Journal of Animal and Veterinary Advances 10 (5): 577-580, 2011
ISSN: 1680-5593

Effects of Inactivated Yeast Culture on Rumen Fermentation and Performance of Mid-Lactation Dairy Cows

R. Fortina, L.M. Battaglini, F. Opsi, S. Tassone, M. Renna and A. Mimosi
Dipartimento of Di Scienze Zootecniche, Universita Degli Studi Di Torino, 10095 Grugliasco, Italy

Abstract: The aim of this study was to evaluate the effect of daily supplementation of a devitalized strain of *Saccharomyces cerevisiae* on milk yield and composition of Friesian-Holstein lactating cows. Average Days In Milk (DIM) and Body Weight (BW) of cows were 98±24 days and 619±13.2 kg. Prior to the experiment, the effects of the yeast culture added to the diet were investigated in 2 *in vitro* experiments. In the first experiment, 6 samples of rumen fluid were used to determine the DM, CP and NDF degradability (IVDMD, IVCPD, IVNDFD) of Total Mixed Ration (TMR) at 0 and 48 h with the addition of 0 g (Y_0) and 1 g (Y_1) of yeast culture. In the second experiment, free, total and microbial ATP production were used for the evaluation of microbial activity in rumen fluids after 0, 8, 24 and 48 h incubation. In the 4 weeks trial, 10 multiparous cows were assigned to a Control diet (C) and 10 to the experimental diet (YC = C+35 g/cow/day of yeast culture). TMR on a Dry Matter (DM) basis consisted of corn silage (42.3%), ryegrass hay (14.1%), corn meal (22.1%), soybean meal (17.9%) and a mineral/vitamins mix (3.6%). Crude Protein (CP) and ENl were 14.6% and 1.56 Mcal kg^{-1} DM, respectively. Cows were individually fed once daily and milked at 7 and 19 h. *In vitro* experiments showed that the addition of 1 g YC to the rumen fluid increased IVCPD and IVNDFD after 48 h of incubation but did not influence IVDMD. Free, total and microbial ATP values showed a continuous decrease during the 48 h of the trial but the addition of YC proved to be effective in slowing the decrease. Milk yield (31.9 and 33.4 kg day^{-1}, for the C and YC group), 4% fat corrected milk (FCM: 30.3 and 31.7 kg day^{-1}) and energy corrected milk (ECM: 32.9 and 34.5 kg day^{-1}) were significantly different between groups. Dry Matter Intake (DMI), milk fat and protein percentage were similar in both groups. Feed efficiency (ECM kg^{-1} DMI) was higher in YC group than in C.

Key words: Yeast culture, rumen fermentation, microbial ATP, milk production, rumen fluid, Italy

INTRODUCTION

Yeast and Yeast Cultures (YC) are widely used in diets for lactating dairy cows. The number of yeast products that have undergone substantive evaluation in research studies is somewhat limited (Robinson, 2002) but there is a widespread belief among dairy producers and nutritionists that YC are beneficial enhancing DMI, rumen fermentation and milk production of lactating dairy cows. The exact mechanism responsible for such benefits is not clear from published data.

Improvements in performance have been attributed to changes in rumen pH and VFA, increased number of ruminal cellulolytic bacteria and improvements in fibre degradation (Piva *et al*., 1993).

During the hot season, live yeast supplementation to dairy cows seems to improve the rumen environment in a way that the dry matter intake increases and in consequence the productivity and efficiency are enhanced (Moallem *et al*., 2009; Schingoethe *et al*., 2004). However, results of the addition of YC to rations of lactating dairy cows have been variable and inconsistent because several factors can affect the response: stage of lactation, feeding strategy, type of forage given, heat stress, source of YC product tested (Arambel and Kent, 1990; Dann *et al*., 2000; Williams *et al*., 1991; Yoon *et al*., 2003). The vast majority of the studies published in the scientific literature that have examined the impact of specific commercial YC on rumen fermentation, fibre digestion and animal performance, utilize live strains of the yeast *Saccharomyces cerevisiae*.

Few data on dead or inactivated YC products are available. Aim of this trial was to investigate the effects of daily supplementation of a devitalized strain of *Saccharomyces cerevisiae* on milk production and milk composition of lactating cows during winter season. An attempt to explain the role of inactivated YC on rumen fermentation and microbial activity was performed too.

