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The effects of supplementing diets fed to pregnant and lactating ewes with *Saccharomyces cerevisiae* dried yeast

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Abstract: The objective of this study was to determine the effect of supplementing diets fed to ewes (in late pregnancy and during lactation or only during lactation) with *Saccharomyces cerevisiae* dried brewer's yeast on the yield and chemical composition of milk and on selected parameters of humoral and cellular immunity. The investigated indicators were analyzed on days 28 and 70 of lactation. The addition of dried yeast contributed to a significant increase in milk yield, and the obtained milk was characterized by a lower somatic cell count, suggesting improved mammary gland health. Diet supplementation had a beneficial influence on the health status of ewes, leading to increased nonspecific humoral and cellular immune responses in ewes, including gamma globulin concentrations, lysozyme and ceruloplasmin activity levels, respiratory burst activity and potential killing activity of phagocytes, and the proliferative response of mitogen-stimulated T cells and B cells.

Key words: Sheep, *Saccharomyces cerevisiae*, milk performance, humoral and cellular immunity

1. Introduction

In effective and nutritionally well-balanced animal diets, essential nutrients are often combined with feed additives and supplements that contain microorganisms and their products (1). The results of numerous studies indicate that *Saccharomyces cerevisiae* dried brewer's yeast enhances animal productivity and delivers health benefits (2–6). Those positive effects can be attributed to the presence of mannans and glucose polymers in yeast cell walls (7). Yeasts are unable to colonize the gastrointestinal tract independently (1). By binding to the intestinal epithelium, they compete against pathogenic bacteria and stimulate the immune system of animals (8). β-Glucans and mannan oligosaccharides (MOSs) found in yeast cell walls have immunomodulative properties (9). MOSs are capable of neutralizing pathogenic bacteria, and they support β-glucans in the process of stimulating defense mechanisms (10).

The objective of this study was to determine the effect of supplementing the concentrate fed to ewes with *Saccharomyces cerevisiae* dried brewer's yeast on the yield and chemical composition of milk and on selected parameters of humoral and cellular immunity.

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2. Materials and methods

The study was conducted on a breeding herd of Kamieniec ewes at the Production and Experimental Station in Bałcyny, Poland, at the Zajączki farm. In 2009 and 2010, 2 experiments were carried out to determine the effect of supplementing ewe diets with the Inter Yeast S product containing *Saccharomyces cerevisiae* dried brewer's yeast. In experiment 1, the experimental materials comprised 39 Kamieniec ewes aged 3 to 5 years, divided into 3 equal groups: I – control, II and III – experimental. Group II was created at the end of the third month of pregnancy as confirmed by an ultrasound examination. Group II ewes were fed CJ concentrate in the amount of 0.3 kg animal^{-1} day^{-1}, supplemented with Inter Yeast dried brewer's yeast; therefore, they received 0.015 kg yeast animal^{-1} day^{-1}. The above feeding regime was continued after lambing for 70 days of the lactation period. Groups I and III were formed gradually during the lambing period to ensure that the age of the mothers, litter type, and offspring sex ratio were identical to those in Group II. In experiment 2, the experimental materials comprised 26 lactating Kamieniec ewes aged 3 to 5 years. The ewes were divided into 2 equal groups: I – control and II – experimental.

In both experiments, the composition of diets administered to all groups during 70 days of the lactation period was identical, i.e. meadow hay in the amount of 0.5 kg animal^{-1} day^{-1}, wilted grass and legume silage at 2.4 kg animal^{-1} day^{-1}, and CJ concentrate at 0.6 kg animal^{-1} day^{-1}. Components of the mixture (CJ) were as follows: ground barley (40%), ground wheat (37.5%), ground maize (10%), soybean meal (10%), mineral premix (2%), fodder chalk (0.2%), dicalcium phosphate (0.2%), and salt fodder (0.10%). Uniform feeding standards were applied in all groups.

