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Original Research Article

Effects of dietary fiber and threonine on performance, intestinal morphology and immune responses in broiler chickens

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ABSTRACT

The present experiment was conducted to investigate the effects of fiber and threonine (Thr) on growth performance, intestinal morphology and immune responses of broiler chickens. A total of 420 one-day-old mixed sex broiler chicks (Ross 308) were randomly divided into 6 experimental diets and 5 replicates of 14 chicks based on a factorial arrangement (2×3) from 1 to 42 d of age. Experimental factors included dietary supplemental fiber type (no supplemental fiber [NSF], 30 g/kg sugar beet pulp [SBP] or 30 g/kg rice hull [RH]) and Thr inclusion level (100% or 110% of Thr requirement recommended by breeder company [Ross 308]). Growth performance of broilers was assayed at different periods of the experiment. Intestinal morphometric features were measured at 21 d of age. Antibody titer against sheep red blood cells (SRBC), Newcastle and influenza disease viruses were measured on d 30 of trial. Dietary inclusion of SBP and RH significantly decreased feed intake and weight gain during the entire rearing period ($P < 0.05$). Interaction of fiber and Thr had no beneficial effects on the performance of chickens across the entire rearing phase. Dietary supplementation of 110% Thr required level improved jejunal morphometric features ($P < 0.05$), whereas its inclusion with fibrous materials failed to show the same effects. Dietary supplemental Thr together with SBP significantly increased antibody production against SRBC ($P < 0.05$). In conclusion, although supplemental 30 g/kg fibrous materials impaired growth performance, inclusion of SBP along with 110% Thr level improved the humoral immunity in broiler chickens.

1. Introduction

Fiber is naturally present in plant-based feed ingredients and is an important component in poultry diets. Supplemental dietary fibrous materials have been found to improve digestive organs (Banfield et al., 2002) and enhance the growth performance (Jiménez-Moreno et al., 2010; Kheravii et al., 2017) of broiler chickens, depending upon dietary type and amount of fiber (Jiménez-Moreno et al., 2009). There are natural forms of readily accessible fibrous substances in the market such as rice hull (RH) and sugar beet pulp (SBP) that can favorably simulate effects of fibrous feedstuffs and so help us to better understand the effect of fiber on broiler chickens. As such, RH and SBP were considered as insoluble and soluble fiber sources, respectively in this experiment. Soluble fibers are potential to be fermented by anaerobic microbiota in cecum which may protect the gastrointestinal tract (GIT) from pathogen colonization (Liévin-Le Moal and Servin, 2006). Pectin as a soluble fiber fraction of SBP binds the water and creates the gel forming digesta within the GIT, causing reduced nutrient utilization (Langhout et al., 1999; Serena et al., 2007). However, these types of fiber are mainly investigated for their bacteriostatic effects on pathogenic bacteria (Santos et al., 2013; Wils-Plotz et al., 2013). On the other hand, RH is a by-product of rice milling, rich of insoluble fibers (Feng et al., 2004); mostly known for their effects

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on gizzard development and GIT reflux (Hetland et al., 2004). Diets rich in fiber were reported to influence the mucosa of the small intestine, whereby insoluble fractions favor abrasive effects on the intestinal mucus barrier (Montagne et al., 2003). Intestinal mucosa is a dynamic layer, predominantly composed of mucin, protecting epithelial cells from enzymatic damage and bacterial invasion (Kim and Ho, 2010). It has been demonstrated that dietary fiber undergoes changes in amount and type of mucin (Wils-Plotz and Dilger, 2013).

Mucin structure is composed of proteins and some amino acids such as serine, threonine (Thr) and cysteine. The prominent role of Thr in mucin production is because Thr constitutes up to 11% of mucin amino acids (Gum et al., 1992). As such, mucin dynamics in the gut might be sensitive to Thr availability. Threonine is the third limiting amino acid, particularly in low crude protein diets (Rezaeipour et al., 2012). This amino acid participates in prominent metabolic processes, such as formation of uric acid and protein synthesis. However, poultry do not synthesize enough Thr *de novo*, which makes this amino acid nutritionally necessary to be used in diet of broiler chickens. Also, Thr is known for its role in reducing bacteria adhesion to the mucosa in rats (Faure et al., 2006) since this is an important amino acid for mucin production (Gum et al., 1992). Moreover, Thr helps the mucin to remain intact and subsequently improves the immunity of animals. Evaluation of broilers immunity through antibody assessment is reasonable, owing to the fact that decrease of humoral antibody might increase the susceptibility of chickens to disease (Parmentier et al., 2004). Wils-Plotz and Dilger (2013) declared that dietary inclusion of 70 g/kg purified fiber (cellulose or pectin) does not alter Thr requirement in broiler chickens. Moreover, Thr was reported to influence the intestinal immune response in broiler chicks infected with Eimeria maxima (Wils-Plotz et al., 2013). Insufficient scientific reports exist on the interaction effects of fiber type and Thr on gut development and immune responses in broiler chickens.

