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Japanese quail performance, intestinal microflora, and molecular responses to screened wheat and multienzyme diet

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Abstract: The present paper studied the effect of screened wheat (SW) levels and a multienzyme diet on Japanese quail performance, intestinal performance, and microbial population. A total of 480 unsexed 10-day-old quail chicks were assigned to a multienzyme diet (0 or 0.5 g/kg) and SW (0%, 5%, 10%, or 15%) in a 2 × 4 factorial arrangement of treatments with 4 replications and 15 quails in each in a completely randomized design. No differences were observed in performance between diets at 10 to 36 days of age. Increases in dietary SW levels decreased villus height, villus width, villus surface, and height:crypt depth ratio and increased crypt depth (linear; $P < 0.01$). Increasing SW levels in diets decreased the number of lactobacilli of the cecum and ileum (linear; $P < 0.01$), while it increased the number of Enterobacteriaceae in the cecum and ileum (quadratic; $P < 0.01$). Increasing SW level in diets decreased the population of lactobacilli, increased Enterobacteriaceae, and reduced intestinal morphology growth. Protein synthesis and DNA contents of the jejunal mucosa were decreased linearly with increasing levels of SW in diets ($P < 0.05$). Finally, it was concluded that inclusion of SW up to 15% had no adverse effect on performance, molecular, and intestinal morphological parameters of Japanese quail.

Key words: Intestine, Japanese quail, performance, multienzyme, screened wheat

1. Introduction

Corn, as a major portion of energy in poultry diets, has a high demand (57% to 70%) despite its higher prices. It could be replaced by screened wheat (SW) prepared after harvesting and processing of wheat in flour and macaroni factories. Better balance of proteins and amino acids in SW than in cereals has made it a good alternative to corn. However, there is large variation in the chemical composition of SW due to differences in sources of wheat (e.g., soft vs. hard (1)) and in processing techniques (2). Diet composition has a crucial effect on the microbial community and its activity in the bird's intestinal tract. SW contains nonstarch polysaccharides (NSPs) that modify gut microflora and speed up fermentation in the small intestine, and thus significantly impede the intake of nutrients (3).

The intestine has an inherent ability to create and maintain regional differences with regard to mucosal structure, and especially villus height. These differences are noticeable in mammals and have been observed in poultry (4). Moreover, the morphology of the mucosa in different segments of the small intestine undergoes considerable changes with aging, thereby increasing the efficiency of the intestinal functions (5). Wu et al. (6) reported a similar effect of microbial enzymes on intestinal morphometry with cereal-based diets. Inclusion of cereals rich in NSP increases the viscosity of the digesta, reduces apparent nutrient digestibility (7), and alters bacterial profiles and gut physiology status. Birds do not produce enzymes capable of degrading NSP. Therefore, the lack of enzymatic capacity might be compensated for by supplementation of the diet with exogenous enzymes.

Little information is available on quail performance addressing the morphological and microbial development and the protein synthesis in the small intestine in response to inclusion of SW and a multienzyme diet.

2. Materials and methods

2.1. Birds, housing, and diets

This study was conducted at the Quail Research Farm, Department of Poultry, Faculty of Agriculture, Bu-Ali Sina University. All experimental procedures were carried out according to the local experimental animal care committee and were approved by the institutional ethics committee. A total of 480 unsexed 10-day-old quail chicks were randomly assigned to 8 treatments with the same average body weight. Treatments included 4 replicates with 15 quail chicks in each. Completely randomized designs in a

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factorial arrangement were modulated with 0%, 5%, 10%, or 15% of SW included with 0 or 0.5 kg of a multienzyme supplement (Aras Feed Company: phytase 1,000,000 FTU/kg, lipase 2,000,000 U/kg, xylanase 20,000,000 U/kg, endo-1,3(4)-beta-glucanase 3,000,000 U/kg, cellulase complex 5,000,000 U/kg, alpha-amylase 2,000,000 U/kg, protease 2,000,000 U/kg) per ton. Feed and water were supplied ad libitum and light was provided 24 h/day and gradually reduced up to 23 h a day. The temperature was also gradually reduced by 3 °C per week from the initial 37 °C. Diets were based on yellow corn, soybean meal, and SW (Table 1). Diets were isoenergetic in the whole period and formulated based on the National Research Council's 1994 standards. Body weight (BW), feed intake (FI), feed conversion ratio (FCR), and production index (PI) were measured.