A feeding trial was performed during the 70-day lactation period. The quantities of administered feed and leftovers were monitored to determine feed intake in each group. The nutritive value of diets, determined based on their proximate chemical composition and energy content (Table 1), was expressed in INRA (National Institute of Agronomic Research, Paris, France) units (11): first, the feed unit for milk production (UFL). This is the amount of net energy for milk production (EN_1), which provides for a standard 1 kg of feed barley as feed production during lactation. One unit of milk production is equal to 1700 calories.

UFL/kg = [EM (kcal/kg) × k_1] / 1700 kcal,

where EM = metabolic energy and k_1 = 0.630.

The second unit used was the protein digested in the small intestine when rumen-fermentable nitrogen is limiting (PDIN). It is the total protein from the feed undecomposed in the rumen and the microbial protein with a limiting level of nitrogen in the rumen. The next unit was the protein digested in the small intestine when rumen-fermentable energy is limiting (PDIE). It is the sum of the original feed protein undecomposed in the rumen and the microbial protein with limiting energy levels in the rumen.

The data were processed using WINWAR software. The net energy content of feed was given in oat units and MJ, based on digestibility coefficients reported in the literature (11,12).

The chemical composition of diets was determined by standard methods (13). The addition of *Saccharomyces cerevisiae* dried yeast did not alter the chemical composition of the CJ concentrate; therefore, a pooled sample made up of individual feed samples collected in each of the groups was used in calculations. The results of feed and leftover analyses were used to determine nutrient intake during lactation (Table 2). Similar results were reported in all groups throughout the lactation period.

All procedures related to the animals in this study were approved by Local Ethical Committee for Animal Experiments in Olsztyn (31/2009).

Milk yield, milk composition, and immune parameters in blood on lactation days 28 and 70 were analyzed. Milk production per day was determined based on a morning

Table 1. Chemical composition and nutritive value of diets.

Specification	CJ concentrate	Haylage	Meadow hay
Chemical composition			
Dry matter (%)	88.65	44.26	83.95
Crude ash (%)	4.02	3.9	5.31
Crude protein (%)	15.01	8.1	7.50
Crude fat (%)	1.99	1.50	1.60
Crude fiber (%)	3.52	10.13	28.29
N-free extractives (%)	64.11	20.50	41.25
Gross energy (MJ kg^{-1})	16.11	14.56	17.24
In 1 kg of dry matter			
UFL	0.98	0.36	0.55
PDIN (g)	93.7	47.58	46.69
PDIE (g)	102.1	32.40	57.64
Crude protein (g)	150.10	80.50	75.00
Crude fiber (g)	35.20	101.30	282.90
Oat units	1.23	0.44	0.63
Net energy (MJ)	7.28	2.60	3.73

UFL: feed unit for milk production; PDIN: protein digested in the intestine subject to available nitrogen; PDIE: protein digested in the intestine subject to available energy.

Table 2. Average nutrient intake during the lactation period.

Specification	Nutrient intake		
	Group I	Group II	Group III
UFL	126.89	126.83	126.83
PDIN (kg)	13.19	13.18	13.18
PDIE (kg)	11.30	11.30	11.30
Crude protein (kg)	21.54	21.51	21.51
Crude fiber (kg)	25.63	25.51	25.51

UFL: feed unit for milk production; PDIN: protein digested in the intestine subject to available nitrogen; PDIE: protein digested in the intestine subject to available energy.

test milking performed after a 12-h lactation break. Around 2 min prior to milking, the lambed ewes were administered 5 IU of oxytocin intramuscularly to stimulate the contraction of myoepithelial cells, which surround the alveoli of the mammary gland, and lactiferous ducts. The right half of the udder was milked manually, while the other half was simultaneously sucked by a lamb. The quantity of collected milk was multiplied by 4 to obtain the daily milk yield. Milk was analyzed to determine the percentage content of dry matter, fat, protein and lactose, and somatic cell count per milliliter. The above analyses were performed using the Combi Foss 6000. Somatic cell counts were expressed in thousands per milliliter and in naturally log-transformed units per milliliter of milk.

