Defence mechanisms of the offspring of ewes fed a diet supplemented with yeast (*Saccharomyces cerevisiae*) during pregnancy and lactation

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Abstract

The aim of the study was to determine the stimulating effect of a natural immunostimulator – dried brewer's yeast (Saccharomyces cerevisiae) – on specific and non-specific humoral and cellular immunity of the offspring of ewes fed a diet supplemented with Saccharomyces cerevisiae yeast during pregnancy or lactation. The study involved 66 lambs from ewes divided into 3 groups: I – control group fed a diet not supplemented with yeast, II – experimental group fed a diet containing dried brewer's yeast (Saccharomyces cerevisiae) since the 4th month of pregnancy, and III – experimental group fed a diet with the addition of yeast since lambing. From 28, 56 and 70 days old lambs blood was sampled from the jugular vein to determine the indicators of specific and non-specific humoral and cellular immunity (gamma globulin content, lysozyme activity, ceruloplasmin activity, total protein content and MTT assay of lymphocyte proliferation after stimulation with ConA or LPS, respiratory burst activity of phagocytes – RBA, potential killing activity of phagocytes – PKA). The obtained results are indicative of the immunomodulating properties of dried brewer's yeast (Saccharomyces cerevisiae) in the offspring of ewes fed a diet supplemented with yeast from the time of lambing or from the 4th month of pregnancy.

Key words: *lambs, dried brewer's yeast Saccharomyces cerevisiae, non-specific humoral immunity, non-specific cellular immunity.*

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Introduction

Owing to its specific properties, *Saccharomyces cerevisiae* yeast can be widely used as a natural stimulant in animal feed. Yeast does not colonise the digestive tract, but when administered *per os* it shows a broad spectrum of probiotic and prebiotic activity and, consequently, it has a beneficial effect on the health and productivity of both monogastric animals and ruminants [1]. Yeast has a complex mode of action: by becoming attached to the intestinal epithelium, it is able to effectively fight pathogens by absorbing their antigens and inhibiting their growth, thus stimulating the animals' immune system [2, 3]. The immunomodulatory effect of *Saccharomyces cerevisiae* yeast is principally associated to oligosaccharides which build the cell wall structure, as well as mannan-oligosaccharides (MOS) and beta-glucans [2-6]. An important role in this process could be played by beta-1,3/1,6-D-glucan whose effect on growing lambs has been demonstrated experimentally as regards the indicators of both non-specific humoral immunity [7] and non-specific cellular immunity [8]. Limited research was conducted on lambs to investigate the direct effect of supplementing their diets with *Saccharomyces cerevisiae* yeast cultures [3, 9-11], and the immunological parameters were rarely evaluated [3]. The

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available literature on the subject provides no information on their indirect effect on the offspring of ewes fed a diet containing *Saccharomyces cerevisiae* during pregnancy or immediately after lambing. Aim of the presented study was to determine the effect of dried brewer's yeast (*Saccharomyces cerevisiae*) contained in the diet of pregnant and lactating ewes on the development of selected humoral and cellular immune mechanisms of their offspring.

Materials and Methods

The experiment was performed on Kamieniec sheep from the breeding herd at the Production and Experimental Station in Bałcyny, during the years 2005 and 2006.

Experimental design

The experimental materials comprised 66 suckling lambs, the offspring of 39 ewes, divided into three equal groups: I – control, II and III – experimental. The same feed was administered to all groups, comprising meadow hay, barley straw, hay-silage of grass and legumes, and CJ concentrate. Uniform feeding standards were applied in line with the requirements of the Animal Husbandry Institute [12]. Experimental ewes were additionally fed *Saccharomyces cerevisiae* Inter Yeast®, which accounted for 5% of CJ concentrate, starting from the 4th month of pregnancy in group II and from the moment of lambing in group III. CJ concentrate was administered in the amount of 0.3 kg/animal/day during pregnancy and 0.6 kg/animal/day during lactation; the resulting daily yeast dose amounted to 15 and 30 g/animal, respectively. Group II was formed at the end of the 3rd month of pregnancy after ultrasound examination, while groups I and III were formed successively during the lambing period, analogous to group II. Consequently, every group comprised 22 lambs – 4 single ones and 18 twins – with a similar ratio of gimmers and young rams. On 28, 56 and 70 days of life following the birth , blood was sampled from the jugular vein to determine the indicators of non-specific humoral and cellular immunity.

Leucocytes were isolated from blood by centrifugation at 2000 g for 30 min at 4°C on the Gradisol L or G gradient (Aqua Medica, Poland), washed three times in PBS and resuspended in RPMI 1640 medium (Sigma) supplemented with 10% of FCS (Gibco-BRL) at a stock concentration of 2 × 106 cells/ml of medium. The viability of cells was checked by supravital staining with 0.1% w/v trypan blue.