Table 1. Feed formulation calculated and analyzed contents of main nutrients.

Ingredients	0 g multienzymes/kg				0.05 g multienzymes/kg			
Corn grain	55.75	52.80	49.85	46.90	55.70	52.75	49.80	46.85
Soybean meal	30	30	30	30	30	30	30	30
Screened wheat	0	5	10	15	0	5	10	15
Corn gluten	4.60	3.92	3.24	2.54	4.60	3.92	3.24	2.54
Fish meal	3	3	3	3	3	3	3	3
Oyster shell	2.98	2.57	2.15	1.74	2.98	2.57	2.15	1.74
Dicalcium phosphate	2.78	1.86	0.94	0.02	2.78	1.86	0.94	0.02
Salt	0.32	0.31	0.3	0.3	0.32	0.31	0.3	0.3
Lysine	0.07	0.04	0.02	0	0.07	0.04	0.02	0
Mineral premix [a]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin premix [b]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Multienzyme [c]	0	0	0	0	0.05	0.05	0.05	0.05
ME (kcal/kg)	2800	2800	2800	2800	2800	2800	2800	2800
Protein (%)	23.2	23.2	23.2	23.2	23.2	23.2	23.2	23.2
Crude fiber (%)	3.5	4.02	4.54	5.05	3.5	4.02	4.54	5.05
Lysine (%)	1.26	1.26	1.26	1.27	1.26	1.26	1.26	1.27
Met+Cys (%)	0.79	0.79	0.79	0.79	0.79	0.79	0.79	0.79
Sodium (%)	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Calcium (%)	1.97	1.62	1.26	0.9	1.97	1.62	1.26	0.9
Available phosphorus (%)	0.74	0.61	0.47	0.34	0.74	0.61	0.47	0.34
Electrolyte balance	208.9	216.7	224.3	231.9	208.9	216.7	224.3	231.9
Analyzed								
Dry matter (%)	90.91	90.91	91.22	91.15	90.56	90.84	91.32	91.29
Crude protein (%)	23.22	23.25	23.25	23.19	23.22	23.25	23.25	23.19
Crude fiber (%)	3.98	4.40	4.90	5.15	3.98	4.40	4.90	5.15
Ether extract (%)	4.25	4.25	4.10	4.18	4.28	4.25	4.15	4.19
Ash (%)	6.32	6.65	5.91	5.50	5.98	6.10	5.75	6.31

[a] Vitamin premix (g/kg diet), B1 3.3, B2 0.72, K3 1.6, E 14.4, D 7, A 7.7, pantothenic acid 12, pyridoxine 6.2 mg/kg diet, B12 14.4, choline chloride 440 mg/kg diet. [b] Mineral premix (g/kg diet), Mn 72, Cu 10, Zn 100, Fe 100, I 2, Co 0.2 . [c] Multienzyme mix contains phytase, lipase, β-glucanase, xylanase, α-amylase, protease, pentosanase, hemicellulase, cellulase and pectinase.

PI = (% Livability × live weight/feed conversion × slaughtering age):10

2.2. Intestinal morphology

At 42 days of age, middle sections of the jejunum (3 to 4 cm) of 2 birds per replicate were cut and histological indices were measured according to the method reported by Iji et al. (8). Formalin-fixed jejunum tissue samples were dehydrated, cleared, and saturated with paraffin. The processed tissue was then embedded in paraffin wax and cut into 6-µm sections with a microtome (LEICA RM 2145). The slides were stained with hematoxylin and eosin. Morphometric indices were recorded from these sections using a computer-aided light microscope (Olympus Microscope, Olympus Corporation, Tokyo, Japan) by tenfold magnification with an image analyzer (Motic Images 2000 1.2, Scion Image, Xiamen, China). Twenty villi on each slide were prepared for villus height, width, and surface calculations (9). Morphometric variables analyzed included villus height (from the tip of the villus to the villus crypt junction), crypt depth (defined as the depth of the invagination between adjacent villi), villus width, and villus surface based on the method of Iji et al. (8).

