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Original Research Article

Effects of dietary barley inclusion and glucanase supplementation on the production performance, egg quality and digestive functions in laying ducks

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ABSTRACT

This study evaluated the effects of barley inclusion and glucanase supplementation on the productive performance and digestive function in laying ducks. The experiment used a randomized design with a 5 × 2 factorial arrangement of 5 graded levels of barley (0%, 15%, 30%, 45% and 60%) with or without 1.5 g/kg β-1,3-1,4-glucanase (15,000 U/kg). During the experimental period of 120 d, the weight and total number of eggs within each pen were recorded daily, and egg quality was determined every 4 wk. At the end of the experiment, 3 randomly selected ducks within each replicate were sacrificed, then duodenal digesta and jejunal mucosa was collected. Dietary inclusion of barley had no effects on egg production, daily egg mass or FCR, but supplementation with glucanase improved egg production and FCR ($P < 0.01$). Barley did not affect feed intake of laying ducks, but glucanase tended to increase feed intake ($P = 0.09$). Neither barley nor β-glucanase had effects on the egg quality variables, except for yolk color score, which was decreased with increasing barley supplementation. Glucanase, but not barley, increased the activity of chymotrypsin and amylase in duodenal digesta. Barley inclusion affected the activity of alkaline phosphatase and maltase in jejunal mucosa ($P < 0.05$), but β-glucanase had no effects on the activity of these brush border enzymes. Barley inclusion increased the glucan content in duodenal digesta, but supplementation of glucanase to barley-based diet reduced digesta glucan content and reduced total volatile fatty acids and increased the proportion of acetic acid in cecal contents. The results indicate that, without glucanase, the optimal dietary barley level in the diets of laying ducks is about 13% for maximal production performance; glucanase supplementation of the barley diets improved production performance, probably through enhancing digestive function.

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1. Introduction

Barley is an important crop due to its early maturation making it suitable for areas with a short growing season. As a good source of energy, barley is widely used for human food and animal feed. On a worldwide basis, 30% of the barley produced is used for malting purpose and 70% for feed use (FAO, 2004). In barley with the hull, intact starch is the major constituent accounting for about 600 g/kg of dry matter, followed by total dietary fiber (200 g/kg) and protein (100 g/kg). The fiber in barley, particularly the soluble non-starch polysaccharides (NSP, 23% to 41%), compromises the efficiency of nutrient and energy utilization. β-glucans and arabinoxylans are the main NSP that make up of the major components in the cell walls of the endosperm or in the aleurone layer (Jadhav et al., 1998; Andersson et al., 1999; Han, 2000; Holtekjølen et al., 2006).

For chickens, β-glucans are the main anti-nutritive factors in barley, and they are responsible for increasing viscosity of the digesta (Almirall et al., 1995), and reducing nutrient digestibility (Salih et al., 1991) thereby reducing feed efficiency. For example, a linear decrease in the diet DE and ME of 15 to 22 d broilers were observed with increasing levels of barley (Bolarinwa and Adeola, 2012). Other problems associated with feeding these polysaccharides to poultry are sticky feces (MacGregor and Rattan, 1993; Jadhav et al., 1998). This is especially the case with barley in young birds (Arscott et al., 1960; Arscott, 1963), probably because of their greater sensitivity to anti-nutritive factors in cereals, due to immaturity of their digestive tract during early post–hatch life (Brenes et al., 1993). The negative effects of barley seems to be negligible for older (>8 wk) or adult chickens, however, because of digestive maturation and enhanced function (Salih et al., 1991).

Supplementation with exogenous glucanase has become indispensable in modern poultry diets to improve the efficiency of nutrient utilization and production performance (Ravindran et al., 2007). Supplementation of β-glucanase was reported to alleviate the negative effects of NSP (McNab and Smithard, 1992), most obvious on production performance in young chickens fed barley-based diets. For example, dietary supplementation with recombinant microbial β-1,3-1,4-glucanase at 1,000 U/kg increased feed intake and improved growth performance of 1 to 28 d broilers (Ribeiro et al., 2012). Similarly, supplementation with β-glucanase/pentosanase enzyme complex of diets of 7 to 21 d chickens improved weight gain and feed intake (Brenes et al., 1993) and reduced viscosity of digesta in the gut (Ouhida et al., 2010). The effects of including exogenous enzymes in barley-based diets for adult chickens, however, are controversial. Supplementation with β-glucanase alone or β-glucanase/pentosanase complex had no effects on egg production in adult laying hens that were fed barley-based diets (Brenes et al., 1993; Hamilton and Proudfoot, 1993) yet Benabdeljelil and Arbaoui (1994) showed positive effects of glucanase supplementation on production performance in laying hens when 50% or 60% barley was fed.

In South Asia, barley is extensively used as a feedstuff for egg-laying ducks, an important component of poultry production. Despite the increasing use of barley in the feed of egg-laying ducks, little data are available on this application. Physiological differences between waterfowl and landfowl may be the basis for ducks having higher digestibility of both soluble and insoluble NSP carbohydrates compared to chickens (Jamroz et al., 2002). The objectives of the present study with laying ducks, therefore, was to: 1) determine the optimal content of barley in the feed, and 2) evaluate the effects of β-glucanase supplementation of barley-based diets on the production performance and digestive function.

