

Original Research Article

Effects of calf thymus extract and L-carnitine on immunity and growth performance of broiler chickens

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ABSTRACT

Background: Increasing the bird's wealth and increasing the growth rate are among the goals of increasing the animal wealth so; we studied the effects of L-carnitine and calf thymus extract on growth performance and immunity in broiler chickens.

Methods: Eighty broiler chicks were divided into four groups, each group included twenty chicks. Group 1, was negative control with no treatment of feed or water. Group 2, the regular drinking water was replaced by L-carnitine-infused water (1 gram per liter). Group 3, the regular drinking water was replaced by calf thymus extract- infused water (1 ml per liter). Group 4, the regular drinking water was replaced by both L-carnitine and calf thymus extract- infused water. This treatment was administered from day 1 to day 30 of the cycle. Body weight, feed intake and feed conversion were assessed. The hemogram, leucogram, total protein, globulin, albumin, phagocytic activity, phagocytic index and interleukin 2 were measured.

Results: The hemogram and leucogram parameters recorded a significant increase in treated groups compared to non treated group. The final body weight for all treatments was nearly the same, but slightly higher with application of L-carnitine. Phagocytic activity, index, TP, globulin and IL2 were significantly increased in treated groups.

Conclusions: Both L-carnitine and thymus extract have significantly improved the general health condition, in addition, calf thymus extract improved not only the general body condition but also act has immunomodulatory effect which require more further studies.

Keywords: Growth performance, Immunomodulatory, L-carnitine, Calf thymus extract

INTRODUCTION

The poultry production is the quickest way to produce protein of high quality for human consumption.^{1,2} Broiler chickens require about 3 kg of ration to create 1 kg of meat, but animals need 7 kg of food to produce of red meat.³ So in this inquiry, we enhance growth and immunity of broiler chickens to enhance meat production of good quality.

Natural immunity strengthens the body and protects it against diseases. The immune system identified and

eliminates pathogens with stimulation of natural and adaptive immune responses.⁴

L-carnitine was firstly discovered in muscle extracts & derived from Latin word *carnis* flesh or meat.⁵ L-carnitine is essential for the normal oxidation of long-chain fatty acids in mitochondria for β oxidation and ATP production in tissues.⁶ It is synthesized in liver, kidney and brain from two essential amino acids; lysine and methionine. In poultry L-carnitine is an anti-oxidant and has metabolic activity of fatty acid.^{7,8} In young chick biosynthesis of L-carnitine is less well developed so, L-carnitine supplementation of chicks during the early stage may lead

to faster utilization of yolk sac content. This fast utilization of yolk may result in improvement of performance parameters and immunity functions.⁹ Some studies have shown that supplemental L-carnitine improved body weight gain and reduced the abdominal fat content of broilers it also increased breast muscle and thigh meat yield and fat content of breast muscle, whereas quantity and percentage of abdominal fat was reduced.^{10,11}

Theoretically, adding an a suitable content of carnitine to the broiler diet, facilitate the fatty acids oxidation, and triacylglycerol storage in the adipose tissue.¹² Higher levels of antibodies specific to influenza and pneumococcal vaccines were produced in L-carnitine-supplemented mice than in control mice fed on an unsupplemented diet.^{13,14} L-carnitine and its combination improved broiler performance and decreasing abdominal fat and activated immune system of broiler chickens at 42 d of age.¹⁵

Natural thymic peptides have been isolated from calf thymus by mild acid extraction. Pharmaceutical containing natural peptides, thymalin was put into practice as immune corrector. Thymalin and thymogen were used in persons with chronic pathology and immune dysfunction. The previous results indicated that thymic peptides participate in the regulating mechanisms of inflammatory processes as cytokine antagonists.¹⁶ The researcher reported that thymalin stimulated T cells while acting with immunoglobulins.¹⁷

The thymus gland is the primary lymphoid organ that provide site of T cell production and activation, it represents a key organ of the immune system.¹⁸

The thymus extracts contain amino acids, this acids composition revealed a high content of acidic amino acids and no apparent homology to previously defined growth factors and thymus differentiation hormones.¹⁹

The thymus extracts have been shown to modulate the development, maturation and activation of T cells, stimulate production of interleukin-2 (IL-2), their effect on the specialization and migration of T-lymphocytes throughout the body. The thymus also releases hormones that regulate immune function.²⁰ Pretreatment with the calf thymus extract increased the antioxidant enzymes in a dose-dependent manner.²¹

Administration of calf thymus extract markedly and significantly increased the antibody titers against NDV, serum globulin level, and percentage of lymphocytes in the blood. In addition, thymus extract resulted in definite and significant cellular immunopotential.²²

Further investigations are required to elucidate role of L-carnitine and calf thymus extract in various immune functions and hence the present study was designed to evaluate the effect of L-carnitine and calf thymus extract

on certain immunological functions and performance characteristics in broilers chickens.