2.3. Microbial sampling

In 42 days of age, two quails were randomly selected and slaughtered from each replicate and the complete intestinal tract was removed and transferred into an anaerobic chamber immediately after dressing (10). The intestinal digesta were gently removed into sterile sampling tubes and immediately transferred on ice to the microbial laboratory. Intestinal content of the ileum and ceca were used for microbial study. Media and incubation digesta were homogenized and diluted by physiological salt solution (0.9% NaCl). To separate lactic acid bacteria and Enterobacteriaceae, MRS agar (Merck, Germany) and 0.1% Tween 80 and Violet Red Bile agar media (Merck) were used. The number of lactic acid bacteria and Enterobacteriaceae were calculated after incubation in an anaerobic chamber at 37 °C for 48 h and 24 h, respectively.

2.4. Mucosal protein and nucleic acid content

Cell size and metabolic activity were calculated through measurements of mucosal protein, DNA, and RNA and the ratios of these three factors. Waterlow et al. (11) described the relevance of the assessed biochemical indices.

2.5. Protein assay

The protein content of the mucosal homogenates of the jejunum was tested according to Bradford method (12), which uses bovine serum albumin as a standard. Samples were frozen in liquid nitrogen and ground to a fine powder and indices were measured by spectrophotometer (UV) at a wavelength of 595 nm.

2.6. Mucosal DNA

DNA was extracted from crude mucosal homogenates of the jejunum, using the method described by Doyle and Doyle (13). Samples were collected from the middle part of the jejunum and the mucosa was cut, separated, and transferred to a microtube. DNA was extracted in acetyl trimethyl ammonium bromide. DNA quantity was measured by a spectrophotometer (UV) at a wavelength of 260 nm.

2.7. Mucosal RNA

RNA was extracted with the RNX-Plus Solution Kit (CinnaGen, Tehran, Iran) from mucosal homogenates of the jejunum. For RNA extraction from the jejunum, samples were homogenized by applying liquid nitrogen. RNA was extracted using guanidinium thiocyanate with a spectrophotometer (UV) at a wavelength of 260 nm. This procedure was described by Waterlow et al. (11).

2.8. Statistical analyses

Data were fed into and analyzed by ANOVA using the GLM procedures of SAS software as appropriate for a factorial arrangement of treatments in a completely randomized design. The statistical model included the effects of wheat screening, multienzyme supplementation, and their interactions. Orthogonal polynomial contrast coefficients were used to determine linear and quadratic effects of increasing levels of SW in diets in all measurements. An alpha level of $P < 0.05$ was used as the criterion for statistical significance.

3. Results

3.1. Performance

Table 2 gives data for performance. No differences were found in FI, FCR, or PI for treatments at 10 to 36 days of age.

3.2. Intestinal morphology

Table 3 shows the effect of dietary treatment on intestinal morphology. SW had a linear effect on intestinal mucosal morphology. Increases in SW decreased of villus height ($P = 0.0001$), villus width ($P < 0.05$), villus surface area ($P < 0.0001$), and villus height:crypt depth ($P < 0.0001$) and increased crypt depth ($P = 0.01$). In addition, there was a significant interaction between SW and multienzyme addition for the above-mentioned parameters ($P < 0.05$), except for villus surface. Multienzyme inclusion can revive suppressed intestinal morphology growth.

3.3. Microbial population

SW influenced the distal intestinal microflora (Table 4). Using more SW in the diet decreased the number of lactobacilli in the cecum and ileum (linear) and increased the number of Enterobacteriaceae in the cecum and ileum (linear and quadratic). Multienzymes in diet showed a significant interaction, except for lactobacilli in the ileum.

Table 2. Effects of dietary treatments on performance of quail chicks (10 to 36 days).

