with age to 6 kg/day fed in 3 portions until day 7. Subsequently, as the experimental period lasting 73 days started, whole milk was given to all the calves at the rate of 8 kg for 5 weeks; 6 kg in the 6th and 3 kg in the 7th week with free access to concentrate mixture and meadow hay. After week 7 the calves were fed only concentrate to appetite and 0.3-0.4 kg hay according to the INRA (1988) system. The concentrate mixture consisted of (%): ground barley, 50; ground wheat, 14; soyabean oilmeal, 21; wheat bran, 13 and mineral mixture¹, 2.

The control group (C) was given additionally 200 ml/day of wort without yeast with milk or in the final 24 days – with concentrate. Calves of the experimental groups received the same amount of wort containing the respective yeast culture:

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

¹ Mineral mixture composition, %: Bovimix 50 (commercial mineral preparation), common salt 15, limestone 25 and CaHPO₄ 10. One kg of mixture contained (g): 172 Ca; 73 P; 57 Na; 88 Cl; 19.3 Mg; 1.4 Fe; 0.75 Cu; 1.45 Mn; 0.03 Co and 1.3 Zn

All male calves were sacrificed on the last day of the experiment 3 h after feeding and total content of the abomasum (A), duodenum (D), small intestine (J) and ileum (I) was collected and representative samples were taken, stored at -25°C and freeze-dried before analyzing.

Yeast culture production

Agar cultures of *Saccharomyces carlsbergensis* (strain SK-1; BS-Bratislava or T-81) were obtained from the Institute of Biotechnology of the Agriculture and Food Industry in Warsaw. *Saccharomyces cerevisiae* strain 1026 was isolated using a plate method on Difco malt agar from Yea-Sacc yeast supplied by Alltech Biotechnology Center (USA). The yeasts were cultivated on 12⁰Blg crude malt wort without hops. The portioned wort was stored in plastic bags at -15°C . Portions of wort were thawed, warmed to 25°C and inoculated with a stock culture (4%) and fed after 48h of incubation at 25°C .

Stock cultures of yeast strains were obtained by inoculation of wort, previously sterilized for 30 min in a boiling water bath for 3 consecutive days, from agar slants and incubation for 48 h at 25°C . The obtained cultures were refreshed each week by transferring 4% of strains to fresh sterilized wort, incubating for 48 h and storing in a refrigerator until the next transfer.

Chemical analysis

The density of yeast cultures given to calves was determined once a week by counting in a Burkner chamber the number of yeast cells in samples fixed in formalin-glycerol. The purity of yeast cultures was controlled once a month by malt agar inoculation on Petri dishes and incubation at 25°C for 72h.

Proximate analysis of feeds and nitrogen content in freeze-dried digesta samples was carried out using conventional methods (AOAC, 1975). The nutritive value of feeds was estimated according to the INRA (1988) system using Polish computer software INWAR 1.0 (1993). Concentrate composition was established with INRATION 2.03 (1993) software.

Digesta alpha-amylase activity was estimated according to the Bernfeld method (1955), proteolytic activity according to Kakade et al. (1969). Amino acid proportions in yeast and hydrolyzed digesta were analyzed using a Carl Erba 3A-29 analyzer.

Statistical analysis

The obtained data were subjected to one or two factorial variance analyses using Statgraphics Plus 6.0 software (1992).

RESULTS

The average density of yeast culture ($n \times 10^6$ cells/ml) was: 104.6 – for *Saccharomyces cerevisiae* 1026 and 100.8; 210.4; 156.9 for *Saccharomyces carlsbergensis* strains SK-1, BS-Bratislava and T-81, respectively. Control of yeast culture purity showed that all cultures were satisfactorily pure and bacterial cells were found only sporadically.

The chemical composition and nutritive value of the whole milk, concentrate and meadow hay are shown in Table 1.

