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EFFECTS OF DIETARY PROTEIN AND THREONINE LEVELS ON GROWTH PERFORMANCE AND IMMUNOCOMPETENCE IN BROILER CHICKENS

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ABSTRACT

The present work was conducted to study the effects of feeding low crude protein diets (LCP-diets) with supplementation of threonine (Thr) on growth performance and immunocompetence in broiler chickens. One day old 264 Cobb chicks were divided into 6 equal groups (each of 4 replicates and each replicate was of 11 chicks). The 1st group is considered the control one and fed normal basal control starter, grower and finisher diets containing the recommended levels of CP, threonine, methionine, lysine, tryptophan, valine, arginine and metabolizable energy as specified for Cobb500 broiler performance. The 2nd experimental group was fed ideal protein diets which were formulated to contain the minimum nutrient specifications based upon supplementing digestible essential amino acids (lysine, methionine, tryptophan, threonine, arginine, and valine). The other experimental chick groups (3rd, 4th, 5th and 6th groups) were fed isocaloric diets containing low levels of total dietary CP% (1 and 2% less than the minmum recommended CP%) with supplementation of Thr to 120 or 140% of the minimum recommended levels. The diets were isocaloric (3035, 3108 and 3180 Kcal/kg for starter, grower and finisher diets respectively). All chicks were vaccinated against Newcastle disease virus (NDV) at day 14 of age through eye drops. Growth performance indices (body weight, feed intake, feed conversion ratio and protein efficiency ratio) were measured. Blood samples were collected from the broilers at 14, 21, 28 and 35 days of age to determine the serum titre of antibodies against NDV using haemagglutination inhibition test (HI). At 42 days of age, blood samples were collected for determination of serum metabolites and 12 chickens from each group were slaughtered to obtain some of the carcass traits.

The results revealed that BW and FCR of the broilers fed 2% LCP- diets with 120 or 140% Thr supplementation were significantly lowered than those fed control or ideal protein diets, as well as those fed 1% LCP- diets with Thr supplementation. There were no differences between the groups in dressed carcass percentages. The abdominal fat % in the carcass of the broiler chickens was not significantly affected by decreasing the dietary CP or supplementation of threonine. Liver weight% significantly increased with feeding the 2% LCP- diets. Feeding 2% LCP- diets also resulted in significant increase in serum uric acid and decreased serum total protein, albumin and globulin. The highest HI titer values and lowest serum levels of uric acid were observed in chickens fed ideal protein diets. While, chickens fed LCP- diets (-2%) had reduced HI titer values.

From the present study, it could be concluded that the dietary protein level could be decreased by 1% of the recommended levels with threonine supplementation to 120 % of requirements with no adverse effects on body weight development, FCR, serum metabolites and immune competence in broiler chickens. Formulating broiler diets based upon digestible essential amino acids achieved optimal growth performance and maintained immunocompetence.

INTRODUCTION

Commercial poultry enterprises are growing rapidly and play an important role in developing countries and worldwide as well. Chicken meat is tender, juicy, of high quality protein with low fat content and relatively inexpensive (**FAO, 2015**).

Feeding poultry presents a great challenge to producers and nutritionists in several countries as meeting the nutritional requirements for growing broiler chicks constitutes the majority of costs (70 %) of production (**Dairo et al., 2010**), and certainly is becoming a difficulty of great significance as prices of feed ingredients continue to rise. A large portion of that cost involves in meeting the protein and amino acid requirements (**Awad et al., 2014**).

However, broilers have high dietary CP requirements and protein ingredients are the most expensive in their diets (**Filho et al., 2005 and Jafari et al., 2013**). Excess dietary protein increased heat production, nitrogen excretion and moisture content of the litter (**Hernandez et al., 2013**). While, lowering CP in broiler diet could reduce the feed costs, decreased nitrogen excretion and save the environment. In addition, the use of synthetic amino acids to meet the broiler needs of the most limiting amino acids could result in production of cost effective diets as synthetic AA are becoming commercially available and inexpensive. Therefore, using of LCP-diets supplemented with the limiting EAAs for broiler diets has received great interests in recent years in order to reduce feeding costs, environmental pollution and save protein supplements with achieving optimal broiler production (**Namroud et al., 2008, Orma et al., 2010 &2011, Gheisar et al., 2011 and Kim et al., 2014**).

Several researches had been done to study the effects of decreasing CP levels and supplementation with limiting EAAs of broiler chick diets on growth performance and other treats, however the findings were conflicting. Some studies showed that feeding LCP-diet with amino acids supplementation resulted in inferior performance (**Bregendahl et al., 2002, Waldroup et al., 2005 and Kamran et al., 2008**). Other experiments showed no effect on performance due to feeding LCP-diets (**Aletor et al., 2000, Kamran et al., 2010**). However, **Widyaratne and Drew (2011)** concluded that LCP-diet can support growth performance equal to high- CP diets when highly digestible ingredients are fed. With this concept, our previous researches showed that feeding low protein diets supplemented with either lysine or methionine above requirements maintained growth performance and immunocompetence similar to that of broiler chickens fed basal normal diets (**Orma et al., 2010&2011**).

It has been shown that deficiency or excess of dietary protein (**Wu et al., 1999**) or amino acids (**Quentin et al., 2005, li et al., 2007 and Kogut, 2009**) altered immune responses. The magnitude of an immune response and subsequently alterations in nutrients metabolism is dependent upon a nutritional complete diet (**Maroufyan et al., 2010**).

To greater extent, threonine (the third limiting amino acid especially in low CP diets) plays a great role in modulating immune functions in live stock as Thr is a major component of intestinal mucin and plasma gamma globulin in birds, inhibiting apoptosis, enhancement of antibodies production and stimulation of lymphocytes. Due to its role in immune functions and improving the intestinal health, it should be considered in broiler diets with specific levels because it's excess is costly and its deficiency will decrease the efficiency of sulphur amino acid (SAA) and lysine use (**Kidd, 2000 and Rezaeipour et al., 2012**). In addition, some studies showed that broilers fed low-CP, Thr supplemented diets obtained the same high level of growth performance that was obtained by broilers fed high-protein diets (**Kidd et al., 1997**).