Evaluation of humoral immunity parameters

Lysozyme activity in blood plasma was determined by the turbidimetric method [13] modified by Siwicki and Anderson [14], ceruloplasmin activity in blood plasma – by the method developed by Siwicki and Studnicka [15], total amount of protein in blood serum – by spectrophotometry as described by Lowry et al. [16].

Gammaglobulin levels in blood serum was determined by the precipitation method modified by Siwicki and Anderson [14].

Evaluation of non-specific cellular immunity parameters

The metabolic activity of phagocytes was determined based on respiratory burst activity (RBA) after stimulation with PMA (Phorobol Myristate Acetate, Sigma), by spectrophotometry (OD 620 nm) as described by Chung and Secombes [17], and adapted for dogs by Siwicki et al. [19], the potential killing activity (PKA) of mononuclear (MN) phagocytes and polymorphonuclear (PMN) phagocytes – by spectrophotometry (OD 620 nm) according to Rook et al. [18].

The isolated cells were resuspended in RPMI-1640 medium (Sigma) at 10^6 cells/ml. On 96-well U-shaped microplates 100 µl of isolated blood leukocytes were mixed with 100 µl of 0.2% nitro blue tetrazolium (NBT, Sigma) solution in 0.2 M phosphate buffer at pH 7.2, and 1 µl of PMA at concentration 1 mg/ml in ethanol was added. After 30 min of incubation at 37°C, the supernatant was removed from each well. The cells pellet was washed with absolute ethanol and than three times in 70% ethanol and dried at room temperature. The amount of extracted reduced NBT after incubation with 2M KOH and DMSO (dimethylsulfoxide, Sigma) was measured colorimetrically at 620 nm in a plate microreader (ALAB). All samples were tested in triplicate and the mean value served as the result.

Potential killing activity (PKA) of mononuclear (MN) phagocytes and polymorphonuclear (PMN) phagocytes was determined in isolated blood leukocytes stimulated with killed microorganisms, according to the method presented by Rook et al. [18] and adapted for dogs by Siwicki et al. [19]. On 96-well U-shaped microplates 100 µl of leucocytes were mixed with 100 µl of 0.2 % NBT in phosphate buffer at pH 7.2 and 10 µl of killed *Staphylococcus aureus* strain 209P (containing 10^6 bacteria). The mixture was incubated 1 h at 37°C and the supernatant was removed. The cell pellet was washed with absolute ethanol and three times with 70% ethanol and dried at room temperature. This was followed by the addition of 2M KOH and DMSO to each well. The amount of extracted reduced NBT was measured at 620 nm in a plate microreader (MRX 3 Dynatech). All samples were tested in triplicate and the mean value served as the result.

Evaluation of specific cellular immunity parameters

Lymphocyte proliferation assay after stimulation with mitogens: concanavalin A (ConA) and lipopolysaccharide (LPS) – by MTT spectrophotometry (OD 620 nm) using (3-[4,5-dimethylthiazol-2yl]-2,5-diphenyltetrazolium bromide – 3-[4,5-dimethyl-2-thiazol]-2,5-diphenyl-2H-tetrazolium bromide), as described by Mosmann [20].

MTT (Sigma) was dissolved in PBS at concentration of 5 mg/ml and filtered. On 96-well culture plates (Sarstetd, USA) 100 µl of blood lymphocytes in RPMI 1640 containing 10% FCS, 2 mM L-glutamine, 0.02 mM 2-mercaptoethanol, 1% hepes buffer, penicillin/streptomycin (100 U/100 µg/ml) were mixed with 100 µl of RPMI 1640 containing mitogens ConA (5 µg/ml) or LPS (20 µg/ml). After 72 h incubation at 37°C with 5% carbon dioxide atmosphere (Alab Incubator, Sweden), 50 µl of MTT solution were added into each well and plates were incubated 4 h at 37°C. After incubation the plates were centrifuged (1400 g, 15°C, 5 min). Supernatants were removed and 100 µl of DMSO (Sigma) were added into each well and incubated 15 min at room temperature. After incubation the solubilised reduced MTT was measured colorimetrically at 620 nm in a plate microreader (ALAB). All samples were tested in triplicate and the mean value served as the result.

The results were verified statistically by a one-way analysis of variance (ANOVA), and the significance of differences between the groups was verified with Duncan's test.

Results

The conducted study was the first ever attempt to determine the stimulating effect of a natural immunostimulator – dried brewer's yeast (*Saccharomyces cerevisiae*) – on selected parameters of specific and non-specific humoral and cellular immunity in the applied experimental design involving the investigated animal species (offspring of ewes fed a diet supplemented with *Saccharomyces cerevisiae* during pregnancy and lactation or during lactation only). The obtained results are indicative of the significant effect of *Saccharomyces cerevisiae* on the evaluated parameters of specific and non-specific humoral and cellular immunity in lambs (Table 1).