2. Materials and methods

Animal care procedures outlined by the guidelines of the Animal Care and Use Committee of the Guangdong Academy of Agricultural Sciences were followed for management, housing and slaughter procedures.

2.1. Animals, feed and management

Australian barley was purchased from a commercial trading company and contained approximately 10% β-glucan. The experiment used a randomized design with a 5 × 2 factorial arrangement of 5 graded levels of barley (0%, 15%, 30%, 45% and 60%) with zero or 1.5 g/kg β-1,3-1,4-glucanase (HF131, 15,000 U/kg). The β-1,3-1,4-glucanase (Sunhy Biology Co., Ltd, Wuhan, China) was produced as an extract from the fermentation of *Bacillus licheniformis*, and the assayed activity of β-glucanase provided is 10,000 U/g. Barley was ground and passed through a 3-mm screen before diet mixing. Corn-wheat bran and soybean meals served as the basal control diet. Glucanase was supplemented in place of equivalent weight of zeolite powder from the vitamin/mineral premix. The experimental

Table 1
Composition and analysis of experimental diets (%, as fed).

Item	Level of de-hulled barley, %				
	0	15	30	45	60
Ingredients					
Corn	55	40	25	10	0
Barley	0	15	30	45	60
Soybean meal	24.47	23.70	22.95	22.15	22.10
Wheat bran	8.20	7.68	7.20	6.75	1.70
Lard	0	1.20	2.36	3.55	3.55
DL-Met	0.15	0.17	0.20	0.22	0.20
L-Lys	0.04	0.07	0.11	0.13	0.17
Thr	0	0.03	0.05	0.09	0.11
Arg	0	0.02	0.05	0.06	0.09
Limestone	9.47	9.47	9.47	9.47	9.47
$CaHPO_4$	1.37	1.36	1.31	1.28	1.27
Salt	0.30	0.30	0.30	0.30	0.30
Premix[1]	1.00	1.00	1.00	1.00	1.00
Total	100	100	100	100	100
Nutrient composition[2]					
ME, Mcal/kg	2.50	2.50	2.50	2.50	2.50
CP	17.60	17.84	17.43	17.21	17.36
Ca	3.80	3.80	3.80	3.80	3.80
EE	2.80	3.00	4.60	4.80	4.40
CF	2.80	3.00	3.50	3.90	4.40
Total P	0.60	0.60	0.59	0.59	0.56
Available P	0.35	0.35	0.35	0.35	0.35
Digestible Met	0.37	0.38	0.40	0.41	0.43
Digestible Lys	0.81	0.81	0.81	0.81	0.82
Digestible Met + Cys	0.60	0.60	0.60	0.60	0.60
Digestible Arg	1.09	1.09	1.10	1.09	1.09
Digestible Trp	0.19	0.19	0.19	0.19	0.19
Digestible Thr	0.57	0.57	0.57	0.57	0.57

[1] Premix provided the following minerals in milligrams and vitamins per kilogram of diet: Fe, 52; Cu, 10.4; Zn, 91; Mn, 91; Se, 0.20; I, 0.52; Co, 0.26; riboflavin, 9.6 mg; niacinamide, 114 mg; D-pantothenic acid, 28.5 mg; choline chloride, 500 mg; cobalamin, 30 μg; menadione, 0.96 mg; DL-α-tocopheryl acetate, 6 mg; vitamin A, 12,000 IU; cholecalciferol D_3, 1,800 IU; vitamin E, 6 IU.

[2] Crude protein (CP), ether extract (EE) and crude fiber (CF) are measured values. Metabolizable energy (ME), Ca, total P, available P, digestible Met, Lys, Met + Cys, Arg, Trp and Thr are calculated values.

diets (Table 1) were formulated to be iso-nitrogenous and iso-caloric and were provided in pellet form, with same level of digestible limiting amino acids (Lys, Met, Trp, Thr, and Arg). For each batch of diets (300 kg in total), primary feed manufacturing was processed by mixing individual feed ingredients with the addition of premix containing glucanase, and was then steam-pelleted at 70 °C through a 3-mm die. Chemical analyses were conducted (AOAC, 2000) for determination of CP (method 955.04), ether extract (EE; method 920.39), and CF (method 962.09).

Nine hundred and sixty laying ducks (Shaoxing ducks) aged 42 wk, with similar body weight (1.49 ± 0.17 kg), were randomly allocated to 10 treatments, each with 4 replicate pens containing 24 ducks. The weight and total number of eggs within each pen were recorded daily. Ducks had free access to feed and water, and were subjected to 16 h light and 8 h darkness per day. Feed was provided twice daily (08:00 and 14:30), the remaining feed was weighed at 07:00 the next day and the average feed intake was calculated. The experiment lasted for 120 d.