Therefore, we hypothesized that using different types of fiber in addition to supplemental Thr in diet of broilers may develop their GIT, immunity and growth performance. Thereby, current experiment was aimed to investigate effects of 30 g/kg dietary inclusion of SBP or RH alongside dietary supplemental Thr (100% or 110% recommended level) on production performance, carcass traits, gut morphology and humoral immunity in broiler chickens.

2. Materials and methods

All experimental procedures were evaluated and approved by the Institutional Animal Care and Ethics Committee of the Islamic Azad University, Isfahan (Khorasgan) Branch.

2.1. Fiber sources

Prior to trial commence, SBP and RH were purchased from a commercial supplier, then were ground using a hammer mill (2-mm screen) and analyzed for chemical composition (Table 1). Crude fiber of fibrous materials was measured by sequential extraction with diluted acid and alkali (method 978.10) as indicated by AOAC (2000). Crude protein (CP) of fiber sources were determined using the method 990.03 (AOAC, 2000). The neutral detergent fiber (NDF) was determined sequentially as described by Van Soest et al. (1991) and expressed on ash-free basis. Calcium and phosphorus were analyzed by spectrophotometry (methods 968.08 and 965.17) according to AOAC (2000).

Table 1
Chemical composition of fibrous materials (g/kg, as-fed basis).

Item	Rice hull	Sugar beet pulp
Ash	164	45
Crude protein	50	79
Crude fiber	395	154
Neutral detergent fiber	581	371
Calcium	–	1.7
Phosphorous	–	0.6

2.2. Birds, diets and management

A total of 420 one-day-old mixed sex broiler chicks (Ross 308) were purchased from a commercial local hatchery and used in this experiment. At arrival, chicks were weighed; sexed, wing banded and assigned to treatment groups so that the initial weight was similar among different treatment groups. Male and female chicks were mixed at a ratio of 1:1. Five replicate pens of 14 chicks were randomly allotted to each of 6 treatments based on a factorial arrangement (2 × 3) in a completely randomized design across starter (1 to 14 d of age), growing (15 to 28 d of age) and finishing (29 to 42 d of age) periods. Experimental factors were dietary supplemental fiber type and Thr inclusion levels. Chicks were fed a basal diet (with no supplemental fiber, NSF), or the basal diet supplemented with either 30 g/kg soluble (SBP) or 30 g/kg insoluble (RH) fibers. Threonine inclusion levels in starter (7.8 and 8.58 g/kg), growing (8.6 and 9.46 g/kg) and finishing (7.6 and 8.36 g/kg) periods corresponded to 100% and 110% of total Thr requirements of broiler chickens (Aviagen, 2009). The basal diet included 30 g/kg silica sand that was replaced by the same amount of fibrous materials in the experimental diets. Experimental diets were formulated to meet nutrient requirements provided by Ross Manual (Aviagen, 2009, Table 2) across different periods and fed in a mash form. Chicks housed in 1.2 m × 1.2 m wire floor pens covered with paper roll and had free access to feed and water throughout the trial. Each pen was equipped with a separate feeder and a manual drinker. Broilers were kept in a temperature-controlled house at 32 °C from d 1 to 7, 29 °C from d 8 to 14, 26 °C from d 15 to 21, and 22 °C from d 22 to the end of trial. The lighting program was considered as 23 h light and 1 h darkness.

2.3. Growth performance, carcass traits, digestive organs and gizzard pH

Body weight gain (BWG) and feed intake (FI) of chicks in each pen were recorded at different phases of the experiment. The feed conversion ratio (FCR) was also calculated (FI/BWG). At 21 d of age and the end of the experiment, 2 birds close to the mean body weight of the pen were individually weighed and slaughtered. Carcass, abdominal fat, pancreas, heart and liver were excised, weighed and finally calculated as a percentage of live body weight basis. In order to evaluate the digestive organs, proportions of gizzard, proventriculus, duodenum, jejunum, ileum and cecum to live body weight were calculated at the end of the experiment. The length of intestinal segments including duodenum, jejunum, ileum and cecum were also measured separately. At 42 d of age, gizzard pH was measured by the collection of digesta content of 2 birds per pen, then homogenized in a 50 mL beaker and used to measure the pH of obtained subsamples using a digital pH meter (EDT. Instruments, GP353, ATG pH meter) according to González-Alvardo et al. (2007).

2.4. Morphology of small intestine

Two birds from each pen were slaughtered on d 21. Segments of the small intestine were sampled from midpoint of duodenum; intestine from the gizzard to pancreatic and bile ducts, jejunum;

Table 2
Ingredients and composition of basal diets during different rearing periods (g/kg, as-fed basis).

