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Effects of multi-enzymes supplementation to wheat and soybean meal-based feeds on growth performance, digestibility and carcass characteristics of quails

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Abstract

Japanese quail (*Coturnix coturnix japonica*) is a popular experimental animal model in scientific research. The present study investigated the effects of dietary multiple enzyme supplementation on growth performance, carcass characteristics, nutrient digestibility and small intestinal histomorphology in quails fed diets based on wheat and soya bean meal. A total number of 192 1-day-old quails were assigned to three treatments with 16 replicates in each and four quails *per* replicate for 38 days. The control group received a basal diet, and the treatment groups received a basal diet with 0.10 or 0.20% multi-enzyme, respectively. Growth performance parameters, carcass characteristics, nutrient digestibility and small intestinal histomorphology in quails were evaluated. Dietary supplementation of multi-enzymes to diet significantly increased body weight gain and improved the feed conversion rate. Moreover, quails fed with 0.10 or 0.20% multi-enzymes showed better ash digestibility coefficients and apparent metabolizable energy coefficients than the control quails. Furthermore, quails fed on a diet containing 0.20% multi-enzyme had the highest crude fiber digestibility. The villi length and the villi length/crypt depth ratio of the duodenum were significantly increased and the crypt depth was decreased in quails-fed diets supplemented with both multi-enzyme levels. However, feed consumption, carcass yield, carcass weight, heart weight, gizzard weight, liver weight and total intestine weights were not affected by treatments. In conclusion, our results showed that dietary supplementation of multi-enzymes to a wheat and soybean meal-based diet enhanced the growth performance and nutrient digestibility of quails.

Introduction

Sustainable farming necessitates the use of feed additives that enhance animal digestion resulting in a higher feed conversion ratio and more efficient use of nutrients. The topic of feed additives, which are a variety of substances added to feeds to enhance nutrient absorption and reveal their effects on the intestinal wall, is still one that has received the most investigation.[1,2] Enzymes are biological catalysts that accelerate chemical processes in living organisms with little or no adverse effects and provide a complete product yield. They are highly specific and work under mild conditions making them essential for various biochemical processes in living organisms. The addition of enzymes to chicken diets may improve the feed conversion ratio and reduce the deleterious effect of nonstarch polysaccharides (NSPs) on performance.[3,4] The use of enzyme complexes such as amylase, xylanase and protease is reported to improve the utilization of energy, protein and macro minerals in poultry.[5,6] In addition, supplementing enzymes to diets can improve meat and egg production proficiency by increasing nutrient utilization and reducing animal excreta/waste.[7] Up to now, a variety of enzymes have been investigated for their positive benefits and added to the diets of various animal species. Velázquez-de Lucio *et al*.,[8] reported the digestibility of the fiber substrates of the diet by enzyme supplementation. The NSPs enzymes have been utilized to solve the lack of energy and wet litter problems caused by the addition of barley and wheat to chicken feed since the late 1980s.[9,10] Enzymes are one of the most abundant feed additives in poultry diets to degrade NSPs in the gastrointestinal tract.[11-14] They are primarily used in poultry diets to increase the availability

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of NSPs in feeds like barley, wheat, rye and triticale.[12-16] Dietary supplementation of enzymes to diets can increase growth performance by reducing digestive viscosity and enhance nutrient digestion and absorption improve intestinal microbiota reduce the amount of fluid feces and ammonia in the feces.[17-23] Enzymes can increase the absorption of various NSPs and other nutrients in the gastrointestinal tract of chickens by breaking them down and reduce environmental pollution by preventing their excretion with feces. Enzymes also reduce the side effects of antibiotics.[1,2] Dietary supplementation of multi-enzymes to grain-based poultry diets may increase the digestibility of phytate-bound phosphorus (P), calcium (Ca), zinc and copper as well as the digestibility of crude protein (CP) and amino acids.[10,24,25]

However, there are still conflicting results on the beneficial effect of multi-enzyme supplementation of diets on the growth performance and nutrient digestibility of poultry fed diets containing grains such as wheat and barley. Besides, studies to determine the effects of multi-enzyme supplementation to diets on nutrient digestibility and intestinal morphology of poultry fed with wheat and soybean-based diets are limited. The purpose of this study was to examine how a dietary multi-enzyme could influence growth performance, carcass characteristics, nutrient digestibility and small intestine histomorphology in quails offered wheat-soybean meal-based diets.

