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Effects of dietary yam polysaccharide on growth performance and intestinal microflora in growing Huoyan geese

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ABSTRACT. The present study was carried out to evaluate the effects of dietary yam polysaccharide (YPS) supplementation on growth performance, intestinal morphology, digestive enzyme activities and intestinal microflora of growing geese. A total of 400 Huoyan geese (28 days old, sex-balanced) were allotted to four groups (control, YPS_{100}, YPS_{500} and YPS_{1000}, according to initial body weight), and their diets included 0, 100, 500 and 1000 mg of YPS per kg of basal diet. Each group consisted of 5 replicates, 20 geese each, and the feeding experiment lasted from 28 to 56 days of age. The results showed that compared to the control group, the YPS_{500} group had higher final body weight, average daily gain, villus height, villus height to crypt depth ratio, amylase and trypsin activity, while lower feed to gain ratio and lipase activity in the small intestine ($P < 0.05$). In addition, all experimental groups had a higher number of *Lactobacillus* and *Bifidobacterium*, and a lower number of *Escherichia coli* in the caecal content of growing Huoyan geese ($P < 0.05$). The results indicated that dietary supplementation with 500 mg/kg YPS improved growth performance by regulating digestive enzyme activities, small intestine morphology and intestinal microflora in growing Huoyan geese.

Introduction

Polysaccharides are characterized by good biocompatibility and hypoglycaemic, immunoregulatory, anticancer, cardiomyocyte-protective and antibacterial properties (Yang et al., 2018; Wang et al., 2019; Huang et al., 2020). Previous study showed that supplementation with dietary *Camellia oleifera* cake polysaccharides (CCP) improved the antioxidant capacity, meat quality and the structure of intestinal flora of yellow chickens (Wang et al., 2020a). Dietary polysaccharide from *Astragalus membranaceus* improved growth performance and innate immunity of juvenile crucian carp (*Carassius auratus*) (Wu, 2020). Dietary extract from *Acanthopanax acanthopanax* polysaccharide significantly enhanced growth performance, digestive enzyme activities and the antioxidant capacity of broilers (Longet al., 2021). These results indicated that polysaccharides exerted positive effects on immune function, growth performance and intestinal microflora of animals.

Chinese yam belongs to a type of traditional Chinese medicine plant that is commonly used in the treatment of diarrhoea, asthma and diabetes (Huang et al., 2020). Yam polysaccharide (YPS) is one of the most important bioactive substances in Chinese yam, and it shows hypoglycaemic (Li et al., 2017), anti-aging (Wang et al., 2020c), immunomodulatory (Zhao et al., 2005), antioxidant (Chang et al., 2020) and antitumour activities (Sun et al., 2014).

Previous study has shown that Chinese Huaishan-yam polysaccharides have the ability to inhibit α-glucosidase and relative proliferation rate of B16 murine melanoma cells (Zhu et al., 2018). Chinese yam polysaccharides reduced serum levels of low-density lipoprotein, total cholesterol, interleukin-1β, interleukin-10 and leptin, while improving insulin resistance in C57BL/6 mice (Cheng et al., 2019). In addition, YPS also inhibited gastric inflammation by reducing serum levels of tumour necrosis factor-α, interleukin-1β and interleukin-6 in mice (Guo et al., 2020). These results indicated that YPS can be used as a suitable candidate for animal feed additive.

Huoyan goose is characterized by early rapid growth and good meat quality, and is one of the most famous local goose species (Miao et al., 2020c). Therefore, it is important to look for suitable feed additives to promote the health and growth performance of growing Huoyan geese. Polysaccharides can regulate growth performance, lipid metabolism, immune function and intestinal microflora in chickens, piglets and mice; however, the effects of dietary YPS on growth performance in growing Huoyan geese has not yet been reported. Hence, the present study aimed to evaluate the effect of dietary YPS on growth performance, digestive enzyme activities, intestinal morphology and microflora in growing Huoyan geese.