Item	1 to 14 d of age	15 to 28 d of age	29 to 42 d of age
Ingredients			
Corn	524.0	550.0	580.0
Soybean meal	374.0	346.0	321.0
Soybean oil	27.2	35.4	35.2
Dicalcium phosphate	18.3	16.0	14.5
Calcium carbonate	11.8	10.0	9.4
DL-methionine	3.1	2.5	1.9
L-lysine	2.1	1.1	0.1
L-threonine	1.5	1.0	0.4
Vitamin premix[1]	2.5	2.5	2.5
Mineral premix[2]	2.5	2.5	2.5
Sodium chloride	3.0	3.0	2.5
Silica sand	30	30	30
Total	1,000	1,000	1,000
Calculated composition			
ME, kcal/kg	2,850	2,950	3,000
Crude protein	214.3	196.7	187.0
Digestible lysine	10.7	10.3	9.1
Digestible methionine + cysteine	7.8	7.9	7.1
Digestible threonine	6.8	7.6	6.6
Calcium	10	8.4	8.0
Available phosphorous	4.7	4.2	4.0
Sodium	1.3	1.3	1.3
Crude fiber	37.6	36.3	35.1

[1] Vitamin premix provided the following per kilogram of diet: vitamin A (retinol), 2.7 mg; vitamin D_3 (cholecalciferol), 0.05 mg; vitamin E (tocopheryl acetate), 18 mg; vitamin K_3, 2 mg; thiamine 1.8 mg; riboflavin, 6.6 mg; pantothenic acid, 10 mg; pyridoxine, 3 mg; cyanocobalamin, 0.015 mg; niacin, 30 mg; biotin, 0.1 mg; folic acid, 1 mg; choline chloride, 250 mg; antioxidant 100 mg.

[2] Mineral provided the following per kilogram of diet: Fe ($FeSO_4 \cdot 7H_2O$, 20.09% Fe), 50 mg; Mn ($MnSO_4 \cdot H_2O$, 32.49% Mn), 100 mg; Zn (ZnO, 80.35% Zn), 100 mg; Cu ($CuSO_4 \cdot 5H_2O$), 10 mg; I (KI, 58% I), 1 mg rice husk; Se ($NaSeO_3$, 45.56% Se), 0.2 mg.

midway between the point of entry of the bile ducts and Meckel's diverticulum, Ileum; 10 cm proximal to the ileo-cecal junction. The intestinal samples were evaluated for the villus height, crypt depth and the ratio of villus height to crypt depth (VH:CD). Segments with 1.5-cm length were gently flushed twice with physiological saline solution (1% NaCl) to remove intestinal contents and were placed in 10% formalin in 0.1 mol/L phosphate buffer (pH = 7.0) for fixation. The samples were processed for 24 h in a tissue processor with ethanol as dehydrant and were embedded in paraffin. Sections (5 μm) were made and stained with hematoxylin-eosin. Optical microscope (Olympus CX31, Tokyo, Japan) was used for morphological examination of small intestine samples. Ten intact, well-oriented villus-crypt units were selected for each intestinal cross section (2 cross sections per sample and 20 cross sections per treatment, for a total of 200 measurements per treatment). Villus height (μm) was measured from the tip of the villus to the villus crypt junction, and crypt depth was defined as the depth of the invagination between 2 villi. Villus height to crypt depth ratio was then calculated. The average of value for each cross section was used for further analysis.

2.5. Immune system measurements and heterophil to lymphocyte ratio

At 9 d of age, Newcastle and influenza antigens were injected to chicks with dual vaccine of Newcastle-influenza (H9N2 subtype). Also, chicks were orally vaccinated against Newcastle disease (Lasota) at 19 d of age. Two chicks per pen were selected randomly for intraperitoneal injection with a 1.0-mL of SRBC suspension diluted with phosphate buffer saline at 25 d of age. Five days later, the same wing-banded birds were bled to determine antibody titer against sheep red blood cells (SRBC), influenza disease virus (IDV) and Newcastle disease virus (NDV). Subsequently antibody titer against SRBC was measured by hemagglutination assay (HA) method. Antibody titers against IDV and NDV separately were measured by hemagglutination inhibition method (HI). The HI antibodies were then converted to log2. Antibody titers against SRBC were measured by the microtiter procedure described by Wegmann and Smithies (1966). Heterophil to lymphocyte (H:L) ratio was obtained by blood sampling on d 30. Blood samples were taken from wing vein using syringes containing heparin to avoid blood clot formation. Blood smears were stained by May–Greenwald–Giemsa stain (Lucas et al., 1961). One hundred leukocytes per samples were counted by heterophil to lymphocyte separation under an optical microscope (Nikon, Japan) with 100 × oil immersion lens, and H:L ratio was calculated and recorded (Gross and Siegel, 1983).

