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BREWER'S YEAST AS AN INGREDIENT ENHANCING IMMUNITY

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The research aimed at evaluating the influence of brewer's yeast *Saccharomyces cerevisiae* used in the diet of healthy, growing rats on the selected parameters of research animals' immune system. It was proved that Inter Yeast Vital brewery yeast *Saccharomyces cerevisiae* stabilized basic mechanisms of research animals' immune system. Increased phagocytosis of rats' blood granulocytes in relation to *Staphylococcus aureus* bacterium and *Candida albicans* yeast fungi allows for their use as immunostimulating factor in organisms with infections caused by bacteria and yeast fungi. Moreover, the research proved the advantageous influence of examined yeast on the production of interferon gamma (IFN-γ), thus they may be used in immunotherapy of people with virus infections.

INTRODUCTION

The research conducted in the last 20 years has shown a close relation between the diet and organism self-defence ability against various infections as well as the risk level of developing various diseases. That is why many scientists undertake the research concerning possibilities of modulating human immune system functions in relation to various ingredients of the diet, such as: vitamins, carotenoids, pro- and prebiotics, microelements or polyunsaturated fatty acids [Kelley & Bendich, 1996; Scrimshaw & SanGiovanni, 1997].

The condition and functioning of the human immune system is influenced by many factors including diet's ingredients and their amount. Both shortage and surplus of some elements in the diet may adversely affect the immune system. Moreover, it is known that the immune system is more sensitive to diet changes in the case of elder people rather than the young, and that nutrition mistakes have a synergistic influence on immunologic functions. It has been proved that a high intake of fats or the intake of fats containing incorrect content of fatty acids (lower supply of *n*-3 fatty acids) may result in considerable decrease of human immunity, and consequently increase the risk of infections [Blok *et al.*, 1996]. Similarly, Vitamin B_6 deficiences in the diet causes distemper in maturing and activity of lymphocytes T, thus decreases the production of antibodies [Scrimshaw & San Giovanni, 1997]. Human immunity may also be increased by the increased supply of pre- and probiotics in the diet [Majamaa & Isolauri, 1997].

Brewer's yeast *Saccharomyces cerevisiae* is a natural product of the brewing industry containing various immunostimulating compounds such as beta-glucans, nucleic acids and mannans, and used as a diet additive for various animals. It has been observed to be capable of enhancing immune responses [Siwicki *et al.*, 1994; Ortuño *et al.*, 2002] as well as growth [Lara-Flores *et al.*, 2002] of various fish species and thus may serve as an excellent health promoter for fish culture.

Li & Gatlin [2003] established the beneficial effects of partially autolyzed brewer's yeast on immune responses to *S. iniae* infection.

In search of new pro-health ingredients, brewer's yeast strikes as especially valuable. As they contain many valuable ingredients (beta glucan, nucleic acids, mannans, vitamins from B group), they may be used in human nutrition as a bioactive ingredient, which could naturally enhance human immune system and thus lower the risk of contracting numerous infections, especially diet dependable ones.

The research aimed at evaluating the influence of brewer's yeast *Saccharomyces cerevisiae*, used in the diet of healthy, growing rats, on the selected parameters of the immune system of the experimental animals.

MATERIALS AND METHODS

Dried brewer's yeast *Saccharomyces cerevisiae* under the commercial name - Inter Yeast Vital (from INTER YEAST company) constituted the research material.

The research was conducted on 14 experimental animals – growing Wistar rats of initial weight – 100±10 g, in two groups of 7 animals each: control – fed a diet without the yeast supplement, and experimental – receiving food with

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brewery yeast *Saccharomyces cerevisiae* supplement. Diets (semi-synthetic, isocaloric) were prepared in accordance with the recommendations of the American Institute of Nutrition [Reeves *et al.*, 1993]. The examined diet contained 0.5% of brewer's yeast supplement. The Ethic Commission expressed its approval of the research. Diet intake and body weight gain of research animals were controlled over the research period.

The proper experiment was preceded with a 5-day adaptation period, during which animals were given water and LSM diet *ad libitum*. The proper experiment lasted 6 weeks, after which blood from myocardium was collected. Animals were anaesthetised with the peritoneal injection of Thiopental. Blood necessary for cellular immune response research of rats immune system was collected from heart into test tubes with heparine additive (14 u./mL). The evaluation of lymphocytes T proliferative ability stimulated with phytohemaglutinin (PHA) was conducted with the use of blastic transformation method. *Staphylococcus aureus* bacterium strain and *Candida albicans* yeast fungi were used to estimate the phagocytotic activity of granulocytes.

The determination of IFN-γ and TNF-α cytokine concentration in supernatants of cellular cultures of rat blood was conducted with ELISA immunoenzymatic method, with the use of ready kits (Rat IFN-gamma PromoKine ELISA Kit cat. No.: E-60706 and Rat TNF alpha ELISA Kit Cat No.: E63706). The analyses were carried out according to the procedures recommended by the producer. The absorption was measured at 490 nm using a Dynatech ELISA plate reader. The results obtained were analyzed statistically with STATGRAPHIC ver. 4.1 program.

RESULTS

Over the experimental period, irrespective of the group, the daily diet intake of the animals ranged from from 17 to 20 g on average. This intake provided from 85 to 100 mg of yeast in the experimental group. Body weight of experimental animals after the research reached 375.1±27.4 g in the case of the control group and 410.4±42.48 g in the case of the experimental group.

The results obtained for the total number and individual leukocytes are presented in Table 1. A significant correlation ($p<0.05$) was observed between the average number of leukocytes and the percentage of lymphocytes, neutrophiles, and monocytes in the blood of the rats examined.

