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Research Progress on Physicochemical Properties of Stachyose and Its Application in Animal Production: Postprint

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Abstract

Stachyose is a naturally occurring tetrasaccharide and the major functional component of soybean oligosaccharides, belonging to the category of non-digestible oligosaccharides. This review summarizes the physicochemical properties of stachyose and the advances in its application research in the human food sector and animal production sectors including poultry, swine, and aquaculture, with respect to improving animal gut microbiota composition, enhancing organism immunity, and improving animal production performance.

Full Text

Physicochemical Properties of Stachyose and Research Progress on Its Application in Animal Production

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Abstract: Stachyose is a naturally occurring tetrasaccharide and the main functional component of soybean oligosaccharides, belonging to the category of non-digestible oligosaccharides. This review summarizes the physicochemical properties of stachyose and the research progress in its applications in human food products and animal production, including poultry, swine, and aquaculture, with particular focus on improving intestinal microflora composition, enhancing immunity, and improving animal performance.

Keywords: stachyose; physicochemical property; intestinal microflora; animal production

Stachyose belongs to α -galactooligosaccharides and is primarily found in various plants from the Lamiaceae, Leguminosae, and Scrophulariaceae families, partic-

ularly in the Lamiaceae genus *Stachys* (commonly known as Chinese artichoke). Current preparation methods for stachyose mainly include physical extraction and enzymatic methods. Using materials such as *Lycopus lucidus*, *Eupatorium japonicum*, and Chinese artichoke, physical extraction yields stachyose contents generally ranging from 20% to 70% [1]. Using Chinese artichoke as raw material, biological purification and industrial chromatographic separation technology can produce stachyose with purity exceeding 90% [2]. Soybean oligosaccharides produced from soybeans and their processing by-products typically contain 18%–71% stachyose. According to GB/T 22491-2008, soybean oligosaccharides with 75% purity generally contain 18% stachyose, 6% raffinose, and 24% sucrose. Zhang et al. [3] extracted stachyose with 90% purity through yeast fermentation of soybean protein concentrate whey. Stachyose is also the main functional component of soybean oligosaccharides [4]. In recent years, research on stachyose in China has gradually increased. Beyond traditional applications in food and medicine, its use in animal production has attracted widespread attention.

1 Molecular Structure and Physicochemical Properties of Stachyose

Stachyose consists of one molecule of α -glucose, one molecule of β -fructose, and two molecules of α -galactose, connected in the sequence galactose($1\rightarrow6$)-galactose($1\rightarrow6$)-glucose($1\rightarrow2$)-fructose, thus it is also called a tetrasaccharide [5]. Its molecular structure is shown in [Figure 1: see original paper]. X-ray single crystal diffraction analysis indicates that stachyose belongs to the monoclinic crystal system and forms a three-dimensional layered structure through hydrogen bonding [5–6], as shown in [Figure 2: see original paper].

Pure stachyose is a white powder with a sweetness of 22% that of sucrose, offering a refreshing taste without off-flavors. Stachyose crystallizes as fine crystals bound with four water molecules, is highly soluble in water (130 g at 20 °C), insoluble in organic solvents such as ether and ethanol, and has a melting point of 101 °C. It can lose its crystalline water when heated in vacuum. Anhydrous stachyose has a melting point of 167–170 °C, with moisture retention and hygroscopicity lower than sucrose, and it lacks reducing properties. Guo et al. [7] demonstrated through a series of toxicological tests that stachyose is safe and non-toxic.

2 Applications of Stachyose in the Food Field

Since humans and monogastric animals lack digestive enzymes for α -1,6 glycosidic bonds, stachyose can directly reach the posterior digestive tract where it is fermented and utilized by intestinal bifidobacteria and a few lactobacilli. Therefore, non-digestible oligosaccharides such as stachyose are also called dietary fiber or prebiotics [8]. Developed countries including the United States, Japan, and Europe have extensively developed and utilized stachyose. With

maturing production technology and expanding applications, its development prospects have gradually attracted attention in China. Currently, China has 153 patents related to stachyose, including 29 on production technology, 2 on detection technology, and the remainder on applications [1].