Therefore, the objective of the present study is to further evaluate the effects of feeding LCP-diets and maintaining optimal levels of essential amino acids with supplementation of threonine (above the recommended levels) on growth performance, carcass quality, serum metabolites (total protein, albumin, uric acid and creatinine), immunocompetence (HI titer against NDV).

MATERIALS AND METHODS

Experimental design, chicks and diets:

One day old 264 Cobb chicks obtained from a commercial supplier were used in this experiment. The chicks were divided into 6 equal groups (each of 4 replicates and each replicate was of 11 chicks). The 1st group is considered the control one and fed normal basal control starter, grower and finisher diets containing the recommended levels of CP, threonine, methionine, lysine, tryptophan, valine, arginine and metabolizable energy as specified for Cobb500 broiler performance (Tables 1, 2 &3). The 2nd experimental group was fed ideal protein diets which were formulated to contain the minimum nutrient specifications based upon supplementing digestible essential amino acids (lysine, methionine, tryptophan, threonine, arginine, and valine). The other experimental chick groups (3rd, 4th, 5th and 6th experimental groups) were fed isocaloric diets containing low levels of total dietary CP% (1 and 2% less than the minimum recommended level) with supplementation of threonine to 120 or140% of the minimum recommended levels. All chicks were vaccinated against Newcastle (NDV) at day 14 of age through eye drops.

The chicks were fed according to the 3 phases feeding program: starter (0-10 days), grower (11-22 days) and finisher (23-42 days). The ingredients and nutrients composition of the experimental diets are presented in tables 1, 2 & 3.

The basal control diets (starter 21.4% CP, grower 19.5% CP and finisher 18.5% CP with 3034, 3108 and 3180 Kcal ME/kg respectively) were formulated to cover the recommended minimum nutrient specifications for Cobb broiler performance according to **Cobb-vantress.com** recommendation table. The dietary levels of methionine, lysine, threonine, arginine, tryptophan and valine were checked and supplemented to the recommended minimum percentages. Threonine content in the control diets was 0.86, 0.78 and 0.71 % for the starter, grower and finisher diets respectively.

The ideal protein diets (group 2) were formulated after the recommended minimum nutrient specification for Cobb broilers but depend upon covering the digestible essential amino acids. The diets were isocaloric with the basal control diets.

The low protein diets: diets fed to the 3rd & 4th experimental groups were formulated to contain 1% CP less than the control diets with Thr supplementation at 120% & 140% of the control basal diets, respectively. While, diets fed to the 5th & 6th experimental groups contained 2% less CP with supplementation of Thr to 120 & 140 % of the required levels. The diets were provided regularly in the morning and water supplied ad libitum. Proximate chemical analyses of the experimental diets were carried out for determination of dry matter, crude protein, ether extract and ash according to **AOAC (1995)**.

Indices of broiler performance and carcass traits:

The chicks were weighed at one day old to obtain the average initial body weight then weighed every week and the average body weights for the chicks in each group was weekly calculated. The average feed intake per chick throughout the experimental period (6w) for each group was recorded weekly and feed conversion ratio (FCR) and protein efficiency ratio (PER) were calculated. At the end of the experiment, 12 birds from each group was randomly taken, weighed, slaughtered to complete bleeding, dressed and eviscerated for determination of dressed carcass, abdominal fat, gizzard, heart, liver and spleen weights and percent in relation to live body weight.

Blood sampling, serum metabolites and immune response:

Blood samples were collected from 15 chickens of each group at 14, 21, 28 and 35 days of age without anticoagulant for separation of sera which were used to detect titer of antibodies against Newcastle disease vaccine using haemagglutination inhibition test (HI) as indicative of broiler immune response in the different experimental groups.

Blood samples were also collected from 12 chickens from each group at 42 days of age via wing vein puncture. Sera were obtained by allowing blood samples to coagulate then centrifuged at 3000 rpm for 15 minutes. The separated sera were frozen at -20ºC in a deep freeze until used for determination of total protein (**Gornal et al., 1949**), albumin (**Doumas, 1971**), uric acid (**Young, 2001**), creatinine (**Young, 2001)**, and globulin from the difference between total protein and albumin.

Statistical analysis:

Data obtained in the present work were subjected to analysis of variance (ANOVA) and least significant difference (LSD) as described by **Snedecor and Cochran (1967)** followed by Duncan's Multiple Range Test (DMRT) for testing the significances differences between variables (**M-Stat, 2009**). Results were considered significant only at the level of P (<0.05).

Table 1: Ingredients and chemical composition of the starter experimental diets

Experimental Diets						
	Control[1]	Ideal[2]	Low protein diets			
			(-1%)		(-2%)	
			Threonine supplementation [3](%)			
			20	40	20	40
Ingredients (%)						
Corn, yellow	59.39	60.88	61.74	61.5	63.83	63.34
Soybean meal	30	28.49	28.06	27.83	26.96	27.26
Corn gluten	4.53	4.6	3.84	4	2.6	2.46
Soybean oil	2.60	2.39	2.5	2.6	2.5	2.7
Lime stone	1.9	1.9	1.9	1.88	1.9	1.9
Dicalcium phosphate	0.41	0.47	0.42	0.45	0.46	0.46
Common salts	0.3	0.3	0.3	0.3	0.3	0.3
Min. & Vit. Premix**	0.25	0.25	0.25	0.25	0.25	0.25
DL Lysine HCL	0.39	0.38	0.47	0.48	0.52	0.51
DL methionine	0.13	0.13	0.15	0.15	0.19	0.19
L-Threonine	0.08	0.1	0.3	0.49	0.35	0.51
L-Valine	0.02	0.11	0.07	0.07	0.14	0.12
Chemical composition (%)						
Calculated CP	21.5	21	20.5	20.5	19.5	19.5
Calculated ME(Kcal/kg)	3034	3036	3036	3036	3035	3036
Analyzed CP*	21.32	20.91	20.38	20.35	19.4	19.37
Analyzed EE*	5.40	5.22	5.17	5.31	5.32	5.35
Analyzed Ash*	5.97	6.14	5.93	6.22	6.19	5.85
Ca	0.9	0.9	0.9	0.9	0.9	0.9
Available P	0.46	0.46	0.45	0.45	0.45	0.45
Lysine**	1.31	1.18	1.32	1.32	1.32	1.32
Methionine**	0.50	0.45	0.50	0.50	0.51	0.51
Threonine**	0.86	0.77	1.03	1.22	1.04	1.2

