

Effect of adding L. Carnitine with herbal methionine and sunflower seed oil on the biochemical characteristics of broiler's blood

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Abstract

This study was conducted in the poultry field of the Animal Production Department, College of Agriculture, Kirkuk University from 30/9/2021 to 1/11/2021. The objective of the study was to evaluate the addition of L. carnitine with methionine and sunflower seeds to the broiler's diet and its effect on their productivity. 350 new hatched, unsexed broiler chicks Ross-308, with an average weight 42 g, they were brought from (Kirkuk hatchery) in Kirkuk province. Chicks were randomly distributed into to 8 treatments with 4 replicates for each treatment (10 chicks per replicate). The result of statistical analysis showed that the concentrations of cholesterol and triglyceride in blood serum had significantly decreased ($P \leq 0.05$) in the all treatments of feed additive compared with the control. T4 indicated a decrease in blood cholesterol compared to the other treatments. In addition, T7 and T8 showed a decrease in triglyceride compared to the other experiment treatments, while there were no differences between all treatments in blood glucose. The results showed that the fifth treatment was higher in (HDL) High-Density Lipoprotein, (LDL) Low-Density Lipoprotein, and (VLDL) very Low-Density Lipoprotein compared to other treatments.

دراسة تأثير إضافة L.carnitine مع الميثيونين العشبي وزيت بذور زهرة الشمس في بعض الصفات الكيموحيوية الدم لدجاج فروج اللحم

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الخلاصة

أجريت هذه الدراسة في حقل الدواجن التابع لقسم الإنتاج الحيواني – كلية الزراعة - جامعة كركوك للفترة من 2021/9/30 الى 2021/11/1, وكان الهدف منها معرفة تأثير دراسة مقارنة اضافة L.Carnitine والميثيونين العشبي في أبيض زيت بذور زهرة الشمس المضاف للعليقة في أداء بعض صفات الدم الكيموحيوية الدم لفروج اللحم, اذ تم توزيع وبشكل عشوائي 350 فرخ بعمر يوم واحد من فروج اللحم نوع Ross 308 غير مجنس بمعدل وزن ابتدائي 42 غرام, تم الحصول على الافراخ من (مفقس كركوك) في محافظة كركوك, ربيت هذه الافراخ تربية أرضية في قاعة مغلقة باستخدام 35 قفص ذو أبعاد (190 × 90سم) على فرشاة من نشارة الخشب, وكانت كل قاعة مجهزة بساحتان هواء, وزعت الافراخ عشوائياً على 8 معاملات بواقع 4 مكررات للمعاملة الواحدة (المكرر الواحد 10 افراخ) تم توزيع الافراخ عشوائياً من عمر واحد. بينت نتائج التحليل الإحصائي في الاسبوع السادس من العمر (1-42) يوم اما بالنسبة لصفات الدم الكيموحيوية لم يلاحظ فروق معنوية في تركيز البروتين الكلي لمصل الدم, وكذلك نلاحظ زيادة معاملة السيطرة (الاولى) معنوياً ($0.05 \geq P$) على جميع المعاملات في تركيز كوليسترول مصل الدم, وكذلك الحال وتركيز الكليسيريدات الثلاثية لمصل الدم فنلاحظ أيضاً انخفاضاً معنوياً ($0.05 \geq P$) للمعاملة الرابعة في نسبة كوليسترول في الدم مقارنة بباقي المعاملات وايضا انخفاض معنوي في نسبة الكليسيريدات الثلاثية للمعاملة السابعة والثامنة مقارنة بباقي معاملات التجربة, وتشير النتائج كذلك الى عدم وجود تفوق معنوي ($0.05 \geq P$) للمعاملات في مستوى كلوكوز مصل الدم. وكذلك وجد تفوق معنوي ($0.05 \geq P$) للمعاملة الخامسة في نسبة تركيز البروتينات الدهنية عالية الكثافة HDL و نسبة تركيز البروتينات الدهنية الواطئة الكثافة (LDL) وايضا تفوقت المعاملة الخامسة في نسبة تركيز البروتينات الدهنية الواطئة الكثافة (VLDL), ولوحظ عدم وجود فروق معنوية في مستوى انزيم (Aminotransferase Alanine) ALT و تركيز انزيم ((AST Aspartate aminotransferase و تركيز انزيم (Alkaline phosphatase (ALP).