2.1 Regulation of Intestinal Microflora Balance

Hayakawa et al. [9] reported that soybean oligosaccharides containing 23% stachyose and purified products containing 71% stachyose could be effectively utilized by human intestinal bifidobacteria, significantly increasing fecal bifidobacteria counts. Luo et al. [10] prepared culture media using soybean oligosaccharides, stachyose, raffinose, and sucrose as substrates, then inoculated them with *Bifidobacterium adolescentis* and *Escherichia coli* from human feces, finding that stachyose was the primary factor promoting bifidobacteria growth. Shu et al. [11] demonstrated that adding 0.8% or 1.0% stachyose to Lactobacillus culture medium (MRS) significantly accelerated the growth of *Bifidobacterium bifidum* BB01. Bifidobacteria not only preferentially utilize non-digestible oligosaccharides in the intestine but also preferentially consume stachyose when multiple non-digestible oligosaccharides coexist, enabling stachyose to directly and selectively proliferate bifidobacteria [12].

2.2 Enhancement of Immune Function

The α -1,6-galactose structure in stachyose molecules participates in biological processes such as immune responses, pathogen adsorption, and cell adhesion through molecular mechanisms involving ligand-receptor interactions, endowing stachyose with functions including pathogen infection prevention, toxin neutralization, and immune system modulation [13]. Oral administration of stachyose at 0.2 mL per 10 g body weight to mice once daily for 30 days yielded positive results for both humoral immune function and mononuclear macrophage function [14]. Additionally, under the action of bifidobacteria, stachyose can be decomposed to produce multiple immune function factors that enhance human immunity.

2.3 Alleviation of Constipation and Diarrhea Prevention

Stachyose is a small-molecule water-soluble dietary fiber that, upon reaching the large intestine, is preferentially utilized by bifidobacteria to produce large quantities of short-chain fatty acids (SCFAs) such as acetic acid, propionic acid, and n-butyric acid [15]. Reportedly, 103 constipation patients showed improved defecation status and enhanced intestinal function after daily consumption of 5 g stachyose powder [16]. Moreover, the numerous hydroxyl groups in stachyose molecules enable excellent water absorption in the intestine, helping alleviate diarrhea [17]. Stachyose also possesses hepatoprotective, anticancer, atopic dermatitis-inhibiting, anti-arthritic, caries-preventing, colitis-preventing, lead-excreting, blood pressure- and lipid-lowering, antioxidant, and anti-aging functions [18].

3 Applications in Poultry Production

3.1 Effects of Stachyose on Poultry Intestinal Microflora

Pacifici et al. [19] reported that intra-amniotic injection of 5% or 10% stachyose into chicken embryos at day 17 of incubation, with continued incubation to day 21 (hatching), demonstrated beneficial effects of stachyose or raffinose on intestinal microflora, iron bioavailability, and intestinal mucosal histomorphology and function. Stachyose can be fermented and utilized by bifidobacteria and a few lactobacilli in the cecum of broiler chickens, with a fermentation rate second only to raffinose among soybean oligosaccharides [20]. Yi [21] found that dietary supplementation with 1.0% stachyose preparation significantly increased cecal bifidobacteria and lactobacilli counts while significantly reducing cecal *E. coli* and *Salmonella* counts in broiler chickens. Lan et al. [22] used 14-day-old broiler cecal bacteria as inoculum for in vitro fermentation of soybean oligosaccharides, showing that stachyose produced the greatest gas volume and rate compared with soybean oligosaccharides and raffinose, significantly inhibiting *E. coli* and *Clostridium perfringens* growth. Yang et al. [23] reported that 1.0% stachyose supplementation significantly increased microbial richness in broiler cecal content cultures in vitro and promoted the proliferation of *Blautia*, *Parabacteroides*, and *Ligilactobacillus*.

Through bifidobacterial fermentation, stachyose produces large quantities of SCFAs in the intestine, significantly decreasing intestinal pH and thereby inhibiting harmful bacteria proliferation and regulating microecological balance. However, Jiang et al. [24] reported that stachyose had no positive effects except for significantly influencing cecal butyrate concentration during initial stages. Conversely, Yi [21] found that dietary stachyose preparation significantly increased cecal acetic acid and SCFA concentrations and the molar percentage of butyrate in total SCFAs in broiler chickens, with ileal and cecal pH showing a decreasing trend as stachyose supplementation increased. Additionally, 0.5% stachyose supplementation effectively reduced intestinal volatile basic nitrogen content, thereby inhibiting intestinal putrefactive bacteria proliferation in early-stage broiler chickens.

