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THE EFFECT OF *SACCHAROMYCES CEREVISIAE*[1026] USED ALONE OR WITH VITAMIN-MINERAL PREMIX ON BIOCHEMICAL PARAMETERS OF BLOOD AND MILK IN DAIRY COWS

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When evaluating the effects of yeast culture *(Saccharomyces cerevisiae*[1026]*)* supplied with or without a vitamin premix and mineral bioplexes on some intermediates and end-products involved in the synthesis of milk constituents in 30 early-lactation Black and White Lowland cows, no significant differences were found in the glucose level, mineral contents and enzyme activities of the blood serum. The effect of yeast culture on the availability of minerals for milk synthesis depended upon the dynamics of degradation of mineral bioplexes in the rumen and the cows' mineral status. The insignificant increase found in blood total protein content and the simultaneous small differences in blood urea nitrogen (BUN) and milk urea nitrogen (MUN) values in cows supplied with the yeast culture were probably associated with a high ammonia incorporation into microbial protein in the rumen, which increased protein supply for milk protein synthesis and decreased the nitrogen loss.

Key words: Blood, yeasts, glucose, minerals, protein, blood urea nitrogen, milk urea nitrogen

Data from experiments with dairy cows demonstrate that the addition of yeast culture to the ration can increase milk production (Piva et al., 1993; Gombos et al., 1995; Strzetelski et al., 1996; Wallace, 1996; Iwańska et al., 1999), but the conditions under which maximum responses can be obtained remain unclear. It has been shown that rumen parameters influenced by dietary supplementation with yeast culture include pH (Nisbet and Martin, 1991), changes in the volatile fatty acid (VFA) concentration ratio (Williams et al., 1991; Wallace, 1996), liquid dilution rate (Harrison et al., 1988), rumen ammonia concentration (Wallace, 1994), contents of anaerobic and cellulolytic bacteria (Dawson et al., 1990; Wallace, 1996), rate of rumen fibre degradation (Williams and Newbold, 1990; Wohlt et al., 1991), and total tract nutrient digestion (Wiedmeier et al., 1987; Erasmus et al., 1992). Yeast culture has also been found to stimulate microbial activity and increase the incorporation of nitrogen into microbial protein,

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which confirms the suggestion of Wiedmeier et al. (1987) and Erasmus et al. (1992) that yeast culture may exert an effect on the flow of protein, related to changes in the number and activity of rumen microorganisms.

The efficiency of feed nitrogen utilisation in milking cows supplied with yeast culture involves not only the increase of ammonia incorporation into microbial protein and a higher flow and absorption of amino acids, but also an altered endogenous nitrogen metabolism, expressed mainly by the level of urea excretion. The excess of rumen ammonia concentration, unutilised by rumen bacteria, may result in high endogenous concentrations of urea in blood, milk and urine.

For this reason, milk urea content has been given increased attention (Ferguson et al., 1993; Gustafsson and Palmquist, 1993; Roseler et al., 1993; Harris, 1996). The basis for using blood urea nitrogen (BUN) and milk urea nitrogen (MUN) as indicators of nitrogen utilisation is the high concentration of urea in blood and milk. Roseler et al. (1993) suggested that BUN may serve as an indicator of ruminal protein degradability and postruminal protein supply. Moreover, it was found that BUN and MUN values outside a general range of 12 and 16–18, respectively, indicate insufficient or excessive amounts of protein in the diet and reflect losses of nitrogen, reduced milk production and impaired reproduction (Gustaffson and Palmquist, 1993; Harris, 1996).

No research has examined the relationship between the efficiency of protein utilisation associated with yeast culture action and losses of nitrogen in terms of urea concentration in blood and milk. According to Gustafsson and Palmquist (1993), milk urea determination can be used to make inferences regarding estimation of rumen ammonia.

Therefore, this experiment was designed to evaluate the effect of yeast culture supplementation on some blood and milk components and particularly on the concentration of BUN and MUN as indicators of rumen ammonia utilisation.

Materials and methods

Animals and management

The experiment involved the same 30 Black and White Lowland cows, divided into three experimental groups, that were used in studies on the effect of live yeast culture *Saccharomyces cerevisiae*[1026] and vitamin–mineral premix on milk production and composition.

The details regarding the animals and feeding system used and the doses of supplements are described in the accompanying paper (Iwańska et al., 1999).

Sampling procedures and analytical methods

Milk samples were taken from each cow in every experimental group on Days 7, 21, 28, 42, 56 and 100 of lactation and assayed for calcium by Flapho 4 (Carl Zeiss), for inorganic phosphorus by the method of Fiske-Subbarow (Kokot, 1969), and for magnesium, iron and copper, after dry mineralization, by atomic spectrophotometry (ASA).