Material and methods

Animals and diets

In the present study, all experimental bird handling protocols were approved by the Care and Use Committee (No. 2020HIST018, Henan Institute of Science and Technology, Xinxiang, HA, China). Four hundred growing geese (28-day-old) with similar initial body weight (1126.46 ± 12.28 g) were randomly allotted to 4 groups (control, YPS_{100}, YPS_{500}, and YPS_{1000}, respectively). Each group included 5 replicates, 20 geese each (sex-balanced, 10 male geese and 10 female geese). The control group was fed a diet without YPS supplementation, and the YPS_{100}, YPS_{500} and YPS_{1000} groups received the same diet supplemented with 100, 500, and 1000 mg/kg YPS, respectively. The geese used in this study were similar in breed and age to those used in our previous study (Miao et al., 2020a). Therefore, the experimental diet in this study was the same as in our pervious report (Miao et al., 2020a) and was based on National Research Council (NRC, 1994) recommendations (Table 1). The feeding trial of the experimental geese lasted for 28 days (from 28 to 56 days of age). All birds were reared in concrete pens (3 geese/m^2), and the floor was covered with straw litter (3 cm thickness); the pens were cleaned once a week (Miao et al., 2020b). The experiment was carried out from June to July (average temperature – 27.5 ± 2 °C, relative humidity – 64 ± 6%). The experimental diets were provided *ad libitum*; animals also had unlimited access to water through nipple drinkers. YPS (YPS carbohydrate content is more than 90.40%) in this study was purchased from Shaanxi Hana Biotechnology Co., Ltd (Xian, SN, China).

Table 1. Ingredients and nutrients of the experimental diets (air-dry basis)

Ingredients, %		Nutrition level[b], %	
Corn	66.75	ME, MJ/kg	11.55
Wheat bran	15.00	Crude protein	15.00
Soybean	8.99	Calcium	0.80
Fish meal	3.0	Available phosphorus	0.40
Limestone	0.18	Lysine	0.95
$CaHPO_4$	0.67	Methionine + Cystine	0.67
DL-methionine	0.08	Threonine	0.16
L-lysine-HCL	0.33	DM, %	87.5
Premix[a]	5.00		
Total	100		

ME – metabolizable energy, DM – dry matter; [a] premix supplied per kg: IU: vit. A 30 000, vit. D_3 5 000, vit. E 20; μg: vit. B_{12} 20; mg: vit. K_3 38, vit. B_1 5, vit. B_2 10, nicotinamide 60, vit. B_6 5, D-calcium pantothenate 10, pyridoxol 3, biotin 0.1, choline 1 000, folic acid 1, Cu 5, Fe 100, Mn 80, Zn 100, Se (Na_2SeO_3) 0.1, Co (LCO_4) 0.15, I (KIO_3) 0.4; [b] calculated values

Sample collections

All experimental birds were weighed individually once a week during the feeding trial phases. Average daily gain (ADG), average daily feed intake (ADFI) and feed to gain (F/G) ratio were analysed based on the whole feeding phases. At 56 days, 10 geese in each group (2 geese per replicate, balanced sex) with a similar body weight were selected for weighing and slaughter after fasting for 12 h. The abdominal cavity of the experimental birds was opened, and segments of the duodenum, jejunum and ileum were collected according to the method described in our pervious report (Miao et al., 2020b). Briefly, the small intestine was removed from the abdominal cavity of geese to collect 2- to 3-cm segments from the middle part of the duodenum, jejunum, and ileum, respectively. The samples from the small intestine were rinsed with saline and fixed in 4% formaldehyde for intestinal morphology evaluation. Fresh digesta samples of the small intestine were transferred to 1.5 ml sterile tubes, and subsequently stored at −80 °C until further analysis.