3.2 Effects of Stachyose on Poultry Digestive Organs and Intestinal Mucosal Morphology

Intestinal polyamines, epidermal growth factor, SCFAs, and pH all influence poultry digestive tract morphology, with most of these factors being regulated by dietary nutrients, among which non-digestible oligosaccharides represent important modulators [25]. Yi et al. [26] demonstrated that stachyose supplementation increased the absolute weight of broiler digestive organs to varying degrees, with the most pronounced effects in the cecum and colorectum. Supplementation with 0.5% stachyose preparation significantly increased villus height in the duodenum, jejunum, and ileum, significantly reduced crypt depth in the jejunum and ileum, and increased the villus height/crypt depth ratio in the

jejunum and ileum.

3.3 Effects of Stachyose on Poultry Performance and Nutrient Digestibility

As a non-digestible oligosaccharide, stachyose's unique functional and non-digestible properties significantly influence animal digestive physiology and nutrient metabolism, thereby affecting nutrient digestion and absorption and ultimately animal performance. Jiang et al. [24] found that diets supplemented with 1.2% stachyose showed no significant difference in nutrient digestibility compared with normal soybean meal diets in broiler chickens, though nutrient digestibility decreased slightly with increasing stachyose supplementation. Yi [21] reported that 0.5% stachyose preparation improved apparent utilization of dietary nutrients to varying degrees, whereas 2.0% stachyose supplementation significantly reduced apparent nutrient utilization, with calcium, phosphorus, neutral detergent fiber, and acid detergent fiber being most affected by dietary stachyose level, primarily because high-dose stachyose accelerated feed passage rate through the digestive tract.

Dietary stachyose supplementation in broiler chickens reduced average daily gain and feed utilization efficiency, with chicken growth showing linear decreasing and quadratic increasing trends as stachyose supplementation increased [24]. Yi et al. [27] demonstrated a dose-response relationship between stachyose and broiler performance, with low doses (0.5%) showing slight promotional effects and high doses (2.0%) showing inhibitory effects, and the dose-response being more pronounced during early growth stages than later stages.

3.4 Effects of Stachyose on Poultry Immune Function

Wang et al. [28] reported that certain oligosaccharides including stachyose promoted immune organ development (thymus, bursa of Fabricius) in layer chicks and reduced fecal odor compounds such as ammonia (NH_3) and hydrogen sulfide (H_2S). Dietary supplementation with 0.5% stachyose preparation tended to increase spleen index in 18-day-old broiler chickens and significantly increased serum immunoglobulin A (IgA) content, but significantly reduced spleen index in 36-day-old broilers [29]. Therefore, stachyose can serve as an immune enhancer to improve animal immune function, likely primarily through modulation of intestinal microecology [30].

4 Applications in Swine Production

4.1 Effects of Stachyose on Swine Intestinal Microflora

The mechanism of stachyose action in pig intestines is similar to that in broiler chickens, with comparable effects. Krause et al. [31] first reported that stachyose could be completely fermented by hindgut microorganisms of weaned piglets under in vitro conditions, with this fermentation process being accelerated by

high dietary lactose levels. Zhang et al. [32] found that 1.0% dietary stachyose significantly increased lactobacilli counts in the ileum and bifidobacteria counts in the cecum and colon, significantly reduced bacterial counts in the colon, and significantly increased volatile fatty acid content in the ileum, cecum, and colon. However, high-dose stachyose decreased lactobacilli and bifidobacteria counts in the jejunum, ileum, cecum, and colon [33].