Milk urea nitrogen (MUN) was determined in deproteinized milk using a colorimetric diacetyl monoxine procedure (No. 535; Sigma Diagnostics, St. Louis, MO) and spectrophotometry with absorbance at 535 nm.

Blood samples were taken from the jugular vein prior to the morning feeding two weeks prepartum and on Days 7, 56 and 100 of lactation.

Serum was separated by centrifugation at 2000 × *g* for 15 min and stored at –20 °C until analysed. Serum samples were analysed for total protein, glucose and BUN level as well as for alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities using the tests of Alpha Diagnostics Methods (Catalogue no. A-6525-200, A-6560-200, B-6550-200, G-6519-400, T-6528-500; 1993–1994) and an Epoll 200 spectrophotometer. The mineral concentrations (Mg, Fe, Cu) of the blood serum were analysed by atomic spectrophotometry (ASA), except for calcium content which was measured using Flapho 4 (Carl Zeiss) and inorganic phosphorus, which was estimated by the method of Fiske-Subbarow (Kokot, 1969).

Analysis of variance (Backsalary et al., 1977) followed by least significant difference and Duncan's multiple range tests were used to determine the variations in blood and milk composition due to time of blood and milk sampling and group interaction. The significance of this variation for each variable was also determined.

Results

The analysis of nutrient concentration in dry mass of the daily ration showed that the level of energy (MJ), crude protein, ADF and NDF contents met or slightly exceeded the requirements for cows at the milk production level obtained in this experiment. The dietary contents of calcium, inorganic phosphorus and magnesium were in the required range, except for high iron and lowered copper levels (Table 1).

The results of mineral content estimations in blood serum showed that the concentrations of calcium, inorganic phosphorus and iron were relatively constant in all groups of cows throughout the experiment, although an insignificant increase in the serum magnesium and copper levels of cows supplied with yeast culture with or without a vitamin premix and mineral bioplexes could be found after calving (Table 2). Despite an elevation of serum copper level, copper con-

centration was found to decrease in the milk. This decrease was the lowest in cows supplied with yeast culture combined with a vitamin premix and mineral bioplexes (Table 3). Although the differences in the concentrations of milk minerals were not statistically significant, from the comparison of control and experimental cows it appears that yeast culture and premix supplementation could influence the mineral element concentrations of the milk.

Table 1

Nutrient concentrations of the diet (in 1 kg of dry mass)

Components		
Net energy	MJ	6.0
Crude protein	%	13.7
Crude fibre	%	23.0
NDF	%	48.2
ADF	%	26.6
Hemicellulose	%	21.6
Cellulose	%	23.7
Ca	%	0.59
P	%	0.30
Mg	%	0.20
Fe	mg	250
Cu	mg	7.1

Premix composition (1 gram contains vitamin A – 10,000 IU; vitamin D_3 – 800 IU; vitamin E – 4.0 mg; vitamin K_3 – 0.6 mg; vitamin B_1 – 0.8 mg; vitamin B_2 – 2.0 mg; vitamin PP – 2.4 mg; vitamin B_5 – 2.4 mg; vitamin B_6 – 1.6 mg; choline – 4.0 mg; methionine – 2.0 mg; lysine – 4 mg; mineral chelates: Mn – 5.0 mg; Fe – 1.0 mg; Cu – 0.25 mg; Co – 0.06 mg; Zn – 3.0 mg; Mg – 1.0 mg; Se – 0.15 mg

It is evident from the results that all groups of cows maintained similar concentrations of glucose and similar activities of AST and ALT in the blood serum throughout the experiment. There were no significant differences between the control and experimental groups and no direct effect of yeast culture on glucose level could be observed. The only marked differences found were an increase of serum total protein content with a simultaneous decrease in blood urea concentration (Tables 4 and 5) in the experimental cows after calving. Only cows supplied with yeast culture in combination with vitamin premix and mineral bioplexes (Group 3) maintained serum total protein and urea at the same level up to Day 100 of lactation.

In order to evaluate the effect of yeast culture supplementation on the efficiency and improvement of nitrogen utilisation or loss, BUN and MUN were

estimated. The results shown in Table 5 suggest that the values of BUN and MUN can be compared. The MUN values of control cows were found to be only 66 and 73 per cent of BUN on Days 7 and 56 of lactation, respectively, whereas in the experimental groups supplied with yeast culture the MUN values were higher, ranging from 83 to 97 per cent of BUN. The highest levels of BUN and MUN in cows supplied with yeast culture were found at the beginning of lactation. A decrease of these values was observed on Days 56 and 100 of lactation, although in cows of Group 3 no changes in BUN values could be found. It appears that the levels of BUN and presumably those of MUN suggest that yeast culture supplementation exerted an influence on nitrogen utilisation and reduced the nitrogen losses.