* According to AOAC (1995)

** Amino acid calculated according to feed composition tables of NRC (1994)

** Mineral and vitamins premix each 2.5kg/ton contained: Vit A, 12,000,000 IU; Vit D3. 2,000,000 IU; Vit E 10,000 mg;
Vit B12.,10 mg; Riboflavin, 5000 mg; Pantothenic acid 10000 mg; Niacin, 30,000 mg; Menadione, 2000 mg;; Thiamine, 1000 mg; Folic acid, 1000mg; Pyridoxine,1500 mg; d-biotin, 50 mg;, Manganese, 60,000mg; Zinc, 50,000 mg; Selenium, 100mg;; Copper sulfate 4,000 mg; Iodine 1000mg; Iron 30,000mg ,cobalt 100mg, ca carbonate untill 3kg..

1- Control diet containing 21.5 CP% & 3035 Kcal/kg ME as recommended to cover minimum nutrient specifications.
2- Ideal protein diet formulated to cover specified levels of digestible essential amino acids.
3- Threonine supplementation to 120 and 140% of the recommended minimum specifications.

Table 2: Ingredients and chemical composition of the grower experimental diets

	Experimental Diets					
	Control[1]	Ideal[2]	Low protein diets			
			(-1%)		(-2%)	
			Threonine supplementation [3](%)			
			20	40	20	40
Ingredients (%)						
Corn, yellow	63.46	67.26	66.73	67.04	69.26	69.55
Soybean meal	26.83	21.72	23.47	23.93	21.57	20.77
Corn gluten	3	5.28	3.2	2.87	2.5	3
Soybean oil	3.4	2.2	2.9	2.8	2.69	2.56
Lime stone	1.83	1.8	1.84	1.85	1.8	1.8
Dicalcium phosphate	0.33	0.44	0.33	0.34	0.44	0.44
Common salts	0.3	0.3	0.3	0.3	0.3	0.3
Min. & Vit. Premix**	0.25	0.25	0.25	0.25	0.25	0.25
DL Lysine HCL	0.35	0.41	0.45	0.44	0.53	0.54
DL methionine	0.15	0.12	0.16	0.18	0.18	0.18
L-Threonine	0.08	0.1	0.28	0.43	0.33	0.48
L-Valine	0.02	0.12	0.09	0.07	0.15	0.13
Chemical composition (%)						
Calculated CP	19.5	19	18.5	18.5	17.5	17.5
Calculated ME (Kcal/kg)	3107	3108	3109	3109	3109	3109
Analyzed CP*	19.38	19.41	18.45	18.40	17.39	17.41
Analyzed EE*	5.82	4.93	5.22	5.36	5.28	5.40
Analyzed Ash*	6.36	5.86	5.39	6.19	6.16	6.31
Ca	0.84	0.84	0.84	0.84	0.84	0.84
Available P	0.43	0.43	0.41	0.42	0.43	0.42
Lysine**	1.19	1.05	1.19	1.19	1.20	1.19
Methionine**	0.48	0.42	0.48	0.49	0.48	0.48
Threonine**	0.78	0.69	0.94	1.08	0.95	1.09

According to AOAC (1995)

** Amino acid calculated according to feed composition tables of NRC (1994)

** Mineral and vitamins premix each 2.5kg/ton contained: Vit A, 12,000,000 IU; Vit D3. 2,000,000 IU; Vit E 10,000 mg; Vit B12.,10 mg; Riboflavin, 5000 mg; Pantothenic acid 10000 mg; Niacin, 30,000 mg; Menadione, 2000 mg;; Thiamine, 1000 mg; Folic acid, 1000mg; Pyridoxine,1500 mg; d-biotin, 50 mg;, Manganese, 60,000mg; Zinc, 50,000 mg; Selenium, 100mg;; Copper sulfate 4,000 mg; Iodine 1000mg; Iron 30,000mg ,cobalt 100mg, ca carbonate untill 3kg..

1- Control diet containing 19.5 CP% & 3108 Kcal/kg ME as recommended to cover minimum nutrient specifications.
2- Ideal protein diet formulated to cover specified levels of digestible essential amino acids.
3- Threonine supplementation to 120 and 140% of the recommended minimum specifications.

Table 3: Ingredients and chemical composition of the finisher experimental diets

	Experimental Diets					
	Control[1]	Ideal[2]	Low protein diets			
			(-1%)		(-2%)	
			Threonine supplementation [3](%)			
			20	40	20	40
Ingredients (%)						
Corn, yellow	69.17	69.7	70.08	70.02	72.51	72.38
Soybean meal	18.92	19.08	19.44	19.14	18.05	17.78
Corn gluten	6.3	5.29	4.1	4.33	3.06	3.25
Soybean oil	2.6	2.8	3.1	3.1	2.95	2.99
Lime stone	1.74	1.72	1.75	1.73	1.76	1.76
Dicalcium phosphate	0.2	0.22	0.2	0.2	0.18	0.19
Common salts	0.3	0.3	0.3	0.3	0.3	0.3
Min. & Vit. Premix**	0.25	0.25	0.25	0.25	0.25	0.25
DL Lysine HCL	0.38	0.36	0.39	0.4	0.45	0.46
DL methionine	0.08	0.11	0.12	0.11	0.15	0.15
L-Threonine	0.06	0.09	0.25	0.38	0.27	0.42
L-Valine	0	0.08	0.02	0.04	0.07	0.07
Chemical composition (%)						
Calculated CP	18.5	18	17.5	17.5	16.5	16.5
Calculated ME (Kcal/kg)	3180	3180	3180	3180	3180	3180
Analyzed CP*	18.41	17.92	17.38	17.41	16.45	16.44
Analyzed EE*	5.80	5.91	6.11	6.20	6.05	5.93
Analyzed Ash*	5.00	4.02	5.95	6.09	5.74	5.35
Ca	0.76	5.27	0.76	0.76	0.76	0.76
Available P	0.38	0.38	0.38	0.38	0.37	0.37
Lysine**	1.05	0.95	1.05	1.05	1.06	1.06
Methionine**	0.43	0.39	0.43	0.43	0.44	0.44
Threonine**	0.71	0.65	0.86	0.99	0.85	0.99