4.2 Effects of Stachyose on Swine Performance and Nutrient Digestibility

Raffinose and stachyose in soybean meal are considered anti-nutritional factors for monogastric animals, primarily because their fermentation in the hindgut does not positively contribute to metabolizable energy provision [34]. Smiricky et al. [35] also reported that stachyose and raffinose reduced nitrogen and amino acid digestibility in pigs, with dry matter digestibility showing significant linear decreases as supplementation increased. Research found that adding α -galactosidase to stachyose-containing diets could eliminate these negative effects [36], as this enzyme hydrolyzes 80% of stachyose in the small intestine of piglets, significantly improving dietary α -galactose digestibility. Although stachyose negatively affects nutrient digestibility in pigs, compared with normal soybean meal diets, 1.0% stachyose supplementation in soybean meal-free diets had no significant effect on piglet performance but significantly reduced diarrhea incidence in weaned piglets; however, when supplementation reached 2.0%, piglet performance during the first two weeks post-weaning significantly declined [32]. This indicates a clear dose-effect relationship for stachyose's impact on piglet performance and demonstrates that its anti-nutritional effects primarily manifest in nutrient digestibility.

5 Applications of Stachyose in Aquaculture and Other Animal Production

Cai [37] reported that feed intake in Japanese flounder (*Paralichthys olivaceus*) increased significantly with increasing dietary stachyose content, while apparent digestibility of dietary dry matter and protein tended to decrease. Dietary supplementation with stachyose, raffinose, or their combination in Atlantic salmon (*Salmo salar*) resulted in body weight and growth rates intermediate between fish meal-based diets and soybean meal-substituted diets, with no significant effects on protein and lipid digestibility [38]. Compared with fish meal diets, 300 g/kg soybean meal supplementation significantly reduced growth performance in juvenile allogynogenetic silver crucian carp (*Carassius auratus gibelio*), but equivalent supplementation of stachyose, raffinose, or stachyose+raffinose did not significantly alter growth status or intestinal morphology in juvenile fish during the 8-week trial [39]. Stachyose had no significant effects on body composition or intestinal microflora counts in allogynogenetic silver crucian carp [40], though it could improve non-specific immune function to some extent [41]. Functional feeds containing stachyose (patented products) have been developed

for preventing red skin disease in grass carp [42]. Hu et al. [43] found that 1.25% dietary stachyose significantly improved growth performance, feed utilization, and digestive enzyme activity in juvenile turbot (*Scophthalmus maximus*), while 1.25% and 5.00% stachyose significantly increased the abundance of intestinal cellulolytic bacteria related to digestion and enhanced intestinal mucosal barrier function. However, stachyose supplementation (2.5%-5.0%) also increased feed intake and reduced feed efficiency in turbot, and at 5.0% supplementation, while increasing beneficial bacteria, it also increased some potentially pathogenic bacteria [44]. Thus, the dose-effect relationships of stachyose differ across various functions (growth, digestion, intestinal barrier) even within the same species.

Beyond chickens, pigs, and fish, stachyose application in silkworms, macaques, and mice has also shown positive effects. Chen et al. [45] reported that 0.50% stachyose supplementation in silkworm diets significantly improved unified life rate of larvae and pupae, whole cocoon weight, cocoon shell weight, and cocoon shell ratio. Li [46] found that stachyose supplementation in macaque diets increased intestinal lactobacilli counts and reduced *E. coli* counts; after 6 weeks of feeding, macaques showed normal defecation with yellow, loose, strip-shaped feces, indicating improved intestinal function, while immunity and disease resistance were also significantly enhanced. Li et al. [47] discovered that soybean oligosaccharide preparations containing 55.3% stachyose, 25.8% raffinose, and 9.7% verbascose promoted proliferation of beneficial bacteria and inhibited pathogen proliferation in mouse intestines, significantly promoting intestinal peristalsis and excretion. Wei et al. [48] demonstrated that stachyose-*Lactobacillus plantarum* synbiotics significantly improved specific cellular immunity, humoral immunity, and non-specific immune function in immunocompromised mice, increasing serum levels of some immune factors and enhancing immune function. Feeding stachyose to Type 2 diabetic rat models for 4 weeks altered intestinal microflora balance by changing microflora mRNA expression [49]. Dou et al. [50] found that 50 and 100 g/mL stachyose significantly enhanced viability and phagocytic capacity against *Staphylococcus aureus* in rabbit neutrophils cultured in vitro.