Intestinal morphology analysis

Fixed intestinal segments were dehydrated using a graded ethanol series (70–100%), washed with xylene (Damao Chemical Reagent Factory, Tianjin, China) and embedded in standard paraffin wax (Shanghai Huayong Paraffin Wax Co., Ltd, Shanghai, China). Three cross sections (thickness 5 μm) in each intestinal segment were cut using a Leica RM2135 rotary microtome (Leica Microsystems GmbH, Wetzlar, Germany) after staining with haematoxylin (Beijing Solarbio Science & Technology Co., Ltd, Beijing, China) and eosin (Beijing Solarbio Science & Technology Co., Ltd, Beijing, China). At least, ten intact crypt-villous units were examined in triplicate from each intestinal section according to the methods described in earlier studies (Lu et al., 2011). Briefly, each slide was measured under a light microscope (CK40, Olympus, Tokyo, Japan), and digital image was captured. Villus height and crypt depth were evaluated using an image analysis system (Leica Imaging System Ltd, Cambridge, England).

Analysis of digestive enzyme activities

Digesta samples from the duodenum, jejunum and ileum were diluted 10-fold using ice-cold PBS (phosphate buffered saline) based on sample weight and homogenized for 1 min. The homogenates were centrifuged at 2500 rpm for 10 min at 4 °C, and then the supernatant was isolated for digestive enzyme activity assays (Yu et al., 2019; Miao et al., 2020b). The activity of trypsin, lipase and amylase were carried out using 722 UV visible spectrophotometer (Coregitech Holding Group, Nanjing, JS, China) according to the instructions included in the detection kits (Amylase assay kit, C016-1-1; Lipase assay kit, A054-1-1; Trypsin assay kit A080-2-2; Jiancheng Bioengineering Institute, Nanjing, JS, China). In this study, enzyme activities in the digesta of the small intestine in geese were expressed as units per mg of protein in the digesta.

Microbiological analysis

At 56 days of age, ten geese were separated from each group and slaughtered based on average body weight. Ceacal content samples of the experimental geese were transferred to sterile flasks for microbial culture. Microbiological analysis was performed according to the method described previously (Qorbanpour et al., 2018). Briefly, ceacal content was transferred to Petri dishes, and *Lactobacilli*, *Escherichia coli*, and *Bifidobacterium* were cultured in Man Rogosa Sharpe (MRS) (Beijing Solarbio Science & Technology Co., Ltd, Beijing, China), Eosin Metilan-Blou (EMB), and BBL agar media (Qingdao High-tech Industrial Park Haibo Biotechnology Co., Ltd, Qingdao, SD, China). Sample suspension (1 ml) was added into 9 ml of PBS after shaking for 30 min, and diluted 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} times. Subsequently, 0.1 ml was removed from 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} dilutions, and transferred to Petri dishes for ceacal microbial population determination. *Lactobacilli* were incubated under anaerobic conditions for 48 h at 37 °C, *E. coli* was incubated under anaerobic conditions for 24 h at 37 °C, and *Bifidobacterium* was incubated under anaerobic conditions for 72 h at 37 °C. Bacterial counts in the digesta were calculated using a colony counter, and converted to a 1g sample.

Statistical analysis

All analyses in this study were carried out using SPSS 17.0 for Windows (SPSS, Inc., Chicago, IL, USA). The data were analysed using the one-way ANOVA procedure implemented in SPSS 17.0; the means were compared with Duncan's multiple range tests; differences were considered significant at $P < 0.05$. Linear and quadratic polynomial effects were analysed to measure the impact of dietary YPS supplementation. The replicate in the present study was used as an experimental material unit (10 geese per group) to assess growth performance, digestive enzyme activities, small intestinal morphology and caecal microbiota. All data in this study are expressed as mean ± SEM.

Results

Growth performance

As shown in Table 2, no differences in IBW and ADFI were found between the control, YPS_{100}, YPS_{500} and YPS_{1000} groups ($P > 0.05$). Geese from the YPS_{500} group had higher FBW and ADG compared to the control group ($P < 0.05$), and no differences were observed between the YPS_{100}, YPS_{500} and YPS_{1000} groups ($P > 0.05$). Meanwhile, the F/G ratio in the YPS_{500} group was lower than that in the control group, while no differences were found between the YPS_{100}, YPS_{500} and YPS_{1000} groups ($P > 0.05$).

