

Adding wheat and rapeseed meal to corn-soy diets affects intestinal morphology and nutrient digestibility in broilers

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Abstract

This study was conducted to investigate the effects of thyme essential oil and enzyme supplementation on rations with two different energy (wheat or corn) and protein resources (soy or rapeseed meals) on gut morphology and protein digestibility of broiler chickens. Three hundred eighty-four male Ross-308 broiler chickens were used with a 2³ factorial arrangement with treatments according to a completely randomized design. The morphological examinations of the intestine were carried out on 1-cm long excised segments from duodenum, jejunum and ileum. Adding wheat to the diet increased small intestine length in birds compared to other feedstuffs ($P < 0.05$). Duodenum crypt depth and jejunum villi length were also higher in groups fed with rations containing wheat ($P < 0.05$). Groups that received thyme essential oil and enzyme supplementation had increased length of the duodenum ($P < 0.05$). However, the two- and three-factor interaction effects were not significant. Protein digestibility was not affected by treatment ($P > 0.05$). Feeding wheat to broiler chickens may increase the surface area for nutrient absorption in the small intestine as compensation for the anti-nutritive activity of non-starch polysaccharides in wheat-based diets. Wheat based diets are expected to be acceptable for broilers when supplemented with appropriate enzymes or thyme essential oil.

Keywords: enzyme supplementation, protein, rapeseed meal, soybean meal, thyme essential oil

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Introduction

Non-starch polysaccharides (NSP) of cereal grains exhibit anti-nutritive activity when present in broiler diets (Annison & Choct, 1991). In poultry, soluble NSP can increase digesta viscosity, reduce the digestibility of starch and protein and thus reduce growth performance (Choct *et al.*, 2010) and increase the moisture content of litter (Dida, 2016; Tsiouris *et al.*, 2020). Phytic acid found in wheat prevents the absorption of minerals such as iron, calcium, manganese, and zinc by binding to them before they can be absorbed in the small intestines (Lopez *et al.*, 2002). Additionally, excess phosphorus in wheat grain is excreted and aggravates environmental problems (Nobakht *et al.*, 2010). Chickens do not secrete the enzymes necessary to breakdown the NSP and phytate that are present in wheat and thus increase their utilization (Haribhau *et al.*, 2020). In order to reduce these problems and increase the efficiency of wheat for use in poultry diets, different nutritional additives have been used as supplements. These supplements include organic acids and specific enzymes can be used in poultry diets especially containing highly levels NSP from grains such as wheat and rye (Shahir *et al.*, 2012). Sharma *et al.* (2018) studied the use of enzyme supplementation in broiler diets substituting rice for corn on performance and the economics of meat production finding that higher doses of enzymes (50 g on 100 kg feed) resulted in improved performance and reduced production costs.

As previously discussed by Nunes *et al.* (2015), the use of enzyme supplementation improved the performance of broilers by reducing the amount of nutrients and energy required. Moftakharzadeh *et al.* (2019) indicated that the use of enzymatic supplementation increased energy utilization from wheat and soybean based diets. Hussein *et al.* (2019) stated that adding enzymes to low-energy diets is an effective strategy to improve meat quality parameters and small intestine characteristics.

With the elimination of growth-promoting antibiotics from poultry diets, medicinal plants and their derivatives have been studied as alternatives to them (Hashemi & Davoodi, 2010; Grashorn, 2010). These

substances derived from medicinal plants have been shown to increase growth performance, improve health status (Dhama *et al.*, 2014; Yadav & Jha, 2019), and improve the intestinal microbiota (Al-Kassie, 2010; Kyaw *et al.*, 2017).

Thyme (*Thymus vulgaris*) is a plant of the mint family. Its active constituents are thymol and carvacrol (Rabiei *et al.*, 2011; Gursul *et al.*, 2019) which can affect animal metabolism and physiology (Bouhtit *et al.*, 2019). The active substances in thyme can stimulate secretion of pancreatic enzymes, thereby improving digestion efficiency and absorption of protein and other nutrients from the bird's gastrointestinal tract (El-Hakim *et al.*, 2009).

It is well known that small intestine is an important digestive organ and the main absorptive site of the gastrointestinal tract, but it is also immunological barrier in the gut to protect against incursion of endogenous microorganisms (Pickard *et al.*, 2017; Cui *et al.*, 2019). Fibre supplementation, such as would be provided by NSP, enhanced development of intestinal morphology and functional development in chickens (Yu *et al.*, 2018; Saadatmand *et al.*, 2019).