Corresponding Author: R. Fortina, Dipartimento of Di Scienze Zootecniche, Universita Degli Studi Di Torino, 10095 Grugliasco, Italy

MATERIALS AND METHODS

The devitalized YC used in this trial (Thepax 100 R, Dox-Al Italia SpA, Sulbiate, Italy) was composed of selected strains of *Saccharomyces cerevisiae*, grown in suitable medium to a final concentration of 10×10^9 yeast cells g^{-1}. According to the producer, cells were devitalized by an osmotic treatment in order to have <1000 cfu g^{-1} in the final product (European patent n. 0904701 and 98116181.3). Prior to the trial, the effects of YC daily added to the TMR were investigated in 2 *in vitro* experiments.

In the first experiment, DM, CP and NDF degradability (IVDMD, IVCPD, IVNDFD) of TMR fed to the dairy cows of the field trial was determined at 0 and 48 h by an Ankom Daisy Incubator (Ankom Technology Corp., Fairport, NY). About 6 samples of TMR (0.25±0.01 g) were weighted into F57 filter bags and placed into the digestion jars containing 1330 mL of buffer solution A (10 g L^{-1} KH_2PO_4+0.5 g L^{-1} $MgSO_4$*$7H_2O$+0.5 gL^{-1} NaCl+0.1 g L^{-1} $CaCl_2$*$2H_2O$+0.5 g L^{-1} urea) about 266 mL of buffer solution B (15 g L^{-1} Na_2CO_3+1 g L^{-1} Na_2S+ $9H_2O$) and 400 mL of filtered rumen fluid. Jars were added with 0 g (Y_0 = 3 samples) and 1 g (Y_1 = 3 samples) of YC, corresponding to 0 and approximately 35 g day^{-1} of YC in the cow's diet.

The experiment was replicated 6 times using different rumen fluids collected from the cows used in the trial. The pH of the buffered inoculum was measured at 0 and 48 h incubation. In the second experiment, ATP production was used for the evaluation of microbial activity in 6 rumen fluids with the addition of 0 g (Y_0) and 1 g (Y_1) of YC after 0, 8, 24 and 48 h incubation. ATP was extracted and determined by the firefly bioluminescence technique with a Biocounter M1500 (Lumac, NL) and Microbial Biomass Kit (Celsius, NL) (Ciardi and Nannipieri, 1990).

A sample of filtered rumen fluid (1 g) was added to 20 mL of acid solution (27.3 mL L^{-1} H_2SO_4 and 44.5 g L^{-1} Na_2PO_4*$2H_2O$) and stirred 15 min; about 50 μL of acidified rumen fluid were added to 1.5 mL of buffer solution (Trizma PRE-SET crystal-T4128 Sigma, 37.7 g L^{-1} and Triplex III-EDTA-Merck, 1.5 g L^{-1}). Total ATP was recovered adding 100 μL of NMR solution and 100 mL of luciferine/luciferase complex to 50 μL of buffered rumen fluid solution. No NMR was added for free ATP analysis. Microbial ATP production was calculated as difference between total ATP and free ATP.

The effects on milk production and quality of the devitalized YC were studied on 20 multiparous Holstein-Friesian cows (DIM: 98±24; BW: 619±13.2 kg).

Table 1: Diet composition and characteristics

Samples	DM (%)
Corn silage	42.30
Corn meal	22.10
Soybean meal	17.90
Ryegrass haylage	14.10
Minerals and vitamins[1]	1.70
Buffer salts[2]	1.20
$NaHCO_3$	0.70
Dry Matter (DM)	48.10
Ash (DM%)	9.10
Crude Protein (CP, DM%)	14.60
Soluble Protein (CP%)	44.10
RDP (CP%)	53.10
Fat (DM%)	3.50
NDF (DM%)	41.60
ADF (DM%)	24.20
ADL (DM%)	2.70
NE_L (Mcal kg^{-1} DM)	1.56
NE_L (MJ kg^{-1} DM)	6.51