Current research on stachyose in animal production primarily focuses on its effects on intestinal microflora, digestive capacity, and immune function in poultry, swine, and fish: (1) Similar to its effects on human intestinal environment and microflora, stachyose positively regulates intestinal microflora balance in chickens, pigs, fish, macaques, and rodents; (2) Stachyose can increase digestive organ weight, improve small intestinal villus morphology, and enhance intestinal mucosal barrier function to varying degrees; (3) Stachyose possesses functions in preventing pathogen infection, neutralizing toxins, and modulating the immune system. The main research results regarding stachyose's effects on intestinal microflora, digestive capacity, and immune function in poultry, swine, and aquatic animals are summarized in .

6 Summary

Derived from natural plants, stachyose is highly safe and widely applicable. Rational application of stachyose can significantly improve gastrointestinal microflora balance and enhance animal immunity, though its effects on animal performance are limited and dose-dependent. Many issues remain in animal production applications, including cost concerns, variations in stachyose sources and supplementation levels, effects of basal dietary stachyose content, and the need for further research on potential physiological functions such as effects on animal product quality and fecal odor compound content, especially regarding molecular mechanisms. Although research on stachyose started relatively late in China, its development and application are gaining increasing attention. With decreasing preparation costs and deepening research, the application scope of stachyose will continue to expand, promising broader market prospects.

References

- [1] ZHANG Jinze. Research overview and application status of water-soluble dietary fiber stachyose[J]. Food Safety Guide, 2014(13): 62-63.
- [2] ZHANG Jinze, ZHOU Zhiqiao, ZENG Ming, et al. Method for preparing high-purity stachyose using industrial chromatographic separation technology: China, CN: 101633676[P]. 2010-01-27.
- [3] ZHANG Shanshan, WANG Liping, WANG Xichang. Fermentation conditions for stachyose preparation from soybean protein concentrate whey[J]. Soybean Science, 2008, 27(1): 137-140.
- [4] SHI Yun. Study on purification technology and mechanism of oligosaccharides in soybean molasses[D]. PhD Thesis. Wuxi: Jiangnan University, 2015.
- [5] WU X Y, CHAO Z M, WANG C, et al. Extraction absolute crystal structure stachyose[J]. Chinese Journal of Structural Chemistry, 2014, 33(1): 65-70.
- [6] MA Yucui, WANG Chun, WANG Wei, et al. Development of stachyose reference material[J]. Chinese Journal of Experimental Traditional Medical Formulae, 2017, 23(14): 68-73.
- [7] GUO Meili, ZHOU Yanping, FENG Xuan, et al. Toxicological safety test of stachyose[J]. Journal of Toxicology, 2012, 26(3): 236-238.
- [8] PATEL S, GOYAL A. Functional oligosaccharides: production, properties and applications[J]. World Journal of Microbiology and Biotechnology, 2011, 27(5): 1119-1128.
- [9] HAYAKAWA K, MIZUTANI J, WADA K, et al. Effects of soybean oligosaccharides on human faecal flora[J]. Microbial Ecology in Health and Disease, 1990, 3(6): 293-303.
- [10] LUO Yu, MAO Lina, CAI Fangqin. Growth-promoting effects of soybean oligosaccharides on intestinal bifidobacteria and enterobacteria[J]. Modern Biomedical Progress, 2007, 7(3): 399-400.
- [11] SHU Guowei, JI Liyuan, CHEN He, et al. Effects of stachyose, xylo-oligosaccharides and galactose on bifidobacteria growth[J]. Food Science and Technology, 2011, 36(6): 6-8, 17.
- [12] MILANI C, LUGLI G A, DURANTI S, et al. Bifidobacteria exhibit social behavior through carbohydrate resource sharing in the gut[J]. Scientific Reports, 2015, 5: 15782.
- [13] HASHIMOTO H, YAMASHITA A, FUJITA K, et al. Enzymatic synthesis α -linked galactooligosaccharide (α -GOS) functions[J]. Journal Applied Glycoscience, 2004, 51(2): 169-176.
- [14] LIU

