

Interactive effects of threonine levels and protein source on growth performance and carcass traits, gut morphology, ileal digestibility of protein and amino acids, and immunity in broilers

I. Ahmed,* S. N. Qaisrani,*¹ F. Azam,* T. N. Pasha,* F. Bibi,[†] S. Naveed,* and S. Murtaza*

*Department of Animal Nutrition, Faculty of Animal Production and Technology, University of Veterinary and Animal Sciences, Lahore, Punjab 54000, Pakistan; and [†]Department of Livestock and Poultry Production, Faculty of Veterinary Sciences, Bahauddin Zakariya University, Multan, Punjab 66000, Pakistan

ABSTRACT An experiment was executed to test the hypothesis that supplementation of dietary threonine (**d-Thr**), above NRC recommendation to diets containing poorly digestible protein source (**PS**) may compensate its detrimental effects on overall performance of broilers. In total, nine hundred 1-day-old mixed sex broilers (Ross-308) were randomly distributed over 6 (2 × 3) experimental diets comprising 5 replicates of 30 broilers each for 35 d. The experimental diets contain either soybean meal (**SBM**) or canola meal (**CM**) with 3 levels (100, 110, and 120% of NRC recommendation) of d-Thr. During the course of the trial (0 to 35 D), interactions ($P < 0.05$) between PS and d-Thr were observed for feed intake (**FI**), body weight gain (**BWG**), feed conversion ratio (**FCR**), carcass, and gut health parameters. The broilers fed recommended level (100%) of d-Thr had 7 and 5% poorer FCR compared with those fed diets with 110 and 120% d-Thr, respectively. For villus height (**VH**), an

interaction ($P = 0.007$) was found between PS and d-Thr level. Broilers consuming SBM diets had 22% longer villi, 10% deeper crypts, and 30% greater VH to crypt depth ratio (**VCR**) compared to those fed CM. The broilers fed 110% d-Thr diets had 9% lower crypt depth (**CD**) and 15% greater VCR compared with those fed diets containing NRC recommended levels. CM resulted in 9% lower protein digestibility with lower ($P < 0.05$) of some AA, whereas it was improved by 7% in broilers fed 120% d-Thr supplemented diets. The bursa and spleen weights were positively affected ($P < 0.001$) by PS. Threonine supplementation (10%) resulted in 25% greater thymus, 18% heavier bursa, and 30% greater infectious bursal disease titer. In conclusion, supplementation of d-Thr, above NRC recommendation, resulted in a better growth performance and carcass traits, improved ileal digestibility of protein and amino acids, better gut health, and immunity in broilers.

Key words: threonine, growth performance, gut morphology, amino acid digestibility, broilers

2020 Poultry Science 99:280–289
<http://dx.doi.org/10.3382/ps/pez488>

INTRODUCTION

To meet the nutritional requirements for optimal production performance, modern broilers require precise levels of dietary nutrients particularly amino acids and energy (Kim et al., 2007). Optimal dietary amino acid contents, additionally, are prerequisite to support gut functions. Various factors including sex, genotype, and age affect the nutrient requirements in growing broilers (Webel et al., 1996). Their improper dietary supply, therefore, may lead to either poor growth performance or contributes to environmental pollution due to undue nitrogen excretion. In addition, these amino acids, besides maintenance, growth, and reproduction, have a major role in the defense mechanisms of birds due

to their involvement in the synthesis of antibodies in immune regulatory pathways (Trevisi et al., 2015).

Threonine (**Thr**), the third limiting amino acid after methionine and lysine in corn-soy diets, is an indispensable amino acid for broilers (Berres et al., 2007) that is involved in the development and proper functioning of the gut. Synthesis of body protein, collagen, elastin, antibody as well as production of uric acid and pancreatic enzymes also requires Thr (Debnath et al., 2019). The influence of Thr on growth parameters, gut health, immunity, and carcass traits was reviewed in broilers (Qaisrani et al., 2018). Threonine, furthermore, is pivotal for optimal utilization of sulphur containing amino acids (Meth+Cys) and lysine (Kidd, 2000). The maintenance of intestinal barrier and mucin synthesis also requires Thr (Wang et al., 2010) since mucus of the digestive tract contains about 40% protein that is majorly Thr (Carlstedt et al., 1993). Dietary Thr, above the requirements, promotes the growth of immune organs, stimulates the synthesis of immunoglobulins, improves

Received March 30, 2019.

Accepted August 6, 2019.

¹Corresponding author: shafqat.qaisrani@uvas.edu.pk

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immune response and alleviates the immune stress caused by *Escherichia coli* or Newcastle disease virus (NDV) in challenged birds (Trevisi et al., 2015).

Canola, also known as double-zero rapeseed, contains low levels of erucic acid (<30 micro moles/gram) and glucosinolates (<2%) (Khajali and Slominski, 2012) with 34 to 38% CP contents that may partially replace soybean meal (SBM) (Gopinger et al., 2014). Several anti-nutritional factors, including glucosinolates, phytic acid, tannins, non-starch polysaccharides (NSPs) (BK et al., 2015) and fiber content, however, decrease its nutritional value for broilers. The NSPs in canola meal (CM), specifically water soluble, cause an increased viscosity of intestinal contents (Shakouri et al., 2009). Canola meal contains more than double (11.5 vs. 5.4%) fiber compared with SBM (Khajali and Slominski, 2012). Most of this fiber in CM is insoluble that causes bulkiness and increased digesta passage rate through the intestine (Hetland et al., 2004) that confines the time required for nutrients breakdown leading to poor digestibility and ultimately compromised performance of the birds (Gopinger et al., 2014). Gut maintenance requirements, moreover, increase as a result of high insoluble fiber proportion in diet because of higher turnover rates of intestinal cells leading to reduced villus height (VH) and villus height to crypt depth ratio (VCR) (Mateos et al., 2012), which results in compromised gut health and ultimately compromised nutrients digestibility.