[1]Containing: Beta-carotene 12 mg; Vit. A 400000 IU; Vit. D3 50000 IU; Vit. E 100 mg; Vit. B1 1.4 mg; Vit. B12 1.5 mg; Vit. B6 0.3 mg; Vit. C 700 mg; Vit. H 0.15 mg; Choline 250 mg; Zn 2 g; Mn 1.2 g; Fe 0.5 g; Cu 0.25 g; I 0.05 g; Co 0.02 g; Se 4 mg, [2]Containing (×100 g): Ca 20 g; Na 2.8 g; Mg 7 g

During a 10 day pre-trial period, 20 cows were paired on the basis of milk yield and DIM then randomly assigned to 2 groups. In the 4 weeks experiment (December 15th, 2008-January 16th, 2009; average temperature: 11°C), 10 cows were assigned to a Control diet (C) and 10 to the yeast culture diet (YC = C+35 g/day/cow of yeast culture). Animals were stabled in single stalls and fed at 9 h; DMI was calcuated daily as difference between the quantity fed and the refusals. The composition and characteristics of TMR are shown in Table 1. Samples of TMR were collected at the beginning of the trial at week 2 and 4; all samples were immediately dried at 55°C in a oven for 48 h then ground through a 2 mm screen of Buhler mill and analysed for crude protein, ether extract, ash according to AOAC (1997) methods. NDF and ADF was determined by Ankom fibre analyzer with the fibre bag technique (Ankom Technology Corp., Fairport, NY).

Milking occurred at 7 and 19 h milk yield of each cow was recorded daily and bulk milk samples from each group were collected and analysed weekly for fat, protein, lactose and total solids (AOAC, 1997) by midinfrared spectrophotometry (Foss Analytical A/S, Denmark). SCC and TB (AOAC, 1997) were determined using a Fossomatic 90 (Foss Analytical A/S, Denmark). Result were analyzed using the GLM procedure of SPSS (SPSS, 2004). In the field trial, main effects included treatment, week and their interaction. The effects of DMI, week and interaction were not significantly different and were consequently dropped from the model. Significance was declared at $p<0.05$ unless otherwise stated.

RESULTS AND DISCUSSION

Yeast culture products have been shown to modify ruminal fermentation and stimulate bacterial growth (Harrison *et al*., 1988; Erasmus *et al*., 1992). Such changes are often associated with increased digestibility of dietary fiber which can lead to higher DMI or animal performance or both. Positive effects of YC on CP degradability and efficiency of aminoacid balance and utilization in dairy cows are not yet demonstrated (Putnam *et al*., 1997).

The results of the *in vitro* trials showed that the addition of 1 g YC to the buffered solution of rumen fluid significantly increased IVNDFD ($p<0.01$) and IVCPD ($p<0.05$) after 48 h of incubation but did not influence IVDMD (Table 2). The average pH value of rumen fluids was 6.51±0.04 at t = 0; at 48 h the pH value for Y_0 and Y_1 were 6.26±0.05 and 6.21±0.06, respectively.

Results of free, total and microbial ATP quantification at successive steps of incubation (0, 8, 24 and 48 h) are shown in Table 3. Free, total and microbial ATP values underwent a continuous decrease during the 48 h of the trial but the addition of YC proved to be effective in slowing the decrease. According to the devitalisation treatment, cells of YC were still active but not viable.

While in the anaerobic environment of Ankom Daisy they are most likely unable to proliferate, nevertheless they can carry ATP amounts that if extracted could contribute to enrich microbial ATP levels from the normal rumen population.

However, the ATP extraction protocol was based on the use of an ATP releasing agent (NMR solution) specifically intended for the bacterial cell wall (made of peptidoglican) with no or low action on fungal cell wall (mainly made of cellulose).

Therefore, the quantitative variations detected in microbial ATP are very likely to be referred to the bacterial microflora whose metabolic activity resulted to be improved by the YC addition. Table 4 shows milk yield and quality, DMI and feed efficiency of cows of group C and YC. DMI was slightly higher in group Y but not statistically different.

Milk yield of lactating cattle fed YC was statistically higher ($p<0.001$) than control group (33.4 vs. 31.9 kg day^{-1}). Feed efficiency as indicated by production of FCM and Energy Corrected Milk (ECM) per kilogram of feed DM consumed was higher ($p<0.03$ and $p<0.04$) when yeast culture was fed. This improvement in feed efficiency reflects a trend toward higher DMI coupled with higher milk production for cows fed yeast culture.