2.6. Statistical analysis

Data were analyzed in a 2 × 3 factorial arrangement of treatments in a completely randomized design using the GLM procedure of SAS 9.2 (SAS Institute Inc., Cary, NC). The pen was considered as the experimental unit for different parameters. The statistical model included the fixed effects of Thr level, fiber type and their interactions. When a significant *F*-test was detected ($P < 0.05$) for the main effects, corresponding means were determined using least significant difference (LSD) test and interaction effects were analyzed by LS-Means. Statements of statistical significance were declared $P < 0.05$ unless noted otherwise.

3. Results

3.1. Growth performance, carcass traits, digestive organs and gizzard pH

Growth performance of chickens is shown in Table 3. Inclusion of dietary Thr more than 100% recommended level along with fibrous materials did not apply positive effects on the performance of chickens across the entire rearing period. In comparison to NSF, dietary supplemental SBP and RH significantly decreased FI of broilers during the entire production period of the experiment ($P < 0.05$). Subsequently, supplementation of fiber decreased BWG considerably during the starter, growing and the entire rearing periods ($P < 0.05$). These alterations in FI and BWG of chicks received fibrous materials impaired FCR from 1 to 14 d of age ($P < 0.05$). However, FCR improved as broiler chickens aged with consumption of diets supplemented with RH in the finishing period of the experiment ($P < 0.05$). Supplemental SBP in the feed significantly decreased heart relative weight compared with broilers receiving NSF at 21 d of age (Table 4; $P < 0.05$). Furthermore, both soluble and insoluble fiber sources increased pancreas relative weight at 42 d of age (Table 4; $P < 0.05$). Broilers given SBP-added diets had higher jejunal length than those given RH at 42 d of age (Table 5; $P < 0.05$). Additionally, supplementation of 110% recommended Thr level resulted in lower duodenum relative weight than those supplemented with 100% Thr (Table 5; $P < 0.05$). Gizzard pH was not affected by fibrous materials, Thr level or interaction of them (Table 5).

3.2. Morphology of the small intestine

The interaction effect of Thr and fiber was significant in jejunum where 110% dietary inclusion of recommended Thr along with SBP had greater villus height than 100% Thr (Table 6; $P < 0.05$) but VH:CD ratio was not influenced. Broilers received RH-containing diets had considerably shorter jejunal villus height than those received NSF and SBP- containing diets (Table 6; $P < 0.05$). On the other hand, RH

Table 3
Effects of dietary treatments on the performance of broiler chickens at different rearing periods.

Item	Threonine level, %	Feed Intake, g/d				Body weight gain, g/d				Feed conversion ratio, g:g			
Fiber type		1 to 14 d	15 to 28 d	29 to 42 d	1 to 42 d	1 to 14 d	15 to 28 d	29 to 42 d	1 to 42 d	1 to 14 d	15 to 28 d	29 to 42 d	1 to 42 d
NSF	100	26.3[ab]	100.2[ab]	154.4	92.7	19.0[b]	63.6[b]	70.6	51.1[ab]	1.39	1.57	2.18[a]	1.81
NSF	110	27.7[a]	103.4[a]	149.1	92.5	21.1[a]	69.1[a]	69.7	53.2[a]	1.32	1.50	2.14[a]	1.73
SBP	100	26[bc]	97.9[abc]	150.8	90.6	18.2[bc]	64.2[b]	74.6	52.3[ab]	1.43	1.52	2.03[ab]	1.73
SBP	110	24.2[d]	94.1[bcd]	142.7	86.1	16.4[cd]	60.1[bc]	67.5	48[b]	1.48	1.56	2.11[ab]	1.79
RH	100	24.5[cd]	88.4[d]	147.2	85.9	16.4[cd]	57.7[c]	77.6	50.6[ab]	1.49	1.53	1.89[b]	1.69
RH	110	23.2[d]	91.3[cd]	146.4	86.1	15.2[d]	59.6[bc]	69.5	48.1[b]	1.52	1.53	2.10[ab]	1.79
SEM		0.81	3.11	1.80	0.97	0.42	2.12	1.07	1.32	0.017	0.011	0.045	0.014
Main effects													
Fiber type													
NSF		26.9[a]	102.1[a]	152.9[a]	93.1[a]	19.92[a]	66.3[a]	71.1	52.4[a]	1.35[b]	1.54	2.15[a]	1.77
SBP		24.9[b]	94.6[b]	142.4[b]	86.8[b]	17.02[b]	61.2[b]	69.8	49.3[b]	1.46[a]	1.54	2.06[ab]	1.76
RH		24.4[b]	90[c]	144.8[ab]	85.6[b]	16.07[b]	59.3[b]	72.6	49.1[b]	1.50[a]	1.51	1.98[b]	1.72
SEM		0.38	1.5	4.11	1.24	0.45	1.12	1.92	0.94	0.022	0.019	0.042	0.023
Threonine level, %													
100		25.6	95.7	149	89.6	18.14	62.2	73.2	51.2	1.42	1.54	2.05	1.75
110		25.04	96.1	144.8	87.9	17.49	62.8	69.4	49.8	1.45	1.52	2.08	1.76
SEM		0.42	1.86	2.53	1.38	0.55	1.23	1.46	0.85	0.023	0.014	0.039	0.02
P-value													
Fiber type		<0.0001	<0.0001	0.0491	0.0009	<0.0001	0.0001	0.7572	0.0187	0.0002	0.5563	0.0300	0.4018
Threonine level		0.3243	0.8382	0.2568	0.1882	0.1972	0.7625	0.0612	0.1011	0.4443	0.6182	0.4488	0.6082
Fiber type × Threonine level		0.0099	0.0129	0.7830	0.1722	0.0021	0.0047	0.2026	0.0080	0.0706	0.1741	0.0414	0.0696