* According to AOAC (1995)

** Amino acid calculated according to feed composition tables of NRC (1994)

** Mineral and vitamins premix each 2.5kg/ton contained: Vit A1, 12,000,000 IU; Vit D3. 2,000,000 IU; Vit E 10,000 mg; Vit B12.,10 mg; Riboflavin, 5000 mg; Pantothenic acid 10000 mg; Niacin, 30,000 mg; Menadione, 2000 mg;; Thiamine, 1000 mg; Folic acid, 1000mg; Pyridoxine,1500 mg; d-biotin, 50 mg;, Manganese, 60,000mg; Zinc, 50,000 mg; Selenium, 100 mg; Copper sulfate 4,000 mg; Iodine 1000 mg; Iron 30,000 mg ,cobalt 100 mg, ca carbonate untill 3kg..

1- Control diet containing 18.5 CP% & 3180 Kcal/kg ME as recommended to cover minimum nutrient specifications.
2- Ideal protein diet formulated to cover specified levels of digestible essential amino acids.
3- Threonine supplementation to 120 and 140% of the recommended minimum specifications.

Table (4): Effects of dietary protein level and threonine supplementation on growth performance parameters. (Means ± SE)

		Experimental diets					
		Control[1]	Ideal [2]	Low protein diets			
				(-1%)		(-2%)	
				Threonine supplementation[3]			
				20	40	20	40
Period (W)	**Initial BW**	**40.21±0.37**	**38.96±0.47**	**39.89±0.46**	**39.58±0.72**	**39.58±0.61**	**38.43±0.72**
0-1	BW (g)	128.25±2.46	126.12 ±2.29	128.38 ±2.52	130 ±2.22	124.12 ±2.34	122.72 ±2.45
	BWG (g)	88.04 ± 2.29	87.16 ± 3.02	88.49 ± 4.16	90.42 ± 1.61	85.04 ± 3.08	84.29 ± 2.47
	FCR	1.16 ± 0.02	1.17 ± 0.01	1.20 ± 0.16	1.15 ± 0.03	1.18± 0.04	1.16 ± 0.06
	PER	4.01 ± 0.12	4.07 ± 0.17	4.06 ± 0.19	4.24 ± 0.14	4.35 ± 0.17	4.42 ± 0.20
1-2	BW (g)	315.33±6.47	306.49± 7.88	313.69 ±7.29	311.43 ±6.56	296.82±6.97	296.50± 8.21
	BWG (g)	187.08 ± 6.83	180.4 ± 11.05	185.31 ± 8.75	181.43 ± 5.35	171.7 ± 6.01	173.78±4.10
	FCR	1.25^{b} ± 0.08	1.27^{b} ± 0.08	1.29^{b} ± 0.09	1.26^{b}± 0.05	1.38^{a} ±0.11	1.39^{a} ± 0.1
	PER	3.72 ± 0.13	3.75 ± 0.20	3.78 ± 0.21	3.87 ± 0.17	3.72 ± 0.20	3.69 ± 0.13
2-3	BW (g)	540.90^{a}±10.72	528.3^{a} ±11.4	538.5^{a} ±10.54	531.61^{a}±16.32	498.4^{b} ± 10.8	501.7^{b}± 11.5
	BWG (g)	225.6^{a} ± 16.7	221.8^{a} ± 8.73	224.84^{a} ± 9.4	220.2^{a} ± 17.7	202.03^{b} ± 7.5	205.2^{b}±10.0
	FCR	1.46^{b} ± 0.19	1.48^{b} ± 0.13	1.47^{b} ± 0.09	1.44^{b} ±0.08	1.59^{a} ±0.22	1.58^{a} ± 0.14
	PER	3.52 ± 0.11	3.56 ± 0.14	3.68 ± 0.13	3.75 ± 0.16	3.59 ± 0.19	4.14 ± 0.20
3-4	BW (g)	881.78^{a}±16.64	872^{a} ± 22.14	873.82^{a} ±26.4	874.14^{a}±22.19	813.19^{b} ±19.1	814.3^{b}± 14.3
	BWG (g)	340.88^{a} ± 22.4	343.71^{a} ±16.9	335.3^{ab}± 16.8	342.53^{a}±31.84	314.84^{b} ± 20.6	312.63^{b}±15.66
	FCR	1.58^{b} ± 0.3	1.54^{b} ± 0.4	1.58^{b} ± 0.13	1.59^{b} ± 0.4	1.76^{a} ± 0.2	1.78^{a} ± 0.35
	PER	3.16 ± 0.20	3.61 ± 0.16	3.62 ± 0.0.18	3.59 ± 0.20	3.44 ± 0.21	3.40 ± 0.17
4-5	BW (g)	1367.95^{a}±36.9	1355.29^{a}±31.8	1346.39^{a}±36.6	1350.48^{a}±36.65	1215.45^{b}±42.2	1230.7^{b}±31.72
	BWG (g)	486.2^{a} ± 31.46	483.29^{a} ±40.1	472.57^{a} ± 18.9	476.34^{a}±19.07	402.26^{b} ± 32.6	416.37^{b}±29.93
	FCR	1.71^{b}± 0.28	1.74^{b} ± 0.29	1.73^{b}± 0.24	1.75^{b} ±0.33	1.91^{a}± 0.39	1.90^{a} ± 0.15
	PER	3.16 ± 0.21	3.19 ± 0.18	3.3 ± 0.16	3.27 ± 0.20	3.43 ± 0.17	3.19 ± 0.13
5-6	BW (g)	1897.56^{a}±38	1899^{a} ±46.20	1880.2^{a} ±48.9	1872.6^{a}±53.16	1644.33^{b}±49.2	1667.4^{b} ±42.4
	BWG (g)	529.61^{a} ± 50.7	543.7^{a} ±26.67	533.83^{a} ± 22.2	522.07^{a}±50.62	428.88^{b} ± 38.7	436.7^{b}±37.34
	FCR	2.05^{b} ± 0.66	2.11^{b} ± 0.52	2.09^{b} ± 0.31	2.10^{b}± 0.44	2.36^{a}±0.312	2.38^{a} ± 0.57
	PER	2.64 ± 0.15	2.68 ± 0.17	2.73 ±0.20	2.72 ± 0.17	2.57 ± 0.17	2.55 ± 0.20

