Reprod Nutr Dev (1993) 33, 43-49

Original article

Effect of a viable yeast culture on digestibility and rumen fermentation in sheep fed different types of diets

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(Received 15 May 1992; accepted 29 October 1992)

Summary — Five mature wethers fitted with rumen fistulas were fed grass hay and a sugarbeet-pulp-based concentrate or maize silage and a cereal-based concentrate (50/50 digestible organic matter basis), or without with 5 g yeast supplement (*Saccharomyces cerevisiae*, Biosaf®) per day in a latin square design. Diets were given for a 28-d adaptation period, followed by a 10-d collection period to determine digestibility and nitrogen retention data. Afterwards, rumen samples were taken on 3 consecutive days and analysed for volatile fatty acids, pH and ammonia.
Digestibility and nitrogen balance were not affected by yeast treatment. Supplementation of yeast increased acetate: propionate ratio, butyrate, isoacids, pH and ammonia. The effects were more pronounced for the maize silage diet. These results demonstrate that the effect of yeast culture on rumen fermentation may depend on the nature of the diet.
Living yeast cell number in the rumen fluid rapidly declined when dietary yeast was ceased. Furthermore, yeast cells survived the passage through the digestive tract.

yeast / rumen fermentation / volatile fatty acids / sheep

Résumé — Effet d'une levure vivante sur la digestibilité et la fermentation dans le rumen des moutons recevant différents types de ration. *Cinq béliers châtrés fistulés ont reçu une ration à base de foin et de concentré riche en pulpe de betteraves sucrières ou de l'ensilage de maïs et du concentré riche en céréales (50/50 sur la base de la matière organique digestible), complémentée ou non avec 5 g de levure (Biosaf®) /j suivant un schéma en carré latin. Les régimes ont été distribués pendant une période d'adaptation de 28 j, suivie d'une période de collecte de 10 j, afin de déterminer la digestibilité et les bilans d'azote. Ensuite, des échantillons du contenu du rumen ont été prélevés pendant 3 j consécutifs pour déterminer les acides gras volatils, le pH et l'ammoniaque. La digestibilité et les bilans d'azote n'ont pas été influencés par la levure, tandis que la proportion acétate/propionate, les concentrations de butyrate et d'ammoniaque et le pH ont été augmentés. L'effet a été plus net avec la ration d'ensilage de maïs. Ces résultats démontrent que l'effet de la levure dé-*

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pend du type de ration. Le nombre des cellules vivantes de levures au niveau du rumen a diminué quand l'administration de levure a cessé. Le dénombrement des cellules vivantes dans les fécès a démontré que les levures peuvent survivre tout au long du tractus digestif de l'animal.

levure / fermentation dans le rumen / acide gras volatil / mouton

INTRODUCTION

Recently, there has been remarkable interest in the use of yeast cultures to improve productivity in livestock husbandry. In comparison with antimicrobial agents, yeast culture offers a natural alternative to manipulate animal performance.

Positive effects of a yeast culture on performances of dairy cows and rearing calves have been reported (Günther, 1989; Gomez-Alarcon *et al,* 1991 and Williams *et al,* 1991).

However, a significant positive effect of yeast on animal performance was not always confirmed (Adams *et al,* 1981; Fallon and Harte, 1987; Gomez-Alarcon *et al,* 1991; Williams *et al,* 1991). Therefore, the mode of action of a yeast culture needs to be unravelled to explain the variable response to yeast supplementation.

The purpose of this study was to evaluate the effect of yeast culture on digestibility and rumen fermentation in 2 different types of diets, and to investigate the survival of yeast cells in the digestive tract.

MATERIALS AND METHODS

Digestibility coefficients and ruminal fermentation characteristics were determined with 5 ruminally cannulated wethers, receiving a diet composed of 50% grass hay and 50% concentrate (diet A) or 50% maize silage and 50% concentrate (diet B) on a digestible organic matter (DM) basis. The concentrates were formulated to contain a low or a high content of rapidly fermentable carbohydrates, respectively, and to yield a 12% crude protein content in the dry matter of the diets. The percentage composition of the 2 concentrates is given in table I. Each diet was fed at maintenance in 2 meals daily, without (control, C) or with 2.5 g yeast (Y, *Saccharomyces cerevisiae*) (Biosaf®, SI Lesaffre, France) per meal. The experiment was carried out according to a 4 x 4 latin square design.