الكلمات المفتاحية: L-carantine, الميثيون العشبي, فروج اللحم

Keywords: L. Carnitine, herbal methionine, broiler

Introduction

Broiler's nutrition is based on nutritional levels that fulfill their body requirements from energy, protein, essential amino acids, essential fatty acids, minerals, and vitamins, as well as feed additives (Souza et al, 2014). Feed additive contains active molecules that support growth and production quantitatively and qualitatively, also has resistant to harmful microorganisms which helps to increase the immunity of the body (Ibrahim, 2014). Researchers started to replace artificial sources of feed addition with plant sources. The amino acids are considered the basic building unit of protein (Abd Ali, 2016), also essential nutrients in poultry nutrition (Guoyao, 2013). Methionine is considered one of the most important amino acids that

plays important role in egg production and enhances its quality (Souza et al, 2014). In addition, Methionine is an essential amino acid for poultry since they cannot make its carbon body, or they make it in a little amount that is not enough for their body requirement (Kalbande, 2009). Furthermore, methionine is an important sulfur amino acid as well as the first determinant in poultry that depends on plant protein in their nutrition, also methionine is the most nutrient that plays a role in a number of important biological processes in the body (Shahzad, 2011). Thus, Also, methionine deficiency in the body has increased continually depending on a diet that contains corn and soybean (Saima, 2009). Therefore, the herbal source of these amino acids was used to replace and avoid the harmful side effects of using artificial sources. In the body, the extra amount of methionine and lysine convert in the liver into L. Carnitine (Hu et al, 2000; Arslan, 2006). L. Carnitine has an important metabolism role for energy via directing the triglycerides, cholesterol, and long-chain fatty acids into the powerhouse (mitochondria through the oxidation process and produce energy ATP; adenosine triphosphate). Based on that, the growth and production improve the quality of the carcass by decreasing the fat ratio (Shahzad, 2011; Makinde, 2017). Therefore, this study aimed to determine the effect of replacing artificial methionine in a broiler diet with herbal methionine on some biochemical characteristics of broiler's blood.

Materials and Methods

The study was conducted in the poultry field of the Animal Production Department, College of Agriculture, Kirkuk University from (30/9/2021 to 1/11/2021). In the study, 320 chicks type Rose were used (308 of them were unsexed) and were randomly distributed to 8 treatments with 4 replicates (10 chicks per replicate) from age one day to 42 days of age. The aim of the study was to determine the effect of a diet that contains herbal methionine and the addition of L. Carnitine in the production performance of a broiler. The experiment consisted of 8 nutritional treatments as follows:

T1: Standard diet (Control)

T2: Standard diet contains 5% of sunflower oil

T3: Standard diet contains 5% sunflower oil + 200 mg/kg of L. Carnitine

T4: Standard diet contains 5% sunflower oil + 250 mg/kg of L. Carnitine

T5: Standard diet contains 5% sunflower oil + 300 mg/kg of L. Carnitine

T6: Standard diet contains 5% sunflower oil + 1/3 of the herbal methionine requirements and 2/3 of the diet.

T7: Standard diet contains 5% sunflower oil + ½ of the herbal methionine requirements and ½ of the diet.

T8: Standard diet contains 5% sunflower oil + 100% of the herbal methionine requirements.

Table 1: Chemical composition and percentage of starter diet from 1 to 10 days of age.

Diet%	Treatments							
	1	2	3	4	5	6	7	8
Ground wheat	56.94	56.94	56.92	56.91	56.91	56.85	56.94	56.94
Yellow corn	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Soybean (47% CP)	30.71	30.71	30.71	30.71	30.71	30.71	30.71	30.71
Soybean oil	5	5	5	5	5	5	5	5
Calcic rock	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85
Di calcium phosphate	2.66	2.66	2.66	2.66	2.66	2.66	2.66	2.66
Salt	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
vitamins and minerals	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
L. Carnitine	-	-	0.020	0.025	0.030	-	-	-
Artificial methionine	0.30	0.30	0.30	0.30	0.30	0.198	0.150	-
Herbal methionine	-	-	-	-	-	0.198	0.150	0.30
Lysine	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24
Total	100	100	100	100	100	100	100	100
Calculated chemical composition *								
M.E. (kcal/kg diet)	2955	2955	2955	2955	2955	2955	2955	2955
Crude protein %	22.37	22.37	22.73	22.73	22.73	22.37	22.37	22.37
C:P Ratio	130	130	130	130	130	130	130	130
Lysine %	1.32	1.32	1.32	1.32	1.32	1.32	1.32	1.32
Methionine %	0.59	0.59	0.59	0.59	0.59	0.49	0.45	0.59

* According to NRC, (1994).