Threonine is considered as the most important amino acid for the maintenance of intestinal mucosa since it is used in synthesis of mucin that covers the surface area of mucosa. For broilers, NRC (1994) recommended total d-Thr requirements are 0.80% for starter (0 to 3 wk), 0.74% for grower (3 to 6 wk), and 0.68% for the finisher (6 to 8 wk) periods. These requirements seem outdated now since NRC requirement levels were established by feeding nutritionally well-balanced diets by using highly digestible dietary ingredients for healthy birds reared in optimal managerial conditions. The modern broilers had made tremendous alterations in various features including genetic selection, management practices, and feed formulation leading to a more faster and improved growth performance than previous years. These broilers are exposed to different kinds of stress including disease, unhygienic conditions, and harsh environment. There are contradictory data, additionally, available regarding Thr requirements about different growth periods in broilers. For instance, Webel et al. (1996) suggested 0.61% digestible Thr during 3 to 6 wk and 0.52% during 6 to 8 wk of age for maximal feed efficiency in broilers. Rosa et al. (2001), in contrast, reported an efficient growth at 0.69% and a maximum feed conversion ratio (FCR) at 0.68% digestible Thr in the starter period. Therefore, NRC (1994) recommended d-Thr levels may be inadequate for modern day broilers particularly when their diets have a poorly digestible protein ingredient as a core protein source (PS). It may, therefore, be necessary to determine the actual Thr requirements in broilers fed poorly digestible protein diets. There

is no direct published study available to evaluate the influence of Thr supplementation on zootechnical performance, carcass traits, morphometric parameters, apparent ileal digestibility of protein and amino acids, and immunity in broilers fed poorly digestible PS.

Keeping in view the above facts, it was hypothesized, therefore, that supplementation of d-Thr, greater than NRC recommendation, in the diets containing low digestible PS would compensate the adverse effects on broiler performance. The aim of the present study was, therefore, to evaluate the influence of d-Thr levels on growth performance and carcass traits, gut morphology, ileal digestibility of protein and AA, and immunity in broilers fed poorly digestible plant PSs such as CM.

MATERIALS AND METHODS

Animal Ethics

The trial protocol coincide with the animal ethics committee of University of Veterinary and Animal Sciences, Lahore, Pakistan. Before execution of the trial, ethical approval was granted.

Husbandry, Diets, and Experimental Design

In total, 900 straight run (Ross-308) 1-day-old mixed sex broilers were separately weighed and randomly distributed over 6 dietary treatments consisting of 5 replicate (pens) with 30 broilers each. The 6 (2 × 3) dietary treatments were tested in a factorial arrangement to evaluate the interactive effect of PS (SBM and CM) and d-Thr level (100, 110, and 120% of NRC recommendation) in a completely randomized design. The high levels of Thr were achieved by supplementing Thr (ThreAMINO) in the basal diet. In the starter period (0 to 21 d), the broilers were fed either corn-soybean or corn-Canola meals based diets containing 0.85% and 2 experimental diets with 0.94 and 1.03% total d-Thr, whereas the grower (22 to 35 d) diets contained 0.75, 0.81, and 0.88% total d-Thr. The nutrient composition as well as analyzed dietary contents are mentioned in Table 1. Rice straw was used as litter material over the concrete floor. Feed and water were provided to the broilers *ad libitum* through the trial. A 23L:1D light and dark schedule was applied for the first 3 d and condensed subsequently to 16L:8D with 20 lux intensity at bird's level during the trial. For the first 3 d, the room temperature was adjusted at 33°C and, thereafter by gradual reduction, retained at 22°C until the end of the trial. The pellet size varies from 2.5 mm for starter (0 to 21 d) to 4 mm for grower (22 to 35 d) periods and did not contain any growth promoter in it. The broilers were vaccinated intraocular against NDV at 6th and in drinking water at 20th d of age. An intraorally live vaccination against infectious bursal disease (IBD) was carried out at 12th d of age. The vaccination titers were measured at 35th d of age during slaughtering of the birds.

Table 1. Ingredients and nutrient composition of the experimental diets (g/kg as-fed basis).

Ingredients (%)	Canola meal						Soybean meal					
	Starter (0 to 21 d)			Grower (22 to 35 d)			Starter (0 to 21 d)			Grower (22 to 35 d)		
	Threonine level (%)											
	100	110	120	100	110	120	100	110	120	100	110	120
Corn 90	41.4	41.3	41.2	51.3	51.3	51.2	51.3	51.2	51.2	56.6	56.6	56.1
Soybean meal 44	0.0	0.0	0.0	0.0	0.0	0.0	30.5	30.5	30.5	24.5	24.5	24.5
Canola meal	34.5	34.5	34.5	25.4	25.3	25.3	0.0	0.0	0.0	0.0	0.0	0.0
Gluten 60	8.0	8.0	8.0	8.0	8.0	8.0	4.0	4.0	4.0	4.0	4.0	4.0
Guar meal	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Canola oil	5.0	5.0	5.0	5.0	5.0	5.0	3.3	3.3	3.3	4.7	4.7	4.7
Calcium carbonate	0.7	0.7	0.7	0.6	0.6	0.6	1.0	1.0	1.0	0.85	0.85	0.85
Dicalcium phosphate	2.2	2.2	2.2	1.8	1.9	1.8	2.2	2.2	2.2	1.85	1.84	1.85
L-lysine sulphate	1.1	1.1	1.1	0.9	0.9	0.9	0.6	0.6	0.6	0.5	0.5	0.5
DL-methionine	0.1	0.1	0.1	0.1	0.1	0.1	0.3	0.3	0.3	0.2	0.2	0.2
ThreAMINO	0.16	0.25	0.34	0.12	0.19	0.26	0.14	0.24	0.32	0.12	0.17	0.20
L-isoleucine	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.05	0.05	0.05
NaCl	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
NAHCO ₃	0.2	0.2	0.2	0.2	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1
Celite	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Vitamin premix ¹	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Calculated composition (%)												
ME (kcal/kg)	3,006	3,009	3,006	3,160	3,162	3,165	2,996	2,996	2,996	3,156	3,156	3,158
CP	23.0	23.0	23.0	20.0	20.0	20.0	23.0	23.0	23.0	20.0	20.0	20.0
CF	5.1	5.1	5.1	4.7	4.7	4.7	4.1	4.1	4.1	3.7	3.7	3.7
Lysine	1.27	1.27	1.27	1.08	1.08	1.08	1.28	1.28	1.28	1.08	1.08	1.08
Methionine	0.50	0.50	0.50	0.46	0.46	0.46	0.50	0.50	0.50	0.47	0.47	0.47
Threonine	0.85	0.94	1.03	0.75	0.81	0.88	0.85	0.95	1.03	0.75	0.81	0.88
M+C	0.88	0.88	0.88	0.81	0.81	0.81	0.88	0.88	0.88	0.81	0.81	0.81
Analyzed composition (%)												
CP	22.4	22.5	22.4	19.5	19.5	19.4	22.5	22.5	22.4	19.5	19.4	19.4
CF	5.0	5.0	5.0	4.5	4.5	4.4	4.0	4.0	4.0	3.5	3.5	3.6
Lysine	1.25	1.25	1.25	1.04	1.04	1.04	1.26	1.26	1.26	1.04	1.04	1.04
Methionine	0.48	0.48	0.48	0.44	0.44	0.44	0.48	0.48	0.47	0.45	0.45	0.44
Threonine	0.84	0.93	1.01	0.74	0.80	0.87	0.84	0.93	1.01	0.74	0.80	0.86
M+C	0.86	0.86	0.86	0.80	0.80	0.80	0.86	0.86	0.86	0.80	0.80	0.80