These results support other field reports and results (Swartz *et al*., 1994; Huber, 1998; Yoon *et al*., 2003) that indicated tendencies for improved response of lactating cattle when fed yeast culture. The results of the *in vitro* trials showed some influence of YC on rumen fermentation but they did not explain the mode of action by which the feeding of YC improved feed efficiency of dairy cows.

A possible mode may result from interactions between yeast culture supplementation and diet composition as observed by Masek *et al*. (2008) on dairy ewes. An improved ration digestibility (Gomez-Alarcon *et al*., 1990; Wohlt *et al*., 1991) is another possible explanation.

In fact, the *in vitro* experiment showed an increase of IVCPD and IVNDFD at 48 h when 1 g of YC was added to the rumen fluid. The addition of 35 g/cow/day of YC to the diet may have enhanced rumen fermentation thus increasing feed efficiency and milk yield. Data from *in vitro* experiments also showed an increase in bacterial metabolic activity and a better CP and NDF degradation of TMR when rumen fluid was added with the inactivated yeast culture.

The field trial showed that the addition of YC in the diet of mid-lactation Holstein-Friesian cows was beneficial in improving production of milk, 4% FCM and ECM but all parameters of milk composition and quality (fat, protein and lactose percentage and yield and SCC) were unaffected. Further research to elucidate the mechanisms involved is required to better understand the benefits of feeding YC in the diets of lactating dairy cows.

Table 2: *In vitro* DM, CP and NDF degradability at 48 h

Factors	IVDMD	IVCPD	IVNDFD
Y_0	60.1±2.9	48.2±3.8^{a}	42.6±5.5^{A}
Y_1	61.6±4.2	51.3±2.8^{b}	47.4±1.5^{B}

Y_0 = Rumen fluid+0 g YC; Y_1 = Rumen fluid+1 g YC; $^{A, B}$ = $p<0.01$; $^{a, b}$ = $p<0.0$

Table 3: Free, total and microbial ATP (%, mean of 3 replications) at 0, 8, 24 and 48 h of incubation (SD<10%)

	Free ATP				Total ATP				Microbial ATP			
H	0	8	24	48	0	8	24	48	0	8	24	48
Y_0	100	26.9^{A}	3.5^{A}	0.3	100	47.2^{A}	07.3^{A}	3.1	100	51.3^{A}	8.4^{A}	4.4
Y_1	100	99.8^{B}	47.6^{B}	0.1	100	92.1^{B}	40.8^{B}	2.3	100	86.7^{B}	55.5^{B}	1.6

Y_0 = Rumen fluid+0 g YC; Y_1 = Rumen fluid+1 g YC (^{A,B} = $p<0.01$; ^{a,b} = $p<0.05$)

Table 4: Milk yield and composition, DMI and feed efficiency

	Diet			
Composition	C	YC	SE	p-value
Milk (kg day^{-1})	31.90	33.40	0.23	0.001
4% FCM (kg day^{-1})	30.30	31.70	0.25	0.001
ECM (kg day^{-1})	32.90	34.50	0.24	0.001
Fat				
(%)	3.67	3.68	0.05	0.760
kg day^{-1}	1.17	1.23	0.03	0.680
Protein				
(%)	3.23	3.22	0.02	0.750
kg day^{-1}	1.03	1.07	0.03	0.650
Lactose				
(%)	5.05	5.10	0.03	0.450
kg day^{-1}	1.61	1.70	0.05	0.470
SCC×10^{3} mL^{-1}	231.00	227.00	170.00	0.560
DMI (kg day^{-1})	22.10	23.20	0.70	0.250
FCM/DMI	1.37	1.41	0.02	0.030
ECM/DMI	1.49	1.53	0.03	0.040

CONCLUSION

The present findings indicate that the inactivated yeast culture may have enhanced rumen fermentation thus increasing feed efficiency and milk yield of mid-lactation Friesian-Holstein cows.

ACKNOWLEDGEMENT

The researchers wish to acknowledge Dr. Alberto Rovera of Dox-Al Italia for supplying the yeast culture product used in the trial.