The analysis of humoral immunity parameters in lambs (Table 1), i.e. lysozyme activity, ceruloplasmin activity and the serum levels of gamma globulins, revealed that their values were statistically significantly ($p \leq 0.01$) higher in the experimental groups (II and III) fed a yeast-supplemented diet throughout the experiment than in the control group (I) fed diet without yeast supplementation. Statistically significant differences between the experimental groups and the control group were not observed only in respect of the serum levels of total protein. A statistically significant ($p \leq 0.01$) difference between group II and group III was reported only with regard to ceruloplasmin activity, which was higher in the offspring of ewes fed a yeast-supplemented diet since lambing, compared to the offspring of ewes fed such a diet since the 4th month of pregnancy.

As regards the investigated indicators of specific and non-specific cellular immunity in lambs (Table 1), i.e. the proliferative response of lymphocytes (MTT), the metabolic activity of phagocytes (RBA) and the potential killing ability (PKA) of phagocytes, a statistically significant ($p \leq 0.01$ or $p \leq 0.05$) increase in their values was reported in the experimental groups (II and III) throughout the experiment than in the control group (I). Differences between the experimental groups (II and III) were noted only in re-

Table 1. Parameters of specific and non-specific humoral and cellular immunity in lambs ($\bar{x} \pm s$)

Investigated parameters	Group		
	I	II	III
Lysozyme activity (mg/l) at the age of (days):			
28	0.82 ± 0.03^{B}	0.98 ± 0.04^{A}	0.97 ± 0.03^{A}
56	0.80 ± 0.04^{B}	0.98 ± 0.03^{A}	0.99 ± 0.02^{A}
70	0.81 ± 0.03^{B}	1.00 ± 0.04^{A}	0.99 ± 0.03^{A}
Ceruloplasmin activity (IU/l) at the age of (days):			
28	28.20 ± 1.44^{C}	31.20 ± 1.42^{B}	32.97 ± 1.46^{A}
56	28.97 ± 1.29^{C}	32.08 ± 1.71^{B}	31.99 ± 1.61^{A}
70	29.97 ± 1.07^{C}	31.46 ± 1.23^{B}	33.45 ± 1.43^{A}
Total protein content (g/l) at the age of (days):			
28	63.70±4.00	63.60±4.35	65.00±4.62
56	64.22±4,51	65.74±4.12	64,89±3.97
70	65.30±3.30	64.80±3.15	64.70±2.63
Gamma globulin content (g/l) at the age of (days):			
28	34.3 ± 1.2^{B}	39.7 ± 1.0^{A}	38.9 ± 0.9^{A}
56	$33,9 \pm 1,6^{B}$	39.3 ± 1.1^{A}	38.6 ± 1.0^{A}
70	34.6 ± 1.7^{B}	40.1 ± 0.9^{A}	38.4 ± 0.9^{A}
RBA (OD 620 nm) at the age of (days):			
28	0.37 ± 0.01^{B}	0.45 ± 0.03^{A}	0.43 ± 0.02^{A}
56	0.37 ± 0.02^{B}	0.44 ± 0.02^{A}	0.46 ± 0.02^{A}
70	0.38 ± 0.01^{C}	0.47 ± 0.02^{A}	0.44 ± 0.01^{B}
PKA (OD 620 nm) at the age of (days):			
28	0.33 ± 0.02^{B}	0.37 ± 0.02^{A}	0.36 ± 0.02^{A}
56	0.30 ± 0.01^{B}	0.36 ± 0.02^{A}	0.35 ± 0.01^{A}
70	0.32 ± 0.02^{B}	0.37 ± 0.01^{A}	0.34 ± 0.02^{A}
MTT-ConA (OD 620 nm) at the age of (days):			
28	0.42 ± 0.02^{Bc}	0.52 ± 0.03^{Aa}	0.47 ± 0.02^{Ab}
56	0.45 ± 0.01^{B}	0.51 ± 0.02^{A}	0.48 ± 0.02^{A}
70	0.45 ± 0.03^{C}	0.53 ± 0.02^{A}	0.47 ± 0.01^{B}
MTT-LPS (OD 620 nm) at the age of (days):			
28	0.25 ± 0.02^{C}	0.34 ± 0.01^{A}	0.32 ± 0.01^{B}
56	0.23 ± 0.03^{B}	0.35 ± 0.02^{A}	0.32 ± 0.03^{A}
70	0.24 ± 0.02^{B}	0.36 ± 0.01^{A}	0.31 ± 0.04^{A}

A, B, C – $p \leq 0.01$; a, b, c – $p \leq 0.05$

spect to the proliferation capacity of lymphocytes, where both LPS ($p \leq 0.01$) and concanavalin A ($p \leq 0.01$; $p \leq 0.05$) proved to be more effective mitogens in the lambs of ewes fed a diet containing yeast since the 4th month of pregnancy than in the offspring of ewes fed a yeast-supplemented diet from the time of lambing.

