Kafkas Univ Vet Fak Derg
22 (2): 165-172, 2016
DOI: 10.9775/kvfd.2014.12002

Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

Research Article

The Effects of Increase in Threonine to Lysine Ratio on Performance, Blood Parameters and Humoral Immune Responses of Male Broiler Chickens Challenged with *Salmonella*

Mohammad Reza VALIZADE [1,2] Ali Asghar SADEGHI [1]
Mohammad CHAMANI [1] Parvin SHAWRANG [3] Nasser KASHAN [1]

[1] Department of Animal Science, Science and Research Branch, Islamic Azad University, Tehran, IRAN
[2] The Health of Plant and Livestock Products Research Center, Mazandaran University of Medical Sciences, Sari, IRAN
[3] Nuclear Agriculture Research School, Nuclear Science and Technology Research Institute, Atomic Energy Organization of Iran, Karaj, IRAN

Article Code: KVFD-2014-12002 Received: 17.07.2014 Accepted: 10.01.2016 Published Online: 17.01.2016

Abstract

The aim of this study was to assess the effect of threonine to lysine (Thr/Lys) ideal ratio for optimum performance, blood parameters and immunity of broiler chicks. Supplemental threonine (equal or 25% more than breed's threonine/lysine ratio requirement) was added to a control, threonine deficient and high crude fiber diet, then fed to 288 one-day-old male Ross 308 broiler chicks (three treatments and eight replications per treatment). On day 11 and 21 of age, infectious bursal disease virus and infectious bronchitis virus vaccines were orally administered individually, respectively. Then, 21 days after each administration, blood antibody titers against viruses were measured. On day 32 of age, four birds in each treatment group were infected orally with equal numbers of *Salmonella* Paratyphi A (5×10^4 cfu/bird). As a result of this study, challenging with *Salmonella* led to increase in mortality rate and increase in Thr/Lys ratio could not decrease it. Increase in Thr/Lys ratio decreased feed intake and weight gain of ensemble challenged and non-challenged groups, however improved feed conversion ratio of challenged group in finisher period. Feed consumption cost increased by salmonellosis and increase in Thr/Lys ratio could not improve salmonellosis based economic loss. Salmonellosis increased serum urea, uric acid and AST and decreased serum glucose and cholesterol and increase in Thr/Lys ratio did not alleviate triglyceride. Increase in Thr/Lys ratio improved non-significantly humoral immune response in the challenged and non-challenged groups. These findings indicate that higher Thr/Lys ratio in infected birds improved production performance, however could not be an economical lucrative medicative agent.

Keywords: *Broiler, Humoral immune responses, Lysine, Salmonella, Threonine*

Salmonella'ya Maruz Kalan Erkek Broyler Piliçlerde Threonine-Lysine Oranındaki Artışın Performans, Kan Parametreleri ve Humoral İmmun Tepki Üzerine Etkileri

Özet

Bu çalışmanın amacı, etlik piliçlerde ideal lisin (Thr/Lys) oranının optimum performans, kan parametreleri ve immunite üzerine etkisini değerlendirmekti. 288 adet bir günlük erkek Ross 308 etlik civcivlere kontrol, treonin yönünden yetersiz ve yüksek ham selüloz diyetine treonin (türün treonin/lizin oranı gereksinimine eşit veya %25 daha fazla) eklendikten sonra verildi (üç uygulama ve her bir uygulama için sekiz tekrarlı tarzda). Bireylere 11. ve 21. günlükken sırasıyla bulaşıcı bursal hastalık virüsü ve bulaşıcı bronşit virüsü aşıları oral yolla ayrı ayrı uygulandı. Her uygulamadan 21 gün sonra, virüslere karşı kan antikor titreleri ölçüldü. 32 günlükken, her bir tedavi grubundan dört eşit sayıda piliç ağızdan *Salmonella* Paratyphi A (5 x 104 cfu/piliç) ile enfekte edildi. Bu çalışmanın sonucunda, *Salmonella* maruziyeti ölüm oranında artışa yol açarken, Thr/Lys oranındaki artış bu oranı azaltamadı. Thr/Lys oranındaki artış maruziyet olan ve olmayan grupların toplam yem alımı ve ağırlık kazançlarını azaltırken, maruziyet grubunun son periyottaki yem dönüşüm oranını artırdı. Yem tüketim maliyeti salmonellosis ile artarken, Thr/Lys oranındaki artış salmonellosis-kökenli ekonomik kaybı azaltamadı. Salmonellosis serum üre, ürik asit ve AST'yi artırıp serum glukoz ve kolesterolü azaltırken, Thr/Lys oranındaki artış ise trigliseridi baskılamadı. Thr/Lys oranındaki artış maruziyet olan ve olmayan gruplardaki humoral immun tepkiyi nispeten yükseltti. Bu bulgular, yüksek Thr/Lys oranının enfekte kuşlardaki üretim performansını artırdığını, ancak ekonomik yönden kazançlı bir tıbbi ajan olamadığını göstermektedir.

Anahtar sözcükler: *Broyler, Humoral immun tepkiler, Lisin, Salmonella, Treonin*

İletişim (Correspondence)
+98 21 44868536
a.sadeghi@srbiau.ac.ir

INTRODUCTION

Threonine is the third most limiting amino acid in most plant-based broiler diets behind the total sulphur-containing amino acids and lysine [1]. Among the essential amino acids, threonine is particularly important for maintenance of gut barrier integrity and has an important role in the structure and function of gastrointestinal tract [2-4]. A higher threonine to lysine (Thr/Lys) ratio in intestinal infected broilers by coccidiosis or subclinical *Clostridium* infection improved production performance [5,6].

Threonine is found in high concentrations in chicken gamma globulins [7]. Gamma globulins represent the fraction of serum containing the highest concentration of immuno-globulins (antibodies) as determined by electrophoresis [8]. Because immunoglobulins depend on amino acid sequences to form the variable regions for antigen binding and provide structural support [8], threonine deficiency may suppress antibody activity [1].