Blood samples were collected from the jugular vein for immunological assays to determine the parameters of humoral and cellular immunity. The following humoral immunity parameters were studied: activity levels of lysozyme and ceruloplasmin, total protein levels, and gamma globulin concentrations. The following nonspecific cellular immunity parameters were analyzed: the respiratory burst activity (RBA) and potential killing activity (PKA) of phagocytes, and the proliferative response of mitogen-stimulated T cells and B cells.

Lysozyme activity in the blood plasma was determined by the turbidimetric method (14) modified and described by Siwicki and Anderson (15), and ceruloplasmin activity by the method proposed by Siwicki and Studnicka (16). The serum concentrations of total protein and gamma globulins were determined by the colorimetric micromethod proposed by Lowry et al. (17) (Sigma, Diagnostic Kits) and modified by Siwicki and Anderson (15). RBA levels, i.e. the metabolic activity of phorbol myristate acetate-stimulated phagocytes, were measured by spectrophotometry (OD: 620 nm), using the method proposed by Chung and Secombes (18) and modified by Siwicki et al. (19). The PKA of polymorphonuclear and mononuclear phagocytes was determined by spectrophotometry (OD: 620 nm) according to Rook et al. (20). The proliferative response of T cells stimulated with concanavalin A (ConA) and B cells stimulated with lipopolysaccharide (LPS) was determined by MTT-based (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) spectrophotometry according to Mosmann (21).

The collected results of the study were analyzed statistically using the STATISTICA version 9 software program (Stat Soft, Tulsa, OK, USA). The results were processed by one-way ANOVA, and the significance of differences between groups was verified with Duncan's test.

3. Results

The results of test milking in experiment 1 are presented in Table 3. Average daily milk yield was found to be higher in experimental group ewes. On day 28, the milk yield of group II and group III animals increased by 16.5% and 19%, respectively, in comparison with the control, and it differed significantly from the value noted in the control group ($P \leq 0.05$). On day 70, milk yield was 20% higher in the group of ewes fed brewer's yeast only during lactation (group III) as compared with the control group ($P \leq 0.05$). No significant differences in the percentage content of dry matter, fat, protein, or lactose were observed between groups. On day 28 of lactation, the milk of experimental ewes was characterized by insignificantly higher fat and protein concentrations. Protein and fat levels continued to increase as lactation progressed. Yeast supplementation had a significant effect on somatic cell counts in milk. On day 28, somatic cell count values were significantly ($P \leq 0.01$) higher in the milk of control group animals. This trend was maintained until the end of lactation when the differences between groups ceased to be statistically significant. In none of the experimental ewes did somatic cell count values exceed 220,000/mL of milk on the analyzed days.

Milk performance traits and the composition of milk reported in the second experiment are shown in Table 4. On day 28, the average daily milk yield was significantly higher in the experimental group ($P \leq 0.05$) than in the control. Milk yield increased by 16.5% in group II, and it was maintained until the end of lactation. On day 70, ewes whose diets were supplemented with dried yeast produced an average of 1277.92 mL of milk per day, and control group animals produced 998.46 mL of milk. The resulting difference of 279.46 mL (21.87%) was statistically significant ($P \leq 0.05$). Diet supplementation had no significant effect on the chemical composition of milk. An increase in fat, lactose, and, consequently, dry matter concentrations was observed in group II ewes at the peak and towards the end of lactation. On both days,

Table 3. Milk performance traits and milk composition (experiment 1).

