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Effects of Yeast Polysaccharide on Immune Enhancement and Production Performance of Rats

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Abstract: The aims of the present study were to investigate the immune enhancement effect and production performance of Yeast Polysaccharide (YPS) on rats. The results showed that each index in YPS groups was higher than that in blank control group. Any dose of YPS by orally administrated significantly raised spleen and thymus index, serum IgA, IgG, AKP and LZM level, phagocytic index and phagocytic activity of macrophages in the rats. Meanwhile it can increase production performance of rats. Results suggested that any dose of YPS can enhance immunologic function and production performance of rats and the dose of 100 mg kg^{-1} has the most obvious efficacy.

Key words: Yeast polysaccharide, immune enhancement, production performance, rats, cell, China

INTRODUCTION

One of the most promising recent alternatives to classical antibiotic treatment is the use of immunomodulators for enhancing host defense responses (Tzianabos, 2000). Several types of immunomodulators have been identified including mammalian proteins such as Interferon gamma (IFN-γ) (Murray, 1996), granulocyte colony-stimulating factor (Nemunaitis, 1997) etc. In recent decades, polysaccharides isolated from natural sources (plants, fungi, bacteria, algae and animals) have also attracted a great deal of attention in the biomedical arena because of their broad spectrum of therapeutic properties (antitumor, immunostimulant, antiaging, antioxidant, antiviral, antidiabetic, antiatherosclerosis and antiinflammatory activities) and relatively low toxicity (Pang *et al*., 1999; Smestad, 2001; Wasser, 2002; Li *et al*., 2006; Chattopadhyay *et al*., 2008; Dai *et al*., 2009; Sun *et al*., 2009; Khotimchenko *et al*., 2006; De Barba *et al*., 2011; Sohail *et al*., 2011; Thetsrimuang *et al*., 2011).

Fungi is an important source of materials in traditional Chinese medicine. Polysaccharide extracts from many species of fungi exhibit immunostimulating and/or anti-tumor activities (Ohno *et al*., 2001; Wasser, 2002; Zhang *et al*., 2003). Yeast is one of fungi, yeast cell wall is a thick envelope (100-200 nm) representing 15-25% of the dry mass of the cell (Farkas, 1979). The major components of cell wall are polysaccharides (up to 90%), mainly β-D-glucans and α-D-mannans. Yeast Polysaccharides (YPS), β-D-glucans and α-D-mannans have been previously demonstrated to antitumor (Khalikova *et al*., 2005), antioxidant (Kogan *et al*., 2005) and nutrition (Kogan and Kocher, 2007). In this study, rats were treated with different doses of YPS under the same condition. By observing spleen and thymus indexes, the levels of serum IgA, IgG, AKP and LZM, the phagocytic index and phagocytic activity of Macrophages, Average Daily Gain (ADG) and Average Daily Intake (ADI) effects of YPS on immunologic function and production performance of rats were analysed.

MATERIALS AND METHODS

Preparation of polysaccharide: Yeast powder was independently researched and developed by Lanzhou Institute of Animal Science and Veterinary Pharmaceutics, Chinese Academy of Agricultural Sciences, China. Yeast

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powder was mixed with distilled water in the proportion of 1:3 (volume ratio) then break-walled sequentially by autoclaving (15 pounds, 35 min), freezing (-40°C, 2 h) thawing (100°C, 15 min) and ultraphonic (500 W, 25 min), and then 3% of potassium hydroxide (KOH) was added. After a 1.5 h reaction at 100°C and neutralized with 20% of acetic acid immediately then centrifuged at 3500 r min^{-1} for 10 min. The supernatant was precipitated with three volumes of ethanol and stored overnight at 4°C. After centrifugation, the precipitates were collected and then washed sequentially with acetone (2 times) and ether. And after freeze drying, Yeast Polysaccharide (YPS) was obtained. YPS was stored at -20°C and freshly dissolved in distilled water immediately before use.