More threonine requirement was reported for optimal responses in the cellular and humoral immune systems of rats than requirement for optimum growth [9]. Because of threonine participation in immune system functions and influence of nutrition and vaccination programs on diseases prevention, as substitutes for antibiotics, Kidd [1] recommended such researches should evaluate cellular and humoral immune system functions as they are affected by threonine to understand more completely the birds' needs for this essential amino acid [10].

Low Thr/Lys ratio was reported by Ross broiler nutrition specificatio™n [11] compared with NRC [12]; but, Aviagen [11] reported more threonine to metabolizable energy, lysine to metabolizable energy, threonine to crude protein and lysine to crude protein for broilers. This means more amino acid intake per daily feed intake [11]. Kidd [1] deduces that NRC [12] overestimated the threonine requirement of broilers (Thr/Lys ratio admittedly) for optimum performance. Likewise, NRC [12] reported that findings about broilers Thr requirement was insufficient. Lysine and protein requirement of modern broiler strains is more than that reported by NRC [12] as a result of their fast growth rates. Novel researches with new points of view should be done because more Lysine and protein in the diet changes the physiological characteristics and quantity of amino acids requirement [13-19].

Salmonella infects poultry and humans by the oral route through contaminated food or water [20]. *Salmonella* especially Paratyphoid serovar that are motile makes colony on gut epithelium then transmit across from gut mucosal and immune barrier to blood flow and can cause septicemia or tissues infection and damage [20]. Today complementary controls via gut health promotion by non antibiotic therapy method are used to prevent *Salmonella* from entering the food chain; consequently, to improve poultry products consumer's health [21-23].

The objective of this study was to determine variations in performance, blood parameters and humoral immune responses of broiler chickens challenged with *Salmonella* Paratyphi A, fed diet supplemented with L-Threonine to meet various Thr/Lys ratios.

MATERIAL and METHODS

The study was approved by the Ethics Committee of Islamic Azad University, Science and Research Branch (approval date: 16.11.2013; no: 3740, AEC 11).

Experimental Design and Diets

In a completely randomized design 288 one-day old *Salmonella* negative male (Ross 308) broiler chicks were randomized in 3 treatments with 8 repetitions, 12 chicks per repeat. Average body weight (means ± standard error) of chicks at the beginning of the experiment was 40.3±1.6 g. Chicks were housed in floor pen (10 chicks/1 m^2 pen) and had free access to feed and water during the experimental period and 24 h light daily in a temperature-controlled room. The relative humidity was controlled at 65% and temperature was set at 32°C on day 1 and lowered gradually to 24°C for the rest of the experiment period. After 24 h eating similar prestarter pellet, chicks were fed experimental starter (2-10 d), grower (11-24 d) and finisher (25-42 d) rations based on breed's nutritional catalog [11]. Performance assay was accorded with these periods.

Near Infrared Reflectance Spectroscopy (NIRS) (AMINONIR®, 43076) was used to determine amino acids profiles of all ingredients (by: Paya Amin Mehr Co. Ltd., Evonik Animal Nutrition Service, Tehran, Iran).

Treatment 1 had no threonine supplement, treatment 2 had threonine supplement to meet Thr/Lys ratio requirement as pointed in Ross broiler nutrition specification [11] and treatment 3 had more threonine supplement to meet Thr/Lys ratio 25% more. Basal diets ingredients and nutrient analysis and L-Threonine supplement quantity of experimental diets showed in *Table 1*. The difference between threonine supplement content of treatments 1 and 2 with treatment 3, correct with adding grind fine sand as filler to treatments 1 and 2.

Salmonella Challenge and Recovery

Salmonella enterica subsp. *enterica* serovar Paratyphi A (ATCC® 9150™) was used for infection induction. This bacterium was obtained from microorganism's bank of Iranian Biological Resource Centre (IBRC), ACECR, Tehran, Iran (IBRC No.: IBRC-M 10668). The challenge organism for this experiment was grown in tryptic soy broth (Sigma-Aldrich, UK) at 37°C then diluted to 5×10^4 cfu/ml [24].

Table 1. *Composition of experimental diets*
Tablo 1. *Deneysel diyetlerin içeriği*

Items	Starter 2-10 d	Grower 11-24 d	Finisher 25-42 d
Ingredients (g/kg)			
Corn	432.91	472.21	503.00
Wheat	100.00	105.00	105.00
Barley	50.00	60.00	60.00
Wheat bran	25.00	30.00	30.00
Rice bran	25.00	30.00	30.00
Soybean meal (46% CP)	311.20	247.00	209.67
Soybean oil	6.02	11.45	20.60
Choline chloride 60%	1.45	1.40	1.30
L-Threonine suppl. T-1[1]	-	-	-
L-Threonine suppl. T-2[1]	0.59	0.48	0.36
L-Threonine suppl. T-3[1]	2.81	2.44	2.13
L-Lysine monohydrochloride	2.45	2.20	1.93
DL- Methionine	2.97	2.40	2.10
Limestone	11.84	9.60	9.57
Dicalcium phosphate	18.15	15.90	14.90
Sodium bicarbonate	4.10	3.90	3.70
Salt	0.60	1.00	1.10
Vitamin premix[2]	2.50	2.50	2.50
Mineral premix[2]	2.50	2.50	2.50
Maduramycin 1%	0.50	0.50	-
Analysis results of nutrients			
AME_n[3] (kcal/kg)	2720	2820	2920
CP (%)[4]	21.07[4]	18.78	17.34
Thr T. 1 (%)	0.790	0.697	0.641
Thr T. 2 (%)	0.846	0.743	0.675
Thr T. 3 (%)	1.057	0.929	0.843
Lys (%)	1.287	1.110	0.994
Thr /Lys T.1 (%)	61.38	62.82	64.47
Thr /Lys T.2 (%)	65.73	66.93	67.91
Thr /Lys T.3 (%)	82.16	83.68	84.83
Met + Cys (%)	0.965	0.852	0.785
Val (%)	0.990	0.882	0.815
Ile (%)	0.871	0.761	0.693
Arg (%)	1.379	1.201	1.090
Trp (%)	0.258	0.225	0.204
Crude fiber (%)	4.108	3.89	3.70
Ca (%)	0.96	0.80	0.77
Available Phosphorus (%)	0.45	0.40	0.38
Na (%)[5]	0.15	0.17	0.16
Cl (%)[5]	0.15	0.17	0.17
K (%)[5]	0.87	0.78	0.71
DCAD (meq/kg)[5,6]	249	224	207