Indicator	Day of lactation	Control group	Group I	Group II
Milk yield (mL):	28	1603.31 ± 164.89^{b}	1868.46 ± 268.88^{a}	1921.54 ± 439.58^{a}
	70	970.77 ± 158.87^{b}	1078.46 ± 168.22	1164.61 ± 286.84^{a}
Composition of milk (%):				
Fat	28	5.60 ± 1.16	5.42 ± 0.76	5.48 ± 0.74
	70	6.74 ± 0.92	6.80 ± 1.14	6.88 ± 0.85
Protein	28	4.90 ± 0.37	4.77 ± 0.37	4.74 ± 0.32
	70	5.95 ± 0.60	5.92 ± 0.56	6.05 ± 0.31
Lactose	28	5.17 ± 0.25	5.25 ± 0.15	5.19 ± 0.16
	70	4.81 ± 0.23	4.93 ± 0.19	4.93 ± 0.19
Dry matter	28	15.97 ± 1.26	15.97 ± 0.62	15.72 ± 0.82
	70	17.99 ± 1.15	17.97 ± 1.54	18.04 ± 0.86
Somatic cell count	28	342.46 ± 939.68^{A}	81.31 ± 45.90^{B}	86.61 ± 61.31^{B}
(thousands/mL)	70	271.00 ± 963.00^{a}	71.77 ± 31.20^{b}	78.46 ± 37.74^{b}

Values represent means ± standard deviations. Values within rows with different superscripts are significantly different at: ^{a,b} $P < 0.05$, ^{A,B} $P < 0.01$.

Table 4. Milk performance traits and the composition of milk (experiment 2).

Indicator	Day of lactation	Control group	Experimental group
Milk yield (mL):	28	1596.15 ± 181.90^{b}	1860.15 ± 320.46^{a}
	70	998.46 ± 187.69^{B}	1277.92 ± 175.47^{A}
Composition of milk (%):			
Fat	28	5.04 ± 0.91	5.71 ± 1.14
	70	7.05 ± 1.04	7.64 ± 0.81
Protein	28	4.99 ± 0.43	4.96 ± 0.44
	70	5.55 ± 0.88	5.84 ± 0.58
Lactose	28	5.17 ± 0.33	5.30 ± 0.18
	70	4.72 ± 0.87	4.81 ± 0.25
Dry matter	28	15.78 ± 0.80	16.47 ± 1.23
	70	17.91 ± 1.48	18.61 ± 0.95
Somatic cell count (thousands/mL):	28	316.77 ± 699.63^{a}	120.69 ± 89.89^{b}
	70	236.77 ± 296.34	112.23 ± 73.89

Values represent means ± standard deviations. Values within rows with different superscripts are significantly different at: ^{a,b} $P < 0.05$, ^{A,B} $P < 0.01$.

the milk of experimental ewes had a lower somatic cell count in comparison with the control group, but the noted difference was significant only on lactation day 28 ($P \leq 0.05$).

An analysis of the parameters characterizing the health status of ewes in experiment 1 (Table 5) did not reveal significant correlations between yeast supplementation and total protein levels in the blood serum, whereas gamma globulin fractions were found to be correlated with the dietary inclusion of yeasts. Gamma globulin concentrations were higher in the blood serum of group II ewes, and the noted differences were significant in comparison with groups I and III ($P \leq 0.01$). Lysozyme and ceruloplasmin levels in experimental ewes were elevated, and the observed differences were highly significant, relative to the control group, on both lactation days. The highest lysozyme activity levels were reported in group II animals on both analyzed days of lactation, and the differences resulting from a comparison with the remaining groups were statistically significant ($P \leq 0.01$). Group III

Table 5. Results of immunological assays (experiment 1).

