Effect of a straw-derived xylooligosaccharide on broiler growth performance, endocrine metabolism, and immune response

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Abstract

The aim of this work was to evaluate the effect of 3 levels of supplemental xylooligosaccharides (XOS) from straw on the growth performance, endocrine metabolism, and immune response of broiler chickens. Day-old, healthy Arbor Acres broilers (n = 192) received a basal diet of maize–soybean meal and, depending on the group to which they were allocated, no additive (control group) or the following experimental treatments for 59 d: treatment 1: 5 g XOS/kg; treatment 2: 10 g XOS/kg; and treatment 3: 20 g XOS/kg. By day 59 the body weight gain of the chickens receiving treatment 2 had increased by 9.44% ($P < 0.01$) over the gain of the control group. The levels of serum triiodothyronine, thyroxine, and insulin on day 44 were significantly higher in the treatment groups than in the control group. The titers of antibody to the avian influenza H5N1 virus on day 24 were also significantly higher in the treatment groups than in the control group, and on day 59 the titer of the chickens receiving treatment 2 were still significantly increased ($P < 0.05$). Thus, the addition of XOS to feed can increase growth performance, enhance endocrine metabolism, and improve immune function in broiler chickens.

Résumé

L'objectif de ce travail était d'évaluer les effets de trois niveaux d'un supplément de xylooligosaccharides (XOS) provenant de la paille sur les performances de croissance, le métabolisme endocrinien, et la réponse immunitaire de poulets à griller. Des poussins à griller en santé âgés d'un jour (n = 192) de race Arbor Acres ont reçu une alimentation de base maïs-soya et, selon le groupe auquel ils ont été assignés, aucun additif (groupe témoin) ou pendant 59 j le traitement expérimental suivant : traitement 1 : 5 g XOS/kg; traitement 2 : 10 g XOS/kg; et traitement 3 : 20 g XOS/kg. Au jour 59, le gain de poids corporel des poulets recevant le traitement avait augmenté de 9,44 % de plus (P < 0,01) que le gain du groupe témoin. Les niveaux sériques de triiodothyronine, de thyroxine et d'insuline au jour 44 étaient significativement plus élevés dans les groupes de traitement que dans le groupe témoin. Les titres d'anticorps contre le virus de l'influenza aviaire H5N1 au jour 24 étaient également significativement plus élevés dans les groupes de traitement que dans le groupe témoin, et au jour 59 les titres des poulets recevant le traitement 2 étaient encore significativement augmentés (P < 0,05). Ainsi, l'ajout de XOS à l'alimentation peut augmenter les performances de croissance, augmenter le métabolisme endocrinien et améliorer la fonction immunitaire des poulets à griller.

(Traduit par Docteur Serge Messier)

Introduction

Antibiotics as growth promoters have been used for decades in poultry production to improve farm performance and control intestinal pathogens. With increasing interest in discontinuing the use of antibiotics as feed additives the search for alternatives has intensified. Ideally these alternatives should improve growth performance and maintain sound health for the chickens. Therefore, the search for new types of feed additives that are pollution-free has become the focus of current research (1). China is a large agricultural country, and substantial amounts of agricultural by-products, such as corn cob, cotton seed hull, and straw, that are rich in cellulose-type xylanase are produced every year (2). Xylooligosaccharides (XOS) can be produced from many edible fungi through the hydrolysis of semicellulose by xylanase (3,4). Straw chaff, the substrate used for cultivating edible fungi, is regarded as XOS after the biologic degradation of fungi through 2 to 3 batches of fungus production. To explore the biologic characteristics of XOS and their application in livestock and poultry production, the authors studied the effects of XOS on growth performance, endocrine metabolism, and immune response in broiler chickens.

Materials and methods

The animal research protocols conformed to those approved by the Yangzhou University Animal Care and Use Committee, Yangzhou, China.

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Table I. Feed formula and nutritional levels of the broiler chickens' basal diet

Composition (% of feed)	Age (d) 1–21	22–42	43–59	Nutritional level	Age (d) 1–21	22–42	43–59
Corn	53.80	62.00	69.72	MJ (mCal/kg)	2.950	3.050	2.966
Soybean meal	38.16	29.97	24.62	Crude protein (%)	21.92	19.06	17.72
Fish meal	1.50	1.50	1.00	Linoleic acid (%)	1.52	1.73	1.73
Limestone	1.10	0.90	1.00	Calcium (%)	1.00	0.85	0.81
Calcium phosphate	1.70	1.60	1.60	Phosphorus (%)	0.47	0.42	0.42
Table salt	0.30	0.30	0.30	Sodium chloride (%)	0.35	0.35	0.35
Lysine	0.02	0.02	0.08	Lysine	1.14	0.96	0.96
Methionine	0.25	0.21	0.18	Methionine (%)	0.54	0.47	0.47
Vegetable oil	2.17	2.50	0.50	Methionine + cystine	0.88	0.78	0.75
Premix[a]	1.00	1.00	1.00				