NSF = no supplemented fiber; SBP = sugar beet pulp; RH = rice hull.
[a, b, c, d] Values in the same column not sharing a common superscript differ significantly ($P < 0.05$).

increased both villus height and crypt depth in ileum ($P < 0.05$) while VH:CD ratio was not affected. The effect of Thr on gut morphology was more observable in jejunum than in ileum since all morphometric features of villus height, crypt depth and VH:CD ratio of chickens received 110% recommended Thr was notably greater than those received 100% recommended Thr (Table 6; $P < 0.05$).

3.3. Immune system measurements and heterophil to lymphocyte ratio

The effect of supplemental SBP on the antibody titer against SRBC was more pronounced when diet included with 110% Thr requirement compared with 100% Thr (Table 7; $P < 0.05$). A significant higher antibody titer against SRBC was observed in broilers fed diets containing 110% recommended Thr compared with 100% Thr ($P < 0.05$). Supplementation of diet with RH increased H:L ratio (Table 7; $P < 0.05$). On the other hand, broilers given 110% dietary recommended Thr showed attenuated H:L ratio (Table 7; $P < 0.05$).

4. Discussion

In this trial, we expected that fiber inclusion would increase Thr requirement of broiler chickens because dietary fibrous materials have abrasive effect on the intestinal mucus barrier and due to the

Table 4
Effects of dietary treatments on carcass traits of broiler chickens.

Item	Threonine level, %	Relative organ weight, % BW								
		Carcass		Abdominal fat	Pancreas		Heart		Liver	
Fiber type		21 d	42 d	42 d	21 d	42 d	21 d	42 d	21 d	42 d
NSF	100	68.81	59.81[b]	1.63[a]	0.37[ab]	0.25	0.72[a]	0.58[a]	2.78	2.24
NSF	110	55.42	62.41[a]	1.20[bc]	0.34[b]	0.24	0.68[ab]	0.51[ab]	2.73	2.19
SBP	100	67.24	62.52[a]	0.99[c]	0.34[b]	0.27	0.62[ab]	0.55[ab]	2.64	2.26
SBP	110	65.95	60.65[ab]	1.53[ab]	0.38[ab]	0.27	0.58[b]	0.48[b]	2.55	2.27
RH	100	68.14	61.86[ab]	1.44[ab]	0.47[a]	0.26	0.62[ab]	0.56[ab]	2.67	2.40
RH	110	67.98	61.40[ab]	1.53[ab]	0.36[ab]	0.28	0.71[a]	0.55[ab]	2.73	2.22
SEM		2.05	0.90	0.097	0.056	0.005	0.056	0.017	0.044	0.031
Main effects										
Fiber type										
NSF		62.11	61.04	1.41	0.36	0.24[b]	0.70[a]	0.55	2.75	2.21
SBP		66.59	61.59	1.31	0.36	0.27[a]	0.60[b]	0.52	2.60	2.26
RH		68.06	61.63	1.49	0.42	0.27[a]	0.66[ab]	0.55	2.70	2.30
SEM		2.43	0.51	0.098	0.027	0.008	0.026	0.018	0.076	0.052
Threonine level, %										
100		68.06	61.39	1.40	0.39	0.26	0.65	0.56[a]	2.70	2.30
110		63.12	61.45	1.42	0.36	0.26	0.66	0.51[b]	2.68	2.22
SEM		2.45	0.42	0.082	0.023	0.007	0.023	0.014	0.063	0.043
P-value										
Fiber type		0.4766	0.7074	0.2540	0.3538	0.0450	0.0471	0.3036	0.4258	0.4724
Threonine level		0.2390	0.8715	0.5499	0.2469	0.6710	0.9485	0.0203	0.8597	0.2402
Fiber type × Threonine level		0.3595	0.0074	0.0030	0.0471	0.7010	0.0334	0.3358	0.8217	0.4425

BW = body weight; NSF = no supplemented fiber; SBP = sugar beet pulp; RH = rice hull.
[a, b, c] Values in the same column not sharing a common superscript differ significantly ($P < 0.05$).

Table 5
Effects of dietary treatments on digestive organs and gizzard pH of broiler chickens.