Materials and Methods

The Animal Research Centre conducted this work in compliance with the Animal Experiments Directive which was approved by the Ethics Committee (No: 2022/07). This research was conducted at the coordinates 37° 53' 27" N 40° 16' 24" E.

Diets and experimental design. The treatment diet was formulated in accordance with the National Research Council (NRC)[26] recommendations to address the dietary needs of quails (Table 1). Totally, 192 1-day-old quails *(Coturnix coturnix japonica)* were randomized into one of three treatments each of 16 replicates (four quails in each) for 38 days. Diet and water were provided to the birds *ad libitum* throughout the experiment. Diets in all groups were iso-nitrogenous and those in the treatment groups had decreased metabolizable energy (ME) levels of 50.00 kcal kg^{-1} feed. The control group received a basal diet and the treatment groups received a basal diet with 0.10 or 0.20% multi-enzyme, respectively. The quails were exposed to fluorescent lighting for 23 hr and had free access to feed and water. The experiment room was kept at 32.00 ˚C for the first week before being gradually decreased to 22.00 ˚C for up to 3 weeks to provide environmental control. All quails *per* cage were weighed and calculated from these data. Body weight (BW) gain and feed intake were measured weekly and the feed

Table 1. Composition of experimental diets.

Ingredients (%)	Control	Multienzyme (0.10%)	Multienzyme (0.20%)
Sunflower oil	2.07	1.47	0.70
Wheat	14.40	20.00	24.77
Maize	42.00	37.00	33.00
Soybean meal	35.00	35.00	35.00
Di-calcium phosphate[a]	1.20	1.20	1.20
Bone meal	4.50	4.50	4.50
DL- Methionine	0.10	0.10	0.10
L-Lysine HCl	0.10	0.10	0.10
Sodium chloride	0.38	0.38	0.38
Vitamin mineral premix[b]	0.25	0.25	0.25
Analyzed nutrient composition, or calculated (%)*			
Dry Matter	89.70	89.60	
Crude protein	22.50	22.50	22.50
ME (kcal kg^{-1})	2900	2850	2850
Ether extract	3.51	3.49	3.44
Ash	6.78	6.78	6.78
Calcium	1.07	1.07	1.07
Sodium	0.19	0.19	0.19
Chlorine	0.22	0.22	0.22
Available phosphorus	0.48	0.48	0.48
L-lysine	1.27	1.27	1.27
Methionine + cysteine	0.80	0.80	0.80

[a] Contains 17.50 g P and 240 g Ca *per* kg; [b] Provided (*per* kg of diet): Vitamin D_3, 1,200 IU; vitamin A, 8,000 IU; vitamin E, 10.00 IU; vitamin K3, 2.00 mg; thiamine, 2.00 mg; vitamin B12, 0.03 mg; riboflavin, 5.00 mg; pantothenic acid, 10.00 mg; pyroxidine, 0.20 mg; niacin, 50.00 mg; biotin, 0.10 mg; 80.00 mg; zinc, folic acid, 0.50 mg; iron, 40.00 mg; selenium, 0.20 mg; manganese, 60.00 mg; iodine, 0.80 mg; copper, 8.00 mg and cobalt, 0.40 mg.

***** Provided (*per* kg of diet): Cobalt, 0.40 mg; selenium, 0.20 mg; copper, 8.00 mg; Iron, 80.00 mg; zinc 40.00 mg; manganese 60.00 mg and iodine 0.80 mg.

conversion rate was calculated as feed consumption *per* BW gain. The multi-enzyme (xylanase, β-glucanase, cellulase, and protease) used in quails diets contained endo-1,3(4)-beta-glucanase (minimum of 1,100 U g^{-1}) and endo-1,4-beta-xylanase (Endofeed® DC, EU Register No. 4a1601; Annex 1, Barcelona, Spain).