[a] Supplied per kilogram of 1% premix: vitamin A, 1500 IU; vitamin D_3, 200 IU; vitamin E, 10 IU; vitamin K, 0.5 mg; thiamine, 1.8 mg; riboflavin, 3.6 mg; pyridoxine, 3.5 mg; vitamin B_{12}, 0.01 mg; pantothenic acid, 10 mg; niacin, 35 mg; choline, 1300 mg; biotin, 0.15 mg; folic acid, 0.55 mg; manganese, 60 mg; zinc, 40 mg; copper, 8 mg; iron, 80 mg.

Production of XOS

Edible fungi were used by the Animal Physiology Laboratory of the College of Veterinary Medicine at Yangzhou University to ferment and degrade straw chaff to make XOS. Briefly, the edible fungus *Pleurotus ostreatus*, obtained from the Yangzhou Academy of Agricultural Sciences, was cultivated in potato dextrose agar (200 g of potato, 15 g of agar, 20 g of glucose, 3 g of KH_2PO_4, 10 mg of VB_1, and 1.5 g of $MnSO_4$; Difco Laboratories, Detroit, Michigan, USA) at 30°C for 7 d, at which time mycelia completely covered the surface of the Petri dishes. Liquid medium was inoculated with 1 disk of agar plate mycelium 6 mm in diameter per 10 mL of medium. The liquid medium contained (per liter) 1.4 g of $(NH_4)_2SO_4$, 2.0 g of KH_2PO_4, 0.1 g of urea, 0.3 g of $MgSO_4 \cdot 7H_2O$, 0.3 g of $CaCl_2$, 5.0 mg of $FeSO_4 \cdot 7H_2O$, 1.56 mg of $MnSO_4 \cdot H_2O$, 2.0 mg of $CoCl_2$, and 1.4 mg of $ZnSO_4 \cdot 7H_2O$. Solid fermentation was carried out in 500-mL plastic flasks containing 350 g of sterile solid medium (pH 5.5) plus 10% of liquid mycelium. The solid medium contained, per 100 g, 96 g of straw, 1 g of $CaHPO_4$, 1.4 g of lime, 1.5 g of gypsum, and 0.6 mg of carbendazole. The flasks were incubated at 30°C for 40 d in a warm room without agitation. Paper chromatography and photoelectric colorimetry were used to determine that the XOS content of the degraded straw medium was 82.48 mg/g and that the other macronutrients consisted mainly of neutral detergent fiber (81.81 mg), acid detergent fiber (56.15 mg), and crude protein (8.30 mg).

Animals and experimental treatments

Day-old Arbor Acres (AA) broiler chickens (n = 192) were provided by Nantong Haian Poultry Breeding Farm, Nantong, Jiangsu, China. Males and females were identified and housed separately in 6 wire cages per group, 8 birds per cage (3 cages for males and 3 for females). Each bird was numbered and weighed. The birds were randomly allocated to 1 of 4 experimental groups for 59 d. All received a basal diet of maize–soybean meal with no additive (control group) or XOS: 5 g/kg (treatment 1); 10 g/kg (treatment 2); or 20 g/kg (treatment 3). The basal diet was prepared by the authors according to the recommendations of the US National Research Council (5). The details of the feed formula and nutritional levels are shown in Table I. The feed was made into pellets for use in 3 periods: the 1st period was from day 1 to day 21, the 2nd from day 22 to day 42, and the 3rd from day 43 to day 59.

Animal management

The chickens were kept in cages in a chicken house during the entire period of the experiments. Infrared lights and heaters were used to maintain warmth; natural ventilation and a sustained lighting system were also implemented. The temperature was kept at 23.4°C to 29.0°C, and the relative humidity ranged from 50% to 80%. All the chickens had free access to feed and water.

Vaccination and sample collection

On day 14 all the chickens were given an inactivated vaccine against the H5N1 subtype of avian influenza (AI) virus via a single intramuscular injection. Starting on day 24 blood samples were collected every 5 d from the plantar vein of 6 chickens (3 male and 3 female) selected randomly from each group.

Measurement of parameters

To assess growth performance the weight of the chickens was measured on days 1 and 59. Body weight gain, feed intake, and feed conversion ratio were determined.

The radioimmunoassay (RIA) technique was used to determine the serum concentrations of triiodothyronine (T_3), thyroxine (T_4), and insulin on day 44. The RIA assay kits were produced by Beijing Kemei Dongya Biotechnology Company, Beijing, China.