The average consumption of concentrate mixture during the experimental period did not differ among the groups and equalled 59 in group C; 63 in group S and 61 kg per calf in the remaining two groups ($P > 0.05$). Average daily intake of concentrate mixture was 0.82 ± 0.03 kg; crude protein – 300 ± 4 g; PDI – 241 ± 3 g; UFL – 2.13 ± 0.03 g and 24.33 ± 0.22 MJ ME. Daily weight gain of calves (Table 2) in group S, receiving SK-1 yeast culture, was higher than in groups C, Yp and T ($P \leq 0.05$) with slightly better, but not significantly, crude protein and concentrate utilization per 1 kg body weight gain. Average daily weight gain was about 8% higher and nutrient utilization 6% higher in group S compared with the control group C. Calves of group B gained more and utilized nutrients about 4% better as compared with group C.

TABLE 1

Chemical composition (%) and nutritive value of feeds

Feeds	Dry matter	Crude protein	Ether extract	Crude fibre	N-free extractives	Ash
Whole milk	12.13	2.90	3.81	–	4.67	0.75
Ground barley	87.10	8.01	2.08	4.45	70.56	2.00
Ground wheat	87.40	9.70	1.76	2.64	71.44	1.86
Soyabean oilmeal	87.30	40.77	2.32	6.28	30.68	7.25
Wheat bran	87.20	13.25	3.32	6.98	59.03	4.62
Concentrate mixture	87.20	15.70	2.20	4.71	59.45	5.14
Meadow hay	85.90	11.46	2.52	25.03	42.81	4.08

according to INRA system (PAN-INRA, 1993) content in 1 kg DM of meadow hay 83 g PDI (PDIN = PDIE) and 0.73 UFL; concentrate mixture – 124 g PDI (PDIN = PDIE) and 1.20 UFL; whole milk – 140g PDI=PDIE (PDIN = 165)

TABLE 2

Specification	Group of calves ¹						Sex	
	c	Yp	S	B	I	+ heifers	bulls	SE
Initial liveweight, kg	39.1	38.5	39.3	39.5	40.3	39.2	39.5	4.31
Final liveweight, kg	92.4	92.7	96.9	94.8	92.7	93.1	95.0	5.00
Daily gain ² , g	730 ^b	742 ^b	789 ^a	746 ^{ab}	730 ^b	739	760	37.25
Feed utilization (per 1 kg gain)								
concentrate mixture, kg	1.11	1.13	1.08	1.11	1.14	1.12	1.10	0.07
dry matter, kg	2.23	2.21	2.10	2.20	2.25	2.27	2.20	0.09
crude protein, g	407.0	407.0	384.9	402.6	411.5	406.5	397.4	16.81
PDI ³ , g	326.4 ^b	326.3 ^b	308.1 ^a	323.2 ^{ab}	330.1 ^b	330.1	318.5	13.56
NE (UFL) ³	2.89 ^b	2.88 ^b	2.72 ^a	2.85 ^{ab}	2.92 ^b	2.92	2.81	0.12
ME ³ (MJ)	33.03 ^b	32.93 ^{ab}	31.11 ^a	32.61 ^{ab}	33.33 ^b	33.27	32.16	1.34

a, b - P ≤ 0.05

¹ strain of yeast; C - control; Yp - *Saccharomyces carlsbergensis* 1026; S - *Saccharomyces carlsbergensis* SK-1; B - *Saccharomyces carlsbergensis* BS-Bratislava; T - wine yeast strain T-81S

² experimental period 73 - days

³ PDI - protein digestible in the small intestine, NE (UFL) - net energy in feed units for milk production, ME - metabolizable energy (according to INRA, 1988)

TABLE 3

Amylolytic (P) and proteolytic activity (P), crude protein (CP) content and total amino acid (AA) profile in digesta of total intestine and parts of digestive tract in calves fed different strains of yeast