REFERENCES

AOAC, 1997. Official Methods of Analysis. 16th Edn., Association of Official Analytical Chemists Intl., Gaithersburg, MD USA.

Arambel, M.J. and B.A. Kent, 1990. Effect of yeast culture on nutrient digestibility and milk yield response in early to midlactation dairy cows. J. Dairy Sci., 73: 1560-1563.

Ciardi, C. and P. Nannipieri, 1990. A comparison of methods for measuring ATP in soil. Soil Biol. Biochem., 22: 725-727.

Dann, H.M., J.K. Drackley, G.C. McCoy, M.F. Hutjens and J.E. Garrett, 2000. Effects of yeast culture (*Saccharomyces cerevisiae*) on prepartum and postpartum intake and milk production of Jersey cows. J. Dairy Sci., 83: 123-127.

Erasmus, L.J., P.M. Botha and A. Kistner, 1992. Effect of yeast culture supplement on production, rumen fermentation and duodenal nitrogen flow in dairy cows. J. Dairy Sci., 75: 3056-3065.

Gomez-Alarcon R.A., C. Dudas and J.T. Huber, 1990. Influence of cultures of *Aspergillus oryzae* on rumen and total tract digestion of dietary components. J. Dairy Sci., 73: 703-710.

Harrison, G.A., R.W. Hemken, K.A. Dawson, R.J. Harmon and K.B. Barker, 1988. Influence of addition of yeast culture supplement to diets of lactating cows on ruminal fermentation and microbial population. J. Dairy Sci., 71: 2967-2975.

Huber, J.T., 1998. Yeast products help cattle handle heat. Hoard's Dairyman, 143: 367-367.

Masek, T., Z. Mikulec, H. Valpotic, N. Antunac and N. Mikulec *et al*., 2008. Influence of live yeast culture (*Saccharomyces cerevisiae*) on milk production and composition and blood biochemistry of grazing dairy ewes during the milking period. Acta Vet., 77: 547-554.

Moallem, U., H. Lehrer, L. Livshitz, M. Zachut and S. Yakoby, 2009. The effects of live yeast supplementation to dairy cows during the hot season on production, feed efficiency and digestibility. J. Dairy Sci., 92: 343-351.

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

Putnam, D.E., C.G. Schwab, M.T. Socha, N.I. Whitehouse, N.A. Kierstead and B.D. Garthwaite, 1997. Effect of yeast culture in the diets of early lactation dairy cows on ruminal fermentation and passage of nitrogen fractions and amino acids to the small intestine. J. Dairy Sci., 80: 374-384.

Robinson, P.H., 2002. Yeast products for growing and lactating cattle: Impacts on rumen fermentation and performance. Proceedings of the 12th International Meeting in Milk and Meat Production in Hot Climates. Mexicali, Mexico.

SPSS, 2004. SPSS 13.0 Base Users Guide. Statistical Package for Social Sciences Inc., Chicago, IL, USA.

Schingoethe, D.J., K.N. Linke, K.F. Kalscheur, A.R. Hippen, D.R. Rennich and I. Yoon, 2004. Feed efficiency of mid-lactation dairy cows fed yeast culture during summer. J. Dairy Sci., 87: 4178-4181.

Swartz, D.L., L.D. Muller, G.W. Rogers and G.A. Varga, 1994. Effect of yeast cultures on performance of lactating dairy cows: A field study. J. Dairy Sci., 77: 3073-3080.

Williams, P.E., C.A. Tait, G.M. Innes and C.J. Newbold, 1991. Effects of the inclusion of yeast culture (*Saccha romyces cerevisiae* plus growth medium) in the diet of dairy cows on milk yield and forage degradation and fermentation patterns in the rumen of steers. J. Anim. Sci., 69: 3016-3026.

Wohlt, J.E., A.D. Finkelstein and C.H. Chung, 1991. Yeast culture to improve intake, nutrient digestibility and performance by dairy cattle during early lactation. J. Dairy Sci., 74: 1395-1400.

Yoon, I., G.M. Kamande, C.R. Belknap, M.A. Engstrom and M.A. Kujaiva, 2003. The role of yeast culture in transition cow nutrition. J. Dairy Sci., 86: 3803-3811.