Serum antibody titers against the AI H5N1 vaccine virus were determined by hemagglutination inhibition (HI) (6) with use of a preparation of 1% chicken erythrocytes made by conventional technique. The antibody titers were expressed as the average of $\log_2$.

Statistical analysis

A Microsoft Excel database was established for all the experimental data. One-way analysis of variance was used in SPSS 11.5 statistical software (SPSS, Chicago, Illinois, USA) to determine the

Table II. Effects of xylooligosaccharides (XOS) added to the feed on the growth performance of the chickens in 59 d

	Growth performance, mean ± standard error[a]		
Treatment group	Body weight gain (kg)	Feed intake (kg)	Feed conversion ratio
Control: basal diet	3.486 ± 0.052	6.402 ± 0.110	1.844 ± 0.044
Treatment 1: 5 g XOS/kg	3.694 ± 0.085	6.475 ± 0.057	1.767 ± 0.031
Treatment 2: 10 g XOS/kg	3.815 ± 0.099**	6.479 ± 0.063	1.716 ± 0.045*
Treatment 3: 20 g XOS/kg	3.510 ± 0.050	6.171 ± 0.048*	1.765 ± 0.028

[a] Significant difference from the mean for the control group at *P*-values of * < 0.05 and ** < 0.01.

Table III. Effects of XOS on serum hormone concentrations after 44 d

	Hormone concentration, mean ± standard error[a]		
Treatment group	Triiodothyronine (ng/mL)	Thyroxine (ng/mL)	Insulin (ng/mL)
Control	1.422 ± 0.159	4.654 ± 0.317	7.095 ± 0.333
Treatment 1	2.327 ± 0.189**	6.288 ± 0.136**	7.984 ± 0.357*
Treatment 2	1.829 ± 0.171	6.375 ± 0.170**	7.762 ± 0.190
Treatment 3	2.709 ± 0.272**	5.689 ± 0.148**	8.250 ± 0.275**

[a] Significant difference from the mean for the control group at *P*-values of * < 0.05 and ** < 0.01.

Table IV. Changes over time in titer of hemagglutination inhibition antibody to the H5N1 subtype of avian influenza virus

Treatment	Average $\log_2$ titer, mean ± standard error;[a] age (d)							
group	24	29	34	39	44	49	54	59
Control	4.1 ± 0.2	5.8 ± 0.3	4.6 ± 0.2	5.4 ± 0.3	5.0 ± 0.3	6.5 ± 0.4	4.8 ± 0.5	5.1 ± 0.5
Treatment 1	5.4 ± 0.3**	5.3 ± 0.3	4.7 ± 0.4	4.8 ± 0.3	5.3 ± 0.3	5.0 ± 0.3	5.2 ± 0.3	5.9 ± 0.6
Treatment 2	5.3 ± 0.3**	5.5 ± 0.5	4.8 ± 0.3	5.1 ± 0.5	5.6 ± 0.5	5.6 ± 0.2	5.7 ± 0.2	6.8 ± 0.4**
Treatment 3	4.9 ± 0.3*	4.3 ± 0.3	4.6 ± 0.2	5.1 ± 0.3	4.3 ± 0.3	5.0 ± 0.4	4.9 ± 0.2	5.1 ± 0.5

[a] Significant difference from the mean for the control group at *P*-values of * < 0.05 and ** < 0.01.

differences between groups. Results for the multiple-range test variant of the least significant difference method are shown as mean ± standard error. Differences between means were considered significant at $P < 0.01$ and $P < 0.05$.

Results

As shown in Table II, the chickens receiving treatment 2 had a 9.44% greater gain in body weight ($P < 0.01$) and a 4.18% lower feed conversion ratio ($P < 0.05$) than the control group. In addition, the chickens receiving treatment 3 had a 3.61% lower feed intake ($P < 0.05$) than the control group.

As shown in Table III, on day 44 the serum level of T_3 in treatment groups 1 and 3 was greater than that in the control group by 63.64% ($P < 0.01$) and 90.51% ($P < 0.01$), respectively, and the serum level of T_4 in treatment groups 1, 2, and 3 was greater than that in the control group by 35.11% ($P < 0.01$), 36.98% ($P < 0.01$), and 22.24% ($P < 0.01$), respectively. In addition, the serum insulin level in treatment groups 1 and 3 was greater than that in the control group by 12.53% ($P < 0.05$) and 16.28% ($P < 0.01$), respectively.

