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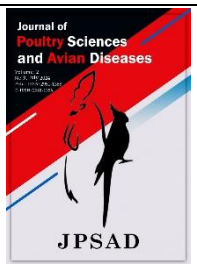

Growth performance and gut function of broiler chickens received barley-based diets with or without an enzyme mixture

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A B S T R A C T

An experimental study was performed to evaluate the responses of broiler chickens to dietary substitution of barley for corn. A total of 252 one-day-old broiler chicks were randomly distributed among 18 floor pens. Six such pens were then randomly assigned to each dietary treatment. Dietary treatments consisted of a corn-based diet, a barley-based diet, and the barley-based diet plus an enzyme mixture. Results indicated that barley substitution for corn significantly (p< 0.0001) impacted broiler performance criteria. However, enzyme supplementation significantly (p= 0.02) improved the feed conversion ratio to the extent obtained in the corn control. The villus absorptive surface area was shrunk in birds fed a barley diet compared to the corn diet. Nevertheless, supplementation of the barley diet with the enzyme mixture significantly (p= 0.02) restored the situation. The expression of the TLR2 gene was significantly down-regulated by replacing barley with corn. Enzyme supplementation of the barley diet significantly (p< 0. 001) up-regulated TLR2 gene expression but not to the extent observed in the corn control group. Significant increases were observed in circulatory levels of uric acid (p< 0.05), total protein (p<0.01), and albumin (p< 0.001) when barley was substituted for corn. Enzyme supplements did not make any significant difference to these variables. In conclusion, the substitution of barley for corn was associated with the poor performance of broilers. However, supplementing barley-based diets with the enzyme mixture improved chicken's performance through enhanced villus absorptive surface area. It reduced the turnover of enterocytes, as reflected in a lower crypt depth in the jejunum.

Keywords: *Cereal type, chicken, enzymes, gut, performance*

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1 Introduction

Barley (*Hordeum vulgare* L.) is a cereal grain that can be used as an energy source in poultry diets. The fiber content of barley, however, is much higher than that of corn grain, and it is composed of non-starch polysaccharides (NSPs), mainly β-glucans and pentosans, which are not efficiently utilized by poultry (1-3). This is because poultry does not have adequate enzymatic and microbial activities to efficiently degrade NSP constituents of barley (3, 4). Nowadays, the development of exogenous NSP-degrading enzymes such as β-glucanases has provided the opportunity to substitute barley for corn. Barley has several advantages to corn. First, it has a higher protein content, allowing less soybean meal use. Second, barley production is more sustainable, requiring much less water and fertilizers (N, P, K). These benefits make the production of barley more environmentally friendly and less resource-intensive when compared to that of corn (5).

The use of β-glucanase enzymes in barley-based diets could improve the feeding value of barley. Despite the development of exogenous enzymes and their usage in barley-based diets, the responses of poultry to such diets have been inconsistent. In the Teymouri et al. (2018) study, hull-less barley was replaced by corn at 0, 25, 50, 75, and 100% with or without β-glucanase enzymes, respectively. The results showed that barley could be substituted for corn up to 75% (6). However, in the study of Shakouri et al. (2009), the replacement of corn by barley resulted in poorer weight gain, feed intake, and feed conversion efficiency of broiler chickens (7). Similarly, Rodriguez et al. 2012 evaluated the use of barley instead of corn in broiler diets and reported a decrease in overall performance. In another study, however, using β-glucanase enzymes significantly improved broiler performance due to enhanced nutrient digestibility and a better microbial balance in the gut (8).

One main mechanism by which NSPs impact broiler performance is elevating digesta viscosity. Increased viscosity *per se* results in the proliferation of undesirable microbiota in the gut (9). Application of NSP-degrading enzymes decreases digesta viscosity and alleviates the deleterious effect of viscous fiber on the intestinal mucosa in poultry. A thorough insight into how dietary NSP influences gut function, and health is required to get the most out of cereal grains such as barley in poultry nutrition.

In the present study, the response of broiler chickens to barley-based diets was evaluated with or without β-glucanase enzymes in terms of growth performance and gut function.

2 Materials and methods

2.1 Birds and experimental diets

The present study was performed at the Poultry Research Center of Shahrekord University, Shahrekord, Iran. The experimental protocols were conformed to the Animal Care and Use Committee of Shahrekord University.