2.2. Sample collection

During the experimental period, 3 eggs, with similar weight to the average egg weight of each replicate pen, were randomly sampled from each pen every 4 wk for egg quality assay. At the end of the experiment, 3 randomly selected ducks from each replicate pen were weighed and killed by cervical dislocation for tissue collection. The small intestine, from the proximal end of the duodenum to the ileocecal junction was excised. Duodenal digesta was collected and stored at −80 °C. After rinsing residual digesta with ice-cold saline, the mucosa from the mid-jejunum was collected by scraping and stored at −80 °C. The gizzards were emptied, rinsed and weighed and cecal contents from both sides were collected.

2.3. Egg quality determination

Egg quality variables (shape index, yolk color score, albumen height, Haugh units, proportions of albumen, yolk and shell) were determined as described previously (Chen et al., 2015; Luo et al., 2018).

2.4. Enzyme activity measurement

Chymotrypsin, lipase, amylase and trypsin in duodenal digesta were assayed as described by Almirall et al. (1995). Protein concentration in supernatants of digesta was assayed using a BCA kit (Thermo Scientific, Waltham, MA).

Activities of jejunal mucosal brush border enzymes were assayed in homogenates (50 mg tissue/mL of saline) with a unit of activity of alkaline phosphatase being the amount liberating 1 mmol of p-nitrophenol/h. Sucrase (EC 3.2.1.48) and maltase (EC 3.2.1.20) were assayed colorimetrically using sucrose and maltose, respectively, as substrates (Dahlqvist, 1964); results are expressed as micromoles of glucose released per hour.

2.5. Assay of β-glucan in barley and digesta

The content of β-glucan in barley and duodenal digesta was assayed using a commercial kit (Megazyme Co., Wicklow, Ireland). About 100 mg of barley (ground to <0.5 mm) were weighed then dispersed in aqueous ethanol (50% vol/vol) for 5 min at 50 °C. Two-step enzyme reactions (lichenase then β-glucosidase) were used to release glucose which was measured colorimetrically using glucose oxidase and peroxidase and glucose standards; the content of β-glucan in barley was expressed as percentage of feed (%). For duodenal digesta, lyophilized samples (approximately 100 mg) were first weighed and extracted twice, as described above, with centrifugation (1,000 ×*g*, 10 min) before assay; glycan was expressed as a percentage of digesta DM.

2.6. Determination of viscosity in the duodenal content

The duodenal contents were centrifuged at 12,000 × *g* for 5 min at 25 °C. The viscosity of the supernatants was measured using a Brookfield digital viscometer (Model DV3T, Middleboro, MA) at 40 °C, as described (Bedford and Classen, 1993). The value for viscosity was expressed in centipoise (cp = 1/100 dyne s per centimeter^2).

2.7. Volatile fatty acid (VFA) analysis

Volatile fatty acids in cecal contents were measured, as described previously (Yu et al., 2002; Clarke et al., 2018). Cecal VFA concentration (mg/g) was calculated using the peak area of the VFA and internal standard against the reference curve, and the percentage of individual VFA was calculated accordingly.

2.8. RNA extraction and real-time PCR

Total RNA was extracted from jejunal mucosa using Trizol reagent (Invitrogen, Carlsbad, CA). After RNA samples were treated with DNAase (Takara, Biotechnology Co. Ltd, Dalian, China), RNA concentration and quality were determined by OD260/280. Complementary DNA (cDNA) was synthesized by reverse-transcription from 2.0 μg of high-quality RNA in a final volume of 30 μL according to the manufacturer's instructions (Takara). The primers employed (Table 2) were designed from GenBank sequences using Primer Premier 5.0 and prepared by Shanghai Shenggong Biological Company (Shanghai, China).

Quantitative real-time PCR was performed using the Bio-Rad iQ5 Real Time PCR Detection System (Bio-Rad, San Diego, CA) with 1 μL of the cDNA product in a total volume of 20 μL, which contained 10 μL of SYBR-green PCR master Mix (Takara) and 0.5 μL (10 mmol/L) of gene-specific forward and reverse primers. The specificity of the reactions was assessed from the product melting curves. The following protocol was used: denaturation for 30 s at 95 °C, followed by 40 cycles of 20 s at 95 °C, 30 s at 60 °C, and 20 s at 72 °C. The relative abundance of each mRNA was calculated by the ΔCt method as described by Livak and Schmittgen (2001).

2.9. Statistical analysis

All the data are presented as means and they were initially examined for normality using the UNIVARIATE procedure of SAS. The data on production performance and egg quality, gizzard weight, digestive enzymes, nutrient transporter gene expression and VFA concentrations were analyzed as 5 × 2 factorials using the PROC GLM procedures of SAS (Littell et al., 1996). The model included the fixed effects of barley level, enzyme inclusion and the associated two-way interaction while replicate pens and animals in pens were random effects. Orthogonal contrasts were employed to test for linear and quadratic effects when significant effects of dietary barley were demonstrated (Chen et al., 2017). Due to the

Table 2
Oligonucleotide polymerase chain reaction primers used.