Experimental rats: About 120 male, healthy and unvaccinated rats (provided by laboratory animal center of Lanzhou University, China), each weighing around 220 g were randomly allotted into 12 cages. Every 3 cages formed a group, 10 in each cage. Rats were housed and maintained under pathogen-free conditions in microisolator cages.

Animal care was in compliance with recommendations of The Guide for Care and Use of Laboratory Animals (National Research Council). About 120 rats were randomly divided into 4 groups.

Groups division: Groups I-III were the YPS groups. Group IV was the blank control group. Polysaccharide contents: rats were administered with YPS at the doses of 200,100 and 50 mg kg^{-1} body weight marked as I-III, respectively. Group IV contained no polysaccharide in its distilled water. YPS groups were orally administrated daily with YPS solution according to design dose and weighed in time every day; equivalent physiological saline was orally administrated at the same doses in the blank control group during 30 days. Rats were sacrificed by femoral bloodletting on day 31. Spleen and thymus were collected and weighed. The immunological parameters and relevant indexes were described in this study.

Effect of YPS on spleen and thymus indexes: After bloodstain of spleen and thymus without fat was washed with 4°C equivalent physiological saline and sipped up with filter paper then weighted. The immune organs index was calculated as follows:

$$\text{Immune organs index}\,(\text{mg g}^{-1}) = \frac{\text{Immune organs weight (mg)}}{\text{Body weight (g)}}$$

Effect of YPS on serum IgA, IgG, AKP and LZM: On termination of the experiment, blood samples were collected; serums were separated and stored at -80°C before assay. Two-site sandwich Enzyme-Linked Immunosorbent Assays (ELISA) were performed for quantify IgA and IgG (Sigma, USA) and colorimetry assays detected quantify Alkaline Phosphatase (AKP) and Lysozyme (LZM) (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) with kit according to the manufacturer's instruction.

Macrophage phagocytosis assay (carbon clearance): The test of carbon clearance was carried out according to the method of Yang *et al.* (2007) and Han *et al.* (2010). Normal rats were divided into four groups (8 per group) and orally administrated with distilled water 2.0 mL or with YPS 2.0 mL (50, 100 and 200 mg kg^{-1} body weight, respectively) for 10 days. After 10 days of gastric feed, Indian ink according to 0.1 mL/10 g body weight was injected into the tail vein of the rats.

A total of 20 μL blood was respectively collected through eye orbit after 2 min (t_1) and 10 min (t_2) and added to 2.0 mL 0.1% Na_2CO_3 at once and its absorbance was determined at 620 nm (Jayathirtha and Mishra, 2004). At the same time, rats were sacrificed by femoral bloodletting; the spleens and liver were weighed. The carbon particle clearance's capacity was expressed by phagocytic index. Calculate out the phagocytic index (k) and phagocytic activity (a) as follow:

$$k = \frac{\lg OD_1 - \lg OD_2}{t_2 - t_1}$$

$$\alpha = \sqrt[3]{k} \times \frac{\text{Body weight}}{\text{Liver weight} + \text{Spleen weight}}$$

Production performance indexes: From days 1-30, production-index of each rat was tested:

$$\text{ADG (Averagedailygain)} = \frac{\text{(Post-weight)} - \text{(Pre-weight)}}{\text{Days}}$$

$$\text{ADI(Average daily intake)} = \frac{\text{Experimental period intake}}{\text{Days}}$$

$$\text{G/I} = \frac{\text{ADG}}{\text{ADI}}$$

Statistical analysis: All the data were expressed as mean±SD. Statistical differences were analyzed by Analysis of Variance (ANOVA) followed by LSD's Multiple Range test using SPSS Version 17.0 Software. The treatment effects were considered significant if the p-value was at or below 0.05.