Group	Activity [▲]		Percent of DM	
	A	P	crude protein	total amino acid
<i>Bulked sample of digesta of abomasum, duodenum, small intestine and ileum</i>				
Control	617 ^{Aa}	2346 ^a	37.7	31.9 ^{ab}
Yp 1026	720 ^a	2950 ^{ab}	36.9	30.1 ^{ab}
SK-I	1017 ^{Aa}	3380 ^{ab}	37.2	29.1 ^a
BS	1780 ^{Bb}	3486 ^b	38.3	31.9 ^{ab}
T-81	961 ^{Aa}	2702 ^{ab}	41.5	35.5 ^b
<i>Digesta from reseceptive parts of digestive tract</i>				
Abomasum	118 ^{Aa}	360 ^{Aa}	13.1 ^{Aa}	9.8 ^{Aa}
Duodenum	1149 ^{Bb}	2966 ^{Bb}	53.4 ^{Cc}	46.3 ^{Cc}
Small intestine	1697 ^{Bb}	4536 ^{Cc}	59.5 ^{Cc}	50.6 ^{Cc}
Ileum	1112 ^{Bb}	4030 ^{Cc}	25.4 ^{Bb}	20.1 ^{Bb}
SE	422	1121	5.1	5.8
Interaction	**	**	NS	NS

▲ - 1 unit activity = absorbance increase by 0.01 at 550 nm (A) or 280 nm (P) wave length in both tests
 abc - $P \leq 0.05$; ABC - $P \leq 0.01$

Average amylolytic and proteolytic activities in whole digestive tract contents were higher in calves fed yeast than in the control group, but significant differences were demonstrated only for group B ($P \leq 0.01$) for amylolytic activity and ($P \leq 0.05$) for proteolytic activity (Table 3).

Enzymatic activity differed along the digestive tract and reached the highest values in digesta from the small intestine (J) and ileum (I), lower in the duodenum (D) and the smallest in the abomasum (A) ($P \leq 0.01$). Interaction between yeast strain and enzymatic activity in digesta from different part of digestive tract was significant ($P \leq 0.01$).

Average crude protein content in the dry matter of bulked digesta of whole intestines did not differ significantly among treatments, including the control group ($P > 0.05$) but reached the highest value in the duodenum and small intestine, the lowest values were obtained in the abomasum (Table 3). Total amino acid content in dry matter of bulked samples from the digestive tract was the highest in animals of group T and the lowest in group S ($P \leq 0.05$).

Crude protein and total amino acid were the lowest in abomasal digesta dry matter (Table 3) but increased in the duodenum and small intestine ($P \leq 0.01$) while declining again in ileal digesta ($P \leq 0.01$). The amino acid proportion was similar in all of the used yeast cultures.

Feeding rations with yeast cultures did not significantly change the amino acid profile in relation to the control group ($P > 0.05$), except tyrosine, the level of which was higher in the digesta of group B (Table 4). Changes in amino acid proportion along the digestive tract were found to be significant for glutamine, which was higher in the ileum than in the abomasum ($P \leq 0.05$), alanine was higher in the abomasum and ileum than in the duodenum and small intestine ($P \leq 0.05$ or $P \leq 0.01$) and arginine was the lowest in the abomasum ($P \leq 0.05$).

DISCUSSION

The obtained results indicate that the type of yeast played a role in stimulating the amylolytic and proteolytic activity of the intestinal content but not that of the abomasum and, in some cases, influenced the amino acid profile in the digesta of calves. The degree of stimulation depended on the type of yeast used, as the influence of other nutritional factors was eliminated by feeding the calves the same basal ration in all of the groups.

Fallon and Harte (1987a,b) fed calves dry cultures of *Saccharomyces cerevisiae* 1026 in the form of the Yea-Sacc preparation and found that feed intake and body weight gain depended on the composition of the concentrate mixture. Williams (1988) suggested that the advantageous effect of feeding yeast to calves was better visible when the rations contained more readily fermented carbohydrate. Such carbohydrates in the diet normally decrease the appetite of animals, but yeast either reduced this effect or directly stimulated feed intake. In the present experiment, the liquid culture of *Saccharomyces cerevisiae* 1026 isolated from the Yea-Sacc preparation (group Yp) did not affect either feed intake or body weight gain as compared with the control group (C), although the starch content in the concentrate amounted to about 60%. It can be presumed that the activity of the liquid culture differed from that of the dry Yea-Sacc preparation due to the different method of yeast preparation. When Williams and Newbold (1990) analyzed the results of other authors, they concluded that changing the cultivation conditions of fungal culture might significantly affect their metabolic activity.