Digestibility assay. On the 35th day of the experiment, 12 quails from each group were selected and placed in separate cages for 3 days. Quails were fed the same experimental diets on days during this period. The feces were promptly frozen and lyophilized after collection. Lyophilized feces samples were crushed through a 0.50-mm screen and kept at – 4.00 ˚C in sealed containers for chemical analysis. Diets and feces samples were analyzed for gross energy (GE), crude protein (CP), Ca, P and acid-hydrolyzable fat. The apparent fecal nutrient digestibility coefficients were calculated as the difference between the concentration of nutrients ingested and excreted divided *per* concentration of the ingested nutrient.

Chemical analysis and calculations. An adiabatic bomb calorimeter (Gallenkamp Autobomb, London, UK) calibrated with benzoic acid was used to calculate GE. Ether extract, Ca and P contents were analyzed by Association of Official Analytical Chemists (AOAC).[27] The CP contents were analyzed using an automatic nitrogen/ CP analyzer (FP-528; Leco Corp., St. Joseph, USA). In order to calculate CP digestibility, the total nitrogen in the feces was subtracted from the excreta uric acid concentration.[28] The equation proposed by Nkukwana *et al.* as follows for the nutrient digestibility (%):[29]

$$Digestibility = \frac{Nutrient\ intake - Nutrient\ in\ excreta}{Nutrient\ intake} \times 100$$

It was used to determine the digestibility of dry matter (DM), organic matter (OM), CP and ether extract. Dry matter is the only form of value expression. According to Adeola[30] the following formula was used to determine nitrogen-corrected apparent metabolizable energy (AME).

The apparent digestibility (%) values were calculated by measuring dietary nutrient input and excreta nutrients as shown below:

$$Digestibility = \frac{Nutrient\ in\ diet - Nutrient\ in\ excreta}{Nutrient\ in\ diet} \times 100$$

The AME (kcal kg^{-1}) of the basal and test diets used in the present study were calculated using the formula shown below with appropriate corrections for differences in DM content.

$$AME = \frac{(Feed\ consumption \times GE\ diet) - (Excreta\ output \times GE\ excreta)}{Feed\ consumption}$$

At the end of the experiment, (day 38) 12 quails in each group (male:female = 1:1) were slaughtered as a representative sample. After that, the quails were dissected and de-feathered. The hot carcass, liver, gizzard, proventriculus, intestine and abdominal fat were weighed. The results for the slaughter features were shown as relative values (1.00 g *per* 100 g BW) in relation to the pre-slaughter BW. Hot carcass yield was calculated by rating the hot carcass weight to pre-slaughter BW. After weighing the intestinal tract, it was divided into sections (duodenum, jejunum and ileum) and placed in 10.00% formaldehyde liquid for histological analysis.

Intestinal histomorphology. The tissue samples were fixed in buffered neutral 10.00% formaldehyde for histopathological examination. The samples were passed through xylole (Tekkim, Bursa, Türkiye) and alcohol series (50.00, 75.00, 96.00 and 100%) and embedded in paraffin blocks. Then, 5.00-µm-thick sections were cut from each block and stained with Hematoxylin & Eosin. All sections were evaluated under light microscopy (AxioCam ERc 5s; Zeiss Primo Star, Oberkochen, Germany) and ZEISS image processing software (ZEN 2012 SP2; Carl Zeiss, Jena, Germany) at 40 - 400-fold magnification. The measured parameters included villus height to crypt depth ratio, crypt depth and villus height which were evaluated according to previously published grading system.[31] In this regard, the crypt depth was measured from the base upward to the region of transition between the crypt and villus. The villus height was measured from the top of the villus to the top of the lamina propria.[31]

Statistical analysis. All data were analyzed by using the One-way ANOVA as a completely randomized design using the GLM procedure of the SPSS Software (version 16.0; SPSS, Inc., Chicago, USA). Differences were considered significant at $p < 0.05$ and significant differences between means were separated by Tukey's test assuming the following mathematical model:

$$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$$

where, Y_{ij} = Observation of dependent variable recorded on *ith* and *jth* treatment, μ = Population means, α_i = Effect of i^{th} composting system (i = 1, 2,3), ε_{ij} = Residual effect associated k^{th} observation on i^{th} and j^{th} treatment.