*[1] L-Threonine supplement of treatment 1&2&3. Feed grade and 98.5% purity; [2] Breed's special supplement made as Ross nutrition catalog suggested (Anonymous, 2009), contain: 4.400.000 IU/kg of Vit. A, 2.000.000 IU/kg of Vit. D_3, 30.000 IU/kg of Vit. E, 1.200 mg/kg of Vit. K (Menadione), 1.200 mg/kg of B_1, 3.200 mg/kg of B_2, 24.000 mg/kg of Nicotinic Acid, 6.000 mg/kg of Pantothenic Acid, 1.600 mg/kg of B_6, 60 mg/kg of Biotin, 800 mg/kg of Folic Acid, 6 mg/kg of B_{12}; 6.400 mg/kg of Copper, 500 mg/kg of Iodine, 16.000 mg/kg of Iron, 48.000 mg/kg of Manganese, 120 mg/kg of Selenium, 40.000 mg/kg of Zinc; [3] AME_n: apparent metabolizable energy corrected for nitrogen excretion; [4] CP: crude protein; All limiting essential amino acids were supplied in basal diet by increase in ration crude protein content; [5] by calculation; [6] **DCAD:** dietary cation anion difference*

All chickens of four replications of each treatment individually infected by oral gavage using an animal feeding needle with equal numbers of *Salmonella* Paratyphi A (5×10^4 cfu/ml per bird) at the age of 32-d of old [25]. In a 2×3 factorial arrangement, other four replications of each treatment received normal saline. Challenge pens were separated by 2 meter distance and separated instrument from unchallenged pens.

Three cloacae swab samples from each pen of challenged and non-challenged chickens were cultured on day 39 to confirm that no *Salmonella* was present in the unchallenged group and success of *Salmonella* present in challenged group. A resistance again tylosin observed in an antibiogram test that initially done on this *Salmonella* serovar. Cloacae samples were streaked for isolation onto xylose lysine deoxycholate (XLD, Sigma-Aldrich, UK) agar plates containing tylosin (20 µg/mL) and incubated for 24 and 48 h at 37°C. Plates were evaluated for the presence or absence of *Salmonella*, which grow as red colonies on this selective medium [25-27].

Blood Sampling and Measurements

On days 10, 17, 24, 33 and 42 of age growth efficiency (all birds/pen) and on day 42, blood parameters (2 birds/pen) measured. Blood samples (without fasting) had been caught from wing vein after washing skin with distilled water and then drying. Serum glucose, urea, uric acid, cholesterol, triglyceride and aspartate aminotransferase (AST) measured by human Roche diagnostics kits with automatic analyzer COBAS INTEGRA 400 plus, (Roche Diagnostics Ltd. CH-6343 Rotkreuz, Switzerland). Principle of these test's methods was enzymatic-spectrophotometric of hexokinase, urease/glutamate dehydrogenase, uricase/peroxidase, cholesterol esterase/cholesterol esterase/peroxidase, Lipoprotein lipase/Glycerol kinase/GPO/Peroxidase respectively and AST method was according to the IFCC but without P-5'-P [28].

Humoral Immune Assay

On day 11 of age, infectious bronchitis H 120 strain live vaccine product of MERIAL, 17 rue Bourgelat 69002 (Lyon, France) (Batch no.: L395281) and on day 21 of age, Gumboro D78 live vaccine product of Intervet International B.V. (Boxmeer, Holland) (Batch no: 12648BM01) were diluted by disinfectant-free drinking water then orally administered individually (1.1 dose per chicken). These vaccines were the only vaccine throughout the study. Blood antibody titer against each vaccine measured with ELISA method on 21 days after each administration in serum. Antibody test kit used for Infectious Bronchitis Virus (IBV; code: CK119; Lot no: FS5674) and Infectious Bursal Disease (IBD; code: CK113; Lot no: FS5709) was product of BioChek veterinary diagnostics, (BioChek (UK) Ltd., 11 Mill farm business park, Millfield Road, Hounslow, London TW4 5PY).

Statistical Analysis

The statistical normality of all data were tested in MINITAB software, confidence level=95% [29]. Statistical normal data of each variable with normal distribution (P>0.05) used for ANOVA procedure and statistical un-normal data (P<0.05) normalized by especial equations according to each variable properties [29,30]. Then treatments analyzed by ANOVA procedure using the GLM procedure of SAS software [31]. When significant differences among means were found, means were separated using Duncan's Multiple Comparison test (α=5%) for post hoc multiple comparisons.

RESULTS

Salmonella Recovery

Salmonella culture of cloacae samples showed that only one chicken of challenged group in treatment 1 was not infected and no *Salmonella* were present in unchallenged group.

