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EFFECT OF FEEDING BROILERS ON LOW PROTEIN DIETS BY WITHDRAWAL PROTEIN CONCENTRATES AND FORTIFIED WITH DIFFERENT ESSENTIAL AMINO ACIDS, CHOLINE AND CARNITINE ON BLOOD BIOCHEMICAL PARAMETERS AND INTESTINAL MORPHOLOGY

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Abstract

The aim of current study was to conducted in the poultry farm of the Department of Animal Production in the College of Agricultural Engineering Sciences - University of Baghdad (Abu Ghraib site) for 42 days from 27/9/2019 to 11/8/2019 to study the effect of low-protein level and replacement of protein concentrates by methionine, threonine, lysine, arginine, choline and carnitine in broiler diets on blood parameters and Intestinal morphology. Amino acid mixture Consists of methionine, lysine, threonine, arginine, choline and carnitine (17, 16.6, 16.6, 16.6, 16.6 and 16.6 %) respectively. Then added to the broiler diets by ratios as follows: T1, is a control treatment containing the Protein concentrate at 5% (starter period, growth period and finisher period), T2 a second control treatment containing a 5% Protein concentrate and low protein 2% in (starter period, growth period and finisher period), T3 withdrew the Protein concentrate and low protein 2% level in all stages (starter period, growth period and finisher period) and the mixture is added 1%, T4 withdraw the Protein concentrate and low protein 2% in all stages (starter period, growth period and finisher period) and add the mixture 1.5%, T5 withdraw the Protein concentrate and low protein 2% in all stages (starter period, growth period and finisher period) and the mixture is added 2%. The result of blood parameters showed decrease Significant on triglycerides for T4 compared with T1 but no significant differences T3, T4 and T5 compared with controls treatments T1 and T2 in total protein and uric acid. Intestinal Morphology result showed was a significant difference in thickness of the mucous layer for T3 and T5 compared with T2 and no significant crypt depth between all treatments, but showed significant difference for T2 compared with T4 in villus length.

Key words: low protein diets, essential amino acids, choline, carnitine

Introduction

The accuracy in meeting broiler nutritional protein requirement, as well as the search for an ideal profile of amino acids (AA) to obtain the maximum protein utilization of protein has guided the research in this area. New formulation approaches started to be applied when measuring AA in feeds became feasible, allowing the transition from using crude protein values to the use of total AA. Subsequently, feed formulations for poultry recommended of digestible (dig) AA values, based on the ideal protein concept; this was aided by the development and availability of synthetic AA. On the other hand, the genetic progress of broilers, including gender and strain, can influence the ideal AA ratio needed; any alteration in the proportion of body protein, feathers and maintenance requirements may result in changes in the ideal AA ratios (Hackenhaar and Lemme, 2005). Feeding high amino acid density diets improves feed conversion and increases weight gain and breast meat yield of broiler chickens (Kidd *et al.,* 2004).

Methionine has a vital role in the metabolic functioning which is why it is also known as functional amino acid (Bunchasak, 2009). It plays a vital role in the production of energy through the synthesis of protein, Also, a methyl group that is provided by sulfur-adenosyl methionine is required for many metabolic reactions such as epinephrine, carnitine, choline and creatine synthesis (Binder, 2003; Elnesr. 2019) L-Met is directly used by

Table 1: Composition and nutrient content of the starter diet (day 0–10).

Ingredients	Treatments				
	T1	T2	T3	T4	T5
Maize	47.7	48.8	43	42.9	45
Wheat	10	15.2	17.8	17	13.4
Soybean 44 %	33	27.3	31.9	32.1	32.5
Protein concentrate 1	5	5	-	-	-
Oil	2	1.4	2.6	2.8	3
Limestone	1.1	1.1	1.2	1.2	1.2
DCP 2	0.7	0.7	2	2	2
Vitamins and minerals	0.2	0.2	0.2	0.2	0.2
Salt	0.3	0.3	0.3	0.3	0.3
Amino acids 3	-	-	1	1.5	2
Total	100	100	100	100	100
CP%	23	21	21	21	21
M. Energy (kcal/kg)	3000.5	3006.51	3008.22	3002.79	3001.88
Methionine	0.51	0.48	0.32	0.32	0.32
meth+Cys	0.86	0.82	0.66	0.66	0.66
Lysine	1.32	1.17	1.11	1.11	1.12
Threonine	0.87	0.76	0.78	0.77	0.78
Calcium	0.92	0.91	0.99	0.99	0.99
Available phosphorus	0.47	0.46	0,46	0,46	0.47