Table 2

Effect of yeast culture on mean concentrations of minerals in blood serum

Groups of cows	Ca		Inorganic P		Mg		Fe		Cu	
	mmol/l						µmol/l			
	Days of blood sampling									
	14 days prepartum									
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
1	2.50	0.34	2.02	0.27	1.22	0.19	26.0	5.0	18.6	3.4
2	2.51	0.25	2.18	0.42	1.11	0.29	28.4	4.7	19.5	4.7
3	2.63	0.53	2.28	0.39	1.26	0.19	26.3	6.5	19.2	6.0
	Day 7 of lactation									
1	2.69	0.75	1.77	0.19	1.35	0.05	28.1	9.5	18.3	2.8
2	2.75	0.40	2.24	0.19	1.36	0.07	30.8	5.5	20.2	7.6
3	2.73	0.61	2.31	0.35	1.40	0.08	30.0	9.6	20.4	4.7
	Day 56 of lactation									
1	2.69	0.66	2.11	0.25	1.22	0.04	20.2	4.3	19.8	3.2
2	2.74	0.53	2.16	0.27	1.37	0.05	25.7	5.9	21.0	3.7
3	2.57	0.48	2.25	0.10	1.40	0.07	27.0	6.7	21.9	4.4
	Day 100 of lactation									
1	2.55	0.63	2.23	0.23	1.30	0.11	24.4	5.0	22.9	4.3
2	2.74	0.47	2.27	0.17	1.49	0.08	23.2	4.7	27.8	3.6
3	2.62	0.51	2.32	0.20	1.51	0.10	28.2	5.3	27.3	3.9

1 – control; 2 – yeast culture; 3 – yeast culture + premix

Discussion

When evaluating the effects of yeast culture addition on some intermediates and end-products involved in the synthesis of milk constituents, no significant differences were found between the control and experimental cows in the concentration of blood metabolites and minerals and in enzyme activities.

Table 3

Effect of yeast culture on mean concentrations of minerals in milk

Minerals			Days of milk sampling					
			Day 7 of lactation			Day 100 of lactation		
			Groups of cows					
			1	2	3	1	2	3
Ca	mmol/l	$\bar{x}$	27.6	28.9	28.9	27.5	28.8	30.0
		s	3.0	3.2	3.3	2.1	2.1	1.6
P	mmol/l	$\bar{x}$	26.2	27.5	28.2	25.5	27.6	27.8
		s	3.4	2.7	1.9	2.2	1.3	0.7
Mg	mmol/l	$\bar{x}$	6.7	6.5	6.7	7.1	8.4	8.3
		s	0.4	0.4	0.7	0.1	0.6	0.4
Fe	µmol/l	$\bar{x}$	10.3	13.8	13.7	10.6	12.5	13.7
		s	2.9	3.3	2.0	3.5	1.7	1.0
Cu	µmol/l	$\bar{x}$	6.0	5.2	5.3	3.6	4.7	4.9
		s	1.3	0.4	0.5	0.5	1.2	0.6

1 – control; 2 – yeast culture; 3 – yeast culture + premix

Although the requirement for milk glucose is high and obviously a decrease of blood glucose content could be observed at the peak of lactation, in cows supplied with yeast culture the concentration of blood glucose tended to be maintained nearly at the same level throughout the experiment. As the availability of blood glucose depends largely on propionate synthesis, it appears that rather stable levels of blood glucose could be associated with the tendency of lowered acetate-propionate ratio in the rumen, resulting from an increased production of propionate rather than from a reduced synthesis of acetate (Williams and Newbold, 1990; Nisbet and Martin, 1991).

The differences in blood serum mineral contents between the groups were insignificant and of minor biological significance, because the values were in the

normal physiological range. Despite the basically unchanged levels of Ca, inorganic P, Mg and Fe in the blood serum, an increase was found in the level of these minerals in milk, suggesting a high utilisation rate and optimal mineral availability from the diet and premix.

Table 4

Effect of yeast culture on some indices of blood serum

Groups of cows	Glucose, mmol/l		Total protein, g/l		AST, IU/l		ALT, IU/l	
	Days of blood sampling							
	14 days prepartum							
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
1	3.62	1.2	77	17	35	4	16	6
2	3.23	0.5	78	14	32	5	14	3
3	3.38	0.4	76	8	27	6	16	5
	Day 7 of lactation							
1	2.94	0.6	82	15	41	9	20	6
2	3.08	1.0	81	12	41	4	15	5
3	3.00	0.8	81	6	36	8	17	2
	Day 56 of lactation							
1	2.97[a]	0.6	82	9	38	8	19	5
2	3.32[b]	0.4	83	10	36	6	20	4
3	3.51[b]	0.6	90	18	38	7	18	5
	Day 100 of lactation							
1	2.91[a]	0.6	86	8	40	5	15[a]	3
2	2.75	0.5	103	13	39	5	21[b]	3
3	3.34[b]	0.6	96	12	40	4	18[a]	4

1 – control; 2 – yeast culture; 3 – yeast culture + premix; Significance of differences: ab – $P \leq 0.05$

It may be assumed that simultaneous supplementation with yeast culture and minerals in the form of bioplexes made minerals more available which, in turn, increased the transport and uptake rate of minerals by trace cells of the mammary gland (Peterson et al., 1987).