Intestinal morphology

The effect of dietary YPS supplementation on the intestinal morphology of growing Huoyan geese is shown in Table 3. Geese from the YPS_{500} group

Table 2. Effect of dietary yam polysaccharide (YPS) on growth performance in growing Huoyan geese

Item	Groups				SEM	Contrast		
	control	YPS_{100}	YPS_{500}	YPS_{1000}		T	L	Q
IBW, g	1123.83[a]	1129.51[a]	1128.17[a]	1124.33[a]	13.39	0.931	0.515	0.998
FBW, g	2530.33[b]	2872.84[ab]	3013.50[a]	2738.16[ab]	17.16	0.022	0.014	0.059
ADG, g/d	50.23[b]	62.26[ab]	67.34[a]	57.65[ab]	0.839	0.026	0.011	0.056
ADFI, g/d	148.12[a]	155.42[a]	159.01[a]	151.78[a]	2.84	0.073	0.062	0.132
F/G, g/g	2.94[a]	2.49[ab]	2.36[b]	2.63[ab]	0.064	0.001	0.002	0.053

control – group fed the basal diet; the YPS_{100}, YPS_{500} and YPS_{1000} groups were fed the basal diet with 100, 500 and 1000 mg/kg yam polysaccharide, respectively; T – treatment, L – linear, Q – quadratic, IBW – initial body weight, FBW – final body weight, ADG – average daily gain, ADFI – average daily feed intake, F/G – feed-to-gain ratio, SEM – standard error of the mean; [ab] – means within a row with different superscripts are significantly different at $P < 0.05$

Table 3. Effect of dietary yam polysaccharide (YPS) on the small intestinal morphology in growing Huoyan geese

Item	Groups				SEM	Contrast		
	control	YPS_{100}	YPS_{500}	YPS_{1000}		T	L	Q
Duodenum								
villus height, μm	701.31[b]	751.34[ab]	823.12[a]	763.62[ab]	19.29	0.034	0.025	0.071
crypt depth, μm	243.47[a]	225.36[ab]	208.17[b]	223.56[ab]	5.54	0.042	0.032	0.089
V/C	2.88[b]	3.35[ab]	3.96[a]	3.43[ab]	0.28	0.002	0.001	0.064
Jejunum								
villus height, μm	656.14[b]	708.63[ab]	779.18[a]	706.39[ab]	20.80	0.023	0.014	0.063
crypt depth, μm	209.58[a]	197.55[ab]	183.27[b]	210.20[ab]	6.26	0.014	0.011	0.054
V/C	3.14[b]	3.59[ab]	4.27[a]	3.47[ab]	0.34	0.003	0.002	0.058
Ileum								
villus height, μm	579.61[b]	630.60[ab]	683.16[a]	641.71[ab]	17.96	0.037	0.022	0.094
crypt depth, μm	219.45[a]	200.74[ab]	183.58[b]	201.09[ab]	8.37	0.018	0.013	0.066
V/C	2.65[b]	3.15[ab]	3.74[a]	3.21[ab]	0.22	0.001	0.001	0.052

control – group fed the basal diet; the YPS_{100}, YPS_{500} and YPS_{1000} groups were fed the basal diet with 100, 500 and 1000 mg/kg yam polysaccharide, respectively; T – treatment, L – linear, Q – quadratic, V/C – villus height/crypt depth ratio, SEM – standard error of the mean; [ab] – means within a row with different superscripts are significantly different at $P < 0.05$

had higher villus height and villus height to crypt depth ratio (V/C ratio), while lower crypt depth in the duodenum, jejunum, and ileum in comparison to the control group ($P < 0.05$). However, there were no differences in villus height, V/C ratio and crypt depth of the small intestine between the YPS_{100}, YPS_{500} and YPS_{1000} groups ($P > 0.05$).

Digestive enzyme activities

The YPS_{500} group showed higher amylase and trypsin activities, while lower lipase activity in the digesta of the duodenum, jejunum and ileum compared to the control group ($P < 0.05$); no differences were determined in amylase, trypsin and lipase activities between the YPS_{100}, YPS_{500} and YPS_{1000} groups ($P > 0.05$) (Table 4).