Item	Multienzyme	SW[a]	FI [b] (g)	BW [c] at 36 days (g)	FCR [d]	PI [e]
Treatment	(g/kg)	(%)				
	0	0	497.9	228.8	2.52	348.7
		5	476.6	231.5	2.59	315.7
		10	470.8	225.5	2.60	323.4
		15	466.3	221.3	2.65	304.7
	0.05	0	522.2	234.2	2.84	305.2
		5	520.8	238.7	2.61	332.7
		10	510.6	228.9	2.85	276.6
		15	463.7	224.2	2.49	346.0
SEM			25.73	6.25	0.11	19.45
P-value						
Multienzyme			0.36	0.53	0.54	0.50
SW						
Linear			0.09	0.07	0.60	0.53
Quadratic			0.69	0.17	0.64	0.37
Multienzyme × SW			0.79	0.64	0.19	0.09

[a] Screened wheat, [b] feed intake, [c] body weight, [d] feed conversion ratio, [e] production index = (% Livability × live weight/feed conversion × slaughtering age):10. SEM, standard error of the mean. Orthogonal polynomial contrast coefficients were used to determine the linear and quadratic effect of dietary treatments.

Table 3. Effects of different treatments on quail intestine mucosal morphology at 42 days of age.

Item	Multienzyme	SW[a]	VH[b] [mm]	VW[c] [mm]	CD[d] [mm]	VSA[e] [m^2]	VH:CD[f]
Treatment	(g/kg)	(%)					
	0	0	1010.4^{ab}	132.5	129.2	0.134^{a}	7.83^{ab}
		5	972.6^{b}	130.7	144.2	0.127^{a}	6.76^{bc}
		10	841.5^{c}	125.0	155.0	0.105^{b}	5.50^{c}
		15	779.8^{c}	124.7	147.0	0.097^{b}	5.37^{c}
	0.05	0	1067.6^{a}	134.7	129.8	0.142^{a}	8.90^{a}
		5	941.0^{b}	137.2	147.8	0.128^{a}	6.40^{bc}
		10	840.3^{c}	129.1	159.0	0.109^{b}	5.28^{c}
		15	796.1^{c}	125.4	157.1	0.099^{b}	5.20^{c}
SEM			30.46	9.42	4.61	0.005	0.62
P-value							
Multienzyme			0.49	0.15	0.07	0.16	0.5
SW							
Linear			0.0001	0.0300	0.0100	< 0.001	<0.0001
Quadratic			0.500	0.7200	0.1100	0.7700	0.6600
Multienzyme × SW			0.0100	0.0300	< 0.001	0.1100	<0.0001

[a] Screened wheat. [b] Villus height. [c] Villus width. [d] Crypt depth. [e] Villus surface area. [f] Villus height:crypt depth. Means without superscripts in the same column are not different at P < 0.05. SEM, standard error of the means. Orthogonal polynomial contrast coefficients were used to determine the linear and quadratic effect of dietary treatments.

Table 4. Distal intestinal microflora [log CFU/grams of digesta] by SW and multienzyme inclusion at 42 days of age.

Item	Multienzyme	SW [a]	Cecum		Ileum	
Treatment	(g/kg)	(%)	LAC[b]	ENT[c]	LAC	ENT
	0	0	5.29[a]	4.55[d]	5.19[a]	4.12[c]
		5	5.28[ab]	4.62[cd]	5.22[a]	4.26[c]
		10	5.19[bc]	4.83[bc]	5.11[ab]	4.69[b]
		15	5.13[c]	4.38[ed]	4.95[bc]	4.06[c]
	0.05	0	5.22[abc]	4.89[b]	5.09[bc]	4.76[ab]
		5	5.16[c]	5.21[a]	4.95[ab]	5.06[a]
		10	4.96[d]	4.42[ed]	4.85[bc]	4.11[c]
		15	4.95[d]	4.18[e]	4.77[c]	4.01[c]
SEM			0.03	0.16	0.06	0. 12
P-value						
Multienzyme			<0.0001	0.1900	0.0001	0.0200
SW						
Linear			0.0001	<0.0001	0.0001	0.0008
Quadratic			0.7900	0.0002	0.4500	0.0020
Multi-enzyme× SW			0.0400	<0.0001	0.4500	<0.0001

[a] Screened wheat, [b] *Lactobacillus*, and [c] Enterobacteriaceae. Means without superscripts in the same column are not different at $P < 0.05$. SEM, standard error of the means. Orthogonal polynomial contrast coefficients were used to determine the linear and quadratic effect of dietary treatments.

Microbial populations did not experience a favorable effect in the cecum or ileum microflora.