Therefore, it is reasonable to posit that effects of replacing corn with wheat on intestinal morphology require study. The condition of this part of the gastrointestinal tract can affect feed consumption and is useful for better understanding effects on measures of performance. The purpose of this research is to investigate the possibility of using wheat in poultry diets by also using enzyme and thyme essential oil supplements to mitigate the negative effects of NSP. Thus, the effects of replacing corn with wheat, soybean meal with rapeseed meal and using enzyme and thyme essential oil as supplements on intestinal morphology and nutrient digestibility were evaluated in broilers.

Materials and methods

The research animal ethics committee of Islamic Azad University approved this experimental protocol. A total of 384 male Ross-308 broilers (one-day-old) weighed before starting the trial. They were divided to treatments in a 2³ factorial arrangement consisting of grain type (corn and wheat), meal type (soybean and rapeseed) and additive type (0.2 ml thyme essential oil and 100 gr/ton enzyme) with 32 plots.

Table 1 Nutritional contents of diets fed to broilers during the growing and finishing periods

	Growing period (11 to 24 days of age)				Finishing period (25 to 42 days of age)			
Corn	54.87	47.75	33.61	31.09	54.39	53.16	33.60	29.15
Soybean meal	38.59	36.52	30.60	22.82	36.85	30.75	30.40	24.09
Wheat	-	-	30.00	30.00	-	-	30.00	30.00
Rapeseed meal	-	10.00	-	10.00	-	10.00	-	10.00
Oil	1.90	1.40	1.78	2.20	3.50	2.50	2.50	3.18
Di-calcium phosphate	1.95	1.90	1.60	1.47	1.45	1.50	1.40	1.36
Calcium carbonate	0.99	1.49	1.33	1.28	1.20	1.20	1.20	1.20
Salt	0.24	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Mineral	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.37	0.14	0.28	0.30	0.01	0.001	0.018	0.17
L-Lysine	0.29	-	-	0.04	0.01	-	-	0.03
Metabolisable energy, kcal/kg	2950	2950	2950	2950	3000	3000	3000	3000
Crude protein, %	21.20	21.20	21.20	21.20	18.75	18.75	18.75	18.75
Calcium, %	0.96	0.83	0.83	0.95	0.84	0.84	0.84	0.84
Available phosphorus, %	0.45	0.41	0.41	0.44	0.33	0.33	0.35	0.38
Lysine, %	1.41	1.18	1.15	1.10	1.05	1.05	1.10	1.10
Methionine, %	0.49	0.49	0.48	0.49	0.36	0.36	0.36	0.36
Methionine + Cysteine, %	0.85	0.83	0.87	0.87	0.68	0.68	0.75	0.75

At the end of the experiment when the birds were 42 days old, two birds were selected from each experimental unit for slaughter based on their body weight. The birds were fasted for 6 hours prior to slaughter. After slaughter, lengths of the duodenum, jejunum and ileum were recorded and samples were collected for subsequent histological evaluation.

The middle sections of the duodenum (10 cm away from the pyloric junction), jejunum (proximal half of the remaining small intestine), and ileum (15 cm before the ileocecal junction) were aseptically isolated, flushed with 0.9% physiological saline solution. After fixation in 10% neutral buffered formalin, a single 0.5-cm sample was cut from each section, dehydrated with increasing concentrations (70, 80, 95, and 100%) of ethanol, cleared with xylene (Sub-X, Surgipath Medical Industries, Richmond, IL), and placed into polyfin embedding wax (Polysciences, Warrington, PA). Tissue sections (5 μ m) were cut, floated onto slides, stained with hematoxylin (Gill #2, Sigma, St. Louis, MO) and eosin (Sigma), and measured for villus height and crypt depth using light microscopy and a micrometer. Measurements for villi length were taken from the tip of the villus to the valley between individual villi and measurements for crypt depth were taken from the valley between individual villi to the basolateral membrane (Burkholder *et al.*, 2008).

The diets were formulated using the UFFDA software program (Pesti & Miller, 1993) to provide the advised levels of nutrients as specified for the Ross-308 broiler. To measure digestibility, on day 42 of growth period, 2 birds were randomly selected from each experimental unit and after slaughter, ileum contents from 5 cm left in cecum, was collected.