abc means in the same row followed by different superscripts are significantly different ($P<0.05$).
BW= body weight; BWG= body weight gain; FCR= feed conversion ratio; PER= protein efficiency ratio.

1- Control diets containing 21.5, 19.5 & 18.5% CP & 3035, 3108 & 3180 Kcal/kg ME as recommended to cover minimum nutrient specifications.
2- Ideal protein diet formulated to cover specified levels of digestible essential amino acids.
3- Threonine supplementation to 120 and 140% of the recommended minimum specifications.

Table (5): Effects of dietary protein level and threonine supplementation on carcass quality

	Experimental diets					
	Control[1]	Ideal [2]	Low protein diets			
			(-1%)		(-2%)	
			Threonine supplementation[3]			
items			20	40	20	40
Live body weight,g	1890 ±73.25	1895 ± 65.52	1874 ± 91.12	1863 ± 85.06	1640 ± 78.95	1658 ± 64.19
Eviscerated carcass%	66.08 ± 1.54	66.02 ± 0.5	65.12 ± 1.23	67.09 ± 1.02	65.56 ± 1.53	66.80 ± 3.45
Bursa %	$0.16^{b} \pm 0.02$	$0.17^{ab} \pm 0.01$	$0.15^{b} \pm 0.01$	$0.16^{ab} \pm 0.01$	$0.19^{a} \pm 0.02$	$0.15^{b} \pm 0.01$
Liver %	$2.70^{ab} \pm 0.1$	$2.64^{ab} \pm 0.08$	$2.6^{b} \pm 0.12$	$2.6^{b} \pm 0.07$	$2.7^{ab} \pm 0.18$	$2.98^{a} \pm 0.18$
Spleen %	$0.15^{ab} \pm 0.01$	$0.17^{a} \pm 0.01$	$0.13^{ab} \pm 0.01$	$0.16^{ab} \pm 0.01$	$0.12^{b} \pm 0.01$	$0.15^{ab} \pm 0.01$
Abdominal fat %	$2.27^{a} \pm 0.19$	$2.38^{a} \pm 0.16$	$1.89^{a} \pm 0.17$	$2.04^{a} \pm 0.21$	$2.29^{a} \pm 0.2$	$2.26^{a} \pm 0.23$
Heart %	$0.61^{a} \pm 0.04$	$0.52^{ab} \pm 0.03$	$0.58^{a} \pm 0.03$	$0.46^{b} \pm 0.02$	$0.54^{ab} \pm 0.03$	$0.55^{ab} \pm 0.04$
Gizzard %	$1.33^{a} \pm 0.07$	$1.37^{a} \pm 0.07$	$1.42^{a} \pm 0.07$	$1.37^{a} \pm 0.05$	$1.34^{a} \pm 0.06$	$1.46^{a} \pm 0.12$

^{abc} means in the same row followed by different superscripts are significantly different (P<0.05).

Table (6): Effects of dietary protein level and threonine supplementation on serum metabolites and HI titer (Geometric mean titer) of broiler chickens

	Experimental diets					
	Control[1]	Ideal [2]	Low protein diets			
			(-1%)		(-2%)	
			Threonine supplementation[3]			
			20	40	20	40
Items **Serum parameters**						
Total protein(g/dl)	$5.46^{b} \pm 0.27$	$5.95^{b} \pm 0.58$	$5.48^{b} \pm 0.27$	$6.93^{a} \pm 0.19$	$2.3^{c} \pm 0.24$	$2.5^{c} \pm 0.22$
Albumin (g/dl)	$1.47^{b} \pm 0.13$	$1.44^{b} \pm 0.08$	$1.29^{bc} \pm 0.13$	$1.99^{a} \pm 0.19$	$1.15^{bc} \pm 0.07$	$1.03^{c} \pm 0.06$
Globulin(g/dl)	$3.99^{b} \pm 0.32$	$4.51^{ab} \pm 0.6$	$4.19^{a} \pm 0.28$	$4.94^{a} \pm 0.29$	$1.24^{c} \pm 0.22$	$1.47^{c} \pm 0.22$
A/G ratio	$0.37^{c} \pm 0.40$	$0.32^{c} \pm 0.12$	$0.31^{c} \pm 0.05$	$0.44^{c} \pm 0.07$	$0.93^{a} \pm 0.23$	$0.70^{b} \pm 0.33$
Uric acid(mg/dl)	$7.60^{a} \pm 0.34$	$3.62^{c} \pm 0.24$	$3.73^{c} \pm 0.24$	$3.26^{c} \pm 0.33$	$8.12^{a} \pm 0.51$	$6.41^{b} \pm 0.46$
Creatinine(mg/dl)	0.61 ±0.02	0.65 ± 0.02	0.64 ±0.03	0.56 ±0.03	0.57 ±0.02	0.58 ± 0.02
HI titer (log 10)						
At 14 days	$0.69^{a} \pm 0.03$	$0.67^{a} \pm 0.015$	$0.66^{a} \pm 0.015$	$0.64^{a} \pm 0.021$	$0.64^{a} \pm 0.04$	$0.66^{a} \pm 0.02$
At 21 days	$0.73^{a} \pm 0.02$	$0.81^{a} \pm 0.01$	$0.81^{a} \pm 0.01$	$0.80^{a} \pm 0.02$	$0.8^{a} \pm 0.02$	$0.82^{a} \pm 0.02$
At 28 days	$0.90^{ab} \pm 0.01$	$0.94^{a} \pm 0.01$	$0.81^{c} \pm 0.02$	$0.89^{b} \pm 0.01$	$0.78^{c} \pm 0.01$	$0.77^{c} \pm 0.03$
At 35 days	$0.40^{b} \pm 0.03$	$0.48^{a} \pm 0.04$	$0.35^{bc} \pm 0.02$	$0.32^{bc} \pm 0.02$	$0.32^{bc} \pm 0.02$	$0.30^{c} \pm 0.00$

