Effects of Ginseng Polysaccharide on Production Performance, Serum Biochemical Indices, and Intestinal Morphology in Male Mink during the Winter Fur Period (Postprint)

Authors: Sun Weili, Zhang Ting, Yang Yahan, Wang Zhuo, 樊燕燕, Li Bin, Du Dongsheng, Gu Haijun, Li Guangyu

Date: 2017-11-07T00:00:00+00:00

Abstract

This experiment aimed to investigate the effects of dietary ginseng polysaccharide supplementation on production performance, serum biochemical indices, and intestinal morphological structure in male mink during the winter fur period. Forty 120-day-old healthy male short-haired black mink (born in the current year) were selected and randomly divided into 4 groups with 10 replicates per group and 1 mink per replicate. Group I (control group) was fed a basal diet, while groups II, III, and IV were fed experimental diets supplemented with 10, 50, and 100 mg/kg (dry matter basis) ginseng polysaccharide, respectively. The experimental period lasted 77 days, including a 7-day pre-trial period and a 70-day formal trial period. The results showed that dietary supplementation with different levels of ginseng polysaccharide had no significant effects ($P>0.05$) on average daily gain, average daily feed intake, or feed-to-gain ratio in male mink during the winter fur period. Pelt length increased with increasing ginseng polysaccharide supplementation levels, with group IV being significantly higher than group I ($P<0.05$); guard hair length reached its maximum value in group IV, which was significantly higher than that in group I ($P<0.05$); underhair height showed no significant difference between groups III and IV ($P>0.05$), but both were significantly higher than groups I and II ($P<0.05$). Dietary supplementation with different levels of ginseng polysaccharide had no significant effects ($P>0.05$) on serum protein indices (such as total protein, albumin, globulin, and urea contents) and lipid metabolism-related indices (such as total cholesterol and triglyceride contents) in male mink during the winter fur period, although total protein content tended to increase with increasing ginseng polysaccharide supplementation levels. Serum alanine aminotransferase activity in group III was significantly higher than that in groups I

and II (P<0.05), with no significant difference from group IV (P>0.05); serum aspartate aminotransferase activity in group IV was significantly higher than that in groups I and II (P<0.05), with no significant difference from group III (P>0.05); serum alkaline phosphatase activity showed no significant differences among all groups (P>0.05). Serum interleukin-6 and tumor necrosis factor-α contents in groups II, III, and IV were all significantly higher than those in group I (P<0.05), with no significant differences among groups II, III, and IV (P>0.05). Dietary ginseng polysaccharide supplementation extremely significantly increased villus height and villus height/crypt depth ratio in the jejunum of male mink during the winter fur period (P<0.01). In conclusion, dietary supplementation with 50–100 mg/kg ginseng polysaccharide can improve intestinal morphological structure in male mink during the winter fur period, which is beneficial for enhancing nutrient digestion and absorption, while also increasing serum interleukin-6 and tumor necrosis factor-α contents, which is conducive to strengthening immune function.

Full Text

Effects of Ginseng Polysaccharide on Performance, Serum Biochemical Indices, and Intestinal Morphology of Male Minks during the Winter Hair Period

SUN Weili[1], ZHANG Ting[1], YANG Yahan[1], WANG Zhuo[1], FAN Yanyan[1], LI Bin[2], DU Dongsheng[2], GU Haijun[3], LI Guangyu[1]*

[1]Jilin Provincial Key Laboratory for Molecular Biology of Special Economic Animals, Institute of Special Animal and Plant Sciences, Chinese Academy of Agricultural Sciences, Changchun 130112, China
[2]Shenyang Boyang Feed Co., Ltd., Liaozhong 110201, China
[3]Jilin Teyan Biotechnology Co., Ltd., Changchun 130112, China

Abstract

This experiment was conducted to investigate the effects of dietary ginseng polysaccharide (GPS) supplementation on performance, serum biochemical indices, and intestinal morphology of male minks during the winter hair period. Forty healthy underyearling short-haired black male minks aged 120 days were randomly allocated into four groups with ten replicates per group and one mink per replicate. Group I (control) received a basal diet, while groups II, III, and IV received the basal diet supplemented with 10, 50, and 100 mg/kg GPS (dry matter basis), respectively. The trial lasted 77 days, including a 7-day pretrial period and a 70-day experimental period. The results showed that dietary GPS supplementation at different levels had no significant effects on average daily gain, average daily feed intake, or feed-to-gain ratio (P>0.05). Skin length increased with higher GPS supplementation levels, with group IV showing significantly greater values than group I (P<0.05). Guard hair length reached its