A total number of 252 one-day-old broiler chicks (Cobb 500) were randomly assigned to 18 floor pens (2.8 × 2.8 m^2). Six-floor pens were randomly assigned to each dietary treatment. Three dietary treatments included a corn diet, a barley diet, and the barley diet plus an enzyme mixture. The enzyme mixture contained 3.6× 10^5 β-glucanase, 1.4× 10^6 cellulase, 1.1× 10^6 xylanase, 3.4× 10^6 protease, and 1.0× 10^5 phytase as well as probiotic activity from *Bacillus subtilis* 1.2× 10^{10} CFU. According to the strain guideline, the experimental diets were isocaloric and isonitrogenous and fed to broiler chicks from 1 to 42 days of age.

2.2 Measurements

Body weight and feed intake were recorded during the feeding trial, and the feed conversion ratio (FCR) was calculated. On day 42, blood samples (3 mL) were collected from two birds per pen (12 birds per treatment). Blood samples were centrifuged at 2500× g for 10 min to separate sera to determine uric acid, total protein, and albumin. The birds were then euthanized to determine carcass characteristics, including carcass and breast yields and relative weight ratios of the spleen, bursa of Fabricius, liver, intestine, and abdominal fat deposition.

2.3 Gut morphometry

The jejunal segments -the region from Meckel's diverticulum to the ileocecal junction- were incised (about 1×1 cm^2), flushed with PBS (pH= 7), fixed in Clarke's solution (75% ethanol & 25% acetic acid) for 45 min, and stored in ethyl alcohol (50%). Segments were then placed in periodic acid-Schiff for staining. Muscle layers were separated from the mucosa. Rows of villi were cut in sagittal sections and transferred onto glass slides, covered with a coverslip. These segments were examined with a microscope using an optical lens of 1,000×. Morphometric variables such as villus length (VL), villus width (VW), and crypt depth (CD) were measured, and the villus absorptive surface

area was calculated using the formula= (2π) × (VW/2) × (VL) (10).

2.4 RNA extraction, cDNA synthesis, and Quantitative Real-time PCR Analysis

Samples of jejunal tissue from two birds per pen (12 birds per treatment) were incised into 2×2 cm^2 and stored in liquid nitrogen until gene expression analysis. The samples underwent homogenization and digestion using RNX-Plus reagent (Sina Clon Bioscience, Karaj, Iran). Homogenates were mixed with 200 µL chloroform, followed by centrifugation at 16,000 g for 15 min at 4°C. Supernatants were collected to extract RNA by mixing with equal volumes of isopropanol, rinsing with 75% ethanol, and subsequent centrifugation at 10000 g. The forming pellets were dissolved in diethyl dicarbonate (DEPC)--treated water and exposed to DNase (Sina Clon Bioscience) to improve RNA purity. Subsequently, RNA was quantified by the absorbance of A260/280 using a biophotometer (Eppendorf, USA). The RNA fraction with the A260/280 ratio between 1.8 and 2.2 was collected for cDNA synthesis using the Prime Script™ RT Reagent Kit (Takara Bio Inc., Japan). The levels of toll-like receptor2 (TLR2), mucin2 (MUC2), and tyrosine 3-monooxygenase/ tryptophan 5-monooxygenase activation protein zeta (YWHAZ) transcripts were determined by real-time RT-PCR using SYBR® Premix Ex Taq™ II kit (TliRnase H Plus; Takara Bio Inc.). YWHAZ was considered an endogenous reference gene to normalize the input load of cDNA across samples (11). Table 1 depicts the primers (forward and reverse), designed with the online software of Primer-Blast.

Table 1. Details of the primers used for quantitative real-time PCR analysis for broiler chickens

Target	Primers	PCR product (bp)	Annealing Temperature (C)	Accession no.
TLR2	F:5´-GGTGTTCCTGTTCATCCTCATCCT-3´ R:5´-TTGGGCTTCCGCTTGGCTT-3´	238	62	XM_01530138001
Muc2	F:5´-CTGGACTTCACGGACACCTG-3´ R:5´-CTCAACACAGCCCCCTCTAC-3´	442	62	XM_421035
YWHAZ	F:5´-AGGAGCGAGCTGTCCAATG-3´ R:5´-TCCAAGATGACCTACGGGCTC-3´	84	60	NM_001031343.1

Abbreviations: TLR2: toll-like receptor2; MUC2: mucin2; YWHAZ, tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta; bp, base pair.

Transcripts (three replications per tissue sample) were amplified in a real-time PCR cycler (Rotor-Gene Q 6000; Qiagen, USA). A mixture of 1 µL cDNA, 10 µL of SYBR Premix Ex Taq II Mix, and 0.5 µmol/L of specific primers was made to a total volume of 20 µL. Thermal cycles were maintained at 95 °C for 30s, 94°C for 40s, 64°C for 35s, and 72°C for 30s, respectively. Data were subjected to LinReg PCR software version 2012.0 (Amsterdam, the Netherlands) (12). The relative transcript levels (gene / YWHAZ) were estimated using the Paffl method (11, 13).