Table 4. Effect of dietary yam polysaccharide (YPS) on digestive enzyme activities in growing Huoyan geese

Item	Groups				SEM	Contrast		
	control	YPS_{100}	YPS_{500}	YPS_{1000}		T	L	Q
Duodenal content, U/mg protein								
amylase	16.45[b]	18.95[ab]	20.12[a]	18.61[ab]	1.23	0.033	0.026	0.084
trypsin	136.21[b]	150.77[ab]	181.42[a]	155.65[ab]	3.01	0.012	0.008	0.061
lipase	182.54[a]	162.07[ab]	150.73[b]	167.82[ab]	2.94	0.024	0.009	0.072
Jejunal content, U/mg protein								
amylase	15.79[b]	18.28[ab]	20.67[a]	18.11[ab]	1.78	0.026	0.014	0.053
trypsin	132.67[b]	145.50[ab]	175.83[a]	154.66[ab]	2.55	0.037	0.022	0.088
lipase	176.54[a]	159.45[ab]	140.51[b]	161.86[ab]	2.48	0.029	0.013	0.074
Ileal content, U/mg protein								
amylase	16.59[b]	19.06[ab]	21.75[a]	18.98[ab]	1.99	0.014	0.005	0.058
trypsin	120.11[b]	149.47[ab]	156.48[a]	140.70[ab]	2.24	0.041	0.025	0.079
lipase	96.71[a]	84.79[ab]	68.03[b]	79.14[ab]	2.01	0.033	0.017	0.064

control – group fed the basal diet; the YPS_{100}, YPS_{500} and YPS_{1000} groups were fed the basal diet with 100, 500 and 1000 mg/kg yam polysaccharide, respectively; T – treatment, L – linear, Q – quadratic, SEM – standard error of the mean; [ab] – means within a row with different superscripts are significantly different at $P < 0.05$

Microbial composition

The YPS_{100}, YPS_{500} and YPS_{1000} groups had lower abundance of *E. coli*, while higher of *Lactobacillus* and *Bifidobacterium* compared to the control group ($P < 0.05$). No differences in *E. coli*, *Lactobacillus* and *Bifidobacterium* counts in the caecal content were identified between the YPS_{100}, YPS_{500} and YPS_{1000} groups ($P > 0.05$).

Table 5. Effect of dietary yam polysaccharide (YPS) on the cecal microbial composition of growing Huoyan geese (CFU/g)

Item	Groups				SEM	Contrast		
	control	YPS_{100}	YPS_{500}	YPS_{1000}		T	L	Q
Escherichia coli, 10^5	1.8[a]	1.4[b]	1.1[b]	1.2[b]	0.18	0.033	0.022	0.074
Lactobacillus, 10^7	4.2[b]	5.3[a]	5.7[a]	5.4[a]	0.23	0.012	0.007	0.061
Bifidobacterium, 10^7	3.9[b]	4.7[a]	4.9[a]	4.6[a]	0.19	0.028	0.011	0.054

control – group fed the basal diet; the YPS_{100}, YPS_{500} and YPS_{1000} groups were fed the basal diet with 100, 500 and 1000 mg/kg yam polysaccharide, respectively; T – treatment, L – linear, Q – quadratic, CFU – colony forming units, SEM – standard error of the mean; [ab] – means within a row with different superscripts are significantly different at $P < 0.05$

Discussion

The present study found that the YPS_{500} group had higher ADG and lower F/G ratio compared to the control group, which indicated that dietary YPS supplementation increased growth performance in growing geese by regulating ADG and F/G ratio. These results were consistent with the previous report (Gao et al., 2015), which found that dietary YPS supplementation (200 and 400 mg/kg diet) increased growth performance in Konmin mice. Rats fed a diet with 0.25 g/kg YPS supplementation showed higher ADG and lower F/G ratio compared to animals on a basal diet (Yang et al., 2010; Kong et al., 2010). Another study also observed that dietary supplementation of 400 and 600 mg/kg YPS increased ADG and F/G ratio in Ningdu Yellow Chickens (Tan et al., 2014). In addition, supplementing the diet with astragalus polysaccharide (APS) also significantly improved ADG of weaned piglets (Yuan et al., 2006). We also observed in the current study that the positive effect of dietary YPS supplementation tended to disappear when YPS level was increased to 1000 mg/kg. The reason could be that the presence of high YPS concentration in the gastrointestinal tract decreased nutrient digestion, and thus reduced growth performance of growing geese. The data suggest that an appropriate dietary YPS level can be used as a potential feed additive to improve growth performance of growing geese. However, the exact mechanism behind this phenomenon still needs to be evaluated in further studies.