Xiuying, HU Yixiu, HU Yuming, et al. Experimental study on immune enhancement by stachyose and jujube seed extract oral liquid[J]. *China Tropical Medicine*, 2009(3): 571-572, 576. [15] TUOHY K M, ROUZAUD G C, BRÜCK W M, et al. Modulation of the human gut microflora towards improved health using prebiotics-assessment efficacy[J]. *Current Pharmaceutical Design*, 2005(11): 75-90. [16] LI T, LU X S, YANG X B. Evaluation of clinical safety and beneficial effects of stachyose-enriched α -galacto-oligosaccharides on gut microbiota and bowel function in humans[J]. *Food & Function*, 2017, 8(1): 262-269. [17] DUAN Sufang. Functions and applications of stachyose[J]. *Beverage Industry*, 2016, 19(5): 74-78. [18] HUANG Weizhi, ZHONG Xianfeng, PENG Jiawei, et al. Brief introduction to stachyose production, functions and applications[J]. *Food Industry Science and Technology*, 2018(1): 327-332. [19] PACIFICI S, SONG J, ZHANG C, et al. Intra amniotic administration of raffinose and stachyose affects intestinal brush border functionality alters microflora populations[J]. *Nutrients*, 2017, 9(3): 304-313. [20] GRMANOVÁ M, RADA V, SIROTEK K, et al. Naturally occurring prebiotic oligosaccharides in poultry feed mixtures[J]. *Folia Microbiologica*, 2010, 55(4): 326-328. [21] YI Zhonghua. Effects of stachyose on growth performance, intestinal physiology and immune function in broilers[D]. PhD Thesis. Beijing: China Agricultural University, 2006. [22] LAN Y, WILLIAMS B A, VERSTEGEN M W A, et al. Soy oligosaccharides in vitro fermentation characteristics and its effect on caecal microorganisms of young broiler chickens[J]. *Animal Feed Science and Technology*, 2007, 133(3/4): 286-297. [23] YANG Guiqin, YANG Hang, LIU Jizhe, et al. Effects of soybean oligosaccharides and their functional components on skatole production and microflora composition in broiler cecal content in vitro[J]. *Chinese Journal of Animal Nutrition*, 2017, 29(11): 4058-4068. [24] JIANG H Q, GONG L M, MA Y X, et al. Effect of stachyose supplementation on growth performance, nutrient digestibility and caecal fermentation characteristics in broilers[J]. *British Poultry Science*, 2006, 47(4): 516-522. [25] ZHANG Pei, YANG Guiqin, LIU Haiying. Research progress on effects and mechanisms of soybean oligosaccharides on poultry intestinal microecology[J]. *Feed Industry*, 2016, 37(5): 60-64. [26] YI Zhonghua, MA Qiugang, WANG Xiaoxia, et al. Effects of stachyose on digestive organ development and intestinal mucosal morphology in broiler chickens[J]. *Journal of Jiangxi Agricultural University*, 2010, 32(3): 566-570, 576. [27] YI Zhonghua, JI Cheng, MA Qiugang, et al. Effects of stachyose supplementation in low-oligosaccharide diets on broiler performance[J]. *China Feed*, 2008(16): 12-13. [28] WANG Li, SU Ning, SU Jun, et al. Effects of oligosaccharides on laying performance and eggshell quality in laying hens[J]. *China Poultry*, 2002, 24(3): 11-13. [29] YI Zhonghua, JI Cheng, WANG Xiaoxia, et al. Effects of stachyose on immune organ indices and serum immunoglobulins in broiler chickens[J]. *Poultry Husbandry and Disease Control*, 2009(2): 4-9. [30] GUO Yuming, YANG Xiaojun. Nutrition-immune interaction[J]. *Chinese Journal of Animal Science*, 2005, 41(5): 3-5, 9. [31] KRAUSE D O, EASTER R A, MACKIE R I. Fermentation of stachyose and raffinose by hind-gut bacteria of the weanling pig[J]. *Letters in Applied Microbiology*, 2010, 18(6): 349-352. [32] ZHANG L Y, LI D F, QIAO S Y, et al. Effects of stachyose on performance,