Performance

Salmonellosis led to increase (P<0.01) in mortality and addition of Thr level did not alleviate it significantly *(Table 2)*. Feed intake (FI) was not affected by salmonellosis significantly; but, the increase in Thr/Lys ratio over catalog recommendation decreased (P<0.05) feed intake in whole period *(Table 3)*. Salmonellosis and addition of Thr/Lys ratio over catalog recommendation decreased (P<0.05) weight gain *(Table 2, Table 3)*. In *Salmonella* positive group, recommended Thr/Lys ratio increased (P<0.05) weight gain than other Thr/Lys ratio level *(Table 3)*; but, increase in Thr/Lys ratio in *Salmonella* positive group at finisher period did not improve weight gain significantly *(Table 2)*. Feed conversion ratio (FCR) and protein efficiency ratio

Table 2. *Response to different Thr/Lys ratio and Salmonella challenge for performance*
Tablo 2. *Farklı Thr/Lys oranı ve Salmonella maruziyetine karşı performans tepkisi*

Traits[1]	Starter (2-10 d)				Grower (11-24 d)				Finisher (25-42 d)			
	Mortality %	FI (kg)	WG (kg)	FCR	Mortality %	FI (kg)	WG (kg)	FCR	Mortality %	FI (kg)	WG (kg)	FCR
Treatments[2]												
T.1	2.59	0.272	0.218	1.255	3.89	1.532^{ab}	0.943^{ab}	1.624	4.77^{b}	2.546	1.327^{a}	1.918^{c}
T.2	0.00	0.271	0.224	1.212	5.19	1.588^{a}	0.997^{a}	1.593	0.00^{b}	2.553	1.311^{a}	1.932^{c}
T.3	0.00	0.269	0.216	1.255	4.02	1.463^{b}	0.901^{b}	1.626	0.00^{b}	2.453	1.340^{a}	1.831^{c}
T.4	-	-	-	-	-	-	-	-	19.09^{a}	2.526	1.005^{b}	2.509^{a}
T.5	-	-	-	-	-	-	-	-	19.39^{a}	2.590	1.076^{b}	2.405^{ab}
T.6	-	-	-	-	-	-	-	-	19.09^{a}	2.397	1.034^{b}	2.320^{b}
Factors[3]												
Sal. -	-	-	-	-	-	-	-	-	1.59^{b}	2.511	1.326^{a}	1.894^{b}
Sal. +	-	-	-	-	-	-	-	-	19.19^{a}	2.504	1.038^{b}	2.412^{a}
Thr.1	-	-	-	-	-	-	-	-	10.90	2.537	1.210	2.171^{a}
Thr.2	-	-	-	-	-	-	-	-	8.31	2.557	1.209	2.135^{a}
Thr.3	-	-	-	-	-	-	-	-	8.18	2.429	1.189	2.040^{b}
SEM[4]												
Treatments	0.522	0.002	0.003	0.016	1.003	0.015	0.011	0.006	3.496	0.073	0.022	0.041
Sal.	-	-	-	-	-	-	-	-	2.018	0.042	0.013	0.023
Thr.	-	-	-	-	-	-	-	-	2.472	0.051	0.16	0.029
P-value[5]												
Treatments	0.119	0.906	0.657	0.516	0.863	0.017	0.021	0.150	0.001	0.614	0.0001	0.0001
Sal.	-	-	-	-	-	-	-	-	0.0001	0.925	0.0001	0.0001
Thr.	-	-	-	-	-	-	-	-	0.781	0.221	0.523	0.022
Sal.×Thr.	-	-	-	-	-	-	-	-	0.756	0.769	0.204	0.388
CV[6]	295.8	4.48	8.53	6.33	112.4	4.81	6.14	2.06	76.55	5.85	3.78	3.90

*[1] **FI:** Feed Intake (kg/41d), **WG:** Weight Gain (kg/41d), **FCR:** Feed Conversion Ratio (feed intake/weight gain); [2] Comparison of treatments effects: **T.1** = Low Thr/Lys ratio+ no Salmonella challenge, **T.2** = Standard Thr/Lys ratio + no Salmonella challenge, **T.3** = High Thr/Lys ratio+ no Salmonella challenge, **T.4** = Low Thr/Lys ratio + Salmonella challenge, **T.5** = Standard Thr/Lys ratio + Salmonella challenge, **T.6**= High Thr/Lys ratio + Salmonella challenge; [3] Comparison of factors effects: **Sal. -** = Salmonella negative group, **Sal. +** = Salmonella positive group, **Thr.1** = Low Thr/Lys ratio group, **Thr.2** = Standard Thr/Lys ratio group, **Thr.3**= High Thr/Lys ratio group; [4] Standard error of mean for treatments or factors (Sal. = Salmonella grouping; Thr. = Threonine grouping); [5] Significance level of calculated F in analysis of variance; [6] Coefficient of variation (%); [abc] Means without a common superscript letter differ within each part of a column (P<0.05)*

Table 3. *Response to different Thr/Lys ratio and Salmonella challenge (whole period)*
Tablo 3. *Farklı Thr/Lys oranı ve Salmonella maruziyetine karşı tepki (tüm periyot)*