METHODS

Drugs

L-carnitine: It was obtained as pure white powder from Mepaco Company (Arab company for pharmaceutical & Medicinal plants) (Egypt).

Thymus extract: It was obtained from Biomedica Company for Biological and Veterinary Products, Egypt, under a trade name (CYTOIMMUNE®). It is a liquid preparation, each bottle of 500 ml capacity of calf thymus gland extract. Main ingredients of this extract are natural thymic peptides, thymalin & polynucleotides.

Birds

A total of 80, one-day old non vaccinated cobb broiler chicks were purchased from local provider, On arrival, chicks were weighed and randomly housed in metal battery in a controlled temperature environment, and were allowed free access to water and feed throughout the growth period (1 to 30 days). The protocol of the study has been approved by the Ethical Committee of the College of Medicine or Faculty of Veterinary Medicine, University of Sadat City, Egypt.

Experimental design

The birds were divided into four groups (20 chicks each) as the following:

Group 1 (control): was negative control with no treatment of feed or water. *Group 2:* the regular drinking water was replaced by L-carnitine- infused water. *Group 3:* the regular drinking water was replaced by calf thymus extract- infused water. *Group 4:* the regular drinking water was replaced by both L-carnitine and calf thymus extract-infused water.

The infusions were prepared daily, L-carnitine- infused water (1 gram per liter). The calf thymus extract- infused water (1 ml per liter) as recommended by provider. The container was sealed and placed at environmental temperature for 8 h. and the prepared infusion was freshly added to drinkers till the end of the experiment.²³

Performance data

Body weight, feed intake and feed conversion were assessed.

The initial and final body weight and the feed intake were weekly recorded for each group. Average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) were also calculated for each

treatment. Live body weight (LBW) was individually measured on day 30.

For performance data, pen means served as the experimental unit for statistical analysis. For data on relative weight, individual birds were considered as the experimental unit.

Blood sampling

At day 30, blood samples were collected from wing vein from each bird in tubes, each blood sample was divided into three portions. The first portion was collected in a small labeled dry and clean vial containing one drop of heparin as anticoagulant to determine the phagocytic activity and phagocytic index. The second portion of blood was collected in a small labeled dry and clean vial containing EDTA for hematological studies. The Third portion of blood was collected in plain clean dry and sterile centrifuge tubes and were allowed to coagulate and then centrifuged at 3000 rpm for 10 min. to separate sera used to determine total proteins and globulin concentration in the serum.

Hematological examinations

RBCs, WBCs count and differential leukocytic count were measured.

Biochemical analysis

Electrophoresis (Ig), phagocytic activity, phagocytic index and IL2 were measured.

Candida phagocytosis

The phagocytic activity of heterotrophs performed according to the method.²⁴ In clean dry tube, the following aliquots were mixed; 100ul poultry serum, 100 ul heat killed *Candida albicans* (5×10^6 /ml) and 100 ul of heparinized tested blood. The tube was mixed and then incubated at 37°C for 30 minutes, after which it was centrifuged at 1500 rpm for 5 minutes. The supernatant was discarded leaving a droplet into which the sediment was re-suspended. Smear was prepared from the deposit, dried in air, fixed with methyl alcohol for about 3 min and stained with Giemsa stain for 30 min. The slides were then washed well with water and dried in air. The slides were then examined under oil immersion lens. One hundred heterotrophs were examined and the number of heterotrophs ingesting *Candida* was counted and expressed as percentage.

No of heterotrophs ingesting *Candida*

Phagocytic activity% = ----- $\times 100$

Total number of heterotrophs

Phagocytic index = Total no of ingested *Candida*/ phagocytic activity%

Body weight and immune-related organs relative weight

Just before killing at the end of experiment (at 30 days of age), final body weight of bird in all experimental groups was recorded. Upon being killed, the spleen was removed aseptically, weighed and their relative organ weights (ROW) using the following formula:

ROW = [Absolute organ weight (g) / body weight of bird on sacrifice day (g)] $\times 100$. Also weighed relative weight of bursa of fabricius.²⁵

Statistical analysis

Data obtained in this study were statistically analyzed for variance (ANOVA) with confidence limits set as 95% (significance at $p < 0.05$ probability level).²⁶ The results were reported as mean \pm standard error "SE", multiple range tests should be performed to compare among different groups of experiment. Statistical analysis was performed using SPSS statistical package.

RESULTS

Effect of L-carnitine and CTE on body weight

Day-old chick weights were similar between the treatment groups. Table 1 shows average chick weights according to age and treatments. At 7 day of age, chicks of control group were lighter ($p < 0.05$) than those of group 2 and group 4 which were comparable but higher than group 3. At day 15 of age, chick weights of control group were slightly lower than those of group 2 and the same as group 4 and higher than group 3, but the difference was not significant.