Indicator	Day of lactation	Control group	Group I	Group II
Gamma globulin level (g/L)	28	30.23 ± 2.51^{B}	36.68 ± 2.56^{A}	30.76 ± 1.39^{B}
	70	29.93 ± 2.50^{B}	36.33 ± 1.34^{A}	30.83 ± 0.94^{B}
Lysozyme activity (mg/L)	28	0.93 ± 0.04^{C}	1.21 ± 0.08^{A}	1.02 ± 0.06^{B}
	70	0.94 ± 0.03^{C}	1.25 ± 0.05^{A}	1.01 ± 0.05^{B}
Ceruloplasmin activity (IU/L)	28	32.58 ± 4.71^{C}	37.96 ± 2.76^{B}	41.57 ± 1.53^{A}
	70	32.88 ± 1.36^{C}	37.36 ± 2.32^{B}	41.57 ± 4.28^{A}
Respiratory burst activity	28	0.41 ± 0.03^{C}	0.57 ± 0.04^{A}	0.50 ± 0.02^{B}
	70	0.41 ± 0.02^{C}	0.57 ± 0.03^{A}	0.50 ± 0.01^{B}
Potential killing activity	28	0.33 ± 0.02^{C}	0.44 ± 0.03^{B}	0.40 ± 0.01^{B}
	70	0.33 ± 0.01^{C}	0.44 ± 0.02^{B}	0.40 ± 0.01^{B}
MTT-ConA (RI)	28	0.48 ± 0.02^{C}	0.61 ± 0.03^{A}	0.53 ± 0.02^{B}
	70	0.46 ± 0.01^{C}	0.61 ± 0.02^{A}	0.53 ± 0.02^{B}
MTT- LPS (RI)	28	0.26 ± 0.02^{C}	0.39 ± 0.02^{A}	0.35 ± 0.02^{B}
	70	0.25 ± 0.02^{C}	0.39 ± 0.02^{A}	0.34 ± 0.01^{B}

MTT-ConA: proliferative response of T cells stimulated by mitogen concanavalin A;
MTT-LPS: proliferative response of B cells stimulated by mitogen lipopolysaccharides; RI: reactivity index.
Values represent means ± standard deviations. Values within rows with different superscripts are significantly different at: ^{a,b} $P < 0.05$, ^{A,B} $P < 0.01$.

ewes were also characterized by higher levels of lysozyme activity than control ewes ($P \leq 0.01$). The highest activity levels of ceruloplasmin, an acute phase protein, were noted on lactation days 28 and 70 in group III ewes, compared with ewes of the remaining groups ($P \leq 0.01$). In group II ewes, ceruloplasmin activity levels were significantly higher than in control group animals on both studied days ($P \leq 0.01$). Throughout the experiment, the highest values of nonspecific cellular immunity parameters, RBA, PKA, MTT-ConA, and MTT-LPS, were observed in group II animals receiving dried yeast during late pregnancy and lactation. In this group of ewes, the values of all analyzed immunity parameters were higher compared with the remaining animals ($P \leq 0.01$). On lactation days 28 and 70, the studied parameters were also higher in group III than in control group ewes ($P \leq 0.01$).

The immunological parameters of ewes in the second experiment are presented in Table 6. Similarly to the first experiment, total serum protein concentrations were comparable in ewes from all groups, whereas significant or highly significant differences were observed between groups as regards the remaining immunity parameters on lactation days 28 and 70. In group II ewes receiving the yeast supplement, the analyzed parameters were elevated, excluding ceruloplasmin activity.

4. Discussion

The results of both experiments suggest that feed supplementation with *Saccharomyces cerevisiae* dried brewer's yeast increases milk yield. The stimulating effect of yeasts on milk production was also noted by Milewski and Sobiech (22). In their study, the milk yield of lactating ewes whose diets were supplemented with *Saccharomyces cerevisiae* dried brewer's yeast increased by 18.19% and 15.53%, respectively, on lactation days 28 and 70. The efficacy of the above supplement was also demonstrated in a study of cows whose milk yield increased by approximately 12% (2). In the works of Dobicki et al. (23), Robinson and Garrett (24), and Swartz et al. (25), *Saccharomyces cerevisiae* dried yeast was not found to influence the protein content of milk from high-yielding cows. Similar results were obtained in sheep by the present authors. Dobicki et al. (2) demonstrated a significant increase in the percentage fat content of milk obtained from cows whose diets were supplemented with dried brewer's yeast, whereas no changes were noted in the levels of the remaining milk components. Lower somatic cell counts in the milk of sheep fed dried yeast suggest that the supplement improved the animals' overall health condition, including udder health. Somatic cell counts in milk are widely used to monitor udder condition. Mastitis leads to a deterioration of the physical properties of milk and, in consequence, of its quality and processing suitability. Milk from inflamed glands is characterized by higher albumin and globulin levels and lower lactose concentrations (26).