¹Premix composition (per kg of diet): 12,000 IE retinol, 2,400 IE cholecalciferole, 50 mg dl- α -tocopherole, 1.5 mg menadione, 2.0 mg thiamine, 7.5 mg riboflavin, 3.5 mg pyridoxine, 20 mcg cyanocobalamine, 35 mg niacin, 12 mg D-pantothenic acid, 460 mg choline chloride, 1.0 mg folic acid, 0.2 mg biotin, 80 mg iron, 12 mg copper, 85 mg manganese, 60 mg zinc, 0.8 mg iodine, 0.1 mg selenium, 125 mg anti-oxidant mixture.

Traits Measured

Growth and Carcass Parameters Feed intake (**FI**), body weight gain (**BWG**), and FCR, which was obtained by dividing total FI by BWG including the dead bird(s), per pen were measured weekly (7, 14, 21, 28, and 35 D of age), while mortality was documented daily.

At the completion of the trial (35 d), 3 broilers from each pen were randomly selected and slaughtered by the halal method and processed for determination of carcass weight (excluding giblets, % of live weight), breast and leg (drumstick with thigh) weight (% of carcass). Dressing percentage was determined by dividing eviscerated weight to BW. Breast and leg muscles were weighed and yield was calculated in terms of g/100 g of carcass yield.

Tissue Collection and Morphometric Analysis

To examine the duodenal morphology, a 2 cm longer duodenal sample, from middle of the duodenum, was collected, rinsed with cold normal saline (0.9% saline),

and immediately placed in Bouin's fluid. Within 24 h, the samples were shifted to ethanol (70%), fixed in paraffin and segmented at a 5 μ m thickness. For histological evaluation, 6 cross-sections per bird were processed (Owusu-Asiedu et al., 2002). Villus length (the distance from the apex of the villus to the junction of the villus and crypt) and crypt depth (**CD**) (the distance from the junction to the basement membrane of the epithelial cells at the bottom of the crypt) were determined on 10 intact, well-aligned villi (from the collected sample) per broiler assisted by a compound light microscope furnished with a video camera.

Three broilers from each pen were randomly chosen, slaughtered, as described in previously, and abdominal cavity opened. On the dissection day, all the broilers had *ad libitum* availability of feed and water until slaughtering. The different segments like immune organs (spleen, thymus, bursa), breast meat, leg quarter, and carcass were segmented and weighed. The ileal (from Meckle's diverticulum to ileo-cecal junction) digesta from 3 broilers of each pen was gently squeezed,

pooled into a plastic container, and frozen, thereafter, at -20°C till the pending analysis.

Ileal Digestibility Measurements of Protein and Amino Acids Crude protein quantity in the diets and in ileal digesta was measured as $N \times 6.25$, with N being quantified by the Kjeldahl method with CuSO_4 as a catalyst (ISO 5983). Following equation was applied for measurement of apparent ileal digestibility (AID) of CP and AA;

$$\text{AID} = [100 - (\text{CP}_d/\text{CP}_f) \times (\text{Ti}_f/\text{Ti}_d)] \times 100$$

where CP_d and Ti_d are the quantities of CP and Celite in the ileal digesta, respectively, and CP_f and Ti_f are the quantities of the same dietary item in the feed, respectively, all shown on a DM basis.

The AA quantities of the test diets and ileal digesta were analyzed by the procedures used by Ullah et al. (2017) with an AA analyzer (Biochrom 30+, Biochrom Limited, Cambridge, UK). Briefly, the sample was finely ground up to 500 micron and, thereafter, oxidized with performic acid for methionine and cysteine conservation. This oxidation modifies the mentioned AA to met-methionine sulphone and cys-cystic acid. The samples were hydrolyzed, thereafter, with 6 M hydrochloric acid/phenol for 24 h and, afterwards, pH adjusted to 2.2. The filtered samples were poured in sample vials for quantification of AA in Biochrom 30+ AA analyzer by using ion exchange chromatography.