Growing evidence indicates that villus length, crypt depth and V/C ratio are associated with intestinal health and its absorption capacity (Turner et al., 2009; Liao et al., 2021). Higher villus length and V/C ratio usually reflect stronger intestinal absorption capacity and better growth performance of animals (He et al., 2015; Miao et al., 2020b). In the present study, dietary YPS_{500} supplementation increased villus height, and V/C ratio, while decreased crypt depth in the duodenum, jejunum, and ileum, which suggested that the structure and morphology of the small intestine of growing geese could be improved by proper dietary YPS addition. Similar results were obtained by other researchers (Liao et al., 2021), who found that dietary APS improved villus length and V/C ratio in the small intestine of Muscovy ducklings. Another study found that dietary APS and GPS (ginseng polysaccharide) increased jejunal villus length and V/C ratio, while decreased jejunal crypt depth in weaned piglets (Yang et al., 2019). In turn, supplementing the diet with APS or GPS was also shown to increase jejunal villus height in piglets (Wang et al., 2020b). These results indicated that plant polysaccharides could improve growth performance of animals by increasing villus length and V/C ratio, and regulating intestinal morphology (Liao et al., 2021).

Previous results have demonstrated that nutrient digestion is associated with the activity digestive enzyme, which in turn affect animal growth (Qian et al., 2016; Yang et al., 2017). In this study, amylase and trypsin activities were enhanced by dietary YPS_{500} supplementation, which indicated that the proper YPS supplementation could regulate the function of digestive enzymes in growing Huoyan geese. Similar results were reported by other authors (Long et al., 2020), who found that dietary LBP (*Lycium barbarum* polysaccharide) supplementation (1000 or 2000 mg/kg) increased the activity of amylase, protease and lipase in the small intestine digesta of broilers. Another study also observed that the proper AMP (*Astragalus membranaceus* polysaccharides) addition to the diet enhanced amylase, protease, and lipase activities in juvenile broilers

(Wu et al., 2018). In contrast, lipase activity was decreased by dietary YPS_{500} supplementation in this study. The inconsistent results regarding lipase activity could be due to differences in animal species, polysaccharide types and dosage applied during the feeding period. The underlying mechanism of YPS action in regulating intestinal digestive enzymes in growing geese still requires further investigations.

In this study, dietary supplementation with YPS could selectively promote the growth of *Lactobacillus* and *Bifidobacterium*, while reducing the growth of pathogenic bacteria, such as *E. coli*, which would imply an important function of YPS in improving the intestinal flora in growing geese. The mechanism of this effect may include competitive binding of YPS with pathogenic bacteria, and subsequent inhibition of their proliferation. Certainly, future studies are required to confirm this hypothesis. Similar results were reported by earlier studies (Zhang et al., 2019) showing that the addition of high doses of Chinese yam promoted the proliferation of *Lactobacillus* and *Bifidobacterium*, while decreasing the count of *Enterococcus* and *Clostridium perfringens* in mice. Another study also found that dietary CCP supplementation increased the growth of *Lactobacillus* and decreased the number of *E. coli* in the caecum of broilers (Wang et al., 2020a). These results demonstrated that the structure of the intestinal bacterial community could be regulated by plant-derived polysaccharides.

Conclusions

Adequate dietary supplementation with YPS (500 mg/kg) can improve growth performance of growing geese by regulating morphology, digestive enzyme activities and bacterial community in the small intestine.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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