Each collection period lasted 10 d and was preceded by an adaptation period of 28 d. Faeces and urine were collected to determine apparent nutrient digestibility and nitrogen (N) retention. Analyses of feeds and faeces were conducted according to standard methods. After the collection period, rumen liquid samples were taken on 3 consecutive days at 2 h postprandial to measure pH, volatile fatty acid (VFA) concentration and ammonia. VFA's were determined by gas chromatography (Supelco, 1991). Ammonia was analysed according to the method of Voigt and Steger (1967).

Digestibility and fermentation data were presented as means, using a 2-way analysis of variance to detect differences (Snedecor and Cochran, 1980).

At the end of the yeast administration rumen samples were taken from 3 animals per diet at the time of the last feeding of Y and at 2 and 8 h after this meal. Furthermore, rumen samples were also taken after 24, 32 and 48 h only from 2 animals, one on each diet.

Faeces samples were taken from the same animals at 2 h after the last feeding of Y and after 24 and 48 h. One animal, fed the control diet, was also investigated. Because of the negligible amount of yeast cells in the rumen fluid no further measurements were carried out. The number of viable yeast cells in rumen fluid and faeces was determined by counting the colony forming units on YM agar (Difco) containing 1% chloramphenicol.

Table I. Composition of the experimental concentrates and chemical analysisof the feeds.

	Diet A		*Diet B*	
	Concentrate	*Grass-hay*	*Concentrate*	*Maize silage*
Ingredients (%)				
Sugarbeet pulp	96.3	–	–	–
Wheat	–	–	43	–
Barley	–	–	43	–
Soybean meal	–	–	9.4	–
Mineral-vitamin premix	3.7	–	4.6	–
Chemical analysis (g/kg DM)				
Dry matter	865	824	860	362
Crude protein	107	157	157	69
Ether extract	5	17	14	26
Crude fibre	201	270	45	210
N-free extract	643	485	739	650
Total ash	44	71	45	45
Sugars	59	95	29	5
Starch	–	6	535	331

RESULTS

The chemical composition of the feeds is shown in table I. Due to the rather low protein content of maize silage (69 g/kg DM), the diets were not exactly isonitrogenous. They contained 133 and 110 g crude protein per kg DM, respectively. Starch content of diet B averaged 426 g per kg DM, while it was negligible in diet A.

Apparent digestibility coefficients (table II) were not significantly affected by yeast addition. Diet type exerted a significant effect on organic matter, ether extract and crude fibre. Organic matter ($P = 0.07$) and crude fibre ($P < 0.001$) were better digested in diet A than in diet B.

Nitrogen balance data are presented in table III. They were not affected by diet type or yeast treatment.

The inclusion of yeast in the diet provoked a more pronounced effect on ruminal fermentation characteristics than on nutrient digestibility (table IV). Total VFA concentration was not modified. However, molar concentrations of butyric, isobutyric and isovaleric acid were increased by Y, while propionic acid concentration was reduced. Isoacids (branched VFA and valeric acid) were slightly but non significantly increased by yeast. Furthermore, pH and ammonia concentration were higher for Y than for C treatment.

Most fermentation parameters also differed between diets A and B. Only pH was similar for both diets.

The number of viable yeast cells remained at $\approx 10^5$–10^6 per ml rumen fluid for at least 8 h. After 48 h the number decreased to a negligible amount of 3.10^2 cells per ml.

In the faeces a concentration of 10^5–10^6 yeast cells per g was found after 8 h. However, yeast cell number decreased more slowly in the faeces than in the rumen and amounted to 15.10^3 after 48 h.

Table II. Effect of diet and yeast culture on nutrient digestibility (%).