Gene	Accession number	Primer sequence	Product size, bp
CD36	XM_005016709.3	F: GCCTGAGCCCAAATGAGAAG R: GGACCCGACCAGAGACTTTT	240
FABP2	NM_001310343.1	F: AGCAACTTCCGTACCATCGA R: GCCGACGATTTCTCTGTATGC	174
SLC2A1	XM_005014490.3	F: GGAGATGAAGGAGGAGAGCC R: CAATGGTGGCATAGACAGGC	209
SLC15A1	NM_001310803.1	F: ATCCTGAAGAAACTCCCGCA R: GTGTGACCTGCTGCTTTCAA	241
SLC5A1	XM_005026696.3	F: GCAGTGGGACTATGGGCTAT R: CTGCTGCTGTTCCTGCTATG	187
SLC7A1	NM_001310833.1	F: GTGTGGATTCTTGCTGCGAT R: GCATCCAGACAGCAAATCGT	190
Y^+L2	XM_021278685.1	F: TTGTGGGTCTGTCTATCGCA R: CACTGCAGACATCTAGGCCT	247
B0	XM_005012939.3	F: TTCCACCCAGAGAAGAGCTG R: CGTGACTGCAGTGTGTGAAA	223
ACTB	NM_001310421.1	F: GCTATGTCGCCCTGGATTT R: GGATGCCACAGGACTCCATAC	174

CD36 = CD36 molecule; *FABP2* = fatty acid-binding protein 2; *SLC2A1* = facilitated glucose transporter member 1; *SLC15A1* = solute carrier family 15 member 1; *SLC5A1* = sodium/glucose cotransporter 1; *SLC7A1* = high affinity cationic amino acid transporter 1; *Y^+L2* = *Y^+L* amino acid transporter 2; *B0* = B (0, +)-type amino acid transporter 1; *ACTB* = β-actin.

nonlinear responses for egg production and daily egg mass, nonlinear regression analysis was used to estimate the optimal dietary barley level. The following nonlinear equation was applied: $y = ax^3 + bx^2 + cx + d$, in which y = dependent variable (egg production or daily egg mass), x = independent variable (dietary barley level, %); a, b, c = slopes corresponding to x^3, x^2, x, respectively, d = intercept of the line. $P < 0.05$ was chosen to indicate significant differences.

3. Results

3.1. Effects of barley and glucanase on production performance in laying ducks

The egg production and daily egg mass were significantly affected by dietary barley content. Highest egg production was obtained with 15% barley (Table 3); other levels had no effect. Glucanase supplementation increased egg production by 10%; there was no interaction between barley content and glucanase. The average egg weight was not affected by barley or enzyme supplementation. Dietary content of barley did not affect feed intake of ducks, but glucanase tended to decrease feed intake ($P = 0.09$). FCR was affected by barley content, the lowest (most efficient) being observed with 15% barley. Overall, supplementation with glucanase decreased FCR ($P < 0.001$). Gizzard weight relative to BW was not affected either by barley or glucanase. Based on the regression equation, the estimated optimal dietary barley level, without supplementation of glucanase, is 13.54% for maximal egg production and 13.14% for maximal daily egg mass in laying ducks (Fig. 1A). For diets containing glucanase, the estimated optimal dietary barley level is 15.08% for maximal egg production and 12.88% for maximal daily egg mass (Fig. 1B).

Table 3
Effects of barley content and β-1-3,1-4-glucanase supplementation on the production performance of laying ducks.

Item	β-glucanase[1]	Dietary barley level, %					Analysis of variance					
		0	15	30	45	60	S.E.[2]			*P*-value		
							B	G	B × G	B	G	B × G
Egg production, %												
	+	65.9	71.4	66.0	65.9	69.1	5.18	4.42	2.85	0.0001	0.002	NS
	–	61.5	69.5	62.0	54.9	59.1						
Average egg weight, g												
	+	69.0	68.7	68.6	69.0	68.1	0.47	0.13	0.27	NS	NS	NS
	–	68.7	67.9	68.6	69.0	68.1						
Daily egg mass, g												
	+	45.5	49.1	45.1	45.1	46.8	3.45	3.05	0.96	<0.0001	0.001	NS
	–	42.1	47.2	42.5	37.9	40.3						
Feed intake, g												
	+	160	162	161	163	163	1.01	1.06	0.96	NS	0.10	NS
	–	163	165	163	163	163						
FCR												
	+	3.71	3.36	3.68	3.70	3.58	0.30	0.38	0.16	0.02	<0.0001	NS
	–	4.15	3.72	4.01	4.53	4.34						
Gizzard weight relative to BW, %												
	+	3.37	3.02	3.07	3.29	3.72	0.20	0.06	0.37	NS	NS	NS
	–	3.31	3.26	3.59	3.41	3.37						

NS = not significant.
[1] +: with β-glucanase (1.5 g/kg); –: without β-glucanase.
[2] S.E: pooled standard error of fixed effects; B: barley; G: β-glucanase; B × G: interaction of barley and β-glucanase.