maximum in group IV, which was significantly higher than in group I ($P<0.05$). Villus height in groups III and IV did not differ significantly ($P>0.05$) but was significantly higher than in groups I and II ($P<0.05$). Dietary GPS supplementation did not significantly affect serum protein indices (total protein, albumin, globulin, and urea) or lipid metabolism markers (total cholesterol and triglyceride) ($P>0.05$), though total protein tended to increase with higher GPS levels. Serum alanine aminotransferase (ALT) activity in group III was significantly higher than in groups I and II ($P<0.05$) but did not differ from group IV ($P>0.05$). Serum aspartate aminotransferase (AST) activity in group IV was significantly higher than in groups I and II ($P<0.05$) but did not differ from group III ($P>0.05$). Serum alkaline phosphatase (ALP) activity showed no significant differences among groups ($P>0.05$). Serum interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) contents in groups II, III, and IV were significantly higher than in group I ($P<0.05$), with no significant differences among the supplemented groups ($P>0.05$). Dietary GPS supplementation significantly increased jejunum villus height and villus height-to-crypt depth ratio ($P<0.01$). In conclusion, supplementation with 50–100 mg/kg GPS improved intestinal morphology in male minks during the winter hair period, enhanced nutrient digestion and absorption, and increased serum IL-6 and TNF-α contents, thereby strengthening immune function.

Keywords: mink; ginseng polysaccharide; winter hair period; serum biochemical indices; intestinal morphology

Introduction

Ginseng polysaccharide (GPS) is a major bioactive component of ginseng with proven immunomodulatory effects [1]. Compared with other polysaccharides such as those from *Ganoderma*, *Astragalus*, and *Lentinus*, GPS demonstrates more pronounced immunoenhancing properties [2]. Studies in aquatic species including *Litopenaeus vannamei* [3] and *Carassius auratus gibelio* [4] have shown that GPS can improve digestive function by enhancing digestive enzyme activity and boost immunity by modulating hepatic superoxide dismutase (SOD) activity. In fur animal production, Wang et al. [5] reported that 0.9% fructooligosaccharide (FOS) supplementation in growing mink diets could replace 150 mg/kg oxytetracycline. Minks are valuable fur-bearing animals with relatively short intestinal tracts, rapid food transit times, and quick intestinal emptying [6]. Alterations in small intestinal villus structure can facilitate nutrient digestion and absorption. No previous studies have investigated GPS application in fur animal diets. Given the demonstrated benefits of GPS in other animal production systems, this experiment was designed with three GPS dosage levels to evaluate its effects on production performance, serum biochemical indices, and intestinal morphology in male minks during the winter hair period, thereby providing theoretical support for GPS application in mink production and opening new avenues for functional feed additive development in fur animals.

1.1 Experimental Design

Forty healthy underyearling short-haired black male minks aged 120 days were randomly divided into four groups with ten replicates per group and one mink per replicate. Group I (control) received a basal diet, while groups II, III, and IV received the basal diet supplemented with 10, 50, and 100 mg/kg GPS (dry matter basis), respectively. The trial lasted 77 days, including a 7-day pretrial period and a 70-day experimental period from September 23 to December 8, 2015. Minks were fed twice daily at 07:00 and 14:00 with free access to water.

1.2 Basal Diet Formulation and Management

The basal diet was formulated according to NRC (1982) standards for mink nutrition combined with our research group' s established nutrient requirements for the winter hair period. The diet consisted primarily of fresh ingredients including chicken skeleton and fish, with composition and nutrient levels shown in Table 1 . GPS was provided by the Medicinal Plant Resources Innovation Team at the Institute of Special Animal and Plant Sciences, Chinese Academy of Agricultural Sciences. The extraction procedure involved soaking dried ginseng roots, hot water extraction, and two-stage ethanol precipitation, yielding a 10% extraction rate. Using glucose as the standard, the phenol-sulfuric acid method [7] determined a total sugar content of 90%.

1.3.1 Dietary Nutrient Content Analysis

Dietary moisture, crude protein, crude fat, crude ash, calcium, and phosphorus contents were determined according to reference methods [8]. Metabolizable energy (assuming 85% protein digestibility, 90% crude fat digestibility, and 60% carbohydrate digestibility) and carbohydrate content were calculated using the following formulas:

Metabolizable energy (MJ/kg) = [Crude protein content (%) × 0.85 × 18.82 (MJ/kg) + Crude fat content (%) × 0.90 × 39.73 (MJ/kg) + Carbohydrate content (%) × 0.60 × 17.56 (MJ/kg)] / 100

Carbohydrate content = Dry matter content (%) - Crude protein content (%) - Crude fat content (%) - Crude ash content (%)

1.3.2 Growth Performance

Body weight was measured every two weeks after overnight fasting to calculate average daily gain (ADG). Daily feed intake was recorded to determine average daily feed intake (ADFI). Feed-to-gain ratio (F/G) was calculated from ADG and ADFI.