T1, was first control treatment containing the Protein concentrate at 5% in all periods, T2 a second control treatment containing the Protein concentrate at 5% and low protein 2% in all periods, T3 withdrew the Protein concentrate and low protein 2% level in all periods and added the mixture 1%, T4 withdraw the Protein concentrate and low protein 2% in all periods and add the mixture 1.5%, T5 withdraw the Protein concentrate and low protein 2% in all periods and added the mixture 2%. 1-BROCON - 5 SPECIAL W Protein concentrate added in broiler diets each Kg contains: 40% crude protein, 5% fat, 2.26% fiber, 5% calcium, 4.68% phosphorus, 3.85% lysine, 3.7% methionine, 4.12% methionine + cysteine, 2.4% sodium, 2107 kilograms of energy represented / Kg . 2- DCP : Di-calcium phosphite 3- amino acids : methionine 33.4%, lysine 33.3% and threonine 33.3%. broiler diets chemical analysis according to NRC 1994.

the animal as a precursor for protein synthesis and metabolized through the trans-sulfuration pathway to produce cysteine and glutathione (Fang *et al.,* 2010).

Threonine is amino acid that has functions of the thyroid gland, protect the intestinal mucosa and improves the absorption of nutrients, it was addition to broiler diets to improve body weight (Sturkie, 1986; Schutte and Pack and 1995; Leclercq *et al.,* 1998; Si *et al.,* 2001). Low protein level and adding amino acids reduces energy for digestion and improves absorption, which improves broiler performance also reduces the cost of feed and improves environmental conditions, The aim study to find the effect of low protein diets and replace protein concentrates by amino acid mixture on broiler diets in broiler performance.

Arginine plays roles in different metabolic, pathophysiological and immunological in poultry (Cline *et al.,* 2016) to the lack of a functional urea cycle (Takenaka *et al.,* 2000). Broilers are not able to synthesize endogenous L-arginine and therefore it is considered as an essential amino acid. Indeed, chickens exclusively rely on the dietary sources of arginine and hence a proper amount of it should be provided with the diet's. L-arginine can be converted into citrulline and nitric oxide by the enzyme nitric oxide synthase (Cline *et al.,* 2016). Nitric oxide has shown marked vasodilator properties and it could enhance blood flow to the breast muscle alleviating the hypoxic condition usually observed in breasts affected by severe woody breast (WB) or white striping (WS) myopathies (WU, 2014; Mutryn *et al.,* 20015).

Choline, is a water soluble colorless compound with vitamin-like properties as not a metabolic catalyst but forms an essential structural component of body tissues (McDonald *et al.,* 2011). Choline is ubiquitously distributed in all plant and animal cells, mostly in the form of the phospholipids, phosphatidylcholine (lecithin), lysophosphatidyl- choline, cholineplasmalogens and sphingomyelin - essential components of all membranes (Zeisel, 1990) Choline degrades in hot alkali creating trimethylamine. Choline has ability to form salts with many organic and inorganic acids. It is well soluble in water and ethanol, but not in ether. Choline is chemically a strong alkali and hygroscopic nature. Cholines is amino ethyl alcohol and have three methyl groups on the nitrogen atom, chemically termed as (2- Hydroxyethyl) trimethylammonium. Chemical formula of choline is C_5H_14NO+ and of choline chloride is ($HOCH_2CH_2N(CH_3)3HCl$) (Chan, 1991).

L-carnitine is a vital micronutrient required for lipid metabolism and energy production for poultry. Furthermore, dietary L-carnitine supplementation may have a beneficial effect on broiler nutrition status, mainly due to its sparing effect on its precursor's lysine and methionine. Providing sufficient amount of L-carnitine to broilers resulted in more efficient utilization of dietary energy and protein (Ossininezhad *et al.,* 2011). L-carnitine acts by reducing the availability of lipids for per-oxidation through transportation of fatty acids into the mitochondria for β-oxidation to produce ATP energy (Ossininezhad *et al.,* 2010).