Results

The supplementation of different levels of multi-enzyme to the diet had positive effects on the growth performance, carcass characteristics and internal organ weights of quails (Table 2). Our study indicated that multi-enzyme supplementation at 0.10 and 0.20% kg of the diet significantly enhanced BW gain compared to the control treatment ($p < 0.05$). Also, the addition of multi-enzyme addition to the diets resulted in significant improvements in feed conversion rate ($p < 0.05$). The groups had no differences in carcass yield, carcass weight, heart weight, gizzard weight, liver weight or total intestinal weight ($p > 0.05$). There was a significant difference in proventriculus weight among the treatments ($p < 0.05$). Proventriculus weight was 0.38% (g *per* BW) in the 0.20% enzyme fed

birds, 0.40% (g *per* BW) in the 0.10% enzyme fed birds and 0.33% (g *per* BW) in the control diet fed birds. The results of this study showed that adding multi-enzymes to quails' diet did not affect carcass yield or weight. Similarly, multi-enzyme addition to complex diets had no significant effect on gizzard, heart, liver, or total intestinal weights ($p > 0.05$). There was no difference between the treatment groups in terms of DM and CP digestibility as well as P and Ca utilization ($p > 0.05$). Dietary multi-enzyme supplementation increased AME and ash digestibility compared to control quails ($p < 0.05$). Moreover, quails fed on a diet containing 0.20% multi-enzyme had the highest crude fiber digestibility ($p < 0.05$; Table 3). Dietary multi-enzyme supplementation caused some changes in the small intestinal histomorphological parameters of quails (Table 4).

Dietary multi-enzyme supplementation decreased crypt depth, increased duodenal villi length and the villi length/crypt depth ratio ($p < 0.001$). The supplementation of a high-level enzyme (0.20%) increased the villi length of the jejunum compared to the control ($p < 0.001$). Also, both multi-enzyme levels decreased the thickness of the lamina muscularis mucosa, villi length and crypt depth in the ileum compared to the control ($p < 0.001$). The villi length/crypt depth ratio in jejunum and ileum was unaffected by multi-enzyme levels ($p > 0.05$; Fig. 1).

Table 2. The effects of dietary supplementation of multi-enzyme on the growth performance, carcass characteristics and internal organ weights of growing quails.

Parameters	Control	Multi-enzyme (0.10%)	Multi-enzyme (0.20%)	*p*-value
Body weight gain (g *per* quail)	234.6 ± 2.80^{b}	246.1 ± 3.97^{ab}	253.1 ± 4.02^{a}	0.005
Feed consumption (g *per* quail)	539.8 ± 5.01	526.8 ± 6.90	527.9 ± 4.82	0.191
Feed conversion rate (g *per* g)	2.31 ± 0.02^{a}	2.14 ± 0.03^{b}	2.09 ± 0.03^{b}	0.005
Carcass yield (%)	66.5 ± 0.54	67.2 ± 0.48	66.6 ± 0.88	0.755
Carcass weight (g)	158.9 ± 3.69	156.5 ± 5.37	160.5 ± 4.89	0.829
Proventriculus weight (g *per* BW; %)	0.33 ± 0.02^{b}	0.40 ± 0.02^{a}	0.38 ± 0.02^{a}	0.048
Heart weight (g *per* BW; %)	0.66 ± 0.06	0.75 ± 0.06	0.75 ± 0.02	0.321
Gizzard weight (g *per* BW; %)	2.15 ± 0.06	2.27 ± 0.09	2.23 ± 0.10	0.616
Liver weight (g *per* BW; %)	2.14 ± 0.05	2.00 ± 0.07	2.17 ± 0.10	0.256
Total intestinal weight (g *per* BW; %)	2.98 ± 0.07	2.87 ± 0.12	2.92 ± 0.13	0.792

^{abc} Different letters in the same row mark statistical difference between values ($p < 0.05$).