2.5 Statistical analysis

Data were performed to the General Linear Model (GLM) procedure using SAS (2007) software in a completely randomized design. Means were compared by Duncan's multiple range test at $p \leq 0.05$.

3 Results

Barley substitution for corn significantly ($p <= 0.001$) reduced feed intake and body weight gain irrespective of enzyme supplementation (Table 2). However, enzyme supplementation of a barley-based diet significantly increased these performance variables and resulted in a feed conversion ratio comparable to the corn diet.

Table 2. Substitution of barley for corn with or without exogenous enzymes on broiler performance (1-42 days of age)

	Corn	Barley	Barley+enzyme	SEM	*p*- value
Weight gain (g/b)	2023^{a}	1630^{c}	1776^{b}	22.30	<0.001
Feed intake (g/b)	3884^{a}	3219^{c}	3363^{b}	43.40	<0.001
Feed conversion ratio	1.91^{b}	1.97^{a}	1.91^{b}	0.02	0.02

^{a,b,c}Means in the same raw with heterogenous letters indicate significantly different probability levels shown in the table.

Table 3 depicts morphometric measurements of jejunum. Jejunal villus length was significantly decreased (p= 0.001), whereas crypt depth (p=0.007) significantly increased in birds fed barley compared to the corn control. The villus

length to crypt depth ratio (p= 0.0001) and the villus absorptive surface area (P= 0.02) were more significant in birds fed corn than those fed with barley diet. However, supplementation of barley diet with enzyme mixture significantly increased the villus length to crypt depth ratio and the villus absorptive surface area to the extent observed in the corn group.

Table 3. Substitution of barley for corn with or without exogenous enzymes on broiler jejunal morphometry

	Corn	Barley	Barley+enzyme	SEM	*p*- value
Villus length (µm)	1131[a]	938[b]	1122[a]	32.1	<0.001
Villus width (µm)	399	371	397	17.2	0.42
Crypt depth (µm)	111[b]	136[a]	107[b]		0.007
Villus length/crypt depth	10.4[a]	7.3[b]	10.3[a]	0.47	0.0001
Villus absorptive area (mm^2)	1.4[a]	1.1[b]	1.4[a]	0.09	0.02

[a b]Means in the same raw with different letters indicate significantly different at probability levels shown in the table.

Table 4 represents the effects of replacing corn with barley on jejunal gene expression in broiler chickens. TLR2 gene was significantly (p< 0.001) down-regulated when barley was replaced with corn. Enzyme supplementation of the barley diet significantly up-regulated TLR2 gene expression but not to the extent observed in the corn diet. The expression of MUC2 did not show a significant change. The relative weight of intestines was significantly increased when barley was fed to broilers.

Table 4. Substitution of barley for corn with or without exogenous enzymes on relative intestine weight and jejunal gene expression in broiler chickens

	Corn	Barley	Barley+enzyme	SEM	P- value
TLR2 gene	1.676[a]	0.497[c]	0.792[b]	0.08	<0.001
MUC 2 gene	2.956	2.344	2.934	0.03	0.28
Intestine/BW (%)	5.1[b]	6.7[a]	6.0[a]	0.28	0.002

[a,b,c]Means in the same raw with heterogenous letters indicate significantly different at probability levels shown in the table.

Table 5 shows the effects of substitution of barley for corn with or without exogenous enzymes on circulatory levels of uric acid, total protein, and albumin in broiler chickens. Serum levels of uric acid (p< 0.05), total protein (p< 0.05), and albumin (P= 0.005) were significantly increased when barley was substituted for corn, respectively. Enzyme supplements did not make any significant difference to these variables.

Table 5. Substitution of barley for corn with or without exogenous enzymes on circulatory levels of uric acid, total protein, and albumin in broiler chickens

	Corn	Barley	Barley+enzyme	SEM	*p*- value
Uric acid (mg/dL)	7.8[b]	8.7[a]	8.2[ab]	0.26	0.05
Total protein (mg/dL)	3.4[b]	3.8[a]	3.6[ab]	0.07	0.01
Albumin (mg/dL)	1.31[b]	1.53[a]	1.48[a]	0.04	0.005

[a,b,c]Means in the same raw with heterogenous letters indicate significantly different at probability levels shown in the table.