As shown in Table IV, on day 24 the serum HI antibody titer to the AI H5N1 vaccine virus was greater in treatment groups 1, 2, and 3 than in the control group by 31.77% ($P < 0.01$), 27.71% ($P < 0.01$), and 19.61% ($P < 0.05$), respectively. In addition, comparing with the titers in the control group, the titer in treatment group 2 was 33.78% higher ($P < 0.05$) on day 59.

Discussion

In this study, broilers with XOS-supplemented diets had greater body weight gain than those with a basal diet, along with decreased feed conversion, in agreement with the Food and Agriculture Organization of the United Nations (7), which suggested that XOS could be considered emerging prebiotics and defined a prebiotic as "a nonviable food component that confers a health benefit on the host associated with modulation of the microbiota". Made up of xylose units, and approximately half as sweet as sucrose, XOS can be produced by enzymatic hydrolysis from xylan, the main component of plant hemicelluloses and therefore readily available in nature (8). The fungal genera *Trichoderma, Aspergillus, Fusarium,* and *Pichia* are considered great producers of xylanases (9–13). White-rot fungi have also been shown to produce extracellular xylanases that act on a wide range of hemicellulose materials and are also useful as food sources (14) and metabolites of interest to the pharmaceutical, cosmetic, and food industries (15,16). In this study, the edible fungus *P. ostreatus* was used to ferment and degrade straw chaff to make XOS. During the process, other nutritional agents were produced (e.g., neutral and acid detergent fiber, crude protein, and minerals)

that could help to modify feed intake (17,18). The fact that XOS are relatively stable in acidic conditions may protect XOS from decomposition when passing through the stomach (19). Degradation in the intestine has been studied *in vitro* with an artificial model of digestive enzymes (α-amylase, pancreatin, gastric juice, and intestinal brush border enzymes), and no hydrolyzation of XOS (xylobiose) was observed (20). This suggests that XOS may be nondigestible or of low digestibility and would reach the colon intact. In addition, XOS can decompose harmful substances, produce organic acids and other beneficial substances, reduce fecal stench, and improve the farming environment (21).

The effects of T_3 and T_4 on avian metabolism and growth rates have been well-documented (22–24). The biologic activity of T_3 is several times greater than that of T_4, and T_3 functions much faster than T_4. Therefore, T_3 is believed generally to be the main thyroid hormone with respect to physiological function (25). Kühn et al (26) found that the level of T_3 in poultry is related positively to growth. Growth is slowed significantly in an animal with hypothyroidism. The relation between thyroid secretion rate and growth demonstrated in chickens (27) suggests that the poorer growth performance of the control birds in the present study could be partly attributed to decreased T_3 activity. Comparison of the serum concentrations of T_3 between the control and treatment groups indicated that XOS increased the T_3 activity, though not always significantly. The level of T_4 in the serum was significantly increased in the birds receiving supplemental XOS compared with the control group. These results indicate that XOS can improve thyroid function and that it helps the thyroid hormones participate in the growth and metabolism of poultry. The main mechanism of action of T_3 involves control of the gene expression and synthesis of growth hormone. It also increases insulin and the RNA content of muscle, which further promote protein synthesis (28). In the group receiving a supplement of 20 g XOS/kg, although the serum levels of T_3 and T_4 increased significantly, there was no notable difference in body weight gain compared with the control group. This may be explained by excessive supplementation; however, this question needs further exploration.

The main functions of insulin are to decrease the blood glucose level, to regulate growth, and to participate in a wide range of metabolic processes. One of the most important mechanisms of action of insulin is to facilitate the transport of glucose and amino acids into cells. This promotes protein synthesis and increases the concentration of glucose in the cells, which facilitates glycolysis and, further, improves the synthesis of fatty acids and the breakdown of triglycerides. In the meantime, insulin inhibits the degradation of glycogen, proteins, and triglycerides. An increased level of insulin assists glucose transport into cells, decreases the concentration of cyclic adenosine monophosphate, and enhances glycogen synthesis (29). This study showed that the insulin level in the groups treated with XOS was higher than that of the control group. The possible reasons are that XOS is rich in amino acids, mainly lysine (1.2%) and tryptophan (0.8%), and that XOS can facilitate intestinal and bowel movement and further increase digestibility. Other studies have shown that insulin can facilitate the transformation of T_4 to T_3 (30). Therefore, XOS supplementation can improve the metabolism of broilers.

Avian influenza is one of the most damaging diseases affecting the poultry industry. In the present experiment the serum levels of antibody against the AI H5N1 vaccine virus were significantly greater in the treatment groups than in the control group. This suggests that XOS can strengthen humoral immunity in poultry. Because the level of maternal antibody may have had an influence on the experimental results, we will avoid the interference of maternal antibody and other external factors in future experiments.

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