1.3.3 Serum Biochemical Indices

At the end of the feeding trial, eight minks per group were selected for cardiac blood collection (10 mL each) into procoagulant tubes. After serum separation, samples were centrifuged at 3,500 r/min for 10 min at 4°C, aliquoted into 1.5 mL Eppendorf tubes, and stored at -80°C for subsequent analysis. Serum AST, ALT, and ALP activities and albumin (ALB), total protein (TP), globulin (GLO), urea (UREA), total cholesterol (TC), and triglyceride (TG) concentrations were measured using an automatic biochemical analyzer (Selectra E, Netherlands) with assay kits from Zhongsheng Beikong Biotechnology Co., Ltd., following manufacturer protocols. Serum interleukin-2 (IL-2), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) were determined using ELISA kits from Nanjing Jiancheng Bioengineering Institute.

1.3.4 Fur Quality Assessment

At the end of the trial, all ten minks per group were slaughtered for pelt collection. After drying, pelt length and guard hair and villus heights were measured.

1.3.5 Intestinal Morphology Analysis

At the end of the trial, all ten minks per group were slaughtered. A 1 cm × 1 cm segment of jejunum was excised longitudinally, rinsed with physiological saline, and fixed in 10% formalin solution. Fixed specimens underwent dehydration, clearing, paraffin infiltration, embedding, trimming, sectioning, and routine hematoxylin-eosin (HE) staining to produce 4–6 μm thick paraffin sections [9]. Qualified sections were examined under a microscope (Leica DM1000, Germany) at 100× magnification, with multiple non-consecutive fields observed and typical fields photographed. Toupview software was used to measure villus length and crypt depth and calculate villus height-to-crypt depth ratio (V/C). Six non-adjacent sections per animal were analyzed, with five measurements per section.

1.4 Data Processing and Statistical Analysis

Results are expressed as "mean ± standard deviation." Data were organized using Excel 2007 and analyzed using one-way ANOVA in SAS V8 software. Duncan' s multiple range test was used for intergroup comparisons. Differences were considered significant at $P<0.05$ and highly significant at $P<0.01$.

2.1.1 Growth Performance

As shown in Table 2 , dietary GPS supplementation at different levels had no significant effects on ADG, ADFI, or F/G in male minks during the winter hair period ($P>0.05$).

2.1.2 Fur Quality

Table 3 shows that dietary GPS supplementation significantly affected pelt length and guard hair and villus heights ($P<0.05$). Pelt length increased with GPS supplementation level, reaching a maximum in group IV (60.21 cm), which was significantly higher than group I ($P<0.05$). Guard hair length also peaked in group IV (23.08 cm), significantly exceeding group I ($P<0.05$). Villus height did not differ significantly between groups III and IV ($P>0.05$) but was significantly higher than in groups I and II ($P<0.05$).

2.2 Effects on Serum Protein and Lipid Metabolism Indices and Enzyme Activities

Table 4 indicates that dietary GPS supplementation did not significantly affect serum protein metabolism indices (TP, ALB, GLO, and urea) or lipid metabolism markers (TC and TG) ($P>0.05$), though TP tended to increase with higher GPS levels. Regarding enzyme activities, ALT activity in group III was significantly higher than in groups I and II ($P<0.05$) but did not differ from group IV ($P>0.05$). AST activity in group IV was significantly higher than in groups I and II ($P<0.05$) but did not differ from group III ($P>0.05$). ALP activity showed no significant differences among groups ($P>0.05$).

2.3 Effects on Serum Immune Indices

Table 5 demonstrates that dietary GPS supplementation significantly affected serum IL-6 and TNF-α contents ($P<0.05$). Groups II, III, and IV showed significantly higher IL-6 and TNF-α contents than group I ($P<0.05$), with no significant differences among the supplemented groups ($P>0.05$). Serum IL-2 content did not differ significantly among groups ($P>0.05$).

2.4 Effects on Intestinal Morphology

Table 6 reveals that dietary GPS supplementation highly significantly affected jejunum villus height, crypt depth, and villus height-to-crypt depth ratio ($P<0.01$). Villus length and villus height-to-crypt depth ratio in group I were significantly lower than in groups II, III, and IV ($P<0.01$), with no significant differences among supplemented groups ($P>0.05$). Crypt depth in group I was significantly higher than in groups II, III, and IV ($P<0.01$), with no significant differences among supplemented groups ($P>0.05$).