The final body weight for all treatments was nearly the same, but slightly higher with application of L-carnitine.

Effect of L-carnitine and CTE on feed intake

Daily feed intake per chick varied between 21 g and 24 g, during the first week of age. Table 1 shows feed consumption according to treatments and age of chicks. Feed intake increased as age of chicks increased ($p < 0.01$). Amount of feed consumed increased with supplementation of L-carnitine ($p < 0.05$). While supplementation of CTE lead to slight lower feed intake, with no severe impact on body weight.

Effect of L-carnitine and CTE on lymphoid organs

Table 2 shows significant elevation in the weight of bursa in group 3 than the other groups, the difference between groups 1,2,4 were non-significant. The relative weight of spleen in all groups was nearly the same.

Table 1: Live body weight, average daily gain, average daily feed intake and feed conversion ratio of experimental male broilers at 1-30 days of age.

Parameters	Age/day	Group 1	Group 2	Group 3	Group 4
Live body weight (g)	7	170	173	166	171
	15	500	510	490	503
	30	1500	1600	1480	1500
Average daily gain (g)	1-7	18.5	19	18	18.7
	7-15	47.1	48.1	46.2	47.4
	15-30	66.6	72.6	66	66.4
	1-30	48.6	52	48	48.6
Average daily feed intake (g)	1-7	21	24	19	21
	7-15	54.4	57	51	55
	15-30	116	120	110	118
	1-30	76	80	73	79
Feed conversion ratio (g/g)	1-7	1.13	1.26	1.0	1.12
	7-15	1.15	1.18	1.10	1.16
	15-30	1.7	1.62	1.6	1.7
	1-30	1.5	1.53	1.5	1.6

Table 2: Relative weights of immune organs.

Organs	Group 1	Group 2	Group 3	Group 4
Bursa of fabricious relative weight (%)	0.21 ^b	0.22 ^b	0.25 ^a	0.21 ^b
Spleen relative weight (%)	0.112	0.113	0.114	0.112

Table 3: Hematological parameters and biochemical analysis (n=10).

Parameters	Group 1	Group 2	Group 3	Group 4
Total Protein (gm/dl)	3.15±0.06	3.10±0.05	3.20±0.04	3.02±0.07
Globulin (gm/dl)	1.18±0.13 ^c	1.53±0.14 ^a	1.40±0.11 ^a	1.35±0.13 ^{ab}
Albumin (gm/dl)	1.97±0.05 ^a	1.57±0.04 ^b	1.80±0.04 ^a	1.67±0.05 ^b
A/G ratio	1.67±0.05 ^a	1.07±0.04 ^c	1.29±0.04 ^b	1.24±0.05 ^b
Lymphocyte (%)	57.17±2.39 ^d	70.17±2.22 ^b	80.17±1.29 ^a	67.17±2.31 ^c
Neutrophil (%)	82.63±3.10	82.07±5.63	84.10±1.57	72.20±15.59
N/L ratio	1.45±0.05 ^a	1.17±0.25 ^b	1.05±0.11 ^c	1.07±0.13 ^c
Phagocytic activity (%)	56.25±0.25 ^d	72.00±1.68 ^b	79.0±1.08 ^a	65.25±1.10 ^c
Phagocytic index	3.74±0.09 ^b	4.77±0.18 ^a	4.93±0.19 ^a	4.58±0.12 ^a
Total RBC (×10 ⁶ µl)	2.32±0.31 ^c	4.32±0.11 ^b	5.32±0.21 ^a	3.32±0.31 ^{bc}
Hemoglobin (g/dl)	9.27±1.42 ^c	10.27±1.02 ^b	11.7±1.1 ^a	10.16±1.3 ^{bc}
Total WBC (×10 ³ µl)	2.05±0.39 ^c	3.05±0.30 ^b	4.1±0.11 ^a	2.5±0.39 ^{bc}
IL2 (Pg/ml)	15.20±1.04 ^d	70.05±5.66 ^a	49.00±2.16 ^b	35.77±1.74 ^c

Haematological parameters and biochemical analysis

Table 3 shows that the mean values of the RBCs, Hb and WBCs were significantly higher in group 3, group 2, respectively than control group. This value was non significantly higher in group4 compared to control one.

Lymphocytes % were significantly higher in group 3 (80.17%) on comparison with other groups as shown in Figure 1.

In Table 3 and Figure 1, we observed that there were significantly increased in phagocytic activity in group 3

(79.00%), group 2 (72.0%), and group 4 (65.25%), respectively on comparison with control group (56.25%) and a significant increase in phagocytic index was observed in group 2, group 3 and group 4 on comparison with control group. Phagocytic activity and phagocytic index mainly were higher in all treated groups than control one.