Immunity The antibody titer for IBD virus and Newcastle disease (ND) virus was evaluated by collecting blood from the wing vein of 3 randomly chosen broilers from each pen at 35 d. The antibody titer against ND virus was measured by hemagglutination inhibition tests in which titer against ND virus was evaluated using 16 hemagglutinin units of the ND antigen (G.D. Animal Health Service, Deventer, Holland) as described by Richtzenhain et al. (1993). The IBD virus antibody titer was tested with the help of ELISA kits (Biochek, Gouda, Holland) as shown by Thayer et al. (1987). Thymus (all lobes on the left side of the neck), spleen, and bursa of fabricius were detached from the dissected broiler. The relative weights of the mentioned organs were determined by dividing the organ weight by live weight of the respective broiler.

Data Analysis

The data were analyzed by PROC MIXED of SAS (version 9.1; SAS Inst. Inc., Cary, NC). The factorial analysis of the treatments was carried out by using the following statistical model:

$$Y_{ijk} = \mu + \text{PS}_i + \text{TL}_j + \text{PS}_i \times \text{TL}_j + e_{ijk}$$

where Y_{ijk} = measured response, μ = overall mean, PS_i = i th fixed PS effect (i = SBM or CM), TL_j = fixed Thr level effect (j = 100, 110, and 120%), $\text{PS}_i \times \text{TL}_j$ =

interaction between PS and Thr level. A 5% probability level was used for significant differences. A post hoc test was used to compare the significant differences between the means.

RESULTS

Growth Performance

Table 2 indicates the results of dietary treatments on growth performance of broilers. The broilers consuming CM based diets resulted in 14% lower FI and 22% reduced BWG during starter period (0 to 21 d), whereas 11% poorer FCR during grower phase (22 to 35 d). Threonine supplementation (10 and 20%) above NRC recommendation, however, resulted in 8 and 6% better FCR during grower phase, respectively. For both grower (22 to 35) and overall (0 to 35 d of age) experimental periods, interactions between PS and d-Thr level were found for FI ($P < 0.001$) and BWG ($P < 0.001$), representing that the broilers consuming CM based diets supplemented (10 and 20%) d-Thr, above NRC recommended level, improved FI and BWG, whereas Thr supplementation in SBM based diets did not influence FI and BWG during the mentioned periods. Likewise, during starter (0 to 21 d) and over the entire (0 to 35 d of age) experimental period, interaction between PS and d-Thr level was detected for FCR, showing that the broilers consuming CM based diets supplemented (10 and 20%) d-Thr above NRC recommendation had a better FCR compared with those fed NRC recommended level, whereas in the broilers consuming SBM based diets, Thr supplementation had no effect on FCR.

Carcass Characteristics

Table 3 represents the effects of various experimental diets on carcass characteristics in broilers. Interactions between PS and d-Thr were observed for carcass weight ($P < 0.001$), breast meat yield ($P = 0.001$), and leg quarter yield ($P = 0.032$). The broilers fed CM based diets supplemented with (10 and 20%) d-Thr above NRC recommendation resulted in an improved carcass weight, breast meat, and leg quarter yield compared with those fed NRC recommended d-Thr level. Thr supplementation (10 and 20%), above NRC recommendation, in SBM based diets; however, the above mentioned parameters remain unaffected.

Gut Morphology

The influence of various experimental diets on duodenal VH, CD, and VCR are presented in Table 4. Interactions between PS and d-Thr level were found for VH ($P = 0.007$), CD ($P = 0.016$), and VCR ($P = 0.043$), representing that the broilers consuming CM based diets with d-Thr (10 and 20%) above NRC recommendation resulted in a higher VH, deeper CD, and an improved VCR compared with those fed NRC

Table 2. Effects of protein source (PS) and dietary threonine levels (d-Thr) on feed intake (FI), body weight gain (BWG), and feed conversion ratio (FCR) in broilers from 0 to 21, 22 to 35, and 0 to 35 d of age.¹

Effects	FI (g/bird/d)			BWG (g/bird/d)			FCR		
	0 to 21	22 to 35	0 to 35	0 to 21	22 to 35	0 to 35	0 to 21	22 to 35	0 to 35
Protein source									
Canola meal	51.5 ^b	135.5 ^b	91.1 ^b	35.3 ^b	73.2 ^b	53.6 ^b	1.46 ^a	1.86 ^a	1.71 ^a
Soybean meal	59.3 ^a	152.5 ^a	105.7 ^a	45.1 ^a	91.6 ^a	68.6 ^a	1.32 ^b	1.66 ^b	1.54 ^b
SEM ²	0.48	2.29	1.00	0.46	1.64	0.69	0.02	0.03	0.02
d-Thr level									
100	56.2	136.4 ^b	92.9 ^b	39.2	75.0 ^c	55.7 ^b	1.45 ^a	1.85 ^a	1.69 ^a
110	54.9	146.4 ^a	100.5 ^a	40.5	86.4 ^a	63.8 ^a	1.36 ^b	1.70 ^b	1.58 ^b
120	55.2	149.3 ^a	101.9 ^a	40.9	85.8 ^a	63.8 ^a	1.36 ^b	1.74 ^b	1.60 ^b
SEM	1.59	2.80	2.23	1.02	3.01	0.85	0.02	0.03	0.03
PS × d-Thr level									
CM 100	52.6	122.4 ^c	80.5 ^c	33.7 ^b	61.0 ^c	44.6 ^c	1.56 ^a	2.01	1.80 ^a
CM 110	50.5	138.5 ^{b,c}	94.7 ^b	35.9 ^b	78.2 ^b	57.4 ^b	1.41 ^b	1.77	1.66 ^b
CM 120	51.5	145.7 ^{a,b}	98.2 ^{a,b}	36.4 ^b	80.3 ^b	58.7 ^b	1.41 ^b	1.81	1.67 ^b
SBM 100	59.8	150.3 ^a	105.2 ^a	44.7 ^a	89.0 ^a	66.8 ^{a,b}	1.34 ^c	1.69	1.57 ^c
SBM 110	59.2	154.3 ^a	106.3 ^a	45.1 ^a	94.6 ^a	70.2 ^a	1.31 ^c	1.63	1.53 ^c
SBM 120	58.9	152.8 ^a	105.5 ^a	45.4 ^a	91.3 ^a	68.9 ^a	1.30 ^c	1.67	1.55 ^c
SEM	0.84	3.96	3.74	1.80	3.85	1.20	0.03	0.16	0.04
<i>P</i> -value									
PS	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
d-Thr level	0.247	<0.001	<0.001	0.635	<0.001	<0.001	0.057	<0.001	<0.001
PS × d-Thr level	0.656	<0.001	<0.001	0.515	<0.001	<0.001	0.022	0.411	<0.001

^{a-c}Means without a common superscript within a column significantly ($P < 0.05$) differ.