Experiment was a factorial design conducted over a growing period from 11 to 24 days of age and finishing period from 25 to 42 days of age. There were four replicates of each treatment, each consisting of 12 birds. The data were analysed using the general linear model procedure of SAS (SAS Institute, Inc., Cary, North Carolina, USA). The linear model was:

$$Y_{ijkl} = \mu + A_i + B_j + C_k + AB_{ij} + AC_{ik} + BC_{jk} + ABC_{ijk} + \varepsilon_{ijkl}$$

where: Y_{ijkl} = a dependent variable,

μ = overall mean,

A_i = the effect of grain type (corn and wheat),

B_j = the effect of meal type (soybean and rapeseed),

C_k = the effect of additive type (0.2 ml thyme essential oil and 100 gr/ton enzyme),

AB_{ij} = the interaction of grain and meal types,

AC_{ik} = the interaction of grain and additive types,

BC_{jk} = the interaction of meal and additive types,

ABC_{ijk} = the interaction of grain, meal and additive types. and

ε_{ijkl} = the residual deviation of the observation from the effects in the model.

Tukey's test at the 5% level of probability was used to compare means.

Results and Discussion

The significance of effects of the experimental treatments on intestinal morphology and protein digestibility are summarized in Table 2. None of the interaction effects were significant. Thus, the presentation of results and their discussion focuses on the main effects.

The lengths of all segments of the small intestine were significantly affected by the grain provided as the dietary base. Birds fed the diet that was based on wheat had a longer duodenum relative to their body weight than those fed the diet that was based on corn (1.48 ± 0.03 cm vs. 1.26 ± 0.03 cm, respectively). The main effect of the basal diet on jejunum length was also significant with the wheat based diet again producing greater length in this part of the small intestine than did the corn based diet (3.84 ± 0.02 cm vs. 3.39 ± 0.02 cm, respectively). Finally, as for the duodenum and jejunum, the length of the ileum was increased in those birds that received wheat relative to those fed corn (2.47 ± 0.04 cm vs. 2.25 ± 0.04 cm, respectively).

The length of the intestinal tract might have been increased in response to feeding wheat because it contains high levels of NSP which can increase the viscosity of the digesta. Increased viscosity of the digesta can lengthen the small intestine in more than one way. First, the more viscous digesta can exert a large amount of pressure on the lining of the intestine causing the muscle layers of the intestinal wall to relax. This relaxation results in increasing the length of the sarcomere and consequently the myofibrils of the muscle to counteract the pressure. In addition to increasing the length of the muscles in the intestinal wall, increased viscosity of the digesta can reduce their thickness is an adaptive mechanism to allow movement of the viscous material through the intestinal tract (Bederska-Łojewska *et al.*, 2017; Kogut *et al.*, 2017; Jha *et*

al., 2019). Second, viscosity may increase the length of the intestine because a greater surface is required to satisfy the bird's need for nutrients (Kaczmarek *et al.*, 2016; Rezaei *et al.*, 2018). It is generally accepted that this increase in gut viscosity reduced the mixing of digestive enzymes and substrates in the intestinal lumen (Kaczmarek *et al.*, 2016).

Table 2 Levels of probability from analyses of variance for the dependent variables that were recorded

Dependent variable		C vs. W (A)	S vs. R (B)	E vs. T (C)	A x B	A x C	B x C	A x B x C
Duodenum	length ¹ , cm	0.008	0.068	0.017	0.715	0.648	0.244	0.974
	Villus height, μm	0.014	0.042	0.876	0.090	0.621	0.714	0.142
	Crypt depth, μm	0.023	0.876	0.988	0.300	0.138	0.416	0.177
Jejunum	length ¹ , cm	0.027	0.097	0.058	0.316	0.945	0.246	0.449
	Villus height, μm	0.011	0.912	0.927	0.918	0.227	0.354	0.149
	Crypt depth, μm	0.215	0.911	0.887	0.087	0.975	0.128	0.235
Ileum	length ¹ , cm	0.001	0.122	0.088	0.784	0.615	0.228	0.061
	Villus height, μm	0.341	0.616	0.936	0.567	0.618	0.587	0.364
	Crypt depth, μm	0.641	0.349	0.967	0.345	0.355	0.114	0.615
Protein digestibility, %		0.310	0.987	0.793	0.649	0.812	0.352	0.150

¹ length measurements were scaled to 100 grams of body weight

C vs W (A): effect of either corn or wheat as the dietary base, S vs R (B): effect of adding rapeseed meal to the diet, E vs T (C): effect of supplemental enzyme or thyme essential oil

Yasar (2003) suggested that the length of the intestine of birds fed mixed rations with milled or whole grain wheat was significantly greater than that of control birds, which is consistent with the results of the present study. In contrast to these results, Wu *et al.* (2004) observed that whole wheat grain, although increasing gizzard weight and had no effect on the relative length of the small intestine. It is well known that the dietary content and amount of different fiber fractions may agitate physiological changes in the small intestine (Zduńczyk *et al.*, 2013; Jankowski *et al.*, 2013). More bioactive components, such as pentosan, may considerably affect intestinal viscosity, transit time, digesta moisture, and other indices of intestinal function (Kosmala *et al.*, 2014).