Table 3. The effect of dietary addition of multi-enzyme on nutrient digestibility of growing quails.

Parameters	Control	Multi-enzyme 0.10%	Multi-enzyme 0.20%	*p*-value
Dry matter (%)	72.30 ± 0.35	73.80 ± 0.34	73.70 ± 0.38	0.056
Crude protein (%)	59.80 ± 0.78	61.20 ± 0.71	62.40± 0.81	0.076
Crude Fiber (%)	44.40 ± 0.35^{b}	45.80 ± 0.58^{ab}	46.50^{a} ± 0.30	0.008
Apparent metabolizable energy (%)	93.70 ± 0.60^{b}	95.20 ± 0.48^{a}	95.50^{a} ± 0.62	0.010
Ash (%)	51.20 ± 0.47^{b}	53.00 ± 0.42^{a}	53.00^{a} ± 0.59	0.020
Calcium (%)	63.80 ± 0.78	63.20 ± 0.73	64.40 ± 0.81	0.124
Phosphorus (%)	53.40 ± 0.35	54.80 ± 0.57	54.60 ± 0.29	0.086

^{abc} Different letters in the same row mark statistical difference between values ($p < 0.05$).

Table 4. The effects of dietary supplementation of multi-enzyme on histomorphology parameters of the small intestine of growing quails.

Intestine section	Groups	Villi length (µm)	Crypt depth (µm)	Thickness of lamina (µm)	Villi/crypt ratio
Duodenum	**Control**	907.02^{c}	53.34^{a}	55.61	17.96^{c}
	Multi-enzyme (0.10%)	1266.14^{a}	48.30^{b}	54.55	27.98^{a}
	Multi-enzyme (0.20%)	1171.70^{b}	48.71^{b}	59.68	25.13^{b}
	***p*-value**	0.001	0.010	0.087	0.001
Jejunum	**Control**	722.60^{b}	44.76^{b}	63.77^{a}	16.54
	Multi-enzyme (0.10%)	701.57^{b}	40.26^{c}	49.45^{b}	18.23
	Multi-enzyme (0.20%)	849.41^{a}	52.67^{b}	58.10^{a}	17.35
	***p*-value**	0.001	0.001	0.001	0.16
Ileum	**Control**	606.59^{a}	48.56^{a}	92.16^{a}	12.91
	Multi-enzyme (0.10%)	438.08^{c}	35.85^{c}	52.25^{c}	12.74
	Multi-enzyme (0.20%)	551.03^{b}	42.67^{b}	66.40^{b}	13.55
	***p*-value**	0.001	0.001	0.001	0.32

^{abc} Different letters in the same row indicate statistically significant differences ($p < 0.05$).