Table 2: Chemical composition and percentage of growth diet from 11 to 24 days of age.

% Diet	Treatments							
	1	2	3	4	5	6	7	8
Ground wheat	34	34	33.98	33.97	33.97	34	34	34
Yellow corn	27	27	27	27	27	27	27	27
Soybean (47% CP)	30.07	30.07	30.07	30.07	30.07	30.07	30.07	30.07
Soybean oil	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Calcic rock	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76
Bi calcium phosphate	2.42	2.42	2.42	2.42	2.42	2.42	2.42	2.42
Salt	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
vitamins and minerals	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
L. Carnitine	-	-	0.020	0.025	0.030	-	-	-
Artificial methionine	0.23	0.23	0.23	0.23	0.23	0.152	0.125	-
Herbal methionine	-	-	-	-	-	0.076	0.125	0.230
Lysine	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22
Total	100	100	100	100	100	100	100	100
Calculated chemical composition*								
M.E. (kcal/kg diet)	3072	3072	3072	3072	3072	3072	3072	3072
Crude protein %	21.22	21.22	21.22	21.22	21.22	21.22	21.22	21.22
C:P Ratio	14.76	14.76	14.76	14.76	14.76	14.76	14.76	14.76
Lysine %	1.28	1.28	1.28	1.28	1.28	1.28	1.28	1.28
Methionine%	0.53	0.53	0.53	0.53	0.53	0.45	0.42	0.53

* According to NRC, (1994).

Table 3: Chemical composition and percentage of final diet from 25 days of age till marketing.

Diet%	Treatments							
	1	2	3	4	5	6	7	8
Ground wheat	20.25	20.25	20.21	20.19	20.19	20.25	20.25	20.25
Yellow corn	44.08	44.08	44.08	44.08	44.08	44.08	44.08	44.08
Soybean (47% CP)	27	27	27	27	27	27	27	27
Soybean oil	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Calcic rock	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69
Bi calcium phosphate	2.19	2.19	2.19	2.19	2.19	2.19	2.19	2.19
Salt	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
vitamins and minerals	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
L. Carnitine	-	-	0.020	0.025	0.030	-	-	-
Artificial methionine	0.25	0.25	0.25	0.25	0.25	0.165	0.125	-
Herbal methionine	-	-	-	-	-	0.082	0.125	0.25
Lysine	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24
Total	100	100	100	100	100	100	100	100
Calculated chemical composition *								
M.E. (kcal/kg diet)	3162	3162	3162	3162	3162	3162	3162	3162
Crude protein %	19.29	19.29	19.29	19.29	19.29	19.29	19.29	19.29
C:P Ratio	164	164	164	164	164	164	164	164
Lysine %	1.21	1.21	1.21	1.21	1.21	1.21	1.21	1.21
Methionine %	0.54	0.54	0.54	0.54	0.54	0.45	0.41	0.54

** According to NRC, (1994).

Blood sample collection: Blood samples were collected at the end of the experiment from 80 birds (10 birds per treatment, randomly). Blood was collected immediately after slaughtering in two types of tubes; Blood serum was collected after placing the tubes which contain the blood in a centrifuge with a speed of 3000 rpm for 15 min. and then placed at -20C until a biochemical test was performed.

Biochemical tests and enzymes of blood serum: Total protein (gm/100ml), Cholesterol concentration (mg/100ml), Uric acid (mg/100ml), Glucose concentration (mg/100ml), Triglycerides, High-density

lipoprotein (HDL), Low-density lipoprotein (LDL), Very low-density lipoprotein (VLDL), Glutamic oxaloacetic transaminase (GOT), Glutamic pyruvic transaminase (GPT) and Alkaline phosphatase (ALP).