4 Discussion

This present study showed that broiler performance, including weight gain and feed intake, was affected by replacing barley for corn. The Feed Conversion Ratio (FCR) also deteriorated in birds that received the barley diet. Previous researchers have reported that feeding barley to broiler chickens caused increased digesta viscosity, trapped nutrients (the cage effect), disrupted intestinal enzyme activities, and reduced nutrient assimilation (3). Such changes could explain the poor growth performance observed in chickens fed the barley diet. Adding the enzyme mixture to the barley diet resulted in a significant and noticeable improvement in growth performance. This

finding was in line with those that reported the inclusion of NSPs-degrading enzymes in barley diets effectively improved weight gain and FCR in broiler chickens (14, 15). Hydrolyzing NSPs exogenous enzymes could reduce digesta viscosity and remove the cage effect, improving nutrient utilization in the small intestine (14, 16, 17).

In the present study, jejunal histomorphology revealed decreased villus length and increased crypt depth following feeding the barley diet. Similarly, the villus length to crypt depth ratio and the villus absorptive surface area were significantly decreased in barley-based diet. These findings are consistent with previous studies (14, 18, 19). Shakouri et al. (2009) reported that jejunal villus length and the villus length to crypt depth ratio in broilers who received a barley-based diet were the lowest compared to those fed corn or wheat (7). Shortening of villi (atrophy) and an increase in the number and size of goblet cells in the jejunum of broiler chickens fed with barley-base diets have been recently reported (3, 9). Jejunum accounts for the most functions in the chicken. Digestion and absorption in the jejunum are rapid processes that last 40 to 60 minutes. The larger the villus length and the higher the villus absorptive surface area, the higher the rate of nutrient absorption (20). These findings suggest poor performance of birds fed a barley-based diet instead of the corn control.

NSP-degrading enzymes could restore the morphometric changes that happened when barley was fed to broilers. Teirlynck et al. 2009 reported that poor growth performance in broilers who received a barley-based diet was attributed to the alteration of intestinal morphology. However, supplementing barley-based diets with β-glucanase enzyme improved intestinal morphology and growth performance (21). The results of this study are also in accordance with those reported by Al-Khalifah et al. in 2022 and Shakouri et al. in 2009 (7, 22). In the present study, the weight of the intestine in relation to body weight was increased in the barley-based diet. Other researchers have reported similar observations (4, 14, 23, 24).

Toll-like receptors (TLRs) are innate immune pattern recognition receptors that play critical roles in immune homeostasis and the defense against toxins and pathogens. Antigen recognition has also been reported to be involved in gut microbial balance. The expression of TLRs is affected by the type of diet and feed supplements (25, 26). In chickens, TLR-mediated regulations maintain the intestinal epithelial barrier's integrity and promote birds' health (27). It has been addressed that intestinal tensions under the influence of cereal type could stimulate the immune system and release multi-pro-inflammatory cytokines, down-regulating the expression of TLR2 as observed in the present experiment (28-30). MUC2 expression did not change significantly when barley was substituted for corn. This observation was comparable to the report of Paraskeuas and Mountzouris in 2019 (25, 26). These researchers evaluated different cereal types with or without a phytogenic feed additive, and they observed that both dietary factors influenced the composition of broiler gut microbiota independently or in combination.

In the present study, circulatory levels of uric acid, total protein, and albumin showed significant elevations in chicken-fed barley compared to those in the corn control. The explanation for this finding may be associated with increased deamination of amino acids under the influence of anti-nutritional NSPs in barley. These findings were agreed with other reports (31, 32). In fairness to this explanation, enzyme supplementation of the barley diet could reduce the serum levels of all those variables. This finding agreed with Sun et al.'s results in 2018 and Ravindran and Abdollahi in 2021 (24, 32).

5 Conclusion

Although corn is currently the most common cereal grain in poultry diets, it is predicted that corn may not meet the demand of the animal feed industry in the future. Barley grain contains non-starch-polysaccharides (NSPs) with anti-nutritional effects. Nevertheless, the anti-nutritional effects of NSPs could be neutralized by an exogenous enzyme mixture including β-glucanase, xylanase, and phytase. In the present study, barley was replaced with corn, which reduced broiler performance. Nevertheless, using the enzyme mixture improved broiler performance fed with the barley-based diet. Better performance in the enzyme-supplemented group was associated with enhanced villus absorptive surface area and a lower turnover of enterocytes, as reflected in a lower crypt depth in the jejunum.

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Conflict of Interest

The authors have no conflict of interest to declare.

Author Contributions

AAT designed the study, conducted the data collection and analysis, interpreted the results, and reviewed the manuscript. HH generated the research idea, participated in the design, conducted the study, collected data, analyzed, and wrote the manuscript. MAK-T, MRA, and FK participated in the study design and manuscript reading.

Data Availability Statement

The data produced and examined during this study are not openly accessible but can be obtained from the corresponding author upon a reasonable request.

Ethical Considerations

Not applicable.

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