Effects of Dietary Lentinan Supplementation on Growth Performance, Antioxidant Capacity, and Intestinal Health in Weaned Rats (Postprint)

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Abstract

This study aimed to investigate the effects of dietary lentinan supplementation on growth performance, antioxidant capacity, and intestinal health in weaned rats. Twenty healthy weaned Wistar rats were selected and divided into 2 groups (n=10 per group) based on similar body weight, and fed either a basal diet or a test diet supplemented with 84 mg/kg lentinan (with an effective lentinan content of 30%). The experimental period lasted 21 days. The results showed that dietary supplementation with 84 mg/kg lentinan significantly increased average daily gain and average daily feed intake ($P<0.05$), and significantly decreased the feed conversion ratio ($P<0.05$) in weaned rats; dietary supplementation with 84 mg/kg lentinan significantly enhanced the total antioxidant capacity in serum and jejunum ($P<0.05$), and decreased malondialdehyde content in serum ($P<0.05$) and jejunum ($P=0.08$) to varying degrees; dietary supplementation with 84 mg/kg lentinan significantly increased jejunal mucosal villus height and the villus height/crypt depth ratio ($P<0.05$); additionally, dietary supplementation with 84 mg/kg lentinan increased the number of lactic acid bacteria in cecal digesta ($P=0.07$), significantly decreased the number of Escherichia coli in cecal digesta ($P<0.05$), and significantly increased the contents of acetic acid, propionic acid, butyric acid, and total volatile fatty acids in cecal digesta ($P<0.05$) in weaned Wistar rats. In conclusion, dietary supplementation with 84 mg/kg lentinan (with an effective lentinan content of 30%) in weaned rats can improve their antioxidant capacity, jejunal mucosal morphology, and cecal microbiota structure, thereby enhancing growth performance.

Full Text

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Abstract: This study was conducted to investigate the effects of dietary lentinan supplementation on growth performance, antioxidant capacity, and intestinal health of weaned rats. Twenty healthy weaned Wistar rats were selected and divided into two groups (n=10) based on similar body weight. The rats were fed either a basal diet or a test diet supplemented with 84 mg/kg lentinan (effective lentinan content of 30%). The experimental period lasted 21 days. The results showed that dietary supplementation with 84 mg/kg lentinan significantly increased average daily gain and average daily feed intake ($P<0.05$), and significantly decreased feed-to-gain ratio ($P<0.05$). Supplementation with 84 mg/kg lentinan significantly enhanced total antioxidant capacity in both serum and jejunum ($P<0.05$), and reduced malondialdehyde content in serum ($P<0.05$) and jejunum ($P=0.08$) to varying degrees. Additionally, 84 mg/kg lentinan supplementation significantly increased jejunal villus height and villus height-to-crypt depth ratio ($P<0.05$). Furthermore, dietary lentinan supplementation increased Lactobacillus counts in cecal digesta ($P=0.07$), significantly decreased Escherichia coli counts ($P<0.05$), and significantly elevated the concentrations of acetate, propionate, butyrate, and total volatile fatty acids in cecal digesta of weaned rats ($P<0.05$). In conclusion, dietary supplementation with 84 mg/kg lentinan (effective content of 30%) can improve antioxidant capacity, jejunal mucosal morphology, and cecal microbiota structure, thereby enhancing growth performance in weaned rats.

Keywords: lentinan; weaned rats; growth performance; antioxidant capacity; intestinal health

Lentinan is a branched polysaccharide extracted from mushrooms, specifically *Lentinus edodes*, consisting of a β-1,3-D-glucan backbone with two β-D-1,3- and β-D-1,6-linked branches, and containing a small portion of internal β-D-1,6 linkages [1-2]. Numerous recent in vivo and in vitro studies have demonstrated that lentinan can regulate various physiological functions, with research primarily focusing on its immunomodulatory and antioxidant effects. These studies indicate that lentinan enhances organism resistance, exerts antiviral, antibacterial, and antiparasitic activities, demonstrates antitumor properties, and thereby maintains and improves health in both animals and humans [3-8].

In addition to immune function and antioxidant capacity, intestinal health represents a crucial factor for maintaining and improving overall health. However, research on lentinan' s effects on intestinal health remains limited. Van Nevel et al. [9] investigated the impact of dietary supplementation with 0.1% lentinan extract (effective lentinan content of 25%) and 5% mushroom powder (effective content unknown) on intestinal health in weaned piglets, but the effects differed substantially between the two forms. Therefore, this experiment was designed to examine the effects of dietary supplementation with 84 mg/kg lentinan (effective content of 30%) on growth performance, antioxidant capacity, jejunal mucosal morphology, and cecal microbiota structure in weaned rats, providing theoretical and experimental support for the application of lentinan in improving health and promoting growth in young animals.