The increased number of neutrophiles and monocytes in the blood of rats receiving yeast supplement with a daily diet affected the number of absorbed bacteria (*Staphylococcus aureus*) and yeast fungi (*Candida albicans*). During the comparison of the phagocytic activity these cells, the increased phagocytosis index was demonstrated in the examined blood samples of the rats fed with yeast supplement, still the differences observed were not statistically significant (Table 2).

TABLE 2. The value of phagocytosis index of leucocytes isolated from the blood of the examined rats.

The value of phagocytosis index	Examined group of rats	
	Diet without brewer's yeast supplement, n=7	Diet with 0.5% brewer's yeast supplement, n=7
The minimal value MIN	0.3	0.3
The maximum value MAX	0 .8	0.9
The arithmetic mean X	0.8^1 0.8^2	0.9^1 0.9^2
Mediana, M	0.5	0.6
Standard deviation, SD	0.1^1 0.1^2	0.1^1 0.1^2

[1]*Staphylococcus aureus*; [2]*Candida albicans*

An analysis of lymphocytes T ability for *in vitro* proliferation under the influence of phytohemaglutinin (PHA), differences in their activity were observed in the examined groups of rats. The percentage of blastic forms of lymphocytes T in the case of rats fed with brewer's yeast supplement manifested the increased cells' mobilization to immunological response (Table 3).

TABLE 3. The comparison of lymphocytes T ability for *in vitro* proliferation under the influence of phytohemaglutinin (PHA).

Examined factors	Examined group of group	
	Diet without brewer's yeast supplement, n=7	Diet with 0.5% brewer's yeast supplement, n=7
The percentage of blastic forms of lymphocytes T in blood cell cultures without induced PHA *in vitro*	3.1±0.82	4.1±0.55
The percentage of blastic forms of lymphocytes T in blood cell cultures with induced PHA *in vitro*	70.7±6.16	78.9±6.11

The number of cytokines produced by macrophages and lymphocytes in PHA-induced cultures was presented in Table 4. The research proved the beneficial effect of the examined yeast on the production of interferon gamma (IFN-γ), thus they may be used in immunotherapy of people with viral infections.

DISCUSSION

The results obtained for the selected parameters of the immune response of rats on the diet with brewer's yeast *Saccharomyces cerevisiae* supplement (Inter Yeast Vital) prove their immunostimulating activity. This is also proved by the increased percentage of lymphocytes and macrophages in

TABLE 1. Total number of leukocytes and the percentage of subpopulation in the blood of the examined rats.

Examined group	Total number of leukocytes (thousand/mm^3)	Neutrophiles (%)	Eosinophiles (%)	Lymphocytes (%)	Monocytes (%)
Rats on the examined diet without brewer's yeast supplement, n=7	10.8±3.2	1.2±0.4	0.3±0.3	7.9±2.3	0.4±0.3
Rats on the examined diet with brewer's yeast supplement, n=7	14.4±3.3	1.9±0.8	0.3±0.2	10.9±2.6	1.2±1.2

TABLE 4. Concentration of the examined cytokines in supernatants of PHA-induced cultures.

Examined group	Interferon gamma (IFN-γ, pg/mL)	Tumor necrosis factor α (TNF-α, pg/mL)
Rats on the examined diet without brewer's yeast supplement, n=7	984.9±151.8	607.1±141.8
Rats on the examined diet with brewer's yeast supplement, n=7	1233.0±217.7	452.9±99.5

rats' blood. The increased activity of cells to bacteria and fungi phagocytosis might be influenced by beta glucan present in yeast cell walls [Tada *et al.*, 2002; Lee *et al.*, 2001].

The presence of the yeast in rats' diet favourably affected the proliferation of lymphocytes into blastic forms in cellular cultures induced by phytohemaglutinin (PHA). The increase of lymphocytes T proliferation from the blood of rats on the diet with the examined yeast supplement was observed to correlate with the amount of interferon gamma (IFN-γ) produced in supernatants from cellular cultures. The intensification of IFN-γ production radically influenced antiviral immunity through the impact on other cells' immunity and the induction of antiphlogistic factors in them [Taylor *et al.*, 1997].

In vitro experiments proved that glucan isolated from brewer's yeast *Saccharomyces cervisiae* activates macrophages to the production of tumour necrosis factor α (TNF-α) [Ishibashi *et al.*, 2001], which was not proved in this research. A lower production efficiency of this cytokine (TNF-) may be explained by poorly soluble glucan form and the presence of other oligosaccharides of yeast cell walls [Zarębski *et al.*, 2001].

The research conducted by Li *et al.* [2003] demonstrated that brewer's yeast used in the diet of hybrid striped bass positively influenced growth performance, body composition and immune response, and may be used for relatively long periods without causing immunopression.

Taking the above into consideration as well as the results obtained in our research, brewer's yeast *Saccharomyces cerevisiae* seems to be a promising ingredient enhancing human immunity.

CONCLUSIONS

1. The examined Inter Yeast Vital brewer's yeast *Saccharomyces cerevisiae* displayed stabilizing activity of basic mechanisms of the immune system of the experimental animals.

2. The use of yeast as a dietary supplement for rats resulted in increasing the number of lymphocytes in rat blood, which enables their use as an immunostimulating factor in the case of organism with lower immunity.

3. The increased phagocytosis in relation to *Staphylococcus aureus* bacteria and *Candida albicans* yeast fungi enables the application of the examined brewer's yeast as an immunostimulating factor of organisms with infections caused by bacteria and yeast fungi.

4. The examined brewer's yeast demonstrates a favourbale influence on the production of interferon gamma (IFN-γ), which points to the possibility of their use in immunotherapy of people with viral infections.

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