diarrhoea incidence and intestinal bacteria in weanling pigs[J]. Archives of Animal Nutrition, 2003, 57(1): 1-10. [33] PAN Baohai, SUN Dongyan. Effects of α -galactosidase and stachyose on growth performance of weaned piglets[J]. Feed Industry, 2011(Suppl. 1): 69-72. [34] HAGELY K B, PALMQUIST D, BILYEU K D. Classification of distinct seed carbohydrate profiles in soybean[J]. Journal of Agricultural and Food Chemistry, 2013, 61(5): 1105-1111. [35] SMIRICKY M R, GRIESHOOP C M, ALBIN D M, et al. The influence of soy oligosaccharides on apparent and true ileal amino acid digestibilities and fecal consistency in growing pigs[J]. Journal of Animal Science, 2002, 80(9): 2433-2441. [36] PAN B H, LI D F, PIAO X S, et al. Effect of dietary supplementation with α -galactosidase preparation and stachyose on growth performance, nutrient digestibility and intestinal bacterial populations of piglets[J]. Archiv Für Tierernaehrung, 2002, 56(5): 327-337. [37] CAI Yinghua. Effects of several soybean anti-nutritional factors on growth and digestive physiology of Japanese flounder (*Paralichthys olivaceus*)[D]. Master's Thesis. Qingdao: Ocean University of China, 2006. [38] SØRENSEN M, PENN M, EL-MOWAFI A, et al. Effect of stachyose, raffinose and soya-saponins supplementation on nutrient digestibility, digestive enzymes, gut morphology and growth performance in Atlantic salmon (*Salmo salar* L.)[J]. Aquaculture, 2011, 314(1/2/3/4): 145-152. [39] KROGDAHL A. The effect of soybean meal (SBM) and its four chemical factors on growth performance and intestinal morphology of juvenile allogynogenetic silver crucian carp (*Carassius auratus gibelio* \times *Cyprinus carpio*) [C]//Program & Abstracts of the International Symposium on Fish Nutrition & Feeding. 2010. [40] CAI C F, WANG W J, YE Y T, et al. Effect of soybean meal, raffinose and stachyose on the growth, body composition, intestinal morphology and intestinal microflora of juvenile allogynogenetic silver crucian (*Carassius auratus gibelio* \times *Cyprinus carpio*) [J]. Aquaculture Research, 2012, 43(1): 128-138. [41] WANG Wenjuan, YE Yuantu, CAI Chunfang, et al. Effects of soybean meal and its anti-nutritional factors on serum biochemical and non-specific immune indices of allogynogenetic silver crucian carp[J]. China Feed, 2010(18): 30-33, 41. [42] JI Guiju, HUANG He, LI Xin. Functional feed for preventing grass carp red skin disease and preparation method: China, CN: 104187171B[P]. 2017-01-04. [43] HU H B, ZHANG Y J, MAI K S, et al. Effects of dietary stachyose on growth performance, digestive enzyme activities and intestinal morphology of juvenile Turbot (*Scophthalmus maximus* L.)[J]. Journal of Ocean University of China, 2015, 14(5): 905-912. [44] YANG P, HU H B, LIU Y, et al. Dietary stachyose altered the intestinal microbiota profile and improved the intestinal mucosal barrier function of juvenile turbot, *Scophthalmus maximus* L. [J]. Aquaculture, 2018, 486: 98-106. [45] CHEN Chuanjie, ZHANG Yaping, GU Yinyu, et al. Effects of stachyose supplementation on unified life rate of larvae and pupae and cocoon quality in silkworm pure breeds[J]. China Sericulture, 2007, 28(2): 30-32. [46] LI Haifang. Determination of intestinal flora in captive non-human primates and effects of stachyose on intestinal microflora[D]. Master's Thesis. Fujian: Fujian Agriculture and Forestry University, 2015. [47] LI T, LU X S, YANG X B. Stachyose-enriched α -galacto-oligosaccharides regulate gut microbiota and relieve constipation mice[J]. Journal Agricultural Chemistry,

2013, 61(48): 11825-11831. [48] WEI Yan, ZENG Xiaoqun, PAN Daodong, et al. Effects of stachyose-*Lactobacillus plantarum* synbiotics on immune function in mice[J]. Journal of Chinese Institute of Food Science and Technology, 2014, 14(1): 14-19. [49] LIU G, BEI J, LIANG L, et al. Stachyose improves inflammation through modulating gut microbiota of high-fat diet/streptozotocin-induced Type 2 diabetes in rats[J]. Molecular Nutrition & Food Research, 2018, 62(6): e1700954. [50] DOU Jiangli, TAN Chengyu, BAI Xuefang, et al. Effects of raffinose and stachyose on rabbit peripheral blood neutrophil function[J]. Fine and Specialty Chemicals, 2008, 16(3/4): 26-27.

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