Although the contents of phosphorus and copper in the diet were marginally deficient, the levels of inorganic P and Cu were slightly elevated in the blood serum of cows supplied with yeast culture. However, the increase of serum copper

concentration had little effect on the availability of copper for milk synthesis, as the level of Cu in milk tended to be lowered by Day 100 of lactation as compared to the early lactation values.

Table 5

Effect of yeast culture on mean concentrations of blood urea nitrogen (BUN) and milk urea nitrogen (MUN)

Groups of cows	BUN mg/dl		MUN mg/dl		Percentage MUN/BUN
	Days of milk sampling				
	Day 7 of lactation				
	$\bar{x}$	s	$\bar{x}$	s	
1	12.2	2.0	8.0^{A}	0.9	66
2	15.3	3.4	14.2^{B}	1.1	93
3	14.8	2.5	13.7^{B}	1.5	93
	Day 56 of lactation				
1	11.3	3.4	9.8	1.1	73
2	13.3	3.9	11.0	1.4	83
3	14.4	2.5	12.5	1.3	87
	Day 100 of lactation				
1	12.6	2.5	11.6	1.6	92
2	12.3	2.4	11.8	1.7	97
3	14.2	3.1	11.9	1.9	84

1 – control; 2 – yeast culture; 3 – yeast culture + premix; Significance of differences: ab – $P \leq 0.01$

Although the magnitude of this decrease was less in the experimental than in the control cows, it may be assumed that the low availability of copper might depend on the dynamics of degradation of copper bioplexes in the rumen and on the necessity to improve Cu status, presumably an adequate copper concentration of the liver (Hemken, 1997). Du et al. (1996) suggested that ruminal bacteria prefer peptides and amino acids from Cu-bioplexes, releasing Cu as free ion, that cannot be absorbed as effectively as Cu bound to amino acids or peptides. In cows given a ration supplemented with yeast culture, the degradation of mineral bioplexes in the rumen might be associated with a high amino acid requirement for bacterial protein synthesis. The higher efficiency of protein synthesis by rumen microorganisms influenced the flow, absorption and supply of nitrogen for milk protein synthesis (Wiedmeier et al., 1987; Erasmus et al., 1992).

Although serum total protein content is generally accepted to be relatively constant and not significantly affected by the diet, the values found in this study suggest that yeast culture supplementation had a favourable effect on that variable. The insignificant increase found in serum total protein content in Group 3 suggests high protein availability resulting from the stimulatory effect of yeast culture on the digestibility and flow of protein, probably attributable to the synthesis of microbial protein. This suggestion is also supported by the small changes found in ALT and AST activities in the physiological range.

At the same time, the crude protein content of the ration and the dynamics of protein degradation to ammonia in the rumen greatly influenced the levels of BUN and MUN. Blood urea may reflect the percentage of dietary crude protein, the ratio of dietary crude protein to rumen-fermentable nitrogen compounds and postruminal protein supply, and hence the values of BUN and MUN may indicate the insufficiency or excess of protein supply (Partschefeld et al., 1982; Roseler et al., 1990; Harris, 1996).

Although in this experiment the crude protein content of the diet was rather low, the levels of BUN were within the normal range in all groups of cows on Day 100 of lactation.

As MUN levels of less than 12 (Gustafsson and Palmquist, 1993; Roseler et al., 1993; Harris, 1996) reflect insufficiency of protein supply or losses of nitrogen and may suggest poorer milk production, the values of MUN found in control cows on Days 7 and 56 of lactation indicate an insufficient supply of protein for milk protein synthesis, which was confirmed by the lowest milk protein content found in the control group (Iwańska et al., 1999). The very small difference in BUN and MUN values found in cows supplied with yeast culture reflect the dynamics of nitrogen utilisation and high magnitude of rumen ammonia incorporation into microbial protein, which might be due to the stimulatory effect exerted on the rumen by the yeast culture.

The results of these studies appear to suggest that the method of estimation of BUN and MUN has made it possible to assess the efficiency of protein supply relative to requirement, the improvement of nitrogen utilisation as well as the reduction of nitrogen losses in milking cows fed yeast culture as a dietary supplement.

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