^{abc} means in the same row followed by different superscripts are significantly different (P<0.05).

RESULTS AND DISCUSSION

Body weight, body weight gain, feed intake, feed conversion ratio, protein efficiency ratio and carcass quality:

The data concerned with body weight development (BW), body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR) and protein efficiency ratio (PER) showed that from the 3rd week to the end of experiment (at 6 weeks), the BW of different groups were mostly similar except that fed 2 % LCP- diets with 20 & 40 % threonine supplementation above the recommended which have the lower body weight means (Table 4). Also, the evaluation of the data concerned with absolute weight gain of broiler chicken groups at the end of the experiment showed that supplementation of Thr at 120 or 140% of recommended levels to the 1% LCP- diets, achieved the same optimal BW development that recorded for the groups of chickens fed the control or ideal protein diets. Similar results obtained by **Abbasi et al. (2014)** revealed that, the best FCR and BWG values were obtained in broilers fed diets containing 95% CP of requirements and supplemented with 120% of Thr requirements and assumed that the availability of free crystalline amino acids are higher than that of the amino acids in intact protein, and Thr supplementation enhanced the growth performance by improving intestinal health as utilizing Thr levels at least 110 % of the recommended level caused increase villus height to crypt depth ratio and that indicated slower tissue turnover and lower demands to compensate for villus atrophy so lower energy will be required to support slower tissue turnover. Also, **Jafari et al. (2013)** obtained the best performance (the highest gain) at levels of Thr supplementation (at 110 and 115 %) by improving nitrogen retention. **Mazraeh et al. (2013)** also found that weight gains and FCR significantly improved in broilers fed high threonine diets compared to control- feeding diets. On the other hand, While reducing the dietary CP by 2% (from 21.5, 19.5 & 18.5%) to (19.5, 17.5 & 16.5%) for starter, grower & finisher respectively with Thr supplementation did not improve growth performance data near to that of the control groups. **Dozier et al. (2002) and Azzam and El- Gogary (2015)** found that increasing the levels of L-Thr in the diet did not affect body weight gain and this in agreement with those of **Penz et al. (1991)** who recommended 0.68 % Thr requirement for optimal feed efficiency at age from 21-42 days.

Using of ideal protein concept in diets of broiler chickens that formulated depending on digestible amino acids also had no adverse effect on weight gain and the results obtained from broilers fed the ideal protein diets were nearly similar to that of the control diet (Table 4). **Kim et al. (2014)** found that utilizing the ideal protein diet concept would be better than the NRC total amino acids (AAs) requirements for supplementing synthetic AAs in low protein diets as it might reduce N input because it made a better amino acid balance and eliminated the excess of AAs and also resulted in obtaining the exact levels of AAs needed by the broilers. With the same concept, **Ghorbanali et al. (2015)** concluded that formulation of diets based on digestible lysine was better than that based on total lysine when diets containing protein sources of low amino-acid digestibility as meat and bone meal.

Decreasing dietary CP by 2% of the recommended levels with Thr supplementation (120 and 140%) resulted in a decrease in weight gain of broiler chickens (Table 4). Similarly **Maloma et al. (2013)** found that decreasing protein level up to 7% of **NRC (1994)** recommended values (23.5%) nearly

1.6% resulted in reduced weight gain due to the low feed intake which resulted in inadequate consumption of essential AAs and under supply of other EAA may result in increase FCR, reduced breast muscle and decrease carcass quality in broilers fed according to **NRC (1994)** requirements.

Feed consumption was not significantly affected during the first 2 weeks. While at the 3rd week FI slightly increased with decreasing dietary CP by 1% with Thr supplementation (120%), but with decreasing DCP to 2% even with Thr supplementation (140%), feed consumption decreased and subsequently the weight gain decreased as shown in Table (4). So the cumulative feed consumption was decreased with decreasing the protein either 1 or 2% of the recommended levels. From these results, it can be concluded that threonine supplementation did not affect feed intake. Similar results were observed by **Azzam and El-Gogary (2015)**. Also, increasing L-Thr level in broiler diets did not affect feed intake up to 42 days of age (**Khan et al., 2006**). On the other hand, **Jafari et al. (2013)** showed that the highest feed intake was achieved in the diets containing Thr at 110 and 115% higher than the required level, and addition of Thr increased growth and breast muscle weight gain and that might lead to consumption of more feed by broiler chickens in order to achieve rapid growth. Furthermore, **Liu et al. (2015)** suggested that feed intake of broiler chickens was affected by maintaining EAAs requirements (AA balance) rather than CP levels of diets. However, **Yamazaki et al. (2006) and Namroud et al. (2008)** found that decreasing dietary CP below a minimum level with maintained EAA levels resulted in decreased feed intake.