Feeding broilers a diet based on corn versus one based on wheat significantly increased the height of villi in the duodenum ($1187 \pm 28 \mu\text{m}$ vs. $1072 \pm 28 \mu\text{m}$, respectively). However, this effect was reversed in the jejunum with the wheat based diet increasing the height of the villi compared to the corn based diet ($388 \pm 19 \mu\text{m}$ vs. $321 \pm 19 \mu\text{m}$, respectively). Feeding rapeseed meal in addition to soybean meal also increased the height of the villi in the duodenum (1143 ± 28 vs. $1054 \pm 28 \mu\text{m}$, respectively). In the ileum, height of the villi averaged $650.5 \mu\text{m}$.

The intestinal villi are specialized structures that are very important for nutrient absorption of nutrients from the small intestine (Li *et al.*, 2017; Groff-Urayama *et al.*, 2019). By increasing the height of the villi, their surface area may become greater which can result in increased nutrient absorption (Marcos *et al.*, 2019).

Regarding the depth of crypt, the main effect of basal diet was significant with the wheat-based diet producing greater crypt depth in the duodenum than the treatments containing corn $388 \pm 19 \mu\text{m}$ vs. $321 \pm 19 \mu\text{m}$, respectively). Average depth of crypts in the jejunum and ileum were 244.5 and $190.5 \mu\text{m}$, respectively. The crypt can be considered the villus factory, and deep crypts indicate rapid tissue turnover and a high demand for new tissue (Wawrzyniak *et al.*, 2017; Kim *et al.*, 2018). Decreased crypt depth indicates a decrease in replacement of enterocytes which form the walls of the villi (Marcos *et al.*, 2019).

In the ileum of the broilers, neither villus height nor depth of crypt was significantly influenced by any of the treatments that were imposed in the present study.

Those birds whose diet was supplemented with thyme essential oil also had a longer duodenum than the birds whose diet was supplemented with enzymes (1.45 ± 0.03 cm vs. 1.32 ± 0.03 cm, respectively). However, beyond this single observation, no other differences in intestinal morphology were observed between birds whose diet was augmented with either supplement. Several researchers reported that feed additives did not affect villus height (Houshmand *et al.*, 2012) or width (Aliakbarpour *et al.*, 2012; Fallah *et al.*, 2013) compared with control groups. It has been reported that dietary exogenous enzymes improved the

gut morphology of broiler chickens (Nabizadeh *et al.*, 2017). However, the results of the present study indicate that the addition of dietary enzymes and thyme essential oil have similar effects on intestinal morphology.

While the basal diets altered intestinal morphology, they had no significant effect on protein digestibility in broiler chickens. Likewise, not significant effects were observed for the other treatments that were applied in this study, either singly or in combination. These results disagreed to previous report by Knap *et al.* (2011) who observed that broiler diets augmented with the probiotic *B. subtilis* significantly improved CP ileal digestibility and nitrogen retention compared to the control. Similarly, Zhang and Kim (2014) found that dietary supplementation with multi-strain probiotics improved ileal digestibility of amino acids and reduced ammonia content of the excreta.

Conclusions

Substitution of wheat for corn in broiler diets alters intestinal morphology with minimal effects on protein digestibility. Effects of adding rapeseed meal as another source of protein had benign effects on intestinal morphology. Effects of enzyme and thyme essential oil supplementation were similar. Interactions among these treatments were inconsequential. Based on these results and reports in related literature, wheat-based diets are expected to be acceptable for broilers when supplemented with appropriate enzymes or thyme essential oil.

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Authors' Contributions

EA collected the data for this study, YM conducted the statistical analyses, EA, AN and ARS developed the original hypotheses and designed the experiments, both authors have read and approved the finalized manuscript

Conflict of Interest Declaration

The authors declare that they have no known competing financial interests or personal relationship that could have appeared to influence the work reported in this paper.

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