¹Each value represents the mean of 5 replicates (30 birds per replicate).

²SEM = Standard error of means.

Table 3. Effects of protein source (PS) and dietary threonine levels (d-Thr) on carcass, breast meat yield (g), and leg quarter yield (g) in the broilers¹ at 35 d of age.

Effects	Carcass ²	Breast meat yield ³	Leg quarter yield ³
Protein source			
Canola meal	64.7 ^b	28.8 ^b	25.8 ^b
Soybean meal	71.3 ^a	33.6 ^a	31.3 ^a
SEM ⁴	0.73	0.68	0.22
d-Thr level			
100	66.1 ^b	29.7 ^b	26.8 ^c
110	68.0 ^a	31.3 ^a	28.7 ^b
120	69.7 ^a	32.6 ^a	30.2 ^a
SEM	0.71	0.68	0.61
PS × d-Thr level			
CM 100	61.7 ^d	26.5 ^d	23.7 ^c
CM 110	64.8 ^c	29.0 ^c	26.1 ^d
CM 120	67.6 ^b	30.8 ^b	27.7 ^c
SBM 100	70.4 ^a	32.8 ^a	29.9 ^b
SBM 110	71.2 ^a	33.6 ^a	31.3 ^{a,b}
SBM 120	71.8 ^a	34.4 ^a	32.7 ^a
SEM	0.72	0.82	0.70
<i>P</i> -value			
PS	<0.001	<0.001	<0.001
d-Thr level	<0.001	<0.001	<0.001
PS × d-Thr level	<0.001	0.001	0.032

^{a-c}Means without a common superscript within a column significantly ($P < 0.05$) differ.

¹Each value represents the mean of 5 replicates (3 birds per replicate).

²Carcass expressed as percentage of body weight at slaughter.

³Breast and leg yield expressed as percentages of carcass weight.

⁴SEM = Standard error of means.

Table 4. Effects of protein source (PS) and dietary threonine (d-Thr) levels on duodenal villus height (μm), crypt depth (μm), and villus height to crypt depth ratio (VCR) in the broilers¹ at 35 d of age.

Effects	VH (μm)	CD (μm)	VCR
Protein source			
Canola meal	1320.2 ^b	169.5 ^a	7.83 ^b
Soybean meal	1688.5 ^a	152.3 ^b	11.1 ^a
SEM ²	29.54	4.40	0.35
d-Thr level			
100	1437.5 ^c	169.4 ^a	8.49 ^b
110	1535.4 ^b	154.0 ^b	9.97 ^a
120	1540.2 ^a	159.3 ^b	9.67 ^a
SEM	36.18	4.02	0.43
PS × d-Thr level			
CM 100	1243.2 ^c	182.4 ^a	6.82 ^c
CM 110	1359.8 ^b	159.2 ^{b,c}	8.54 ^b
CM 120	1357.6 ^b	166.9 ^b	8.13 ^b
SBM 100	1631.8 ^a	156.4 ^c	10.43 ^a
SBM 110	1710.9 ^a	148.8 ^c	11.50 ^a
SBM 120	1702.7 ^a	151.6 ^c	11.36 ^a
SEM	38.2	5.8	0.61
<i>P</i> -value			
PS	<0.001	0.033	0.036
d-Thr level	<0.001	0.011	<0.001
PS × d-Thr level	0.007	0.016	0.043

^{a-c}Means without a common superscript within a column significantly ($P < 0.05$) differ.

¹Each value represents the mean of 5 replicates (3 birds per replicate).

²SEM = Standard error of means.

Table 5. Effects of protein source (PS) and dietary threonine (d-Thr) levels on apparent ileal digestibility of protein and amino acids in the broilers¹ at 35 d of age.