Item	Threonine level, %	Relative weight, % BW						Length, cm				Gizzard pH
Fiber type		Proventriculus	Gizzard	Duodenum	Jejunum	Ileum	Caecum	Duodenum	Jejunum	Ileum	Caecum	
NSF	100	0.38	1.60	0.76	2.25	1.84	0.54	33	88	88	37	3.07
NSF	110	0.37	1.55	0.69	2.23	1.65	0.52	34	89	90	39	3.42
SBP	100	0.41	1.48	0.73	2.45	2.01	0.54	33	90	90	39	3.28
SBP	110	0.41	1.49	0.68	2.67	1.92	0.64	34	93	93	42	3.30
RH	100	0.40	1.51	0.71	2.11	1.75	0.63	33	82	85	39	2.93
RH	110	0.39	1.68	0.67	2.26	2.05	0.59	34	88	91	37	2.96
SEM		0.007	0.037	0.01	0.06	0.04	0.016	0.45	1.18	1.22	0.50	0.073
Main effects												
Fiber type												
NSF		0.38	1.57	0.73	2.24	1.75	0.53	33	89^{ab}	89	38	3.25
SBP		0.41	1.48	0.70	2.55	1.97	0.59	34	92^{a}	92	41	3.29
RH		0.39	1.59	0.69	2.19	1.91	0.61	33	85^{b}	88	38	2.95
SEM		0.012	0.063	0.022	0.11	0.07	0.028	0.78	1.96	1.9	0.83	0.11
Threonine level, %												
100		0.40	1.53	0.73^{a}	2.27	1.87	0.57	33.4	87	88	38	3.09
110		0.39	1.57	0.68^{b}	2.38	1.88	0.58	34.36	90	91	40	3.22
SEM		0.010	0.052	0.017	0.97	0.06	0.023	0.41	1.66	1.55	0.70	0.104
P-value												
Fiber type		0.2335	0.4649	0.4583	0.0664	0.1194	0.1122	0.9765	0.0471	0.4487	0.0627	0.1246
Threonine level		0.5749	0.5888	0.0408	0.4003	0.9291	0.6409	0.3091	0.2050	0.1573	0.2522	0.3793
Fiber type × Threonine level		0.8878	0.4693	0.9330	0.7718	0.0621	0.5315	0.8726	0.7200	0.7349	0.1071	0.5735

BW = body weight; NSF = no supplemented fiber; SBP = sugar beet pulp; RH = rice hull.
[a, b] Values in the same column not sharing a common superscript differ significantly ($P < 0.05$).

fact that Thr is the constituent of mucin (Gum et al., 1992). Even though, interaction results did not show any beneficial effect of providing 110% Thr requirement on the growth performance of chickens fed diets supplemented with SBP or RH. In accord to this study, Wils-Plotz and Dilger (2013) fed broilers diets containing 7 graded levels, i.e. from 0 to 9.6 g/kg supplemental Thr, and observed that purified fiber supplementation not only did not increase the Thr requirement, but pectin decreased the need to Thr. Because dietary fiber inclusion in this study impaired growth performance of broiler chickens, it is likely that the smaller body size of the birds fed experimental fiber-containing diets is the reason for lack of change in their Thr requirement (Wils-Plotz and Dilger, 2013). Broiler chickens gained more weight when received diets supplemented with Thr without supplemental fiber across growing period. Consistent with our findings, Khan et al. (2006) exhibited the improved growth performance of broilers receiving Thr in their feed whereas Kidd et al. (2002) and Rezaeipour and Gazani (2014) failed to observe beneficial effect of dietary Thr supplementation on growth performance of broiler chickens. Supplementation of SBP and RH in diet substantially decreased FI and BWG of broiler chicks at younger ages. This is in agreement with the work of Jiménez-Moreno et al. (2013a,b) who found a slight decrease of FI from 1 to 12 d of age and a significant BWG reduction from d 1 to 6 in broilers fed more than 25 g/kg SBP in their diet. Similarly, Wils-Plotz and Dilger (2013) suggested the BWG reductions in broilers receiving 70 g/kg pectin from 0 to 14 d post-hatch. Furthermore, our results are supported by Sadeghi et al. (2015) who indicated that supplementation of 30 g/kg SBP to the basal diet decreased BWG in broiler chickens. Sugar beet pulp is a soluble fiber source, containing pectin with ability to create viscous environment within

Table 6
Effects of dietary treatments on intestinal morphology of broiler chickens.