Traits[1]	Mortality %	FI (kg)	WG (kg)	FCR	FCC (USD $)	PER	IBV titer	IBD titer	Urea (mg/dL)	Uric acid (mg/dL)	AST (IU/L)	Glucose (mg/dL)	Chol. (mg/dL)	Trig. (mg/dL)
Treatments[2]														
T.1	9.3^{ab}	4.393	2.519^{a}	1.743^{b}	0.691^{b}	3.125^{a}	1412.5	4475.3	2.83^{b}	2.97^{c}	292.5^{bc}	236.4^{ab}	132.8	114.1
T.2	9.0^{ab}	4.412	2.553^{a}	1.727^{b}	0.686^{b}	3.154^{a}	1368.8	4093.7	2.95^{b}	3.53^{bc}	282.2^{c}	228.1^{ab}	136.7	145.2
T.3	4.7^{b}	4.250	2.513^{a}	1.691^{b}	0.680^{b}	3.222^{a}	1505.0	4780.6	3.12^{b}	3.37^{c}	309.3^{bc}	264.0^{a}	148.2	138.3
T.4	21.2^{a}	4.276	2.126^{c}	2.009^{a}	0.796^{a}	2.717^{b}	1235.6	3879.6	4.17^{a}	3.89^{abc}	370.1^{ab}	198.8^{b}	118.9	104.9
T.5	24.2^{a}	4.425	2.271^{b}	1.946^{a}	0.773^{a}	2.805^{b}	1203.9	3561.6	4.34^{a}	4.61^{a}	358.8^{abc}	192.4^{b}	122.7	136.4
T.6	21.2^{a}	4.046	2.077^{c}	1.951^{a}	0.785^{a}	2.799^{b}	1320.9	4162.9	4.58^{a}	4.37^{ab}	393.4^{a}	222.5^{ab}	132.7	127.0
Factors[3]														
Sal. -	7.7^{b}	4.351	2.528^{a}	1.720^{b}	0.686^{b}	3.167^{a}	1428.8	4449.9	2.97^{b}	3.29^{b}	294.6^{b}	242.8^{a}	139.2^{a}	132.6
Sal. +	22.2^{a}	4.249	2.158^{b}	1.969^{a}	0.785^{a}	2.773^{b}	1253.5	3868.0	4.36^{a}	4.29^{a}	374.1^{a}	204.6^{b}	124.8^{b}	122.7
Thr.1	14.4	4.343^{ab}	2.351^{ab}	1.857	0.736	2.950	1336.7	4220.0	3.40	3.37	325.7	220.2	126.8	110.2^{b}
Thr.2	15.5	4.418^{a}	2.432^{a}	1.821	0.724	3.004	1298.1	3865.6	3.55	4.00	315.0	212.8	130.7	141.5^{a}
Thr.3	11.8	4.162^{b}	2.326^{b}	1.802	0.725	3.040	1426.1	4515.9	3.74	3.80	345.3	246.2	141.5	133.5^{a}
SEM[4]														
Treatments	4.25	0.088	0.039	0.024	0.009	0.033	238.0	348.3	0.24	0.28	24.8	14.7	6.8	9.4
Sal.	2.45	0.051	0.022	0.014	0.005	0.019	137.4	201.1	0.13	0.16	14.3	8.5	3.9	5.4
Thr.	3.00	0.062	0.027	0.017	0.006	0.023	168.2	246.3	0.17	0.20	17.5	10.4	4.8	6.7
P-value[5]														
Treatments	0.037	0.117	0.0001	0.0001	0.0001	0.0001	0.963	0.273	0.0001	0.004	0.026	0.034	0.101	0.061
Sal.	0.001	0.210	0.0001	0.0001	0.0001	0.0001	0.409	0.066	0.0001	0.0003	0.0009	0.005	0.022	0.248
Thr.	0.726	0.036	0.037	0.139	0.398	0.069	0.880	0.243	0.411	0.117	0.511	0.107	0.139	0.011
Sal.×Thr.	0.878	0.536	0.209	0.641	0.611	0.562	0.999	0.993	0.973	0.964	0.988	0.983	0.993	0.991
CV[6]	60.9	4.11	3.33	2.70	2.66	2.20	49.73	23.45	19.10	21.65	21.35	18.42	14.59	20.88

*[1] **FI:** Feed Intake (kg/41d), **WG:** Weight Gain (kg/41d), **FCR:** Feed Conversion Ratio (feed intake/weight gain), **FCC:** Feed Consumption Cost (cost of feed intake in Rials/kg of weight gain), **PER:** Protein Efficiency Ratio (kg of weight gain/kg of consumed crude protein), **IBV titer:** ELISA titer of Infectious Bronchitis Virus, **IBD titer:** ELISA titer of Infectious Bursal Disease, **Chol.:** Cholesterol, **Trig.:** Triglycerides; [2] Comparison of treatments effects: **T.1** = Low Thr/Lys ratio + no Salmonella challenge, **T.2** = Standard Thr/Lys ratio + no Salmonella challenge, **T.3** = High Thr/Lys ratio + no Salmonella challenge, **T.4**= Low Thr/Lys ratio + Salmonella challenge, **T.5** = Standard Thr/Lys ratio + Salmonella challenge, **T.6**= High Thr/Lys ratio + Salmonella challenge; [3] Comparison of factors effects: **Sal. -** = Salmonella negative group, **Sal. +** = Salmonella positive group, **Thr.1** = Low Thr/Lys ratio group, **Thr.2** = Standard Thr/Lys ratio group, **Thr.3** = High Thr/Lys ratio group; [4] Standard error of mean for treatments or factors (Sal. = Salmonella grouping; Thr. = Threonine grouping); [5] Significance level of calculated F in analysis of variance; [6] Coefficient of variation (%); [abc] Means without a common superscript letter differ within each part of a column (P< 0.05)*

(PER) negatively affected (P<0.01) by salmonellosis *(Table 3)*. Improved FCR and PER by adding Thr to basal diet was inconsiderable in whole period but Thr/Lys ratio over catalog recommendation in *Salmonella* positive group at finisher improved (P<0.01) FCR than lowest Thr/Lys ratio *(Table 2)*. Salmonellosis led to increase (P<0.01) in feed consumption cost per 1 kg weight gain *(Table 3)*.

Improved feed consumption cost (FCC) by increasing Thr/Lys ratio was inconsiderable. Results on mortality, feed intake, weight gain and FCR at starter and grower are given in *Table 2*. A trend (P<0.05) was observed between increases in Thr/Lys ratio over catalog recommendation and decrease in feed intake and weight gain at grower *(Table 2)*. At grower negative effect of maximum Thr/Lys ratio than catalog recommendation on FCR was inconsiderable and recommended Thr/Lys ratio by catalog has the best FCR non-significantly *(Table 2)*.