3.1 Effects on Production Performance

During the winter hair period, minks primarily develop fur quality, with muscle growth and fat deposition occurring after the bone growth phase, resulting in slow body weight gain. In the final 10 days before pelting in mid-November, body weight typically plateaus or even decreases slightly in some individuals. In this study, no significant differences were observed in ADG, ADFI, or F/G

among groups, indicating that GPS had no obvious regulatory effect on weight gain in male minks during this period. Fur quality serves as the direct indicator for evaluating winter hair period performance. Measurements of pelt length and guard hair and villus heights at the end of the winter hair period revealed that these parameters increased with GPS supplementation level, with the 100 mg/kg dosage showing significant improvements compared to the control group.

3.2 Effects on Serum Biochemical Indices

Serum biochemical parameters are influenced by dietary nutrition levels, functional additives, and endocrine status, serving as important indicators of tissue and organ function, metabolic changes, and nutrient deposition [9]. Serum albumin and globulin constitute total protein, with serum albumin content reflecting protein synthesis metabolism. Higher serum total protein levels generally promote animal growth and improve feed conversion efficiency [10]. Urea, the end product of mammalian protein catabolism, is synthesized in the liver via the ornithine cycle and primarily excreted by the kidneys, with serum urea content mainly affected by renal function and protein intake and catabolism. In aquaculture, Chen et al. [4] reported that GPS promotes digestive enzyme secretion and beneficial intestinal bacterial proliferation, thereby enhancing digestive function in *Carassius auratus gibelio*. Xi et al. [3] found that GPS complexes did not significantly affect relative weight gain in *Litopenaeus vannamei*. In this study, serum total protein tended to increase with GPS supplementation level, but no significant effects were observed on serum albumin, globulin, total protein, or urea contents, suggesting that GPS may promote protein metabolism in minks without reaching statistical significance.

Serum triglyceride and total cholesterol contents reflect lipid metabolism status. This study found no significant improvement in lipid metabolism with GPS supplementation. Serum enzyme activities closely correlate with metabolic levels and functional status of corresponding tissues and organs, as most serum enzymes originate from various tissues and organs whose functions reflect overall regulatory adaptation and stress responses [11]. ALP, ALT, and AST activities primarily reflect liver function status [12]. Under normal physiological conditions, these enzymes are distributed within tissues with low serum activity; however, tissue damage increases cell membrane permeability, releasing cellular enzymes into the bloodstream and elevating serum enzyme activities [13]. In this study, ALT activity in group III and AST activity in group IV were significantly higher than in groups I and II, though no liver damage was observed during slaughter.

Previous studies have demonstrated that GPS and *Polyporus umbellatus* polysaccharides significantly affect intestinal mucosal lymphocyte function in rats, increasing TNF-α and interferon-γ (IFN-γ) contents in peripheral blood mononuclear cell (PBMC) and platelet-poor lymphocyte (PPL) culture supernatants and modulating intestinal mucosal lymphocyte function [14]. Other research indicates that GPS promotes growth and affects non-specific immune function

in black porgy [15]. Collectively, these findings suggest that GPS enhances immunity primarily by modulating immune-related gene expression, stimulating cytokine secretion, or increasing antimicrobial enzyme activity. In this study, GPS supplementation significantly increased serum IL-6 and TNF-α contents in male minks during the winter hair period, indicating that GPS strengthens immune capacity and disease resistance by altering serum immune factor levels.

3.3 Effects on Intestinal Morphology

The small intestine is the primary site for nutrient digestion and absorption, with intestinal morphology closely related to digestive function. As valuable fur-bearing animals with short intestinal tracts, rapid food transit, and quick intestinal emptying [6], structural changes in small intestinal villi can enhance nutrient digestion and absorption in minks. Increased villus height expands the contact area with nutrients, thereby improving absorption efficiency, making villus morphology directly related to animal growth and development [16]. Villus height, crypt depth, and villus height-to-crypt depth ratio all reflect intestinal functional status [17], with the latter generally considered a comprehensive indicator. A higher villus height-to-crypt depth ratio indicates greater nutrient absorption capacity and faster growth [18]. The GPS used in this study is a biologically active polysaccharide mixture extracted from ginseng [8-9]. In aquaculture, Chen et al. [4] reported that GPS enhances digestive enzyme secretion and beneficial bacterial proliferation, promoting digestive function in *Carassius auratus gibelio*. This study demonstrated that GPS supplementation highly significantly increased villus height and villus height-to-crypt depth ratio in male minks during the winter hair period, with groups II, III, and IV showing significantly higher values than group I but no significant differences among supplemented groups. These results indicate that GPS modulates intestinal morphology by increasing villus height-to-crypt depth ratio, thereby expanding nutrient absorption area and promoting nutrient utilization.