	Diet A		*Diet B*		*Pooled SE*	*Significance*		
	C	*Y*	*C*	*Y*		*Yeast* P	*Diet* P	*Interaction* P
Dry matter	76.9	76.8	75.9	75.1	1.9	> 0.1	> 0.1	> 0.1
Organic matter	79.3	79.1	77.7	77.1	1.9	> 0.1	= 0.07	> 0.1
Crude protein	65.4	65.0	67.6	66.1	4.0	> 0.1	> 0.1	> 0.1
Ether extract	0.0	0.0	79.1	78.4	4.6	> 0.1	< 0.001	> 0.1
Crude fibre	80.4	79.6	53.9	52.2	4.3	> 0.1	< 0.001	> 0.1
N-free extract	83.8	83.8	83.9	83.6	1.3	> 0.1	> 0.1	> 0.1
Gross energy	74.6	74.7	75.7	74.7	1.9	> 0.1	> 0.1	> 0.1

Table III. Effect of diet and yeast culture on nitrogen (N) balance.

	Diet A		*Diet B*		*Pooled SE*	*Significance*		
	C	*Y*	*C*	*Y*		*Yeast* P	*Diet* P	*Interaction* P
N intake (g/day)	139.9	142.7	125.3	117.0	12.5	> 0.1	< 0.01	> 0.1
Fecal N (%)	34.6	35.0	32.4	33.9	4.0	> 0.1	> 0.1	> 0.1
Absorbed N (%)	65.4	65.0	67.6	66.1	4.0	> 0.1	> 0.1	> 0.1
Urinary N (%)	51.8	49.6	48.2	52.7	7.3	> 0.1	> 0.1	> 0.1
Retained N (%)	13.6	15.4	19.4	13.4	6.5	> 0.1	> 0.1	> 0.1

Table IV. Effect of diet and yeast culture on rumen fermentation characteristics.

	Diet A		*Diet B*		*Pooled SE*	*Significance*		
	C	*Y*	*C*	*Y*		*Yeast* P	*Diet* P	*Interaction* P
Volatile fatty acids (VFA, Mm/100 ml)	11.32	11.53	10.02	9.49	1.40	> 0.1	< 0.001	> 0.1
Molar % of VFA								
Acetic acid	67.31	67.97	58.18	58.93	2.37	> 0.1	< 0.001	> 0.1
Proprionic acid	20.93	20.32	26.94	22.39	2.95	< 0.001	< 0.001	< 0.01
Butyric acid	8.97	8.85	10.68	14.06	1.64	< 0.001	< 0.001	< 0.001
Isobutyric acid	0.79	0.86	0.86	1.44	0.50	< 0.05	< 0.05	< 0.05
2-Methylbutyric acid	0.31	0.34	0.57	0.58	0.24	> 0.1	< 0.001	> 0.1
Valeric acid	1.32	1.28	1.92	1.56	0.68	> 0.1	< 0.05	> 0.1
Isovaleric acid	0.36	0.38	0.66	1.03	0.14	< 0.001	< 0.001	< 0.001
Caproic acid	0.00	0.01	0.18	0.00	0.19	= 0.09	= 0.09	= 0.06
Isoacids	2.78	2.86	4.01	4.61	0.91	> 0.1	< 0.001	> 0.1
Acetate : propionate	3.22	3.36	2.25	2.67	0.36	< 0.01	< 0.001	= 0.09
pH	5.97	6.03	5.78	6.15	0.28	< 0.01	> 0.1	< 0.05
NH_3 (mg/100 ml)	30.06	30.76	25.77	44.96	8.19	< 0.001	< 0.05	< 0.001

The evolution of yeast cell number is shown in figure 1. For the one animal on the control diet, only 20 yeast cells were found per ml rumen fluid.

DISCUSSION

The lack of an effect of yeast culture on digestibility is in accordance with the experiments of Harrison *et al* (1988) and Oellermann *et al* (1990).

However, Wiedmeier *et al* (1987) reported an increased digestibility. This result can be explained by the incorporation of more structural carbohydrates with a low digestibility in their experimental diets (15% barley straw, 8.5% wheat bran). It may suggest a positive effect of yeast on digestibility of poor-quality roughages.