1.1 Experimental Material

Lentinan was purchased from Sichuan Hengrui Tongda Biotechnology Co., Ltd., with an effective lentinan content of 30%.

1.2 Experimental Animals and Design

Twenty healthy 21-day-old weaned Wistar rats with an average body weight of approximately 75.43 g were obtained from Chengdu Dossy Experimental Animals Co., Ltd. The rats were randomly allocated into two groups (n=10) based on similar body weight, with each rat serving as one replicate. The control group received a basal diet, while the lentinan group received the test diet. The experimental period lasted 21 days. The basal diet was formulated according to the AIN-93G purified diet standard for rats and prepared by Chengdu Dossy Experimental Animals Co., Ltd. The composition and nutrient levels are presented in Table 1 . The test diet was prepared by replacing corn starch in the basal diet with 84 mg/kg lentinan at equal weight. The experiment was conducted at the Teaching and Research Base of the Institute of Animal Nutrition, Sichuan Agricultural University. Rats were housed individually under conventional management with natural lighting and ventilation, with free access to feed and water.

1.3.1 Growth Performance

All rats were weighed after fasting on days 1 and 22 of the experiment, and daily feed intake was recorded to calculate average daily feed intake (ADFI), average daily gain (ADG), and feed-to-gain ratio (F/G).

1.3.2 Sample Collection

On day 22, after weighing, blood samples were collected from all rats via enucleation and placed in centrifuge tubes. After standing at low temperature for 30 minutes, serum was prepared by centrifugation at 3,000 rpm for 15 minutes and stored at -20°C. Following blood collection, rats were euthanized by cervical

dislocation. Intestinal segments were rapidly isolated; a 3-cm jejunal segment was fixed in 10% neutral formalin solution, while jejunal tissue samples and cecal digesta were collected and stored at -80°C.

1.3.3 Antioxidant Indices in Serum and Jejunum

Sample preparation of rat jejunal tissue followed the method described by Tang et al. [10]. Malondialdehyde (MDA) content and total antioxidant capacity (T-AOC) in serum and jejunum were measured using assay kits (catalog numbers A003-1 and A015-1, respectively) from Nanjing Jiancheng Bioengineering Institute, with all procedures performed according to the manufacturer' s instructions.

1.3.4 Jejunal Mucosal Morphology

Jejunal mucosal morphology (villus height, crypt depth, and villus height-to-crypt depth ratio) was determined following the method described by Mao et al. [11].

1.3.5 Microbial Populations and Volatile Fatty Acid (VFA) Content in Cecal Digesta

Microbial populations (total bacteria, Lactobacillus, Bifidobacterium, and Escherichia coli) in cecal digesta were quantified following the method of Mao et al. [12]. VFA concentrations (acetate, propionate, butyrate, and total VFA) in cecal digesta were measured according to the method described by Diao et al. [13].

1.4 Data Processing and Analysis

Experimental data were initially processed using Excel 2003. All results were expressed per rat as the statistical unit. Data were analyzed using SAS 8.1 software with t-tests. Differences were considered significant at $P<0.05$, with a trend toward significance at $0.05\leq P<0.10$, and non-significant at P$ $0.10. Data are presented as "mean ± standard error."

2.1 Effects of Lentinan Supplementation on Growth Performance of Weaned Rats

As shown in Table 2 , compared with rats fed the basal diet, those fed the lentinan-supplemented diet exhibited 14.10% higher ADFI ($P<0.05$) and 36.36% higher ADG ($P<0.05$), while F/G decreased by 15.84% ($P<0.05$).

2.2 Effects of Lentinan Supplementation on Antioxidant Indices in Serum and Jejunum of Weaned Rats

Table 3 shows that compared with the control group, lentinan supplementation increased T-AOC by 203.55% in serum ($P<0.05$) and 41.12% in jejunum

(P<0.05), while decreasing MDA content by 67.18% in serum (P<0.05) and 15.22% in jejunum (P=0.08).

2.3 Effects of Lentinan Supplementation on Jejunal Mucosal Morphology of Weaned Rats

As presented in Table 4 , lentinan supplementation significantly increased jejunal villus height and villus height-to-crypt depth ratio (P<0.05), though crypt depth remained unchanged (P=0.31).