Materials and Methods

Bird management: A total of 225 one-day old unsexed chicks (Ross 308) with initial body weight in

Table 2: Composition and nutrient content of the grower diet (day 11–24).

Ingredients	Treatments				
	T1	T2	T3	T4	T5
Maize	60.3	65.1	44.8	44.5	45.6
wheat	-	1.4	19.3	18.6	16.5
Soybean	30	24.6	28.1	28.3	28.6
Protein concentrate 1	5	5	-	-	-
oil	2.7	1.9	3.5	3.8	4
Limestone	1.1	1.1	1	1	1
DCP 2	0.5	0.5	1.9	1.9	1.9
Vit	0.2	0.2	0.2	0.2	0.2
Salt	0.2	0.2	0.2	0.2	0.2
Amino acids 3	-	-	1	1.5	2
Total	100	100	100	100	100
CP%	21.52	19.5	19.51	19.51	19.5
Energy	3100.04	3101.12	3103.6	3103.59	3100.24
Methionine	0.49	0.47	0.29	0.30	0.30
Lysine	1.24	1.09	1.01	1.01	1.02
Threonine	0.82	0.74	0.72	0.72	0.72
calcium	0.87	0.86	0.89	0.89	0.89
available phosphorus	0.44	0.43	0.44	0.44	0.44

T1, was first control treatment containing the Protein concentrate at 5% in all periods, T2 a second control treatment containing the Protein concentrate at 5% and low protein 2% in all periods, T3 withdrew the Protein concentrate and low protein 2% level in all periods and added the mixture 1%, T4 withdraw the Protein concentrate and low protein 2% in all periods and add the mixture 1.5%, T5 withdraw the Protein concentrate and low protein 2% in all periods and added the mixture 2%. 1-BROCON - 5 SPECIAL W Protein concentrate added in broiler diets each Kg contains: 40% crude protein, 5% fat, 2.26% fiber, 5% calcium, 4.68% phosphorus, 3.85% lysine, 3.7% methionine, 4.12% methionine + cysteine, 2.4% sodium, 2107 kilograms of energy represented / Kg . 2- DCP : Di-calcium phosphite 3- amino acids : methionine 33.4%, lysine 33.3% and threonine 33.3%. . broiler diets chemical analysis according to NRC 1994.

range from 40 to 42g. The chicks were randomly distributed to five treatments with three replicates per treatment by 15 chicks in each replicated. Broilers housed on litter floor system, floor pens measuring 1.9×2.0 m, all chickens received ad libitum access to feed and water throughout the experimental period. The temperature was set at 33°C at first three days of age and then decreased by 1°C every 2 days until a permanent temperature of 24°C was reached. All birds were vaccinated for Newcastle disease virus on days 10, 20 and 30, for Gambaro disease on days 14 of age.

Diets and Diet Analysis: The experimental diets were formulated According NRC (1994) at three-phase feeding program ross nutrition specification 2019: A diet of starter period (0 – 10 day-old) was shown in table 1, the diet of grower period (11-24 day-old) was placed in table 2 and finisher period (25-42 day-old) was shown in table 3.

Intestine histological Measurements

Gut morphology examinations were carried out according to the method described by Iji *et al.,* (2001) with minor modifications. Ileal samples were immersed in formaldehyde before fixation in Bouin's solution and paraffin-embedded. Each sample was sectioned at a thickness of 7 mm, stained with alcian blue/ haematoxylin-eosin and examined by light microscopy. The slides were viewed on a Zeiss Axiophot microscope. Visual measurements of villus height, crypt depth, goblet cell number and epithelium thickness were made at 100 to 200 magnifications using imaging software (Image Pro Plus, Version 4.1.0.9, Media Cybernetics, Silver Spring, MD, USA).

Statistical Analysis

This experiment had a completely randomized (CRD) design, All data were statistically analyzed using Statistical Analysis System (SAS) software (SAS Institute, Cary, North Carolina, USA) (2001). Significant differences among the treatment group means were evaluated using Duncan's multiple range test in all cases, significance was set at $P < 0.05$.