RESULTS AND DISCUSSION

Effects of YPS on spleen and thymus indexes in rats: The spleen and thymus indexes were slightly increased in the rats treated with YPS compared with blank control group ($p<0.05$ or $p<0.01$). A significant increase in weight of thymus and spleen was observed at medium-dose (100 mg kg^{-1}) group when compared with blank control group ($p<0.05$ or $p<0.01$). The test results for details are shown in Table 1. Thymus is the organ in which T lymphocytes develop, differentiate and mature while spleen contains T-cells and B-cells. The weight of the immune organs can reflect immune system state in a certain extent. The weight of the immune organs increase is the performance of immunity enhancement (Grossman, 1985). Thymus and spleen is the important immune organs and participate in the humoral immune and cellular immune. Polysaccharide molecules enter into cells through combining polysaccharide receptors at surface of cells and gather in the organs of spleen, thymus, etc. (Abadi *et al*., 1998). The results showed that YPS medium-dose group could improve the weight of immune organ of rats.

Effect of YPS on serum IgA, IgG, AKP and LZM of rats: Effect of YPS on serum IgA and IgG levels were determined by ELISA. As shown in Table 2, the serum IgA was significantly increased in YPS groups at I-III ($p<0.05$, $p<0.01$ and $p<0.05$, respectively) compared with that in blank control Group IV. The similar observation also indicated in the serum IgG ($p<0.05$, $p<0.05$ and $p<0.05$, respectively) compared with the IV group. The levels of IgA and IgG increased slightly in the YPS groups except serum IgA in II group. YPS markedly augmented serum IgA of rats in dose dependent manner. IgA, IgG and IgM are the most abundant immunoglobulin produced in mammals and their role is against pathogenic and non-pathogenic microorganisms (Macpherson *et al*., 2001). The level of serum immunoglobulins improved in a range can maintain health. This result is agreement with that yeast glucan can improve the level of serum immunoglobulins in pregnant sows (Krakowski *et al*., 2002). Effect of YPS on serum AKP and LZM levels were determined by colorimetry. The serum AKP was significantly increased in I and II groups ($p<0.05$ and $p<0.01$) compared with that in IV group and I group was higher than II group ($p<0.01$). The serum LZM was significantly increased in I and II groups ($p<0.01$) compared with that in IV group but the highest value was in II group ($p<0.01$).

YPS could increase the content of serum AKP and LZM of rats in a dose dependent manner. AKP and LZM play an important role in phagotrophy ability and sterilization ability of macrophages. AKP activity can reflect the growth performance of animals, improving AKP activity is help to improve Average Daily Gain (ADG) (Zhou *et al*., 2010) and AKP can enhance the nonspecific immunity function. LZM is an effector of specific immune and mainly secreted by macrophages. The concentration of serum LZM is important index of nonspecific immune. This increase of the content of IgA, IgG, AKP and LZM may be reached the maximum effect when the rats were administered with the middle dose of YPS (100 mg kg^{-1}).

Effect of YPS on the phagocytic index (k) and phagocytic activity (a) of macrophages: To establish the effect of YPS on carbon particle clearance test, blood samples were taken at different time intervals. There was an enhancement in phagocytic index (k) and phagocytic activity (a) after YPS treatment for 10 days (Table 3). At dose of high and medium-YPS (200 and 100 mg kg^{-1}), a significant increase ($p<0.01$) in phagocytic index (k) was observed but there was no significant difference at low-YPS (50 mg kg^{-1}) group. Phagocytic activity (a) also increased significantly at any dose of YPS treatment when compared with blank control group ($p<0.01$, $p<0.01$ and $p<0.05$, respectively). In these results the values of k and a were characterized in a dose dependent manner.

This result is agreement with that of Yang *et al*. (2007) and they found that pollen polysaccharides could increase the ability of lymphocyte proliferation of tumor-bearing mice in a dose dependent manner. The test of

Table 1: Effects of YPS on spleen and thymus indexes in rats (mg g^{-1})

Groups	Quantity of polysaccharide (mg kg^{-1})	Spleen index	Thymus index
I	200	4.12 ± 0.52^{a}	2.95 ± 0.32^{ab}
II	100	4.45 ± 0.43^{aA}	3.06 ± 0.38^{a}
III	50	4.19 ± 0.62^{a}	3.01 ± 0.43^{ab}
IV	0	3.94 ± 0.68^{bB}	2.83 ± 0.30^{b}