Item	Threonine level, %	Jejunum			Ileum		
Fiber type		Villi height, μm	Crypt depth, μm	VH:CD ratio	Villi height, μm	Crypt depth, μm	VH:CD ratio
NSF	100	610^{b}	173	3.69^{d}	623^{a}	181^{b}	4.20
NSF	110	880^{a}	192	4.73^{a}	535^{b}	182^{b}	3.10
SBP	100	670^{b}	175	4.26^{bc}	481^{c}	151^{c}	3.28
SBP	110	830^{a}	183	4.62^{ab}	609^{a}	195^{a}	3.23
RH	100	630^{b}	169	3.85^{cd}	586^{a}	190^{a}	3.18
RH	110	670^{b}	174	3.95^{cd}	601^{a}	200^{a}	3.16
SEM		10.44	2.14	0.067	7.12	2.95	0.058
Main effects							
Fiber type							
NSF		741^{a}	182	4.18^{ab}	545^{b}	173^{b}	3.65
SBP		767^{a}	179	4.46^{a}	575^{ab}	182^{ab}	3.26
RH		658^{b}	172	3.9^{b}	593^{a}	195^{a}	3.27
SEM		165.18	35.05	0.11	12.19	5.03	0.09
Threonine level, %							
100		641^{b}	172^{b}	3.91^{b}	558	173	3.58
110		795^{a}	182^{a}	4.42^{a}	580	192	3.36
SEM		75	4.09	0.23	11.03	6.09	0.28
P-value							
Fiber type		<0.0001	0.0897	0.0032	0.0075	0.0088	0.7010
Threonine level		<0.0001	0.0114	0.0001	0.1701	0.066	0.5201
Fiber type × Threonine level		<0.0001	0.4159	0.0086	<0.0001	0.0028	0.4362

NSF = no supplemented fiber; SBP = sugar beet pulp; RH = rice hull; VH:CD ratio = villus height to crypt depth ratio.
[a, b, c, d] Values in the same column not sharing a common superscript differ significantly ($P < 0.05$).

Table 7
Effects of dietary treatments on humoral immunity of broiler chickens.

Item	Threonine level, %	Humoral immunity, log_2			H:L ratio
Fiber type		SRBC	NDV	IDV	
NSF	100	4.5[c]	6.2	3.8	0.76
NSF	110	6.4[b]	6.8	3.6	0.54
SBP	100	3.9[c]	5.4	4.0	0.81
SBP	110	8.7[a]	6.2	3.8	0.65
RH	100	5.1[bc]	6.1	4.6	0.98
RH	110	4.2[c]	5.8	4.0	0.85
SEM		0.67	0.21	0.29	0.05
Main effects					
Fiber type					
NSF		5.45	6.5	3.7	0.65[b]
SBP		6.30	8.5	3.9	0.73[b]
RH		5.65	5.9	4.3	0.91[a]
SEM		0.53	0.35	0.51	0.08
Threonine level, %					
100		4.50[b]	5.86	4.13	0.85[a]
110		6.43[a]	6.22	3.80	0.68[b]
SEM		0.39	0.29	0.42	0.07
P-value					
Fiber type		0.3702	0.3827	0.7416	0.0400
Threonine level		0.2419	0.3693	0.6079	0.0385
Fiber type × Threonine level		0.0318	0.6196	0.9578	0.2799

NSF = no supplemented fiber; SBP = sugar beet pulp; RH = rice hull; SRBC = sheep red blood cells; NDV = newcastle disease virus; IDV = influenza disease virus; H:L ratio = heterophile to lymphocyte ratio.
[a, b, c] Values in the same column not sharing a common superscript differ significantly ($P < 0.05$).

the GIT (Jiménez-Moreno et al., 2010). This viscosity has been shown to affect the mechanisms that subsequently regulate and decrease FI (Serena et al., 2007). On the other hand, there are trials pointing out the improved growth performance of broilers in response to moderate intake of fibrous materials irrespective of their solubility, with maximum 15 g/kg crude fiber of basal diet in younger ages (González-Alvardo et al., 2007). In the present trial, we had 30 g/kg of supplemental RH or SBP along with 30 to 40 g/kg of crude fiber within the basal feed. As such, feeding excessive amounts of fibrous materials might had led to distension of the GIT and consequently elevated the maintenance energy requirement of the birds (Zhao et al., 1996). Therefore, deteriorated growth performance of fiber fed broilers in this study may be attributed to their FI reduction and increased maintenance requirement. Moreover, improved FCR in finishing rearing period of chicks fed on fibrous materials is confirmed by the fact that immature GIT of the birds at hatch develop and obtain its maturity from 15 to 21 d of age (Sklan, 2001). Hence, birds might be able to digest more fiber with their age and obtained their BWG with lower FI.

Broilers received SBP had lower heart weight than the bird in other groups at 21 d of age. The reduction in FI and BWG of fiber-fed birds may have resulted in decreased heart activity and declined heart proportional weight. Furthermore, increased pancreas weight in chickens fed SBP is related to the higher digesta viscosity in digestive tract of them (Banfield et al., 2002). Otherwise, higher pancreas weight due to consuming insoluble dietary fiber may primarily attributable to the effect of RH on gastro-duodenal reflux and secretion of pancreatic enzymes (Svihus et al., 2004). These results are in keeping with the findings of Saki et al. (2011) who observed the decreased heart and pancreas relative weights using different dietary ratios of soluble to insoluble fibers.