In conclusion, dietary supplementation with 50–100 mg/kg GPS improves intestinal morphology in male minks during the winter hair period, enhances nutrient digestion and absorption, and increases serum IL-6 and TNF-α contents, thereby strengthening immune function.

References

[1] ZHANG B, LIN R C, FENG F. Research overview of ginseng polysaccharide[J]. Chinese Pharmaceutical Affairs, 2004, 18(9): 566–569.

[2] LI S S, JIN Y P, YAO C L, et al. Research progress on structure and activity of ginseng polysaccharide[J]. China Journal of Chinese Materia Medica, 2014, 39(24): 4709–4715.

[3] XI Q Y, LIU X L, FANG S X, et al. Study on immune enhancement effect of ginseng polysaccharide complex on *Litopenaeus vannamei*[J]. Feed Industry, 2009, 30(6): 15–17.

[4] CHEN Y, ZHOU H Q. Effects of three polysaccharides on intestinal and hepatopancreatic protease and amylase activities in *Carassius auratus gibelio*[J]. Journal of Shanghai Fisheries University, 2005, 14(4): 4468-4471.

[5] WANG Z, SUN W L, FAN Y Y, et al. Effects of fructooligosaccharide on growth performance, nutrient digestibility, nitrogen metabolism and serum biochemical indices of growing minks[J]. Chinese Journal of Animal Nutrition, 2015, 27(11): 3613-3619.

[6] LI G Y, YANG F H. New breeding technology for fox, raccoon dog and mink[M]. Beijing: China Agricultural Science and Technology Press, 2006.

[7] SONG L H, WANG H M, XIAO W. Fractionation and preliminary immunological activity of ginseng polysaccharide[J]. Chinese Journal of Experimental Traditional Medical Formulae, 2012, 18(14): 162-166.

[8] ZHANG L Y. Feed analysis and feed quality detection technology[M]. 2nd ed. Beijing: China Agricultural University Press, 2003.

[9] WANG J P, YOO J S, KIM H J, et al. Nutrient digestibility, blood profiles and fecal microbiota are influenced by chitooligosaccharide supplementation of growing pigs[J]. Livestock Science, 2009, 125(2/3): 298-303.

[10] SAGER S M, LANDRY D, MILLAR W J, et al. Depletion of somatostatin-like immunoreactivity in the rat central nervous system by cysteamine administration[J]. Journal of Neuroscience, 1982, 2(2): 225-231.

[11] GAUGHAN J B, MADER T L. Effects of sodium chloride and fat supplementation on finishing steers exposed to hot and cold conditions[J]. Journal of Animal Science, 2009, 87(2): 612-621.

[12] STRANGE R J, SCHRECK C B, GOLDEN J T. Corticoid stress responses to handling and temperature in salmonids[J]. Transactions of the American Fisheries Society, 1977, 106(3): 213-218.

[13] RADOSTITS O M, GAY C C, BLOOD D C, et al. Veterinary medicine: a textbook of the diseases of cattle, sheep, pigs, goats and horses[M]. 9th ed. London: Saunders Ltd., 2000: 1819-1822.

[14] ZHANG W D, LÜ C, LIU Z L, et al. Effects of ginseng polysaccharide and *Polyporus umbellatus* polysaccharide on intestinal mucosal lymphocyte function in rats[J]. Chinese Traditional and Herbal Drugs, 2007, 38(2): 221-224.

[15] LI C Z, HU K, TANG X L, et al. Effects of ginseng polysaccharide on growth performance and antioxidant enzyme mRNA expression in black porgy[J]. Journal of Huazhong Agricultural University, 2015, 34(6): 94-100.

[16] CASPARY W F. Physiology and pathophysiology of intestinal absorption[J]. American Journal of Clinical Nutrition, 1992, 55(1): 2995-3085.

[17] WINZELL M S, SVENSSON H, ARNER P, et al. The expression of hormone-sensitive lipase in clonal β-cells and rat islets is induced by long-term

exposure to high glucose[J]. Diabetes, 2001, 50(10): 2225-2230.

[18] WU Y B, RAVINDRAN V, THOMAS D G, et al. Influence of method of whole wheat inclusion and xylanase supplementation on the performance, apparent metabolisable energy, digestive tract measurements and gut morphology of broilers[J]. British Poultry Science, 2004, 45(3): 385-394.

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