Discussion

Owing to its structure, polysaccharide beta-1,3/1,6-glucan which is present in the cell walls of brewer's yeast (*Saccharomyces cerevisiae*), ideally fits into the specific receptors (CR3) also TLR-2 and 6 and Lectin-1 of beta-1,3/1,6-glucan in macrophages, and by adhering to them, it activates those cells of the phagocytary defence system. Macrophages constitute the body's first line of defence which ingest and destroys microorganisms, cells recognised as foreign and neoplastic cells. Beta-glucans with a different chemical structure – acquired from other sources – do not activate macrophages, e.g. beta-glucans obtained from grain cereals have mainly 1,4 bonds . Beta-glucans with those types of bonds are highly effective in lowering cholesterol levels in the blood, but they do not activate macrophages. Beta-1,3/1,6-glucan is a natural adjuvant which enhances the activity of other substances – antibiotics, antifungal and antiparasitic substances, vitamins and dietary supplements. It acts rapidly and fully mobilises the immune system after only 72 hours. It is an effective antioxidant and it neutralises free radicals which, by affecting DNA, are one of the causes of cancer. Beta-1,3/1,6-glucan is currently applied as a supplement in the treatment of cancer and other immunological diseases. Mannan-oligosaccharide (MOS), another oligosaccharide which is found only in the cell walls of *Saccharomyces cerevisiae*, not only prevents pathogens from adhering to the intestinal mucosa, but by binding to intestinal wall receptors, it stimulates the immune system in animals. MOS increases the level of immunoglobulins in the intestine and blood plasma, and it absorbs mycotoxins. In cell walls, mannans bind to glycoproteins, lipids, chitin and many other biologically active substances [21-30].

The choice of the investigated immunological parameters resulted from the fact that gamma globulins are effectors of humoral immune response which destroys bacterial cells, and acute phase proteins are the first barrier in fighting the pathogen by cooperating closely with other non-specific immunity indicators, such as the complement, phagocytes, etc. This mutual and relative dependency of the stimulated immune mechanisms has a decisive impact on the body's ability to eliminate infectious agents and, consequently, on the body's immunological status and health condition. The cellular immunity parameters, on the other hand, play a key role in fighting infections caused by intracellular microorganisms. Brewer's yeast has a pronounced positive effect on the phagocytic activity of leukocytes, the body's first protective barrier, which destroy and degrade pathogens. Antigen presenting cells (macrophages and dendritic cells) and target cells present the antigen to T lymphocytes, thus stimulating a specific immune response. The ability of lymphocytes to transform into active, younger blastic forms is equally important in immunological processes. The positive results obtained in this respect, as regards stimulation with both concanavalin A and LPS in this study, lead to the conclusion that brewer's yeast is an important stimulant of non-specific cellular immunity which can be effectively used to fight infections caused by intracellular pathogens, mainly viruses. It indicates that a diet supplemented with yeast can be recommended for the prevention and treatment of infections where an effective remedy is not available. Yeast can also be recommended due to the fact that in addition to its stimulating effect, it is not toxic to the body and may be safely administered.

A comparison of the results obtained in this study with previous research findings [7, 8] shows that the stimulating effect of dried brewer's yeast (*Saccharomyces cerevisiae*), with respect to the investigated parameters of specific and non-specific humoral and cellular immunity in lambs, was lower than that observed for the dietary supplement Biolex-Beta HP. The above could be due to the fact that yeast exerts an indirect effect on the offspring's immunity parameters through the ewe's body. It should also be noted that Biolex-Beta HP comprises a separated fragment of the cell wall of *Saccharomyces cerevisiae* – beta-1,3/1,6-glucan which is characterised by high purity (85%) and high biological activity.

The results of this immunological analysis cannot be compared with literary data because examples of similar studies were not found in the available scientific publications. Yet the findings of other authors confirm that beta-glucan stimulates an immune response in many animal species and when administered, it enhances the body's ability to develop fast and effective protection against bacteria, viruses, fungi and protozoa [1, 21, 23-25, 29].

In view of the results of this study investigating the immunomodulating effect of a natural stimulant – dried brewer's yeast (*Saccharomyces cerevisiae*) – in lambs, it may be concluded that yeast can have a practical immunopreventive effect on those animals, especially in periods marked by increased susceptibility to bacterial and viral infections. The obtained results offer a valuable incentive for further research into the use of yeast in the treatment of impaired immunity in both lambs and adult animals.

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