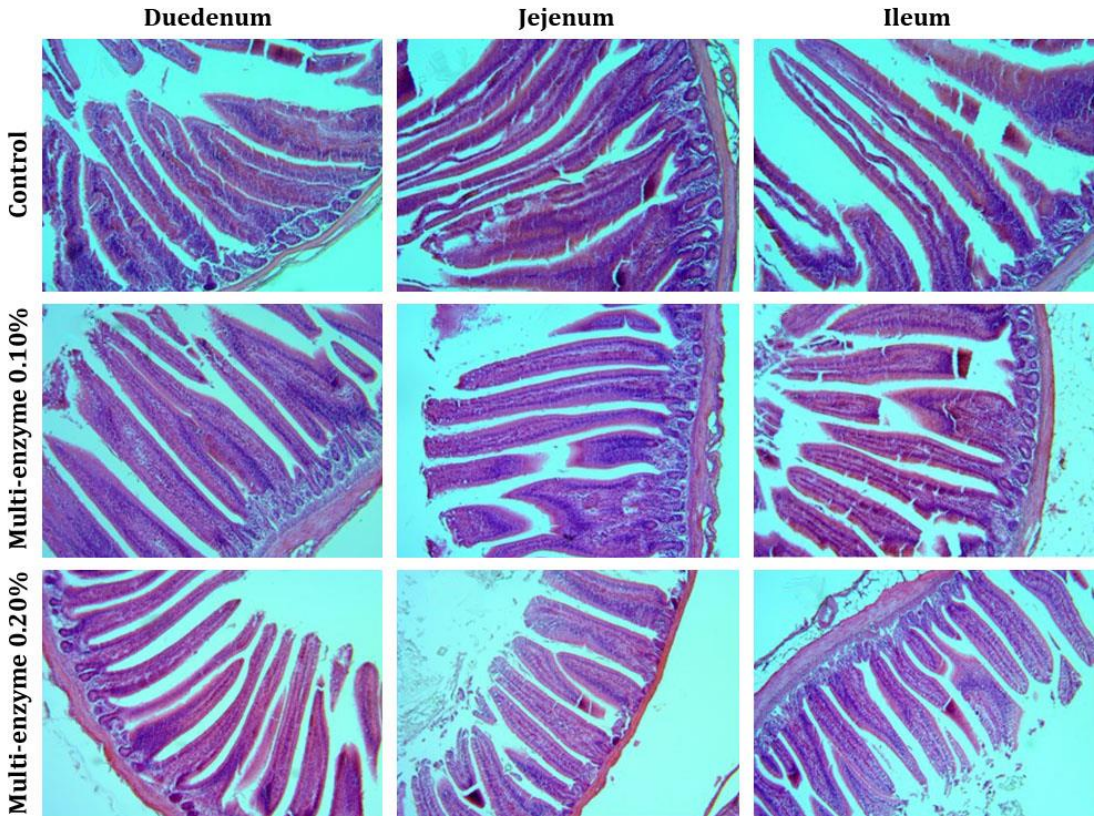

Fig. 1. Histomorphological features of the small intestine of growing quails. In control group, no damage and normal structure are obvious. In 0.10% of multi-enzymes group, decreased crypt depth, increasing duodenal villi length and the villi length/crypt depth ratio were observed. In 0.2% multi-enzymes group, increased the villi length are seen (H&E staining; 40×).

Discussion

Some previous studies have reported that the addition of multiple enzyme complexes to poultry diets increases feed conversion, reduces the negative effects of NSPs and improves the utilization of energy, protein and macro minerals in poultry production.[3-6] The results of this study showed that the addition of various levels of multi-enzyme to quail diets improved BW gain and feed conversion ratio. Nevertheless, no significant change in feed consumption was seen among treatments ($p > 0.05$). The improvement in BW and feed conversion ratio observed in our study might be attributed to the effect of enzymes on the digestion of NSPs. Many other researchers observed similar results to our results on feed consumption and feed conversion ratio.[3,32-35] Nevertheless, Tüzün *et al*.,[23] found that supplementing multi-enzyme supplements to sunflower meal-based quail diets did not have effect on BW increase, feed conversion ratio or feed consumption. On the other hand, Horvatovic *et al*.,[36] observed that adding enzymes to diets containing sunflower meal increased feed consumption, BW gain and feed conversion rate. The different results found among various studies may vary depending on the different dietary materials used in the study, the type and dose of enzyme.[32,37,38]

In this study, dietary multi-enzyme supplementation did not change carcass yield, carcass weight, heart weight, gizzard weight and liver weight, however, increased proventriculus weight. Tüzün *et al*.,[23] did not find any difference in a relative carcass, thigh-baguette, breast, heart, pancreas, gizzard and proventriculus except for only an increase in relative liver weight. Alagawany *et al*.,[37] reported no difference in carcass, heart, gizzard, offal and internal fat, however, they reported decrease in liver weight with enzyme supplementation.[35] There was no difference in live weight in their study with canola meal and enzyme supplementation. Rabie and El-Maaty[34] reported that there was no difference in relative carcass yield, gizzard weight, liver weight and heart weight parameters. Mushtaq *et al*.,[35] did not find any difference in live weight in their study using canola meal and enzyme supplementation. The results of other researchers and the present study were similar in terms of internal organ weights. The fact that the weight of proventriculus was found to be different from some studies might be due to the type of feed, enzyme and its dose used in the study.[39]