3.4. Mucosal protein and nucleic acid content

As Table 5 indicates, SW changed the protein synthesis and DNA contents of the jejunal mucosa, which declined linearly with increasing introduction of levels of SW in diets ($P < 0.05$). No differences were found in protein synthesis, DNA or RNA concentration, and DNA/RNA, DNA/protein, and RNA/protein ratios in jejunal mucosa with different levels of SW and multienzyme inclusion in treatments.

4. Discussion

4.1. Performance

Quail performance was reported in consistence with previous studies. Sarica et al. (14) did not observe differences in quail growth performance when quails were fed wheat-based diets and enzymes. Langhout and Schutte (15) reported that wheat-based diets and enzyme supplementation did not bring changes in broiler chickens feed intake. Lavinia et al. (16) reported that feed intake was significantly decreased with an increase in dietary levels of wheat. The differences observed in these reports seem to be caused by differences in experimental methods, physical and chemical compositions of SW (especially metabolizable energy and NSP content), age and type of birds (17), levels of SW, and other environmental conditions. We hypothesize that the effect of multienzyme supplementation on growth performance may be associated with changes in the metabolic hormone concentrations and metabolites in quails fed with SW diets.

4.2. Intestinal morphology

Mucosa status and microscopic structures are effective indicators of the intestinal tract's response to diet. Changes in intestinal morphology such as shorter villi and deeper crypts have been associated with the presence of toxins. A short villus reduces the surface area for nutrient absorption. However, Khempaka et al. (18) reported that increased villus width did not necessarily lead to higher nutrient intake. The epithelial cells of the villus originate in the crypt and a large crypt indicates fast tissue turnover and a high demand for new tissues. Any additional tissue turnover will increase the need for nutrients for maintenance and will therefore reduce the efficiency of the bird. There is

Table 5. Protein and nucleic acids content in mucosa of quails at 42 days of age.

Item	Multienzyme	SW[a]	DNA[b]	RNA[c]	Protein[d]	DNA/RNA[c]	Protein/DNA[b]	Protein/RNA[b]
Treatment	(g/kg)	(%)						
	0	0	2401	2125	478.43	1.20	198.69	238.38
		5	2344	1975	459.54	1.21	196.98	239.86
		10	2268	1987	453.32	1.15	199.81	230.77
		15	1916	1609	383.98	1.24	200.30	248.16
	0.05	0	2546	2221	514.94	1.20	202.32	244.07
		5	2377	1986	477.05	1.22	200.89	245.84
		10	2324	2037	465.51	1.16	200.50	234.58
		15	2066	1796	416.26	1.17	201.59	237.53
SEM								
P-value								
Multienzyme			0.53	0.61	0.43	0.84	0.11	0.91
SW								
Linear			0.03	0.07	0.04	0.88	0.75	0.91
Quadratic			0.53	0.72	0.62	0.78	0.40	0.69
Multienzyme × SW			0.98	0.98	0.99	0.94	0.80	0.93

[a] Screened wheat, [b]ng/mL, [c]ng/µL, [d]µg/mL. SEM, standard error of the means, Orthogonal polynomial contrast coefficients were used to determine the linear and quadratic effect of dietary treatments.

evidence that lactobacilli and bifidobacteria can speed up the synthesis and secretion of mucin in the gut as a result of an increase in goblet cell number (1). It has been attributed to indigenous microbes that stimulate vascularization and development of the intestinal villi, thereby improving the efficiency of digestion and absorption (19).

Stress factors can lead to rapid changes in the intestinal mucosa due to the proximity of the mucosal surface to the intestinal content, although the performance data reported in this study do not show evidence of stress. Iji et al. (9) reported that inclusion of a viscous product in the diet led to deeper crypts in broilers at 14 days of age. It is thought that the viscous properties of grain have indirect effects on morphology, as improved bacterial activity in the digestive system is in line with changes in intestinal wall morphology (20). Deeper crypts resulting from SW levels may be due to an unusual demand for cell proliferation and tissue renewal. Heightened viscosity can lead to an increase in anaerobic bacterial activity, resulting in changes in villus characteristics. Consequently, reductions in the villus surface area and villus height:crypt depth ratio are expected to happen. Multienzyme inclusion in the diet could stifle intestinal morphological growth. Research has also reported that enzyme supplementation potentially reduces the cell proliferation rate in the crypt depth (21). Sarica et al. (22) reported that lower crypt depths might be related to a reduction in bacterial populations with whole wheat diets. The reduction in crypt depth may be connected to a lower secretion activity, for example a lower mucus production, and goblet cells particularly concentrated in the crypts depth (23). It can be concluded that the negative effects of NSPs are related to their ability to improve digesta viscosity, consequently leading to changes in gut morphology and in efficiency of nutrient intake by the chicks.