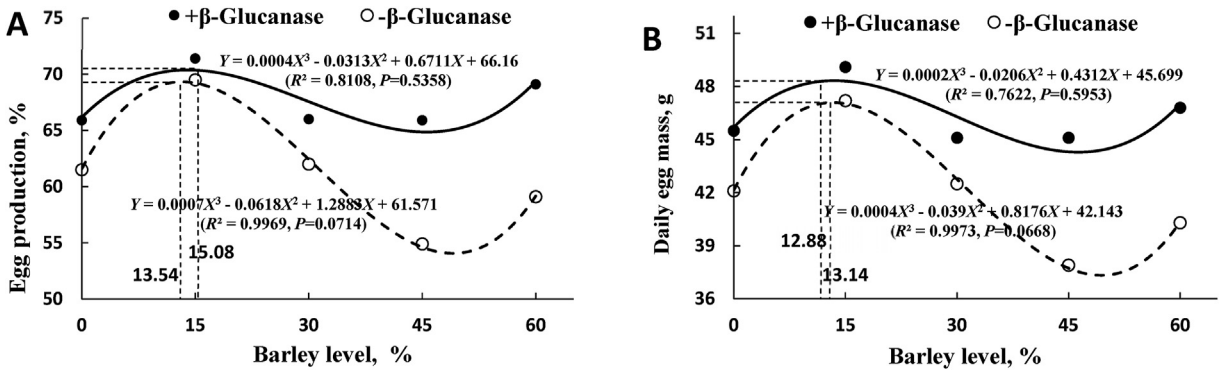

Fig. 1. Regression analysis between (A) egg production or (B) daily egg mass and dietary barley levels. The following estimated regression equation was obtained: $y = ax^3 + bx^2 + cx + d$, in which y = dependent variable (egg production or daily egg mass), x = independent variable (dietary barley level, %); a, b, c = slopes corresponding to x^3, x^2, x, respectively, d = intercept of the line. Based on the regression equation, the estimated value of optimal dietary level was obtained when egg production or daily egg mass had its maximal value.

Table 4
Effects of barley and β-1-3,1-4-glucanase on egg quality of laying ducks.

Item	β-glucanase[1]	Dietary barley level, %					Analysis of variance					
		0	15	30	45	60	S.E.[2]			*P*-values		
							B	G	B × G	B	G	B × G
Egg shape index												
	+	72.8	72.3	72.3	71.2	72.2	0.52	0.29	0.60	NS	NS	NS
	–	72.4	71.3	71.2	72.2	71.6						
Yolk color score												
	+	5.33	5.00	4.08	4.25	2.58	1.46	0.11	0.30	<0.0001 (L, Q)[3]	NS	NS
	–	5.50	5.08	4.75	3.75	2.92						
Albumen height, mm												
	+	6.84	6.14	6.44	6.41	6.73	0.36	0.05	0.08	NS	NS	NS
	–	6.79	6.15	6.28	6.43	6.51						
Haugh unit												
	+	79.7	73.7	75.9	76.0	78.5	0.25-	0.40	3.04	NS	NS	NS
	–	79.1	74.2	74.9	75.6	77.2						
Shell strength, N												
	+	4.11	4.12	4.11	3.99	3.96	0.10	0.02	0.05	NS	NS	NS
	–	4.03	4.17	3.99	3.95	4.00						
Shell thickness, mm												
Blunt												
	+	0.32	0.31	0.31	0.31	0.30	0.005	0.005	0.007	NS	NS	NS
	–	0.32	0.31	0.31	0.31	0.30						
Middle												
	+	0.34	0.34	0.34	0.33	0.34	0.007	0.003	0.009	NS	NS	NS
	–	0.34	0.34	0.33	0.32	0.32						
Sharp												
	+	0.32	0.32	0.32	0.31	0.32	0.008	0.001	0.006	NS	NS	NS
	–	0.32	0.32	0.33	0.32	0.30						
Average												
	+	0.33	0.32	0.32	0.32	0.32	0.006	0.003	0.005	NS	NS	NS
	–	0.32	0.32	0.32	0.32	0.31						
Egg composition, %												
Albumen												
	+	58.4	57.5	57.7	56.9	57.3	0.67	0.36	0.31	NS	NS	NS
	–	57.2	56.9	57.6	56.3	57.1						
yolk												
	+	32.3	33.4	33.1	34.2	33.5	0.86	0.43	0.32	0.08	0.10	NS
	–	33.6	33.9	33.2	34.8	34.0						
shell												
	+	9.28	9.16	9.30	8.92	9.16	0.19	0.04	0.11	NS	NS	NS
	–	9.20	9.24	9.20	9.01	8.89						

NS = not significant.
[1] +: with β-glucanase (1.5 g/kg); –: without β-glucanase.
[2] S.E: pooled standard error of fixed effects; B: barley; G: β-glucanase; B × G: interaction of barley and β-glucanase.
[3] Regression effect of barley level; L: linear effect, $y = bx + c$; Q: quadratic effect, $y = dx^2 + ex + f$; y: trait; x: dietary barley level, %.

3.2. Effects of barley and glucanase on egg quality in laying ducks

The effects of barley or glucanase on egg quality variables were negligible, except for yolk color score (Table 4). Yolk color score decreased with increasing barley content, but was not affected by adding glucanase. Neither barley nor glucanase affected the proportions of albumen, yolk or shell.