The present study revealed a significant increase in the digestion of crude fibre, AME and ash as well as a numerical increase in the digestion of DM, CP, Ca and P. In the present study, the multi-enzyme supplementation

showed an increase in AME and ash digestibility compared to control quails. Consistent with previous research, similar results were observed in our study. Attia *et al*.,[3] found that CP, crude fibre, dry mater and ash digestibility of the multiple enzyme supplemented groups were significantly higher than the control group. Al-Harthi *et al*.,[1] and Ravindran *et al*.,[40] reported similar results in their studies with phytase enzyme. Numerous studies have shown that degradation of NSPs in diets by enzymes benefits protein digestibility and energy utilization.[39,40] This increase in AME and ash utilization can be explained by the fact that multi-enzyme activity reduces the viscosity of the intestinal contents,[11] and glucanase, which is involved in the degradation of cellulose in feeds, breaks the bonds in the glucan chain that are responsible for the increase in AME and ash utilization.

The feed consumed has an important role in the development of the digestive system. Villus length, crypt depth and the ratio of villus length to crypt depth are important parameters for evaluating gut health.[41-43] Longer villi and less depth of the crypt lead to better nutrient absorption. In the present study, multiple enzyme supplementation of the diet decreased crypt depth, increased duodenal villus length and increased villus length/crypt depth ratio. Dietary enzyme supplementation at 0.20% level specifically increased jejunum villus length compared to control (Fig. 1). Furthermore, both levels of multiple enzyme supplementation resulted in a decrease in the thickness of the lamina muscularis mucosa as well as villi length and crypt depth in the ileum compared to the control group. The villus length/crypt depth ratio in both jejunum and ileum was not affected by varying levels of multi-enzyme supplementation.

According to de Oliveira-Bruxel *et al*.,[42] multi-enzyme supplementation increased villus length in the duodenum and jejunum. The same study found that crypt depth was decreased in all three sections of the small intestine. de Oliveira-Bruxel *et al.*,[42] and the data obtained from the present study were similar.[23] It has been reported that ileum villus height and crypt depth were higher and tunica muscularis thickness was lower in groups who received enzymes *versus* those that did not. The results of the current study on ileum data were different from those of Tüzün *et al*.,[23] Ileum data of the current study showed that villus height, crypt depth and lamina muscularis mucosa were all decreased. While the present study investigated the use of enzymes in wheat, Tüzün *et al*.,[23] investigated the enzyme supplementation of sunflower meal. de Oliveira-Bruxel *et al*.'s study and the current study were similar when just ileum histomorphologic data were considered.[42] According to Attia *et al*.,[32] multi-enzyme supplementation at various levels (0.00, 0.10, and 0.20%) increased villus length by 32.60% compared to the non-supplemented group, with the 0.1.00% multi-enzyme group achieving the highest results. The villi are the sites of intensive absorption in the small intestine.[41] Increasing villus length and/or crypt depth are also mechanisms that promote nutrient intake. Morphologic changes in the villi and crypts of the small intestine may be caused by the type of NSPs consumed.[43] In the current study, the improvement in performance parameters was higher in the enzyme-supplemented groups. Similarly, the small intestine histomorphology obtained in the enzyme-treated groups was improved. The data from this study may be attributed to the addition of an exogenous multi-enzyme to the diet. The gastrointestinal system grows fast in chickens during their initial days of life. The villi, which are in charge of nutritional absorption, roughly double in growth every 48 hours reaching their maximum size between 6 and 8 days in the duodenum and 10 days in the jejunum and ileum.[41] Changes in the intestinal mucosa can be caused by the content of poultry diets.

In conclusion, the results of the present study showed that the addition of different levels of multi-enzyme to wheat and soya bean-based diets in quails improved the growth performance by increasing the digestibility of NSPs in wheat.

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Conflict of interest

All authors declare no conflicts of interest.

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