4.3. Microbial population

Since chicken ceca microbial activity is not very critical to nutrient-utilizing ability, little research has addressed the issue. Microorganisms colonizing in the gastrointestinal tract during the early posthatch period form a synergistic

relationship with their poultry host. Gastrointestinal microorganisms have a highly significant effect on the absorption of nutrients, utilization of energy, and response to antinutritional factors.

Stef et al. (24) concluded that increasing NSP-containing cereal had a negative effect on growth and development of lactic bacteria, but it will positively stimulate the growth and development of coliform bacteria in the cecum. Similar results were found in this study, since decreased lactic bacteria was reported with increasing SW due to the negative effect of NSPs. A reduction in the number of anaerobic microorganisms was indicated by stress effect in the intestinal microflora, a direct effect of SW in diet. This condition could bring about higher endogenous corticosteroid levels due to stress and may decrease mucin secretion, where the balance in the gut microflora is adversely affected, resulting in the overgrowth of coliforms and other pathogenic bacteria.

We found that viscosity and a high fiber level in low-quality SW may induce stress and modify the microfloral balance, since many lactic bacteria and Enterobacteriaceae in the ileum and cecum were detected in the control treatment (without SW and multienzyme inclusion). Shakouri et al. (25) found that enzyme supplementation explained the increase in the number of total anaerobic bacteria, lactobacilli, and microbial growth. Birds' responses to enzymes depend mainly on dietary cereal quality and quantity. However, the quantity and quality of fat, microbial status, bird age, and antimicrobial agents can also modify the effects of enzymes. Because of cereal quality (e.g., the complexity of carbohydrates) and the thermolabile nature of enzymes, improvements are not always observed (26).

In general, the present study suggests that an increase in intestinal viscosity may lead to an increase in the activity of certain bacteria capable of changing the villus character. Microbial populations could change the intestinal growth and development.

4.4. Mucosal protein and nucleic acid content

The increase in the mucosal DNA content was likely due to intense mitotic proliferation, which may reflect a compensatory mechanism to counterbalance the reduction in surface area (a consequence of reduced villus height and width) in order to improve the digestive and absorptive functions. The priority role in creating channels to pass nutrients across cell walls is a contributing factor making protein synthesis in the intestine important (27). Even with adequate nutrients in the diet, deficiency in protein synthesis in the intestine can be observed, which could result in lower animal performance (27). The concentrations of DNA and RNA and their ratios to protein content show natural variations during the development of the gastrointestinal tract in the pattern of changes described by Jin et al. (28), Uni et al.(5), and Iji (8). DNA/RNA, DNA/protein, and RNA/protein ratios are reliable parameters to understand the nature of intestinal developments. Waterlow et al. (11) proposed the ratio of RNA/protein as a measure of ribosomal capacity. The present study's results were consistent with those of Gabriel et al. (29), who reported no differences in DNA concentration or protein/DNA, RNA/DNA, and RNA/protein ratios. In their study, chickens were fed a standard feed containing 400 g ground wheat/kg or a similar diet with a portion of wheat given separately as whole grains, being increased progressively from 200 g/kg to 400 g/kg at 8 days and 22 days, respectively.

4.5. Conclusion

The present study found no differences in FI, FCR, and PI with SW and multienzyme inclusion in the diets of quail of 10 to 36 days of age. No differences were found in protein and nucleic acids in jejunum mucosae. No significant changes appeared in intestinal morphology and microbial populations or enzyme effect induced by inclusion of SW in the diet. However, intestinal morphology and microbial populations changed linearly with SW diet. Protein synthesis and DNA contents of the jejunal mucosa decreased linearly with increasing amounts of SW in the diet. It was concluded that inclusion of SW at up to 15% had no adverse effect on performance, molecular, and intestinal morphological parameters of Japanese quail.

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