Effects	Amino acids ²															
	EAA										NEAA					
	CP	Arg	His	Iso	Leu	Lys	Meth	Phe	Thr	Val	Ala	Asp	Cys	Gly	Glu	Ser
PS																
CM	69.3 ^b	0.80 ^b	0.75 ^b	0.75 ^b	0.79 ^a	0.78	0.84	0.84 ^b	0.69 ^b	0.77	0.79	0.76 ^b	0.66 ^b	0.72 ^b	0.83 ^b	0.74
SBM	76.3 ^a	0.85 ^a	0.82 ^a	0.81 ^a	0.85 ^b	0.82	0.86	0.89 ^a	0.73 ^a	0.80	0.82	0.80 ^a	0.70 ^a	0.77 ^a	0.88 ^a	0.78
SEM ³	0.69	0.02	0.02	0.01	0.02	0.02	0.01	0.01	0.02	0.02	0.02	0.01	0.01	0.02	0.02	0.02
d-Thr level																
100	70.1 ^c	0.81	0.76 ^b	0.77	0.80 ^a	0.78 ^b	0.83 ^b	0.85 ^b	0.70	0.77	0.79	0.77	0.66	0.73	0.84	0.74 ^c
110	73.7 ^b	0.83	0.79 ^a	0.79	0.83 ^b	0.81 ^{a,b}	0.86 ^a	0.88 ^a	0.72	0.79	0.81	0.79	0.69	0.76	0.86	0.78 ^a
120	75.1 ^a	0.83	0.80 ^a	0.79	0.83 ^b	0.82 ^a	0.86 ^a	0.88 ^a	0.72	0.79	0.82	0.79	0.69	0.76	0.87	0.77 ^b
SEM	0.85	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.02	0.02	0.01	0.01	0.02	0.02	0.01	0.01
PS × d-Thr level																
CM 100	66.6 ^d	0.78 ^c	0.71 ^c	0.73 ^c	0.76 ^c	0.76	0.81	0.81 ^{b,c}	0.67	0.75	0.77	0.74	0.63 ^b	0.70 ^c	0.81	0.70
CM 110	69.3 ^c	0.81 ^b	0.76 ^b	0.76 ^b	0.81 ^b	0.79	0.85	0.86 ^a	0.71	0.77	0.80	0.77	0.68 ^a	0.75 ^b	0.84	0.76
CM 120	72.1 ^{b,c}	0.81 ^b	0.77 ^b	0.76 ^b	0.80 ^{b,c}	0.80	0.86	0.85 ^b	0.70	0.78	0.80	0.78	0.68 ^a	0.75 ^b	0.85	0.75
SBM 100	73.6 ^b	0.84 ^a	0.81 ^a	0.80 ^a	0.84 ^a	0.82	0.85	0.88 ^a	0.72	0.79	0.81	0.79	0.69 ^a	0.76 ^a	0.87	0.77
SBM 110	77.1 ^a	0.85 ^a	0.82 ^a	0.81 ^a	0.86 ^a	0.82	0.86	0.89 ^a	0.73	0.80	0.82	0.80	0.70 ^a	0.77 ^a	0.88	0.79
SBM 120	78.2 ^a	0.85 ^a	0.82 ^a	0.82 ^a	0.86 ^a	0.83	0.86	0.90 ^a	0.73	0.80	0.83	0.80	0.70 ^a	0.78 ^a	0.88	0.78
SEM	1.20	0.02	0.02	0.01	0.02	0.03	0.03	0.02	0.02	0.01	0.02	0.02	0.02	0.02	0.03	0.02
<i>P</i> -value																
PS	0.001	0.002	0.001	0.001	0.031	0.230	0.350	0.001	0.002	0.47	0.19	0.001	0.001	0.003	0.002	0.311
d-Thr level	0.006	0.431	0.031	0.520	0.830	0.041	0.020	0.002	0.730	0.530	0.430	0.710	0.041	0.491	0.520	0.032
PS × d-Thr level	0.033	0.032	0.002	0.001	0.031	0.385	0.592	0.035	0.430	0.591	0.732	0.531	0.032	0.041	0.431	0.531

^{a-d}Means without a common superscript within a column significantly ($P < 0.05$) differ.

¹Each value represents the mean of 5 replicates (3 birds per replicate).

²EAA = Essential amino acids, NEAA = Non-essential amino acids. Arg = Arginine, His = Histidine, Iso = Isoleucine, Leu = Leucine, Lys = Lysine, Meth = Methionine, Phe = Phenylalanine, Thr = Threonine, Val = Valine, Ala = Alanine, Asp = Aspartate, Cys = Cysteine, Gly = Glycine, Glu = Glutamate, Ser = Serine.

³SEM = Standard error of means.

recommended d-Thr level, whereas in broilers fed SBM based diets, d-Thr did not influence the above mentioned parameters.

Apparent Ileal Digestibility of Protein and Amino Acids

The results of apparent ileal digestibility of protein (AIDP) and amino acids (AIDAA) are presented in Table 5. Interaction between PS and d-Thr level was observed for AIDP ($P = 0.033$). The broilers fed either SBM or CM based diets with d-Thr (10 and 20%), above NRC recommendation, resulted in a higher AIDP compared with those fed NRC recommended d-Thr level. This increase in digestibility was higher in broilers consuming CM compared with those fed SBM based diets. Among EAA, a lower digestibility coefficient for Thr was found in broilers consuming CM based diets in comparison with those fed SBM based diets. Dietary Thr supplementation above NRC recommendation (20%) in broiler diets resulted in a higher digestibility coefficients for Lys (5%) and Met (4%) compared with those fed NRC recommended level regardless of PS. Interactions between PS and d-Thr level were found for Arg ($P = 0.032$), His ($P = 0.002$), Iso ($P = 0.001$), Leu ($P = 0.031$), and Phe ($P = 0.035$). The broilers consuming CM based diets with d-Thr (10 and 20%) above

NRC recommendation resulted in higher digestibility coefficients of the above mentioned amino acids, except Phe where the digestibility was increased in broilers consuming diets with 10% d-Thr only, compared with those fed NRC recommended d-Thr level, whereas digestibility remained unaffected by d-Thr in SBM based diets. Among NEAA, similarly, a lower digestibility coefficient for Asp and Glu was observed in broilers fed CM based diets than those fed SBM based diets. Broilers fed diets supplemented with d-Thr above NRC recommendation (120%) resulted in a 4% improved digestibility of Ser, compared with those fed NRC recommended level. Interactions between PS and d-Thr level were detected for Cys ($P = 0.032$) and Gly ($P = 0.041$), representing that broilers consuming CM based diets with d-Thr (10 and 20%) above NRC recommendation resulted in higher digestibility coefficients of the above-mentioned amino acids compared with those fed NRC recommended d-Thr level, whereas the digestibility of the above-mentioned AA remained unaffected by d-Thr in SBM based diets.

Immunity

Influence of dietary treatments on immune organ weights and antibody titer (ND and IBD) in broilers are shown in Table 6. The broilers consuming CM based

Table 6. Effects of protein source (PS) and dietary threonine levels (d-Thr) on weights (g/100 g of live body weight) of thymus, spleen, bursa, antibody titer of Newcastle disease (ND), and infectious bursal disease (IBD) in the broilers¹ at 35 d of age.