3.3. Effects of barley and glucanase on indices of digestive function

The activities of chymotrypsin and amylase in duodenal digesta were un-affected by dietary barley content but they were increased by glucanase supplementation ($P < 0.05$, Table 5). Activities of lipase and trypsin in digesta were not affected by either barley content or enzyme supplementation. Dietary barley had significant effects on the activities of alkaline phosphatase and maltase in jejunal mucosa, with highest activities when diets containing 30% or 15% barley were fed (Table 5). Enzyme supplementation had no effects on any of the jejunal mucosal enzymes examined.

The transcripts of *CD36* (fatty acid transporter) and *b0* (a neutral amino acid transporter) in jejunal mucosa decreased with increasing barley inclusion (Table 6) but those of Y^+L (amino acid transporter) and *FABP2* (L-type fatty acid binding proteins) increased. Mucosal gene transcripts of glucose transporters (*SLC5A1*, *SLC2A1*) and amino acid transporters (*SLC7A1* and Y^+L2) increased with glucanase supplementation but were not affected by dietary barley. Small-peptide (di and tripeptide) transporter (*PEPT/SLC15A*) transcripts were not affected by either barley or glucanase in the diet.

3.4. Effects of barley and glucanase on glucan and viscosity of digesta and cecal volatile fatty acids

Glucanase supplementation decreased the glucan content of digesta ($P < 0.05$) but dietary barley tended to increase it ($P = 0.1$, Table 7). Glucanase supplementation decreased the viscosity of digesta ($P < 0.05$), whereas barley had no effects. Similarly, barley did not affect the total concentration of volatile fatty acids in cecal contents, whereas they were reduced by glucanase supplementation. Barley did not affect the proportions of individual volatile fatty acids in cecal contents, but glucanase supplementation increased the proportion of just acetic acid.

4. Discussion

In this study, barley inclusion had no effects on either egg production or FCR of laying ducks when dietary barley level was less than 15%, and the estimated optimal dietary barley level is around 13% for maximal production performance of laying ducks. Interestingly, feed intake of the laying ducks was not altered by increasing dietary barley although the content of β-glucan in the experimental diets ranged from 1.5% to 6.17%. This is probably because the digesta viscosity was not affected by the NSP from barley, as digesta viscosity was reported to be negatively related to the passage rate of digesta and feed intake (Almirall et al., 1995). The production performance of laying ducks was compromised when dietary barley level was higher than 15%, although it was reported that adult birds were better able to cope with NSP in barley-based diets than young birds (Salih et al., 1991; Svihus and Gullord, 2002). This indicates that, without enzyme

Table 5
Effects of barley and β-1-3,1-4-glucanase on the activities of duodenal digestive enzymes and jejunal brush border enzymes in laying ducks.

Enzyme	β-glucanase[1]	Dietary barley level, %					Analysis of variance					
		0	15	30	45	60	S.E.[2]			*P*-values		
							B	G	B × G	B	G	B × G
Duodenal digesta												
Chymotrypsin, U/mg prot												
	+	13.0	12.4	7.40	9.74	8.11	1.22	1.18	1.34	NS	0.05	NS
	−	6.43	9.64	5.75	6.89	8.00						
Lipase, U/g prot												
	+	15.9	20.8	15.5	15.9	19.8	5.48	4.0	5.43	NS	NS	NS
	−	15.8	24.5	13.0	15.4	14.3						
Amylase, U/mg prot												
	+	13.3	16.1	12.3	14.3	12.5	1.62	2.42	2.28	NS	0.03	NS
	−	8.53	12.0	10.5	13.0	11.6						
Trypsin, U/mg prot												
	+	3,222	3,334	1,911	3,175	3,025	1.18	1.00	1.26	NS	NS	NS
	−	1,915	2,790	2,622	2,986	2,777						
Activities of jejunal mucosal brush border enzymes												
Alkaline phosphatase, U/mg prot												
	+	6.49	8.49	6.98	5.82	5.61	1.23	0.43	2.77	NS	NS	0.03
	−	7.08	3.87	8.45	4.85	6.05						
Maltase, U/mg prot												
	+	114	136	110	127	120	14.5	2.21	12.2	0.01	NS	NS
	−	104	141	109	117	112						
Sucrase, U/mg prot												
	+	63.3	64.3	73.6	61.8	77.6	7.67	2.59	22.4	NS	NS	<0.01
	−	84.3	82.7	76.0	69.8	56.1						

NS = not significant.
[1] +: with β-glucanase (1.5 g/kg); −: without β-glucanase.
[2] S.E: pooled standard error of fixed effects; B: barley; G: β-glucanase; B × G: interaction of barley and β-glucanase.

Table 6
Effects of barley and β-1-3,1-4-glucanase on transcript abundance in the jejunal mucosa of laying ducks.