Feeding LCP- diets (-2%) with Thr supplementation (120 or 140%) resulted in increase FCR as a result of increased feed intake and decrease weight gain through the period from 1 to 6 weeks of age (Table 4). This result was supported by **Namroud et al. (2008)** who found that decreasing CP below a minimum level (19 %) with maintained EAA resulted in increased FCR. Thr supplementation as shown in table 4 did not affect FCR. Also **Azzam and El-Gogary (2015)** reported that threonine supplementation to broilers diets had no effect on FCR.

The values of protein efficiency ratio (PER) decreased with age in the different experimental groups (Table 4). The results showed that with decreasing dietary CP the PER increased in the treatments fed LCP- diets (-2%) even with either 120 or 140 % Thr supplementation and this was expected due to decreased CP intake resulted in increased protein utilization, although the PER was increased at both groups, the weight gain still lower than that of the broilers fed the control diets and from this finding it could be concluded that we can't decrease dietary CP by 2% even with Thr supplementation at 120 or 140 % of the required. With this concept **Kidd et al. (1996)** demonstrated that low CP diets improved PER by minimizing excesses of indispensable amino acids. Also, **Aletor et al. (2001)** found that male broiler chicks from 3 to 6 weeks of age fed 18% CP-diets supplemented with EAA significantly improved protein retention and PER. In addition, **Awad et al. (2014)** showed significant increase in PER which support the suggestion that formulating diets in ideal protein and digestible amino acids pattern would lead to the best efficiency of protein utilization.

Data of carcass traits of broiler chickens fed diets containing different protein and threonine levels revealed that decreasing dietary protein by 2% with Thr supplementation above requirements, significantly decreased carcass weight percentages with no observable effect on abdominal fat and gizzard weight percentages. While liver weight increased with decreasing CP. However, spleen weight slightly affected by the different treatments but the highest weight was reported in broiler chickens fed the ideal protein diets (Table 5). Similarly, **Djouvinov et al. (2005)** showed that formulating diets for broilers based upon digestible AA lead to reduced feed cost per /kg of yield without negative effect on performance traits as the yield of carcass, breast fillet, thighs, liver, gizzard and heart were similar to those of the control group. While, **Deschepper and Degroote (1995)** did not find differences in carcass yield using AA balance but low CP diets resulted in a higher carcass fat content. In the present study, abdominal fat% slightly increased in chickens fed ideal protein diet. With similar results, **Ghorbanali et al. (2015)** found that formulation of diets based on digestible AA was better than diets based on total AA as the proportion of breast muscle of total live weight and weight of breast muscle increased in broilers fed diets formulated on the basis of digestible compared to total lysine. From the present study it could be concluded that Thr supplementation to low dietary CP (-2%) has no effect on improving carcass weight %. Similar results were reported by **Bregendahl et al. (2002)**.

Liver weight% was the highest and significantly affected by increasing threonine to 140% of the required with decreasing CP by 2% (Table 5). Also **Shirzadegan et al. (2015)** found that the highest liver and pancreas weights observed in broilers fed diet supplemented by 0.5% L-Thr, while the highest gizzard weight% was achieved by 1.0% L-Thr. It was explained by **Swennen et al. (2006)** that lowering dietary CP promotes de novo lipogenesis in liver so resulted in increase in liver weight. In addition, **Namroud et al. (2008)** explained increased liver weight % due to decreased CP to increase ammonia level resulted in activation of ammonia conversion to uric acid that mainly took place in liver so the increase in its weight is to adapt the elevated ammonia production and the low body weight is due to the increase in enzymes activity involved in uric acid synthesis. **Corzo et al. (2007)** reported that low Thr-supplemented diets, decreased thymus weight and **Jahanian and Rasouli (2012)** suggested that Thr might directly influence the process of antibody production or modifying virus replication indirectly. In addition, **Abbasi et al. (2014)** showed that reduction of CP content decreased relative pancreas weight due to synthesis and secretion of exocrine pancreatic enzymes are up-regulated by feed intake in intestine. While, supplementation of Thr to lower CP diet had no effect on weights of liver, gizzard and pancreas (**Azzam and El-Gogary, 2015**).

Serum metabolites and HI titer

Data in Table 6 revealed that feeding broiler chickens Thr supplemented LCP diets (-2%) resulted markedly in low levels of serum total protein, albumin and globulin compared to the chickens fed the control diets. The direct relationship between dietary CP and serum total protein has been documented. **Corzo et al. (2005)** reported that reducing dietary CP caused a reduction in serum albumin and this may be due to decrease of amino acids intake. However, feeding the LCP (-1%)- Thr supplemented diets as well as the ideal protein diets did not decrease serum levels of albumin or total protein (Table 6). In this respect **Liu et al. (2015)** demonstrated that serum total protein concentrations were increased with increasing the levels of DCP. Similar results were reported (**Orma et al., 2010, Teteh et al., 2010, Hernandez et al., 2012 and Awad et al., 2014**). With concept of our results **Jahanian and Rasouli (2012)** reported that serum total protein was increased with increasing dietary Thr up to 110% of **NRC (1994)** requirements. In addition, **Tenehouse and Deutsch (1996)** reported that Thr is a major component of plasma gamma globulins in poultry, rabbits and human. Also, **Wang et al. (2006)** reported that serum specific IgG levels increased in response to increasing intake of true ileal digestible threonine. Thr supplementation of the low 2%-CP diets significantly increased serum levels of uric acid (Table 6). However, serum levels of uric acid decreased in the broiler chickens fed the ideal protein diets as well as those fed the LCP- diets (-1%) with Thr supplementation. Blood uric acid is the main criterion for assessing the bioavailability of protein sources for broilers and dietary protein with good biological value resulted in reduction of serum level of uric acid (**Hevia and Clifford, 1977**).