Result and Discussion

Blood parameters

The data in table 5 showed no significant differences between T3, T4 and T5 compared with control treatments T1 and T2 in Total protein and Uric acid. In triglycerides was a significant difference (<0.05 P) for the treatment T4 and T5 compared with T1 and was no significant difference between other treatments.

Methionine reduce level of fats in body through the events of changes in the composition of fats and analysis of fats in addition to its main role in the metabolism of fats in the liver and the transfer to body tissues, or that the taurine that is manufactured by sulfur amino acids has a role after operations The translation of proteins performs the regulatory modification of the effect on phosphorus or the proteins associated with phosphorus and the removal of phosphorus, which has a role in reducing energy (Takahashi and Akiba, 1995; Mochizuki *et al.,* 1998; Oda, 2006). Low protein diet reduce the effectiveness of the ACC gene, which reduces the level of fat in the body, since increasing the effectiveness of this gene leads to an increase in the formation of fatty substances in the body by increasing the rate of formation of fatty acids by stimulating this gene to malonyl-CoA

Table 3: Composition and nutrient of the finisher diet (25–42 d of age).

Ingredients	Treatments				
	T1	T2	T3	T4	T5
Maize	58.6	62.3	55.3	58.9	60.9
wheat	4.1	6.7	10.9	6	3
Soybean	26.2	20.7	25.1	25.7	26
Protein concentrate 1	5	5	-	-	-
oil	4.1	3.3	4.4	4.6	4.8
Limestone	1.2	1.2	1.1	1.1	1.1
DCP 2	0.4	0.4	1.8	1.8	1.8
Vit	0.2	0.2	0.2	0.2	0.2
Salt	0.2	0.2	0.2	0.2	0.2
Amino acids 3	-	-	1	1.5	2
Total	100	100	100	100	100
CP%	20.02	18	18	18	18
Energy	3204.65	3203.52	3201.07	3204.65	3200.15
Methionine	0.47	0.45	0.28	0.29	0.45
Lysine	1.13	0.99	0.92	0.93	0.94
Threonine	0.76	0.67	0.66	0.67	0.67
calcium	0.88	0.87	0.89	0.89	0.89
available phosphorus	0.41	040	0.42	0.42	0.42

T1, was first control treatment containing the Protein concentrate at 5% in all periods, T2 a second control treatment containing the Protein concentrate at 5% and low protein 2% in all periods, T3 withdrew the Protein concentrate and low protein 2% level in all periods and added the mixture 1%, T4 withdraw the Protein concentrate and low protein 2% in all periods and add the mixture 1.5%, T5 withdraw the Protein concentrate and low protein 2% in all periods and added the mixture 2%. 1-BROCON - 5 SPECIAL W Protein concentrate added in broiler diets each Kg contains: 40% crude protein, 5% fat, 2.26% fiber, 5% calcium, 4.68% phosphorus, 3.85% lysine, 3.7% methionine, 4.12% methionine + cysteine, 2.4% sodium, 2107 kilograms of energy represented/Kg. 2- DCP : Di-calcium phosphite 3- amino acids : methionine 33.4%, lysine 33.3% and threonine 33.3%. . broiler diets chemical analysis according to NRC 1994.

(Hillgartner *et al.,* 1996). Wu *et al.,* (2011) explained that adding arginine contributes to lowering the lipid level by reducing the activity of malate dehydrogenase, glucose-6-phosphate dehydrogenase and fatty acid synthetase in the liver. Choline has a role in metabolism of fats by entering lecithin, thereby reducing the level of triglycerides in the blood (Corbinand and Zeisel; 2013). Carnitine promotes the oxidation of long-chain fatty acids in addition to its role in transporting smooth medium fatty acids in the liver into mitochondria (Van Kempen and Odle, 1993; Zou *et al.,* 2008).

The result was agreement with (Sigolo *et al.,* 2017) when reducing the protein level in the broiler diet in total protein and uric acid level but not agree in triglycerides. The results agreement with (Agbede and Aletor, 2003) in Total protein, and the results did not agree with (Tufarelli and others, 2020) found when studying the effect of adding methionine, lysine, and carnitine in a synergistic manner at different levels to the broiler diet, to the broiler diet in uric acid, triglycerides, and total protein. The results were inconsistent with (Hosseintabar *et al.,* 2015) in uric acid and triglycerides when adding lemethionine, lysine and carnitine in a synergistic manner at different levels to the broiler diet and agree with the same researcher with total protein. The result agrees with (Rezaeipour and Gazani, 2014) by reducing the level of triglycerides when adding threonine and The results were not consistent with (Yang *et al.,* 2016) when adding arginine to the diets of laying hens in triglycerides.