Based on our results, broilers given diets added with SBP had higher jejunal length than those given RH and NSF. The higher length of intestine is associated with rich pectin content of SBP with bulking properties, imposing muscular hypertrophy (Jiménez-Moreno et al., 2013b). This is in contrast to the work of Saki et al. (2011) in which higher dietary ratio of soluble to insoluble fiber had no effects on jejunal length of broilers at 21 d of age but in agreement with Jiménez-Moreno et al. (2013b) who reported the increased intestinal length through SBP supplementation at different ages of broiler chickens.

Villus height is an indicator of prepared surface area for digesta absorption (Montagne et al., 2003) and crypt depth is a criterion to evaluate proliferative potential of the intestine (Iji et al., 2001). Accordingly, measuring VH:CD ratio is a useful method to estimate the absorptive capacity of the small intestine (Montagne et al., 2003). Although supplementation of 110% Thr requirement to diets containing soluble fiber improved jejunal height, the VH:CD ratio was not significantly affected. It may show why the growth performance of broilers did not change following Thr supplementation to the fiber supplemented diets. Broilers given diets supplemented with RH had decreased villus height and VH:CD ratio in jejunum but increased villus height and crypt depth in ileum. Probably, decrease of villus height in jejunum is related to the abrasive effect of insoluble fiber on the intestinal mucus (Montagne et al., 2003). Similarly, Jiménez-Moreno et al. (2013b) reported that diets containing 75 g/kg oat hull decreased intestinal villus height in broiler chickens. Moreover, enlargement in villus height and crypt depth of intestinal ileum in RH fed birds is in line with the work of Rezaei et al. (2011) who suggested increased ileal villus height of chickens supplemented with graded levels of insoluble fiber in their diet. Feeding diets added with 110% Thr requirement increased villus height, crypt depth and VH:CD ratio compared with 100% Thr. The critical effect of Thr on intestinal growth might be due to its prominent role in the structure of mucin (Gum et al., 1992). Beneficial influence of Thr on gut morphology has been reported by Zaefarian et al. (2008). They observed the increased villus height and VH:CD ratio through supplementation of graded levels of Thr in diet of broiler chickens. Results of our study are in agreement with this report.

The beneficial impact of soluble fiber on immunity is established through fermentation in the hind gut, where fiber imposes its bacteriostatic impact on enteric bacteria with the production of short-chain fatty acids (Van der Wielen et al., 2001). In the current trial, dietary Thr supplementation together with inclusion of SBP resulted in the highest level of antibody titer against SRBC. Santos et al. (2013) exhibited that dietary mannan oligosaccharides and

Thr significantly improved intestinal environment and contributed to recovery after Salmonella challenge. Moreover, Wils-Plotz et al. (2013) observed that pectin and Thr may have had synergetic impact on gut immune response against coccidiosis challenge. These results are consistent with the findings of this experiment. Furthermore, Thr supplementation increased antibody titer against SRBC, suggesting the role of Thr in immunity. Protective function of Thr might be applied through maintaining mucus barrier (Wang et al., 2009) and contribution to the normal growth of body lean tissue (Wils-Plotz et al., 2013).

Although fibrous materials increased the H:L ratio, this parameter decreased remarkably using 110% Thr. The H:L ratio is an indicator for the presence of physiological stress (Siegel, 1995). The effect of fiber on H:L ratio in laying hens is well documented, but research in broiler chickens is scarce. El-Lethey et al. (2000) indicated that dietary access to insoluble fiber in laying hens decreased H:L ratio, in contrast to the findings of the current study. Also, Van der Wielen et al. (2001) observed mitigated feather damage and improved welfare using insoluble non-starch polysaccharides. Therefore, it seems that overall prepared fiber, especially RH in this study imposed stress on broilers. Moreover, effect of Thr on reduction of H:L ratio is supported by the work of Abbasi et al. (2012) but is in contrast to increased H:L ratio in the work of Jahanian (2010).

In conclusion, 30 g/kg supplementation of RH or SBP to the basal diet with 30 to 40 g/kg crude fiber had deleterious impact on growth performance of broilers, particularly at younger ages. Furthermore, dietary inclusion of Thr along with SBP or RH supplementation did not affect growth performance of broiler chickens. This is confirmed by data on the morphology of the small intestine whereby inclusion of 110% Thr requirement together with SBP failed to change the VH:CD ratio in jejunum. Dietary supplemental SBP increased antibody titer against SRBC when applied in diets containing 110% Thr. Moreover, H:L ratio increased using RH, suggesting that broilers did not feel comfortable with this amount of fiber in their diet. It seems that further studies are needed to better evaluate the interaction effect of Thr and fiber on immune related parameters in broiler chickens.

Conflict of interest

The authors declare that they have no conflict of interest.

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