Gene	β-glucanase[1]	Dietary barley level, %					Analysis of variance					
		0	15	30	45	60	S.E.[2]			*P*-values		
							B	G	B × G	B	G	B × G
CD36												
	+	1.27	0.94	0.88	0.61	0.70	0.26	0.07	0.366	0.05 (L)[3]	NS	NS
	−	1.00	0.84	0.76	0.86	0.67						
FABP2												
	+	0.69	1.14	1.68	3.29	3.84	1.03	0.26	0.68	<0.0001 (L, Q)[3]	0.08	0.07
	−	1.00	1.35	1.61	2.06	2.17						
SLC5A1												
	+	0.93	1.00	1.40	0.85	1.34	0.155	0.02	0.28	NS	0.05	0.08
	−	1.00	0.92	1.01	0.94	0.75						
SLC15A1												
	+	0.97	1.02	1.45	1.30	1.30	0.34	0.07	0.18	NS	NS	NS
	−	1.00	0.91	1.45	1.86	1.39						
SLC2A1												
	+	1.37	1.40	1.81	1.38	1.55	0.19	0.43	0.16	NS	<0.0001	NS
	−	1.00	0.93	1.07	0.77	0.85						
SLC7A1												
	+	0.93	0.58	0.99	0.96	0.96	0.11	0.27	0.44	NS	0.02	NS
	−	1.00	1.66	1.08	1.32	1.14						
Y^+L2												
	+	1.66	4.65	3.84	9.80	4.03	2.45	1.30	3.24	0.005	0.04	NS
	−	1.00	1.44	5.58	3.30	1.86						
B0												
	+	1.06	0.55	0.49	0.36	0.52	0.40	0.05	0.09	0.001	NS	NS
	−	1.00	0.30	0.46	0.53	0.40						

CD36 = CD36 molecule; *FABP2* = fatty acid-binding protein 2; *SLC5A1* = sodium/glucose cotransporter 1; *SLC15A1* = solute carrier family 15 member 1; *SLC2A1* = facilitated glucose transporter member 1; *SLC7A1* = high affinity cationic amino acid transporter 1; *Y^+L2* = *Y^+L* amino acid transporter 2; *B0* = B (0, +)-type amino acid transporter 1; NS = not significant.

[1] +: with β-glucanase (1.5 g/kg); −: without β-glucanase.

[2] S.E: pooled standard error of fixed effects; B: barley; G: β-glucanase; B × G: interaction of barley and β-glucanase.

[3] Regression effect of barley level; L: linear effect, $y = cx + d$; Q: quadratic effect, $y = ex^2 + fx + g$; y: trait; x: dietary barley level, %.

supplementation, the negative effects of NSP from barley on the production performance should be a great concern when applied in the feed of laying ducks.

It is of great interest to explain here why 15% barley resulted in the best egg production in laying ducks. This is probably because 15% barley stimulated mucosal activities of dissacharidase (maltase) in the intestine of ducks and, therefore, exerted positive effects on the digestion and absorption of dietary carbohydrates. Similarly, in laying hens, dietary inclusion of wheat which contains a high content of NSP increased the activity of aminopeptidase in the small intestine (Mirzaie et al., 2012) and increased intestinal villus height in the jejunum (Shao et al., 2013), indicating a positive role of NSP in improving intestinal morphology and digestive function. Considering that the diets with 15% barley contained 1.8% glucan (measured value) and 1.6% to 4.5% of other NSP (calculated value), it is assumed that the NSP in barley might also play role as prebiotics in modulating gut function. It was reported that dietary β-glucan supplementation at 20 or 40 mg/kg of young broiler chickens increased lymphocyte activity in intestine (Guo et al., 2003). With regard to the role in modulating microbiota composition, oat and barley-derived β-glucans have been demonstrated to increase the intestinal population of beneficial bacteria in pigs (reviewed, Tiwari et al., 2019), older healthy human volunteers (Mitsou et al., 2010) and in patients with high risk for developing metabolic syndrome (Velikonja et al., 2019). Effects of glucan on intestinal microbiota composition in birds, however, are controversial. Some research showed no effects of β-glucan or wheat bran-derived NSP either in broilers (Li et al., 2018; Torki et al., 2018) or in laying hens (Walugembe et al., 2015), but some research reported oral administration of 4 mg/kg β-glucan to 13 d-old chickens for 14 d increased the cecal population of *Bifidobacterium* and *Lactobacillus* (Wang et al., 2019). In the present study, the total cecal VFA concentrations in ducks were not increased by barley inclusion, similar to the findings in growing turkeys (Gonzáonzh-Ortiz et al., 2017). Therefore, whether the NSP in barley would play positive role as prebiotics in laying ducks remains to be elucidated in future study.

In the present study, dietary inclusion of barley higher than 15% compromised egg production performance of laying ducks probably because of high content of NSP in barley. Glucanase supplementation to barley-based diet, however, could reverse the negative effects. As expected, supplementation with β-1,3-1,4-glucanase significantly increased egg production and increased feed efficiency of the laying ducks; dietary barley could reach up to 60% (complete replacement of corn) when glucanase was supplemented. The results support findings in hens that supplementation of barley-based diets with β-glucanase/xylanase enzyme complex improved egg production and FCR (Lázaro et al., 2003; Mathlouthi et al., 2003). Others have found that supplementation of barley-based diets with β-glucanase alone or β-glucanase/pentosanase enzyme complex had no effects on egg production performance in adult laying hens (Brenes et al., 1993; Hamilton and Proudfoot, 1993). These differences are probably related to the enzyme composition and animal ages. The results of the present study with laying ducks demonstrate that supplementation with β-1,3-1,4-glucanase alone did increase egg production (approximately 10%) and feed efficiency (approximately 13%). This is probably because the β-1,3-1,4-glucanase can effectively break down cell wall NSP in barley (Mathlouthi et al., 2003), increasing accessibility of the starch to gut enzymes. It is reported that β-1,3-1,4-glucanase was more effective than β-1,4-glucanase in improving the nutritive value of barley-based diets for poultry because the barley β-glucans are linear homopolysaccharides (of glucose) with approximately

Table 7
Effects of barley and β-1-3,1-4-glucanase on β-glucan content in duodenal digesta and proportions of VFA in the cecal contents.