Blood Parameters

Salmonellosis increased serum urea, uric acid and AST and decreased serum glucose and cholesterol (P<0.01). Salmonellosis did not decrease serum triglycerides significantly. A non-significant trend in challenged and non-challenged groups was observed between increasing Thr/Lys ratio and increased serum urea and uric acid. Increasing Thr/Lys ratio had no significant incremental effect on serum AST and glucose but ratio 25% over catalog recommendation led to a small increasing in challenged and non-challenged groups. Increasing Thr/Lys ratio led to increase (P<0.05) in serum triglyceride but the similar effect on cholesterol was not significant.

Humoral Immune Response

Decreased humoral immune response by salmonellosis was not significant. Increasing Thr/Lys ratio over catalog recommendation did not improve humoral immune response in challenged and non-challenged groups.

DISCUSSION

Salmonellosis inducted by *Salmonella* Paratyphi A, negatively affected production performance (similar to *Clostridium* infection) and mortality rate and increasing Thr/Lys ratio over catalog recommendation could not improve them significantly [5]. However, a trend (P<0.05) was observed between increase in Thr/Lys ratio and improvement of FCR in finisher period that may be because of its small negative effect on feed intake and obtained similar weight gain.

In whole period, recommended Thr/Lys ratio by catalog showed increase in feed intake and weight gain than level 25% more, that was also significant in weight gain of infected chickens *(Table 3)*. Weight gain of infected chickens fed recommended Thr/Lys ratio by catalog was more than other levels, similar to *Clostridium* infected chicken [5,32]. This is may be because low Thr and high Thr led to amino acid imbalance and amino acid imbalance led to decreased feed intake and weigh gain and impairment of FCR [33-35]. Therefore, recommended Thr/Lys ratio by catalog seems to be adequate for optimum performance [11].

Increase in diet's crude fiber can affect intestinal mucosa and consequently digestive tract health that may lead to gut susceptibility to infections [36,37]. Therefore, with the aim of increasing diet's crude fiber and decreasing threonine content of treatment 1 as far as possible, the basal diet was formulated in total amino acids system on base of corn, wheat, barley, wheat bran, rice bran and soybean meal. It is known that a threonine deficiency will affect mucin secretion and, thereby, gut barrier integrity [38]. Mucus contains relatively high threonine levels, suggesting that the threonine requirement in birds with intestinal problems may increase [3]. European Centre for Disease Prevention and Control [39] reported *Salmonella Paratyphi* A was the most commonly identified serotype in human cases of paratyphoid fever in EU/EEA countries. Because of epidemiological importance of this serovar around the world, this serovar was selected for study. In the present study, FCR and PER generally not improved by increasing Thr/Lys ratio. In *Salmonella* challenge group, that may be because of severity of inducted infection by this *Salmonella* serovar similar to *Clostridium* infected chicken or relatively few differences between Thr/Lys ratios of basal diets and recommended Thr/Lys ratio [5,6]. Nevertheless this general resulting, FCR of challenged group in finisher period tended to improve with increasing Thr/Lys ratio; however, no significant effects on feed intake or weight gain of these chickens were observed. This may be because of other beneficial metabolic effect of threonine, like on immune response to *Salmonella* infection as coccidiosis challenge [6].

Some animals, like rats and pigs, have a specific requirement for threonine to optimize immunity that is much higher than that of growth [9,40,41]. In the present investigation no differences in humoral immunity or mortality were observed in challenged and non challenged groups in response to different Thr/Lys ratios. Similar investigations reported that high Thr/Lys ratio over (well-nigh) Ross nutrition specification recommendation had no significant effect on improvement of cellular or humoral immunity [2,42,43]. Nevertheless, improved cellular or humoral immunity in response to increased Thr/Lys ratio were reported in diets with great Thr deficiency and more threonine supplementing than recommended Thr/Lys ratio by Ross nutrition specification had no significant effect [4,5,44].

Identification of the arginine pathway that produces nitric oxide has led to research demonstrating that Arg is a potent immunological modulator [45]. Animal and human studies suggest outcome benefit to the use of supplemental dietary arginine [46]. Complimentary effects of arginine on immune function and health of broiler chickens showed high levels of Arginine accelerated antibody production [47]. Depressed kidney arginase activity by feeding high level of threonine was reported that may leading to more bio-availability of arginine for immune responses and less urea excretion in chickens [48,49]. In the present study no decrease in serum urea in response to increased diets Thr level were observed, whereas serum urea showed a non-significant incremental trend. More investigation about relationship between arginase activity and threonine levels on immune response alteration mechanism is recommendable.

No significant negative effect of highest examined Thr level on AST was observed. Correlation with no significant alteration of serum glucose and cholesterol, showed highest examined Thr level might have had no adverse effect on liver functions. Significant increased of serum triglycerides in response to increased Thr/Lys ratio on ensemble challenged and non-challenged groups might indicate on better absorption of triglycerides from healthier absorptive surface.

Increase in Thr/Lys ratio 25% over catalog recommendation could slightly be economically lucrative; however, Thr/Lys ratio 25% over catalog recommendation significantly improved FCR compare with Thr deficient diet in *Salmonella* challenged group. In severe gastric infection that leading to mortality, increasing Thr/Lys ratio over catalog recommendation seemed to have inconsiderable effect on subjugating the infection and seems cannot be a choice for replacing the antibiotics.

Acknowledgment

The authors are grateful to the Islamic Azad University

for research funding support. Also we thank Samia Momeni Ahangar expert of Babol Razi Pathobiology Lab (Babol, Iran) to carry out biochemistry tests and Dr. Marjan Tafreshi expert of Dr. Rohani veterinary hospital (Babol, Iran) to carry out humoral immune tests.