Result and Discussion

The result of table (4) showed the effect of adding L. Carnitine with herbal methionine and sunflower seed oil on some physiological and biochemical parameters of the broiler's blood. The result showed that there were no differences in blood total protein in all treatments, while T4 showed a lower blood cholesterol compared to other treatments. In addition, all treatments showed no differences in uric acid as well as in blood glucose. The control treatment showed a higher triglyceride, while treatments 7 and 8 showed a lower triglyceride. These results agreed with (Al-Ghazali et al ,2015) they found no differences in glucose, uric acid, and total protein concentration. Therefore, the addition of herbal methionine didn't increase the uric acid in the blood which means there was no higher deamination to get rid of the extra methionine, and there was none of any starving, extreme catabolism in body tissue, kidney diseases as mentioned by (Lien And Horng , 2001) . The results indicated improvement and decrease in cholesterol concentration when methionine was added, and that could be to the optimum level of methionine which played a role in emulsifying agent and breakdown the fats to small molecules, thus the surface area was increased for lipase enzyme to work on fats (Tufft and Jensen ,1992)indicated in a study conducted by adding autoxidation such as L. Carnitine, that L. Carnitine work as an antioxidant which increased the activity of the thyroid gland, and as the activity increases the cholesterol and triglycerides levels decreased.

Table 4: Effect of adding L. Carnitine with herbal methionine and sunflower seeds oil in total protein, cholesterol, uric acid, and triglycerides of broiler at age 42 days (average \pm standard error).

Treatments	Total protein Mg/100ml	Cholesterol Mg/100ml	Uric acid Mg/100ml	Glucose Mg/100ml	Triglycerides
T1	5.15 \pm 0.01	217.50 \pm 1.15 a	4.37 \pm 0.17	186.25 \pm 2.13	82.17 \pm 0.17 a
T2	5.17 \pm 0.02	214.50 \pm 0.64 a	4.40 \pm 0.13	185.25 \pm 2.46	80.62 \pm 0.39 b
T3	5.20 \pm 0.04	195.00 \pm 2.27 c	4.55 \pm 0.19	181.25 \pm 2.95	79.35 \pm 0.05 c
T4	5.35 \pm 0.01	173.00 \pm 1.77 f	4.42 \pm 0.13	183.50 \pm 3.12	78.50 \pm 0.08 d
T5	5.30 \pm 0.07	179.75 \pm 0.94 e	4.40 \pm 0.12	181.25 \pm 3.96	78.62 \pm 0.01 d
T6	5.15 \pm 0.02	208.75 \pm 0.47 b	4.40 \pm 0.14	183.25 \pm 3.17	77.97 \pm 0.02 e
T7	5.35 \pm 0.13	188.5 \pm 1.43 d	4.32 \pm 0.12	182.75 \pm 2.32	77.25 \pm 0.02 f
T8	5.12 \pm 0.10	193.25 \pm 0.47 c	4.25 \pm 0.12	183.75 \pm 3.42	77.00 \pm 0.04 f
Significant level	N.S	*	N.S	N.S	*

* Different letters within the same column indicate of significant differences at ($P \leq 0.05$), N.S= non-significant

T1: Standard diet (Control), T2: Contains 5% of sunflower oil, T3: Contains 5% sunflower oil + 200 mg/kg of L. Carnitine, T4: Contains 5% sunflower oil + 250 mg/kg of L. Carnitine, T5: Contains 5% sunflower oil + 300 mg/kg of L. Carnitine, T6: Contains 5% sunflower oil + 1/3 of the herbal methionine requirements and 2/3 of the diet., T7: Contains 5% sunflower oil + 1/2 of the herbal methionine requirements and 1/2 of the diet., T8: Contains 5% sunflower oil + 100% of the herbal methionine requirements.