2.4 Effects of Lentinan Supplementation on Microbial Populations in Cecal Digesta of Weaned Rats

Table 5 demonstrates that lentinan supplementation tended to increase Lactobacillus counts (P=0.07) and significantly decreased Escherichia coli counts (P<0.05) in cecal digesta, while Bifidobacterium (P=0.41) and total bacterial counts (P=0.11) were not significantly affected.

2.5 Effects of Lentinan Supplementation on VFA Content in Cecal Digesta of Weaned Rats

As shown in Table 6 , lentinan supplementation significantly increased the concentrations of acetate, propionate, butyrate, and total VFA in cecal digesta (P<0.05).

Recent studies have shown that dietary lentinan supplementation can significantly improve growth performance in weaned piglets [4] and broiler chickens [14-15]. The present study demonstrates that 84 mg/kg lentinan supplementation also significantly enhances ADFI and ADG while reducing F/G in weaned rats, thereby promoting growth. Numerous previous studies have revealed that lentinan can regulate immune function and antioxidant capacity in animals and humans, thereby enhancing organism resistance [3-8]. Our findings similarly show that lentinan supplementation improves antioxidant capacity in serum and intestine of weaned rats. Therefore, modulation of immune function and antioxidant capacity may represent important mechanisms through which lentinan enhances animal growth performance.

Beyond immune function and antioxidant capacity, intestinal function constitutes another critical factor affecting animal growth. Intestinal mucosal morphology, including villus height and crypt depth, is closely associated with nutrient digestion and absorption and represents an essential component of the intestinal physical barrier [16-17]. Van Nevel et al. [9] reported that 5.0% mushroom powder supplementation (effective content unknown) increased jejunal villus height and villus height-to-crypt depth ratio in weaned piglets, whereas 0.1% lentinan extract (effective content of 25%) did not produce similar effects. Our results indicate that 84 mg/kg lentinan supplementation improved jejunal villus height and villus height-to-crypt depth ratio in weaned rats. These findings suggest that lentinan supplementation may improve growth performance

in animals, including rats, by enhancing jejunal mucosal morphology, with the form and dosage of lentinan being influential factors for its efficacy.

Van Nevel et al. [9] demonstrated that dietary mushroom powder increased jejunal villus height and villus height-to-crypt depth ratio in weaned piglets, which was associated with reduced apoptotic indices in villus cells. Cellular redox status can influence apoptosis [18]. Our results show that 84 mg/kg lentinan supplementation increased T-AOC and decreased MDA content in both serum and jejunum of weaned rats. Integrating our findings with previous research suggests that lentinan may improve jejunal mucosal morphology by enhancing tissue antioxidant capacity and reducing villus cell apoptosis.

Recent extensive research has highlighted the crucial role of gut microbiota in human and animal health [19-20]. Our study shows that 84 mg/kg lentinan supplementation significantly reduced Escherichia coli counts and tended to increase Lactobacillus counts in cecal digesta of weaned rats. These changes in microbial composition may represent another important factor through which lentinan modulates growth in weaned rats. However, Van Nevel et al. [9] found that 5% mushroom powder (effective content unknown) or 0.1% lentinan extract (effective content of 25%) significantly reduced various bacterial populations (including Escherichia coli, Lactobacillus, and Streptococcus) in jejunal digesta and mucosa of weaned piglets. These discrepant results may stem from differences in lentinan dosage, as excessive supplementation might reduce both harmful and beneficial bacteria, whereas moderate doses may promote beneficial bacteria while inhibiting harmful ones.

As non-direct nutrients produced by intestinal microbiota, volatile fatty acids (VFAs) play important roles in regulating intestinal health by: 1) promoting intestinal epithelial cell proliferation and increasing intestinal DNA, RNA, and protein content, thereby stimulating villus growth [21-22]; 2) reducing intestinal pH and maintaining microecological balance [23]; 3) regulating mucin expression in intestinal epithelial cells and promoting reassembly of intercellular tight junction proteins [24-25]; and 4) alleviating LPS-induced inflammatory responses and reducing pro-inflammatory cytokine expression [26]. Our results demonstrate that 84 mg/kg lentinan supplementation significantly increased acetate, propionate, butyrate, and total VFA concentrations in cecal digesta. Therefore, we hypothesize that lentinan' s effects on jejunal mucosal morphology and cecal microbiota may be partially mediated through increased VFA production in the intestine.

In conclusion, dietary supplementation with 84 mg/kg lentinan (effective content of 30%) in weaned rats can improve antioxidant capacity, jejunal mucosal morphology, and cecal microbiota structure, thereby enhancing growth performance.

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Note: Figure translations are in progress. See original paper for figures.