Effects	Immune organ weight (g/100 g of body weight)			Antibody titer	
	Thymus	Spleen	Bursa	ND	IBD
Protein source					
Canola meal	3.99	2.34 ^b	2.37 ^b	4.57	3,436
Soybean meal	3.82	2.76 ^a	3.47 ^a	4.62	3,746
SEM ²	0.16	0.12	0.11	0.23	280
d-Thr level					
100	3.45 ^b	2.31 ^b	2.64 ^b	4.60	2,831 ^b
110	4.58 ^a	2.49 ^b	3.22 ^a	4.70	4,035 ^a
120	4.37 ^a	2.85 ^a	2.90 ^b	4.50	3,907 ^a
SEM	0.19	0.15	0.13	0.28	344
PS × d-Thr level					
CM 100	3.28	1.73 ^c	1.90	4.26	3,001
CM 110	4.68	2.52 ^b	2.65	4.80	3,940
CM 120	4.02	2.78 ^a	2.56	4.66	3,367
SBM 100	3.62	2.47 ^b	3.38	4.93	2,662
SBM 110	4.48	2.89 ^a	3.80	4.60	4,130
SBM 120	4.37	2.92 ^a	3.24	4.53	4,446
SEM	0.28	0.21	0.19	0.40	486
<i>P</i> -value					
PS	0.461	0.019	<0.001	0.892	0.437
d-Thr level	0.004	0.040	0.015	0.883	0.029
PS × d-Thr level	0.218	0.012	0.128	0.403	0.342

^{a-c}Means without a common superscript within a column significantly ($P < 0.05$) differ.

¹Each value represents the mean of 5 replicates (3 birds per replicate).

²SEM = Standard error of means.

diets resulted in a 32% lower bursa weight compared with SBM based diets. Threonine (10 and 20%) supplementation above NRC recommended level resulted in a higher thymus and bursa weights in comparison with those consuming NRC recommended level. An interaction ($P = 0.012$) between PS and d-Thr level for spleen weight was observed, showing that the broilers consuming CM based diets supplemented with Thr (10 and 20%) above NRC recommendation resulted in a higher spleen weight, compared with those fed NRC recommended level. Antibody titers against IBD was improved by 30% in broilers fed 10% higher d-Thr compared with those fed NRC recommended levels, whereas d-Thr supplementation did not influence antibody titers against ND.

DISCUSSION

The present trial was executed to investigate the influence of different levels of dietary Thr on growth performance and carcass traits, gut morphology, apparent ileal digestibility of protein and AA, and immunity in broilers fed either CM or SBM based diets. A hypothesis that the detrimental influence of low digestible PSs like CM may partially be counterbalanced by d-Thr supplementation above the NRC recommended level. Growth performance and carcass traits, gut morphology, ileal digestibility of protein and AA, and immunity were, consequently, evaluated as explanatory variables.

Greater duodenal VH and VCR, whereas reduced CD were used as signs of a healthy gut (Qaisrani et al., 2015).

The detected poor growth performance in broilers fed CM compared with SBM based diets is in accordance with expectations and is in agreement with previous studies in broilers (Khajali and Slominski, 2012; Gopinger et al., 2014; BK et al., 2015; Qaisrani et al., 2015). This reduced FI in CM fed broilers may possibly be due to the presence of anti-nutritional factors including sinapine, phytic acid, and glucosinolates (BK et al., 2015). Higher bulk density in CM, compared with SBM, may possibly result in lower FI because of higher gut fill effects in broilers consuming CM (Shelton et al., 2005). Lower dietary electrolyte balance in CM, compared with SBM, additionally may also cause a lower FI (Khajali and Slominski, 2012) and an inferior digestion and absorption of nutrients (Wu et al., 2017) resulting in a compromised growth performance. This poorer growth performance of CM fed broilers resulted in a reduced carcass weight compared with those fed SBM based diets. Gopinger et al. (2014) reported 22% lower BW in broilers consuming 40% CM in comparison with those consuming 10% CM based diets. According to these later authors this reduction in BW may be due to higher fiber because of increasing dietary CM contents. The compromised gut health, lower digestibility of protein and AA, and compromised growth performance may also lead to an inferior carcass traits compared with those fed SBM based diets.

Reduced VH and VCR, whereas deeper CD in the broilers consuming CM based diets may be due to high fiber (11.6 vs. 5.4%) content (Rahmatnejad and Saki, 2015; Sadeghi et al., 2015) and aforementioned anti-nutritional factors in comparison with those consuming SBM based diets. These findings are in accordance with published literature in broilers fed CM based diets (Qaisrani et al., 2015). This poorer gut health, a shorter VH and higher CD, may provide less area of contact with nutrients resulting in a lower digestion and absorption of nutrients. This reduced digestibility of protein and AA in broilers fed CM diets (Table 5) is a confirmation of poorer gut health in CM based diets and is in line with the previous findings in broilers (Khajali and solomonski, 2012; Qaisrani et al., 2015). This compromised AA digestibility in CM can be associated with processing techniques including desolventization and toasting during solvent extraction and anti-nutritional factors including fiber, erucic acid, and tannins (Newkirk and Classen, 2002; Khajali and solomonski, 2012). Tannins binds with proteolytic enzymes and proteins in gastrointestinal tract thereby reducing protein and amino acids digestion (Khajali and Slominski, 2012). Fiber content in CM, additionally, may act as laxative resulting in an increased digesta passage rate in the gut leading to a poor nutrient digestibility (Mushtaq et al., 2009).

The greater relative weights of immune organs (spleen and bursa of fabricius) in broilers fed SBM based diets compared with CM diets may be due to a greater biological value and ideal amino acid profile required for proper functioning of immune organs in the broilers consuming SBM based diets in comparison with those consuming CM (Park et al., 2017). These findings are in line with some recent findings in broilers (Eftekhari et al., 2015; Chen et al., 2016). Rabie et al. (2015), similarly, found that substitution of SBM with 20% CM resulted in 32% greater weight of the bursa of fabricius.