Table 5: Effect of adding L. Carnitine with herbal methionine and sunflower seeds oil in high and low lipoprotein of broiler's blood at age 42 days (average \pm standard error)

Treatments	HDL mg/100ml	LDL mg/100ml	VLDL mg/100ml
T1	122.25 \pm 1.31 d	72.25 \pm 0.62 b	41.00 \pm 0.40 a
T2	122.25 \pm 0.75 d	75.50 \pm 1.04 a	42.25 \pm 0.47 a
T3	131.00 \pm 0.40 c	37.75 \pm 1.75 ed	33.75 \pm 0.62 c
T4	140.25 \pm 1.88 b	28.75 \pm 0.75 f	28.75 \pm 0.47 d
T5	145.75 \pm 0.62 a	25.75 \pm 0.85 g	26.75 \pm 0.25 e
T6	125.00 \pm 2.61 d	45.50 \pm 1.04 c	38.00 \pm 0.70 b
T7	124.00 \pm 0.40 d	38.75 \pm 0.25 d	35.00 \pm 0.70 c
T8	124.00 \pm 0.70 d	36.00 \pm 0.91 e	34.50 \pm 0.86 c
Significant level	*	*	*

* Different letters within the same column indicate of significant differences at ($P \leq 0.05$).

T1: Standard diet (Control), T2: Contains 5% of sunflower oil, T3: Contains 5% sunflower oil + 200 mg/kg of L. Carnitine, T4: Contains 5% sunflower oil + 250 mg/kg of L. Carnitine, T5: Contains 5% sunflower oil + 300 mg/kg of L. Carnitine, T6: Contains 5% sunflower oil + 1/3 of the herbal methionine requirements and 2/3 of the diet., T7: Contains 5% sunflower oil + 1/2 of the herbal methionine requirements and 1/2 of the diet., T8: Contains 5% sunflower oil + 100% of the herbal methionine requirements.

The result in table (5) indicated a higher HDL, LDL, and VLDL in treatment 5 compared to other treatments. The addition of different levels of L. Carnitine to the broiler's diet worked on a significant reduction in LDL concentration and a significant inclined in HDL concentration (Hosseintabar et al, 2014). The reason behind that could be related to L. Carnitine. The VLDL and LDL concentration accumulate on the blood vessel walls which causes coronary atherosclerosis and heart attacks, while HDL takes the extra cholesterol from the blood tissue to the liver (Lien and Horng, 2001).

Table 6: Effect of adding L. Carnitine with herbal methionine and sunflower seeds oil in the blood enzymes of broiler at age 42 days (average \pm standard error).

Treatments	GOT-ALT IU/L	GPT-AST IU/L	ALP IU/L
T1	109.25 \pm 3.94	9.50 \pm 0.28	28.00 \pm 2.19
T2	110.25 \pm 3.63	9.25 \pm 0.25	30.75 \pm 2.13
T3	112.50 \pm 5.31	11.25 \pm 0.25	31.25 \pm 2.25
T4	116.25 \pm 3.17	10.75 \pm 0.75	32.50 \pm 2.75
T5	114.25 \pm 4.42	10.75 \pm 1.25	32.00 \pm 3.31
T6	111.25 \pm 4.26	10.00 \pm 0.70	31.00 \pm 2.48
T7	115.25 \pm 4.71	10.25 \pm 0.47	28.50 \pm 2.21
T8	110.25 \pm 4.87	10.50 \pm 1.04	28.00 \pm 2.16
Significant level	N.S	N.S	N.S

N.S= non- significant, T1: Standard diet (Control), T2: Contains 5% of sunflower oil, T3: Contains 5% sunflower oil + 200 mg/kg of L. Carnitine, T4: Contains 5% sunflower oil + 250 mg/kg of L. Carnitine, T5: Contains 5% sunflower oil + 300 mg/kg of L. Carnitine, T6: Contains 5% sunflower oil + 1/3 of the herbal methionine requirements and 2/3 of the diet., T7: Contains 5% sunflower oil + 1/2 of the herbal methionine requirements and 1/2 of the diet., T8: Contains 5% sunflower oil + 100% of the herbal methionine requirements.

The results in table (6) showed no differences in AST, ALP, and ALT enzymes in all treatments. Also, no differences in GOT and GPT enzymes in blood serum. The reason could be related to the fact that the addition of herbal methionine didn't show any negative effect on the activity of some organs such as the heart, liver, kidney, and muscles. These results agreed with (Halder and Roy, 2007; Kalbande et al, 2009; Rekhateh et al, 2010).

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