The obtained results indicate that brewer's yeast had a stimulating effect on immune mechanisms in ewes.

Table 6. Results of immunological assays (experiment 2).

Indicator	Day of lactation	Control group	Experimental group
Gamma globulin level (g/L)	28	35.67 ± 2.87^{b}	40.17 ± 1.17^{a}
	70	31.83 ± 2.23^{B}	39.00 ± 2.19^{A}
Lysozyme activity (mg/L)	28	0.81 ± 0.04^{b}	0.85 ± 0.02^{a}
	70	0.90 ± 0.04^{B}	0.99 ± 0.05^{A}
Ceruloplasmin activity (IU/L)	28	33.00 ± 2.99	34.90 ± 1.86
	70	32.92 ± 2.46	37.36 ± 2.32
Respiratory burst activity	28	0.36 ± 0.02^{B}	0.40 ± 0.02^{A}
	70	0.36 ± 0.02^{B}	0.40 ± 0.02^{A}
Potential killing activity	28	0.32 ± 0.01^{B}	0.35 ± 0.01^{A}
	70	0.32 ± 0.02^{B}	0.36 ± 0.01^{A}
MTT-ConA (RI)	28	0.45 ± 0.02^{b}	0.48 ± 0.01^{a}
	70	0.43 ± 0.02^{B}	0.48 ± 0.01^{A}
MTT- LPS (RI)	28	0.28 ± 0.01^{B}	0.34 ± 0.01^{A}
	70	0.28 ± 0.01^{B}	0.35 ± 0.01^{A}

MTT-ConA: proliferative response of T-cells stimulated by mitogen concanavalin A;
MTT-LPS: proliferative response of B-cells stimulated by mitogen lipopolysaccharide;
RI: reactivity index. Values represent means ± standard deviations. Values within rows with different superscripts are significantly different at: $^{a,b}P < 0.05$, $^{A,B}P < 0.01$.

Our results are consistent with the findings of Wójcik et al. (27), in whose study the use of *Saccharomyces cerevisiae* dried brewer's yeast significantly improved the parameters of nonspecific humoral and cellular immunity. The supplementation of diets fed to late-pregnant and lactating ewes with dried yeast increased gamma globulin concentrations, lysozyme and ceruloplasmin activity levels, and the indicators of nonspecific cellular immunity, RBA, PKA, MTT-ConA, and MTT-LPS. The immunomodulative effect of *Saccharomyces cerevisiae* yeast on suckling lambs was demonstrated by Małaczewska and Milewski (10), in whose study the supplement increased lysozyme and ceruloplasmin levels, gamma globulin concentrations in the blood serum, and cellular immunity parameters RBA, PKA, MTT-ConA, and MTT-LPS. The immunomodulative properties of preparations containing *Saccharomyces cerevisiae* yeast were also described by Milewski et al. (28) and Wójcik (29), who found that the administration of Biolex-MB40 containing 25%–30% β-1,3/1,5-D-glucan and 20%–25% MOSs to lambs significantly stimulated their immune systems and enhanced their disease resistance

The results of this study provide clear evidence that the supplementation of diets fed to lactating ewes with *Saccharomyces cerevisiae* dried brewer's yeast delivers health benefits. Yeast added to diets for pregnant ewes could prepare them better for lactation, but the resulting improvement in their milk performance was similar when yeast was administered in late pregnancy or only during lactation.

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