Table 2: Effect of YPS on serum IgA, IgG, AKP and LZM of rats

Groups	Quantity of polysaccharide (mg kg^{-1})	IgA (μg mL^{-1})	IgG (μg mL^{-1})	AKP (mg 100 mL^{-1})	LZM (U mL^{-1})
I	200	8.60 ± 0.88^{b}	3.83 ± 0.25^{a}	98.71 ± 22.44^{bB}	265.62 ± 23.88^{B}
II	100	9.36 ± 1.05^{aA}	3.90 ± 0.19^{a}	119.57 ± 11.57^{aA}	296.71 ± 21.63^{A}
III	50	8.64 ± 0.49^{b}	3.68 ± 0.16^{a}	86.64 ± 10.21^{cB}	218.56 ± 34.64^{C}
IV	0	8.25 ± 0.59^{cB}	3.36 ± 0.20^{b}	82.36 ± 15.87^{cB}	219.86 ± 22.12^{C}

In the same column, values with different lowercase superscripts mean significant difference ($p<0.05$), values with different capital letter superscripts mean significant difference ($p<0.01$)

Table 3: Effect of YPS on the phagocytic index k and phagocytic activity a of macrophages

Groups	Quantity of polysaccharide (mg kg^{-1})	k	a
I	200	0.0089±0.0012^{B}	5.03±0.64^{aA}
II	100	0.0106±0.0014^{A}	5.16±0.42^{aA}
III	50	0.0061±0.0005^{C}	4.21±0.71^{b}
IV	0	0.0032±0.0003^{C}	2.95±0.48^{cB}

Table 4: Effects of YPS on production performance indexes of rats

Groups	Quantity of polysaccharide (mg kg^{-1})	ADG (g day^{-1})	ADI (g day^{-1})	G/I
I	200	4.53±0.64^{B}	22.09±2.51	0.20±0.03^{b}
II	100	5.63±0.54^{A}	23.12±2.78	0.24±0.02^{a}
III	50	4.73±0.68^{B}	22.35±2.23	0.21±0.02^{b}
IV	0	4.78±0.57^{B}	22.18±1.97	0.22±0.04^{b}

In the same column, values with different lowercase superscripts mean significant difference ($p<0.05$), values with different capital letter superscripts mean significant difference ($p<0.01$)

carbon clearance could reflect the phagocytosis function of monocyte. This study found that oral administration of YPS was associated with significant improvement in macrophage phagocytic function (Table 3). One of the most important immune responses of the body is carried out by macrophages which is called as phagocytic function. Phagocytosis represents the final and most indispensable step of the immunological defense system (Zhao *et al*., 2005). Activated macrophages plays an essential role in building and consolidating immunological defense systems against malignancies like cancer (Blander and Medzhitov, 2004).

Effects of YPS on production performance: Average Daily Gains (ADG) in the II groups was significantly higher than other Groups (I, III and IV) ($p<0.01$). There was no significant difference in Average Daily Intake (ADI) between each group ($p>0.05$). But the G/I in the II groups was significantly higher than other groups (Table 4). ADG in the polysaccharide groups were 5.63 g day^{-1} (maximum) higher than it in the blank control group. It was hard to obtain accurate data if daily measurement was taken so researchers calculated average daily gains with 30 days considered as one experimental period.

This result is disagreement with that of Hiss and Sauerwein (2003) and they found that Average Daily Gains (ADG) of β-glucan treated pigs were not different from the controls feed intake was tendentiously increased at β-glucan without alteration of feed efficiency. The growth-promoting mechanism of YPS has a bearing on the improvement of different body systems, especially digestive system. Relevant indexes are worthy of the deep research.

CONCLUSION

Any dose of YPS can elevate spleen and thymus indexes, serum IgA, IgG, AKP and LZM level and phagocytic index (k) and phagocytic activity (a) of macrophages of the rats. Meanwhile it can increase Average Daily Gain (ADG) and G/I (G/I = Average daily gain/average daily intake) of rats and 100 mg kg^{-1} is the best. YPS can improve immunologic function and production performance of rats in general which ensures its application as an ideal immune potentiator.

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