REFERENCES

1. Kidd MT: Nutritional considerations concerning threonine in broilers. *World's Poult Sci J,* 56, 139-151, 2000. DOI: 10.1079/WPS20000011

2. Abbasi MA, Mahdavi AH, Samie AH, Jahanian R: Effects of different levels of dietary crude protein and threonine on performance, humoral immune responses and intestinal morphology of broiler chicks. *Braz J Poult Sci,* 16, 35-44, 2014. DOI: 10.1590/S1516-635X2014000100005

3. Bertolo RFP, Chen CZL, Law G, Pencharz PB, Ball RO: Threonine requirement of neonatal piglets receiving total parental nutrition is considerably lower than that of piglets receiving an identical diet intragastrically. *J Nutr,* 128, 1752-1759, 1998.

4. Wils-Plotz EL, Dilger RN: Combined dietary effects of supplemental threonine and purified fiber on growth performance and intestinal health of young chicks. *Poult Sci,* 92, 726-734, 2013. DOI: 10.3382/ps.2012-02664

5. Star L, Rovers M, Corrent E, van der Klis JD: Threonine requirement of broiler chickens during subclinical intestinal Clostridium infection. *Poult Sci,* 91, 643-652, 2012. DOI: 10.3382/ps.2011-01923

6. Wils-Plotz EL, Jenkins MC, Dilger RN: Modulation of the intestinal environment, innate immune response, and barrier function by dietary threonine and purified fiber during a coccidiosis challenge in broiler chicks. *Poult Sci,* 92, 735-745, 2013. DOI: 10.3382/ps.2012-02755

7. Tenenhouse HS, Deutsch HF: Some physical-chemical properties of chicken gamma-globulins and their pepsin and papain digestion product. *Immunochemistry*, 3, 11-20, 1966.

8. Tizzard IR: The nature of antibodies. **In,** Tizard IR (Ed): Immunology: An Introduction. 3rd ed., 145-166, Saunders College Publishing, New York, 1992.

9. Lotan R, Mokady S, Horenstein L: The effect of lysine and threonine supplementation on the immune response of growing rats fed wheat gluten diets. *Nutr Rep Int,* 22, 313-318, 1980.

10. Ayasan T, Okan F: Effects of diets containing different levels of threonine and lysine amino acids on fattening performance of broiler chicks. *Süleyman Demirel Üniv Ziraat Fak Derg,* 5, 36-43, 2010.

11. Anonymous: Ross nutrition supplement. 21. Aviagen Technical Service, Newbridge, Midlothian, EH28 8SZ, Scotland, UK, 2009.

12. NRC: Nutrient requirements of poultry. 9th rev. ed., National Academy Press, Washington, DC, 1994. DOI: 10.17226/2114

13. Berres J, Vieira SL, Favero A, Freitas DM, Pena JEM, Nogueira ET: Digestible valine requirements in high protein diets for broilers from twenty-one to forty-two days of age. *Anim Feed Sci Technol,* 165, 120-124, 2011. DOI: 10.1016/j.anifeedsci.2011.01.001

14. Gong LM, Lai CH, Qiao SY, Li D, Ma YX, Liu YL: Growth performance, carcass characteristics, nutrient digestibility and serum biochemical parameters of broilers fed low-protein diets supplemented with various ratios of threonine to lysine. *Asian-Aust J Anim Sci,* 18, 1164-1170, 2005. DOI: 10.5713/ajas.2005.1164

15. Hurwitz S, Sklan D, Talpaz H, Plavnik I: The effect of dietary protein level on the lysine and arginine requirements of growing chickens. *Poult Sci,* 77, 689-696, 1998. DOI: 10.1093/ps/77.5.689

16. Morris TR, Gous RM, Fisher C: An analyses of the hypothesis that amino acid requirements for chicks should be stated as a proportion of dietary protein. *World's Poult Sci J,* 55, 7-22, 1999. DOI: 10.1079/WPS19990002

17. Plumstead PW, Romero-Sanchez H, Paton ND, Spears JW, Brake J: Effects of dietary metabolizable energy and protein on early growth responses of broilers to dietary lysine. *Poult Sci,* 86, 2639-2648, 2007. DOI: 10.3382/ps.2007-00168

18. Baylan M, Canogullari S, Ayasan T, Sahin A: Dietary threonine supplementation for improving growth performance and edible carcass parts in japanese quails, *Coturnix coturnix japonica. Int J Poult Sci,* 5, 635-638, 2006. DOI: 10.3923/ijps.2006.635.638

19. Canogullari S, Baylan M, Ayasan T: Threonine requirement of laying japanese quails. *J Anim Vet Adv,* 8, 1539-1541, 2009.

20. Gast RK: Paratyphoid infections. **In,** Saif YM (Ed): Diseases of Poultry. 12th ed., 636-665. Blackwell Publishing Professional, 2121 State Avenue, Ames, Iowa 50014, USA, 2008.

21. Berndt A, Wilhelm A, Jugert C, Pieper J, Sachse K, Methner U: Chicken cecum immune response to *Salmonella enterica* Serovars of different levels of invasiveness. *Infect Immunol,* 75, 5993-6007, 2007. DOI: 10.1128/IAI.00695-07

22. Chambers JR, Gong J: The intestinal microbiota and its modulation for *Salmonella* control in chickens. *Food Res Int,* 44, 3149-3159, 2011. DOI: 10.1016/j.foodres.2011.08.017

23. Lorenzoni G: Poultry diseases influenced by gastrointestinal health: Traditional treatments and innovative solutions. 73-78. Nottingham University Press, Nottingham, NG11 0AX, United Kingdom, 2010.