Effects of feeding LCP- diets with Thr supplementation on HI titer against NDV are presented in Table 6. The data showed that the proper time of vaccination was at the 14^{th} day of age. At the 7^{th} day post vaccination (21 day of age) there is no significant differences were detected in the HI titer values between the chick groups fed the different diets. By 28 and 35 days of age, the HI titer value was low in the broiler chickens fed the LCP-diet (-2%), with Thr supplementation. The highest HI titer was reported for the chickens fed the ideal protein diet (formulated to cover the digestible EAAs). The present results are in agreement with that of **Carlomagno et al. (1980)** who found that LCP diets inhibited antibodies production and the development of antibody production cells in response to T-dependent antigens. Also, **Takahashi et al. (1994)** found that dietary Thr levels had no effect on the antibody titers to sheep red blood cells in male broilers. In addition, **Kidd et al. (1997)** observed that the cellular and humoral immunity in broilers fed Thr supplemented diets did not improve immunocompetence. Also **Abbasi et al. (2014)** observed that diets with different CP and Thr levels had no effect on Ab titers against NDV. **Jahnian and Rasouli (2012) & Moghaddam and Emadi (2014)** reported similar results.

From the nutritional aspect, amino acids are needed for clonal proliferation of lymphocytes, establishment of germinative centers in the bursa of fabricius to refine immunoglobulin affinity, recruitment of new bone marrow monocytes and heterocytes and synthesis of immunoglobulin and cytokines (**Maroufyan et al. (2010)**). So the results obtained in opposite to our results that Thr modulates immune function by increasing serum IgG levels and jejunal mucosal concentrations of IgA and IgG and also Thr supplementation enhanced primary immune responses and might preserve the memory immune responses for a long time (**Wang et al., 2006, Liu et al., 2007 and Abbasi et al., 2014**). The role of Thr discussed by **Corzo et al. (2007)** that immune response was parallel to the reports of increased health problems in Thr-deficient diets as Thr deficient animals had been shown to be more susceptible to tumors and the thymus weight was decreased in the low Thr-diet because Thr may directly affect the process of Ab production or indirectly by modifying virus replication (**Jahanian and Rasouli (2012)**). As a result, **Porto et al. (2012)** reported that the requirements of Thr for performance were lower than its requirements for optimum immune response as Thr is an important amino acid for intestinal mucus immunity because glycoproteins present in the intestinal mucus are rich in this EAA so, **Babaee (2013)** reported that the wide use of synthetic Thr supplementation in poultry feed (corn-soybean diets) that contain less Thr than the required quantity as with increasing Thr up to 120% of the recommended, may improve the immune response against NDV. However, in the present study dietary Thr supplementation either at 120% or 140% of the required did not markedly improve antibody production against NDV at 3 weeks postvaccination in broiler chickens fed 1% less dietary protein through the 6 weeks experimental period (Table 6).

Generally, from the present study it could be concluded that reducing CP of the starter, grower and finisher broiler diets by 2% with Thr supplementation did not result in growth performance measurements near to that of the control diets. However, Thr supplementation at 120% of the required level to the 1% LCP- diets achieved similar growth performance measurements to those of the control or ideal protein diets. Results obtained from broilers fed ideal protein diets were similar to those of the broiler chickens fed the basal control (high protein) diets, crude protein percentages of ideal protein diets are less than the recommendation of the **NRC (1994)** for total protein and AAs.

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الملخص العربي

تأثير مستوي البروتين والثريونين في علائق بداري التسمين علي معدلات النمو والكفاءة المناعية

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اجريت التجربة علي ٢٦٤ كتكوت عمر يوم من سلالة كب تم تقسيمهم الي ٦ مجموعات وقسمت كل مجموعة الي اربعة تحت مجاميع تكرارية (كل مكررة بها ١١ كتكوت) غذيت الكتاكيت علي علائق ضابطة أساسية تحتوي علي ٢١,٥- ١٩,٥- ١٨,٥ %بروتين و ٣٠٣٥- ٣١٠٨- ٣١٨٠ كيلو كالوري من طاقة التمثيل الغذائي. اما المجموعة الثانية فقد غذيت علي علائق تم حسابها لتوفر المقررات الغذائية لكل الاحماض الامينية الاساسية الضرورية (Ideal protein diets) وكانت متماثلة في الطاقة مع العلائق الضابطة. في علائق المجموعتين ٣&٤ خفضت نسبة البروتين (١%) الي ٢٠,٥- ١٨,٥- ١٧,٥ % مع إضافة الثريونين بنسبة ١٢٠ ، ١٤٠% من المقرر في العلائق الضابطة. أما علائق المجموعتين ٥ & ٦ خفضت نسبة البروتين (٢%) الي ١٩,٥- ١٧,٥ – ١٦,٥ % مع إضافة الثريونين بنسبة ١٢٠ ، ١٤٠% من المقرر في العلائق الضابطة.

-تم تحصين كل الكتاكيت ضد مرض النيوكاسل عند اليوم ١٤، وتم وزن الكتاكيت عند نهاية كل اسبوع من العمر لمتابعة النمو وتم تسجيل العلف المستهلك اسبوعيا ، وكذلك تم حساب معدل التحويل الغذائي اسبوعيا و معدل كفاءة استخدام البروتين. كما أخذت عينات دم من ١٥ كتكوت عند اليوم ١٤،٢١،٢٨،٣٥ وتم فصل مصل الدم لقياس مستويات الاجسام المناعية لمصل النيوكاسل وعند نهاية التجربة جمعت عينات من الدم لقياس بعض مكونات مصل الدم (البروتينات الكلية-الجلوبيولين-الالبيومين-حمض اليوريك-الكرياتينين) .وعند نهاية التجربة (٦ اسابيع) تم أخذ ١٢ دجاجة من كل مجموعة وذبحها وقياس الوزن قبل وبعد الذبح وبعد ازالة الاحشاء الداخلية تم قياس وزن دهن البطن الكلي، وزن المعدة والقلب والكبد والطحال كنسبة من وزن الجسم الحي ، وقد خلصت نتائج هذه الدراسة الي: انه من الممكن خفض نسبة البروتين ١% من المستوي الموصي به مع الاضافة الي ١٢٠ % من الثريونين من المستوي الموصي به أو استخدام البروتين المثالي في علائق بداري التسمين دون اثار سلبية علي معدلات النمو ، مكونات مصل الدم والكفاءة المناعية .