The slightly better calf performance in groups S and B, as compared with the remaining groups, suggests that *Saccharomyces carlsbergensis* brewery strains (SK-1 and BS-Bratislava) have a higher stimulating activity than *Saccharomyces cerevisiae* 1026 and *Saccharomyces carlsbergensis* wine yeast strain T-81. These results indicate that supplementing diets with all of the yeast cultures used in this study increased, but according to a different pattern, the enzymatic activity of intestinal digesta with the exception of the abomasum. Harmon (1993) reported that secretion and activity of pancreatic enzymes may depend to a large degree on

the quantity, quality and proportion of nutrients in the diet; may be the yeast included in the diets affect this activity. Pusztai et al. (1990) suggest that digestion and absorption of nutrients are related to chemical probiosis which is defined as the interaction between feed components, physiological function of the intestine and microbiological spectrum in the digestive tract. Williams (1989) and Williams and Newbold, (1990) demonstrated that, after feeding animals *Saccharomyces cerevisiae* 1026, live yeast cells can be found in the duodenum or small intestine, indicating extraruminal influence of yeast on the digestive processes. The interaction found between type of yeast culture fed and digesta enzymatic activity, depending on the part of digestive tract, suggests that the effectiveness of the yeast additive in our experiment was related to the function of the given segment of the digestive tract (Garvie et al., 1984; Barrow et al., 1989).

The lowest proportion of crude protein in dry matter of abomasal digesta could be explained by the relatively high content of nitrogen free components in this part of the digestive tract. Their higher rate of digestion in the initial parts of the intestine than the rate of protein absorption leads to a higher apparent proportion of protein in digesta of the duodenum and small intestine. The digestion rate of protein in the ileum prevails, leading to a relatively lower protein content than in the small intestine in the digesta.

The lack of changes in the amino acid profile of the digesta content between the control and experimental groups, excluding tyrosine, suggests that adding yeast to the diet of calves did not influence the amino acid composition of the digesta.

In conclusion, it could be said that viable yeast fed with rations to calves affected the amylolytic and proteolytic activity of the digesta. Viable *Saccharomyces carlsbergensis* brewery strains SK-1 and BS-Bratislava used as a supplement to the diet for calves could be of practical significance as they resulted in higher daily body gain of animals in comparison with the control group or those supplemented with *Saccharomyces carlsbergensis* or wine strain T-81 or *Saccharomyces cerevisiae* 1026.

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STRESZCZENIE

Zastosowanie drożdży jako dodatku paszowego w żywieniu bydła.

1. Płynne żywe kultury drożdży w żywieniu cieląt

Doświadczenie trwające 73 dni prowadzono na 40 tygodniowych cielętach rasy ncb, podzielonych na 5 grup po 8 sztuk w każdej. Zwierzęta otrzymywały dziennie po 8 kg pełnego mleka przez 5 tygodni, następnie 5 kg w szóstym, 3 kg w siódmym tygodniu doświadczenia przy stałym dostępie do mieszanki pasz treściwych i siana łąkowego. Po 7 tygodniach zwierzęta otrzymywały jedynie mieszankę treściwą do woli i 0,3-0,4 kg siana dziennie. Podstawowa dieta dla grupy kontrolnej była przez cały okres doświadczenia uzupełniana surową brzoźką, a w pozostałych grupach doświadczalnych płynnymi drożdżami *Saccharomyces cerevisiae* 1026 lub *Saccharomyces carlsbergensis* szczepu piwowarskiego SK-1 lub BS-Bratysława szczepu winiarskiego T-81. Wszystkie szczepy drożdży stymulowały amylolityczną i proteolityczną aktywność treści dwunastnicy, jelita cienkiego i biodrowego. Przyrosty masy ciała cieląt otrzymujących drożdże szczepu SK-1 i BS-Bratysława były większe niż w pozostałych grupach.