Intestine histological

Table 5 showed significant differences for T2 compared with T4 in villi length. Also the result showed no significant differences for T3, T4 and T5 compared with control treatments T1 and T2 in crypt depth. In mucous thickness layer the result showed significant differences for T3 and T5 compared with control treatments T2.

The reason for the increase in the thickness of the mucous layer may be due to the glutathione, which synthesizes the amino acid methionine and threonine, which increases the thickness of the mucous layer and the muscle layer of the intestine (Kidd *et al.,* 1999; Dozier *et al.,* 2001; Hong *et al.,* 2018.). Perhaps the reason for the increase in the thickness of the mucous layer is due to the amino acid threonine, mucous is a glycoprotein substance that includes threonine, serine and proline synthesis, threonine constitutes (28 - 40%) of the composition of the mucous substance (Carlstedt *et al.,* 1993). Threonine plays a vital role in increasing the size and numbers of cells secreting mucosal goblet cells and that raising the level of threonine encourages mRNA to increase gene expression to MUC2, which leads to an increase in the thickness of this layer in the gut of poultry (Chen *et al.,* 2017).

The results not agreement with (Ojediran *et al.,* 2017) when reduce the protein level in the broiler diet in the length of villi and agreed in the depth of crypts for the same researcher. The results do not agree with what he reached (Samiru *et al.,* 2019) when add different sources of methionine on The length of villi, and the results not agree with (Samiru *et al.,* 2019) in the depth of crypts when adding lysine to the flesh of broilers. The results not agree with (Najafi *et al.,* 2017) when adding threonine to the diet in the length of villi but agreement in depth of

Table 4: Effect of feeding broilers on low protein diets by withdrawal protein concentrates and fortified with different essential amino acids, choline and carnitine on Blood biochemical parameters.

Treatments	Total protein	Uric acid	triglycerides
T1	4.05±0.05	87.00±8.00	87.00±8.00 A
T2	4.30±0.10	68.50±0.50	68.50±0.50 BC
T3	4.40±0.36	82.25±2.69	82.25±2.69 AB
T4	4.50±0.12	58.33±5.24	58.33±5.24 C
T5	4.83±0.46	67.33±6.33	67.33±6.33 BC
significant	N.S	N.S	0.05

T1, is a control treatment containing the Protein concentrate at 5% ,T2 a second control treatment containing a 5% Protein concentrate and low protein 2%, T3 withdrew the Protein concentrate and a 2% low protein level in all periods and the amino acids mixture is added 1%, T4 withdraw the Protein concentrate and low protein level 2% in all periods and add the amino acids mixture 1.5%, T5 withdraw the Protein concentrate and low protein level 2% in all periods and the amino acids mixture is added 2%.

Table 5: Effect of feeding broilers on low protein diets by withdrawal protein concentrates and fortified with different essential amino acids, choline and carnitine on intestinal morphology.

Treat-ments	Villi length	crypt depth	mocous thickness layer
T1	570.03±17.75 ab	80.66±2.01	108.45±3.98 a
T2	585.29±11.37 a	75.52±2.72	79.81±3.59 c
T3	540.37±11.00 ab	89.26±4.75	98.10±4.38 ab
T4	526.37±16.70 b	80.57±3.76	90.29±4.17 bc
T5	559.93±20.41 ab	81.09±3.48	94.19±3.52 b
significant	0.05	N.S	0.05

T1, is a control treatment containing the Protein concentrate at 5%, T2 a second control treatment containing a 5% Protein concentrate and low protein 2%, T3 withdrew the Protein concentrate and a 2% low protein level in all periods and the amino acids mixture is added 1%, T4 withdraw the Protein concentrate and low protein level 2% in all periods and add the amino acids mixture 1.5%, T5 withdraw the Protein concentrate and low protein level 2% in all periods and the amino acids mixture is added 2%.

crypts.

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