Variable	β-glucanase[1]	Dietary barley level, %					Analysis of variance					
		0	15	30	45	60	S.E.[2]			*P*-values		
							B	G	B × G	B	G	B × G
Duodenal digesta β-glucan content, %												
	+	1.23	1.96	1.70	2.10	3.15	1.09	0.70	0.39	0.10	0.05	NS
	−	1.49	3.65	3.22	3.60	4.58						
Digesta viscosity, cps												
	+	2.90	2.56	3.17	2.49	2.96	0.02	0.06	0.13	NS	0.001	0.0001
	−	3.13	3.32	2.90	3.53	2.83						
Total VFA, mg/g												
	+	1.10	0.75	1.19	0.75	0.82	0.18	0.22	0.28	NS	0.05	NS
	−	0.92	1.01	1.24	1.52	1.53						
Proportions, %												
Acetic acid												
	+	26.7	29.4	27.0	27.6	32.1	0.56	1.93	2.62	NS	0.01	0.09
	−	26.2	24.9	26.5	27.2	22.3						
Propionic acid												
	+	50.6	33.4	49.9	52.5	40.7	6.22	1.34	5.60	NS	NS	NS
	−	44.7	44.9	48.0	48.1	51.1						
Isobutyric acid												
	+	0.38	2.32	1.64	1.07	1.25	1.51	0.01	0.20	NS	NS	NS
	−	2.31	2.43	0.61	1.47	0.22						
Butyric acid												
	+	20.1	25.8	16.1	14.1	19.6	3.85	0.13	3.35	NS	NS	NS
	−	19.7	19.4	22.0	18.1	21.5						
Isovaleric acid												
	+	0.28	3.33	2.15	1.77	2.43	2.15	0.25	0.67	NS	NS	NS
	−	2.65	3.59	0.66	1.98	0.22						
Valeric acid												
	+	2.03	5.84	3.17	2.92	3.99	2.29	0.09	0.25	NS	NS	NS
	−	4.51	4.75	2.27	3.08	4.61						

VFA = volatile fatty acids; NS = not significant.
[1] +: with β-glucanase (1.5 g/kg); −: without β-glucanase.
[2] S.E: pooled standard error of fixed effects; B: barley; G: β-glucanase; B × G: interaction of barley and β-glucanase.

70% (1 → 4)-linkages and 30% (1 → 3)-linkages (Lazaridou and Biliaderis, 2007; Fernandes et al., 2016). The reduced glucan content and viscosity in the digesta of ducks when glucanase was supplemented in barley-based diets indicates that exogenous glucanase supplementation can effectively break down the barley-derived glucan. The reduced digesta viscosity, however, did not lead to increased feed intake of laying ducks. On the other hand, addition of glucanase decreased the total cecal content of volatile fatty acids, probably indicating decreased fermentable fiber, including glucan, reaching the cecum. This was expected because glucanase reduced glucan content in the intestinal digesta. The reason for glucanase supplementation increasing the proportion of acetate is not obvious but presumably reflects the altered substrates being presented to the cecal microbes.

Increased feed efficiency by adding β-glucanase is probably due to decreasing the anti-nutritive effects of glucan and enhancing digestive function. There were likely positive effects of the β-glucanase on nutrient digestion and absorption, as reflected in increased activities of digestive enzyme (amylase and chymotrypsin), as well as increased mucosal expression of nutrient transporter genes. Similar to the present findings, previous study showed that barley replacement of corn reduced amylase and lipase activities in small intestinal contents in broiler, but β-glucanase addition increased these activities, along with a reduction in intestinal viscosity (Almirall et al., 1995).

5. Conclusion

The estimated optimal dietary barley level in the diets of laying ducks is around 13% for maximal production performance, >15% barley in feed may comprise egg production of laying ducks but dietary supplementation with glucanase increased egg production and feed efficiency, probably by increasing the exposure of feed nutrients and enhancing digestive function of the laying ducks.

Author contributions

Wei Chen: conceptualization, methodology, writing; Shuang Wang: methodology, analysis, project administration; Runsheng Xu: analysis; Weiguang Xia: project administration; Dong Ruan: project administration, investigation; Yanan Zhang: project administration; Khaled F. M. Abouelezz: methodology, writing and editing; Mahmoud M. M. Azzam, Ahmed M. F. Fouad: writing; Kaichao Li, Xuebing Huang: analysis; Shenglin Wang: writing; Chuntian Zheng: supervision.

Conflict of interest

We declare that we have no financial or personal relationships with other people or organizations that might inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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