24. Kallapura G, Morgan MJ, Pumford NR, Bielke LR, Wolfenden AD, Faulkner OB, Latorre JD, Menconi A, Hernandez-Velasco X, Kuttappan VA, Hargis BM, Tellez G: Evaluation of the respiratory route as a viable portal of entry for *Salmonella* in poultry via intratracheal challenge of *Salmonella* Enteritidis and *Salmonella* Typhimurium. *Poult Sci,* 93, 340-346, 2014. DOI: 10.3382/ps.2013-03602

25. Higgins SE, Wolfenden AD, Tellez G, Hargis BM, Porter TE: Transcriptional profiling of cecal gene expression in probiotic and *Salmonella* challenged neonatal chicks. *Poult Sci,* 90, 901-913, 2011. DOI: 10.3382/ps.2010-00907

26. Doyle MP, Busta F, Cords BR, Davidson PM, Hawke J, Hurd HS, Isaacson RE, Matthews K, Maurer J, Meng J, Montville TJ, Shryock TR, Sofos JN, Vidaver AK, Vogel L: Antimicrobial resistance: Implications for the food system. *Comp Rev Food Sci Food Safety,* 5, 71-137, 2006. DOI: 10.1111/j.1541-4337.2006.00004.x

27. Park SH, Ryu S, Kang DH: Development of an improved selective and differential medium for isolation of *Salmonella* spp. *J Clin Microbiol,* 50, 3222-3226, 2012. DOI: 10.1128/JCM.01228-12

28. Bergmeyer HU, Hørder M, Rej R: Approved recommendation (1985) on IFCC methods for the measurement of catalytic concentration of enzymes. Part 2. IFCC method for aspartate aminotransferase. *J Clin Chem Clin Biochem*, 24, 497-510, 1986.

29. Minitab Inc: Statistical software, Release 14.1. Minitab Inc, Pennsylvania State College, USA, 2003.

30. Festing MFW, Altman DC: Guidelines for the design and statistical analysis of experiments using laboratory animals. *ILAR J,* 43, 244-258, 2002. DOI: 10.1093/ilar.43.4.244

31. SAS Institute: SAS/STAT User's guide, Version 9.1. SAS Institute Inc. Cary, NC, 2002.

32. Lensing M, Van Der Klis JD, Le Bellego L, Rovers M: The threonine requirement of broiler chickens during subclinical intestinal infection. **In**, *Proceedings of the 16th European Nutrition Symposium.* 343-346. World's Poultry Science Association Strasbourg, France, 2007.

33. Boorman KN, Ellis GM: Maximum nutritional response to poor quality protein and amino acid utilization. *Br Poult Sci,* 37, 145-156, 1996. DOI: 10.1080/00071669608417844

34. Denbow DM: Food intake control in birds. *Neurosci Biobehav Rev,* 9, 223-232, 1985. DOI: 10.1016/0149-7634(85)90047-8

35. Zaefarian F, Zaghari M, Shivazad M: The threonine requirements and its effects on growth performance and gut morphology of broiler chicken fed different levels of protein. *Int J Poult Sci,* 7, 1207-1215, 2008. DOI: 10.3923/ijps.2008.1207.1215

36. Montagne L, Piel C, Lalles JP: Effect of diet on mucin kinetics and composition: Nutrition and health implications. *Nutr Rev,* 62, 105-114, 2004. DOI: 10.1111/j.1753-4887.2004.tb00031.x

37. Montagne L, Pluske JR, Hampson DJ: A review of interactions

between dietary fibre and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. *Anim Feed Sci Techn,* 108, 95-117, 2003. DOI: 10.1016/S0377-8401(03)00163-9

38. Horn NL, Donkin SS, Applegate TJ, Adeola O: Intestinal mucin dynamics: Response of broiler chicks and White Pekin ducklings to dietary threonine. *Poult Sci,* 88, 1906-1914, 2009. DOI: 10.3382/ps.2009-00009

39. European Centre for Disease Prevention and Control: Annual epidemiological report, 2012. 122-125. Stockholm, ECDC, 2013. DOI: 10.2900/76137

40. Defa L, Changting X, Shiyan Q, Jinhui Z, Johnson EW, Thacker PA: Effects of dietary threonine on performance, plasma parameters and immune function of growing pigs. *Anim Feed Sci Tech,* 78, 179-188, 1999. DOI: 10.1016/S0377-8401(99)00005-X

41. Wang X, Qiao SY, Liu M, Ma YX: Effects of graded levels of true ileal digestible threonine on performance, serum parameters and immune function of 10-25 kg pigs. *Anim Feed Sci Tech,* 129, 264-278, 2006. DOI: 10.1016/j.anifeedsci.2006.01.003

42. Kidd MT, Kerr BJ, Anthony NB: Dietary interactions between lysine and threonine in broilers. *Poult Sci,* 76, 608-614, 1997. DOI: 10.1093/ps/76.4.608

43. Kidd MT, Gerard PD, Heger J, Kerr BJ, Rowe D, Sistani K, Burnham DJ: Threonine and crude protein responses in broiler chicks. *Anim Feed Sci Technol,* 94, 57-64, 2001. DOI: 10.1016/S0377-8401(01)00301-7

44. Ayasan T, Okan F, Hizli H: Threonine requirement of broiler from 22-42 days. *Int J Poult Sci,* 8, 862-865, 2009. DOI: 10.3923/ijps.2009.862.865

45. Collier J, Vallance P: Second messenger role for NO widens to nervous and immune systems. *Trends Pharmacol Sci,* 10, 427-431, 1989. DOI: 10.1016/S0165-6147(89)80001-X

46. Evoy D, Lieberman MD, Fahey TJ: Immunonutrition: The role of arginine. *Nutr,* 14, 611-617, 1998. DOI: 10.1016/S0899-9007(98)00005-7

47. Abdukalykova S, Ruiz-Feria CA: Arginine and vitamin E improve the cellular and humoral immune response of broiler chickens. *Int J Poult Sci,* 5, 121-127, 2006. DOI: 10.3923/ijps.2006.121.127

48. Austic RE, Nesheim MC: Role of kidney arginase in variations of the arginine requirement of chicks. *J Nutr,* 100, 855-868, 1970.

49. Austic RE, Scott RL: Involvement of food intake in the lysine-arginine antagonism in chicks. *J Nutr,* 105, 1122-1131, 1975.