Nitrogen balance data were not affected in our experiment. They were in accordance with the results of Edwards *et al* (1990), obtained with intensively fed bulls.

The inclusion of a yeast culture had a significant effect on the rumen fermentation characteristics. However, these results were not always in agreement with those from other experiments. Yeast increased acetate: propionate ratio, and the concentrations of butyrate and isoacids and ammonia similar to the results obtained previously with Rusitec (Frumholtz *et al*, 1989). The higher pH in our experiment was not confirmed by Frumholtz *et al* (1989) and Carro *et al* (1992), although they reported an elimination of the drop in pH after addition of feed in comparison with control vessels. From other experiments it was reported that yeast culture had no effect on pH or VFA concentrations and ammonia (Fondevila *et al*, 1990; Oellermann *et al*, 1990). Ammonia was not affected when yeast was added to high fibre diets, but yeast yielded less ammonia in low fibre diets (Moloney, 1989). According to Martin *et al* (1989) ammonia production on yeast-supplemented diets may depend on substrate. Harrison *et al* (1988) found that yeast reduced rumen pH, and acetate: propionate ratio and ammonia concentration, but isoacids were increased in cows. Newbold *et al* (1990) reported a higher pH, less acetate, more propionate and less ammonia when a yeast culture was fed.

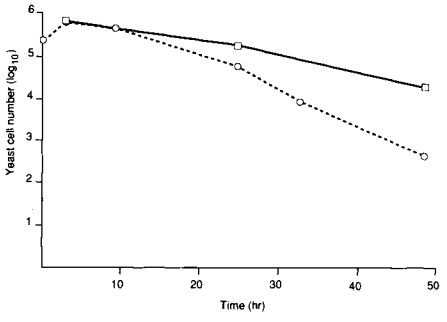

Fig 1. Evolution of yeast cell number per ml rumen fluid (○) and per mg faeces (□) after yeast supplementation.

A higher rumen pH can be partly explained by a lower lactate concentration in yeast supplemented animals (Fondevila *et al*, 1990; Newbold *et al*, 1990). Gedek *et al* (1992) found more amylolytic bacteria in the rumen of dairy cows which received yeast. As they can utilize lactate, the decrease of pH can be prevented by yeast treatment.

Our results (table IV) demonstrated significant interactions between yeast treatment and diet type for some fermentation parameters. Therefore, the diverging effect of yeast culture on rumen fermentation, reported in the literature, may be a consequence of the differences in the nature of the diets used. Williams and Newbold (1990) suggested that the effect of a yeast culture may be associated with the amount of rapidly fermentable carbohydrates in the diets. Diets with high contents of these carbohydrates depress rumen pH. A depressed rumen pH may reduce cellulolysis (Mould *et al*, 1983).

An increased yeast cell number in the rumen fluid (fig 1) is necessary to enable an alteration of rumen fermentation and digestibility. However, total tract digestibility was not affected (table II), while rumen fermentation was differently modified according to the diet (table IV). The results also show that yeast cannot colonize, but only transit through the rumen. The difference between rumen fluid and faeces with regard to the decline of the number of yeast cells is due to the rate of passage. This observation is in agreement with the declining number of viable yeast cells (0.17/h), as shown in the experiments of Newbold *et al* (1990). The reduction of yeast number in the rumen does not necessarily exclude the possibility of a metabolic activity.

Furthermore, the fact that yeast cells can survive the passage through the digestive tract neither excludes a post-ruminal effect. A survival of *Aspergillus oryzae* and *Saccharomyces cerevisiae* in the duodenal digesta was reported by Wanderley *et al* (1985) and Newbold *et al* (1990), respectively. For this reason, a probiotic effect cannot be excluded.

CONCLUSION

This experiment showed no effect of the dietary inclusion of yeast culture on apparent nutrient digestibility. However, there was a significant effect on rumen metabolites.

Furthermore, the interaction between yeast treatment and the nature of diet, found in this study, can help to explain the variable response to yeast culture reported in the literature.

ACKNOWLEDGMENTS

The authors are grateful to N De Paepe, M Martens, L Roels and J Galle for their skilled technical assistance.

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