An improved growth performance in broilers fed diets containing higher, above NRC recommended, levels of d-Thr may be due to more amount of d-Thr available to satisfy optimum Thr requirement for growth and the gut, which resulted in an improved gut health (Table 4) consequently enhanced AIDP and AA (Table 5) compared with those fed recommended level. Zarrin-Kavyani et al. (2018) observed an improved FI during grower and a higher BW during grower and entire experimental period, whereas a better FCR during the starter period in broilers fed 10% extra Thr in comparison with those consuming the control diet. Shirzadegan et al. (2015), similarly, observed a higher BWG, during the grower as well as over the entire experimental phase, in broilers fed 0.75% d-Thr than that of 0.5% and 1.0% dietary Thr. A possible appetitive influence of Thr may result in an increased FI. This appetitive effect may have triggered the appetite regulating mechanisms in broilers. The accelerated growth performance observed in the broilers

consuming Thr supplemented diets, additionally, because of its role in synthesis of protein and maintenance of body protein turnover (Ton et al., 2013). These findings are in agreement with a few recent findings in broilers (Shirzadegan et al., 2015; Min et al., 2017; Zarrin-Kavyani et al., 2018). Min et al. (2017) reported a significant quadratic increase in average daily gain and a quadratic decrease in FCR during the whole experiment (0 to 42 d) with the increase of Thr from 85 to 150% of NRC recommendations. It can be assumed, therefore, that supplemental Thr is freely available to the birds compared with intact Thr found in the diet, resulting in its greater availability for their growth.

An improved carcass yield in the broilers fed diets with greater, above the NRC recommended, levels of d-Thr may be attributed to Thr that interact with lysine, which enhances its utilization for muscle development (Fernandez et al., 1994; Kidd, 2000). Enhanced breast meat yield of broilers fed diets with 110% d-Thr is in line with the studies reported in broilers earlier (Taghinejad-Roudbaneh et al., 2013). These later authors stated a greater (427 vs. 361 g) breast meat yield in the broilers consuming Thr supplemented regimes in comparison with those consuming diet without Thr supplementation. Along with serine, moreover, Thr is involved in muscle building resulting in an improved carcass characteristic.

Extra Thr improved the gut morphology in broilers as supported by higher duodenal VH and greater ratio of VH to CD that resulted in a better digestibility of protein and AA. These findings are in accordance with expectations (Wang et al., 2010; Min et al., 2017; Debnath et al., 2019) since Thr is an essential amino acid involved in mucin production, which constitutes about 40% of gastrointestinal tract proteins (Carlstedt et al., 1993). This mucin makes a thin covering on the inner side of the gut, protects it from pathogens and anti-nutritional factors (Li et al., 2007). Min et al. (2017) reported a significant quadratic increase in duodenal VH in broilers fed diets having varying levels of Thr (85 to 150% of NRC recommendation) with greatest VH in broilers consuming diets with 125% Thr. The increased Thr availability may stimulate mucin synthesis resulting in more intestinal protection and healing, consequently a better gut health. There is a greater, compared with other amino acids, requirements of Thr for the gut maintenance because of faster turnover rates and its high proportions in the gut secretions (Fernandez et al., 1994). About 20% of the energy and 25% of daily synthesized protein is consumed for gut maintenance making it as one of the most expensive organ because of high protein turnover rate. Nutritional imbalance of Thr, therefore, may lead to a severe dysfunctioning of the gut and ultimately broiler performance. It has been stated that about 30 to 50% of Thr and a few other AA including arginine, proline, isoleucine, valine, leucine, methionine, lysine, phenylalanine, glycine, and serine are in direct use of the small intestine (Wu, 1998). It is assumed, therefore, that supplemented

d-Thr levels may have provided sufficient amount of Thr for the more synthesis of mucosal tissues.

The greater immune organs weight of broilers fed diets having high levels of d-Thr is in line with findings already reported in broilers (Chen et al., 2016; Debnath et al., 2019). The greater spleen weight may be indicative of more production of immune cells and antibodies that in turn positively influence immunity of the broilers. The greater relative weight of thymus in d-Thr supplemented diet fed broilers may be due to more T-lymphocyte production from thymus (Debnath et al., 2019) that positively influenced immune status of broilers. Threonine levels influence the immune system response in broilers by manipulating the gut microbial population and moderating immune system by enhancing immunoglobulin A (IgA) secretion and regulating the expression of the inflammatory genes (Chen et al., 2016). It has been reported that excess dietary Thr increases the relative weight of immune organs, synthesize IgA, immunoglobulin G, and secretory IgA in broilers (Ren et al., 2014). Improved antibody production against ND and IBD vaccine in broilers fed diets containing 0.81% d-Thr compared with those fed diets containing NRC recommended level is in line with findings of Eftekhari et al. (2015) in broilers. This improvement in immunity against NDV and IBD is according to the expectation because of the greater weight of immune organs (bursa and thymus) in broilers fed higher levels of d-Thr. This enhanced antibody titer may be attributed to more availability of Thr, which is a major component of immunoglobulins (Kim et al., 2007).

CONCLUSIONS

In conclusion, diets containing greater (10 and 20%), than NRC recommended, levels of d-Thr resulted in a better growth performance and carcass characteristics, improved ileal digestibility of protein and AA, healthy gut and an enhanced immunity in broilers. The broilers fed CM based diets with 20% greater, than NRC recommended, levels of d-Thr performed optimal with higher digestibility of protein and most of the amino acids with a healthy gut and a better immunity, whereas for broilers consuming SBM based diets, d-Thr level for optimal performance was 10% greater than NRC recommended. The detrimental effects of poorly digestible PSs like CM, therefore, can partially be ameliorated by d-Thr supplementation above the NRC recommended levels in broilers. Threonine, additionally, is critical for maintaining gut health, immunity and carcass traits in broilers.

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