Values of total protein, albumin (A) and globulin (G) were slightly higher in group 2, group 3 respectively than control group. Globulin increased in group 2, 3, 4 respectively as illustrated in Figure 2.

Interleukin 2 was shown in Table 3 and Figure 1. The mean values of IL 2 were significantly increased in group 2, group 3 and group 4, respectively on comparison with control group.

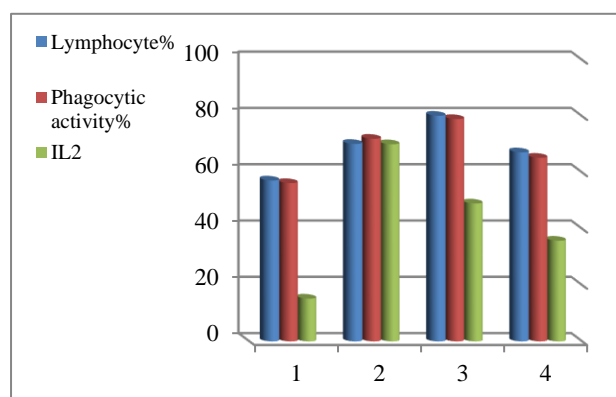


Figure 1: Effect of L-carnitine and calf thymus extract on lymphocyte %, phagocytic activity and IL2.

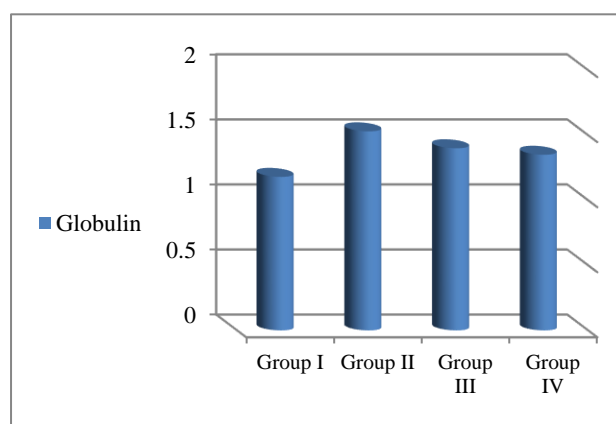


Figure 2: Effect of L-carnitine and calf thymus extract on globulin (gm/dl).

DISCUSSION

This study approved the effect of L-carnitine and calf thymus extract supplementation on the general performance, body weight and immune response of broiler chickens.

Regarding the effect on body weight our results were similar to Rebouche results, who stated the role of L-carnitine as an antioxidant and improves fatty acids metabolism.⁷ L-carnitine administration stimulated CAT activities in renal and liver tissues is highly significant. Similar to that observed in our results, L-carnitine may play a major role in stability of antioxidant status in kidney, liver tissue.²⁷ As mentioned the improvement of body weight may be due to improvement of liver enzymes and may be attributable to an improved utilization of dietary nitrogen, achieved through more efficient fat oxidation.¹² L-carnitine enhances feed intake and feed conversion ratio.²⁸

Regarding the role of L-carnitine in cellular immunity, it was present in lymphocytes at high concentrations, and inhibited apoptosis of those immune cells, there was a very significant increase in number of lymphocytes in CTE treated groups, when compared to other groups.²⁹

In regards to relative Bursa of Fabricius and spleen weights were significantly increased than control, this referred to increase lymphocyte cell%.²⁷ that due to decrease the total number of thymocytes and splenocytes by the effect of stress which is also accompanied by decreasing weight ratio of the spleen.³⁰

In this study, erythrogram, leucogram, phagocytic activity, phagocytic index, total protein, globulin, albumin, A/G ratio, interleukin 2 and relative lymphoid weight were demonstrated.

Our results showed an improvement in RBCs count, Hb concentration after oral administration of L-carnitine in broilers. RBCs count and Hb content were increased in chicks fed diet supplemented by L-carnitine compared to those fed on control diet.³¹ This results may be due to an effect of L-carnitine on erythrocyte stability and survival. Therefore, by this mechanism PCV can be increased. L-carnitine improved anemia and also, L-carnitine improved erythropoietin efficiency.^{32,33}

In this study, L-carnitine treated groups were shown significantly decreased in neutrophil or lymphocyte ratio that may be attributed to increase level of lymphocyte production. Lymphocyte cells% were significantly increased for duckling fed diets supplemented with L-carnitine than control group.³⁴

In this study, L-carnitine significantly increased globulin and significantly decreased A/G ratio. This agree with the previous findings reporting that L-carnitine significantly increased globulin, phagocytic activity, phagocytic index and significantly decreased A/G ratio.³⁵

Our findings revealed that L-carnitine significantly increased interleukin 2 producing from T-helper cells. This agree with the other studies demonstrating that L-carnitine in mice exhibiting a modification in production of a number of cytokines (interferon, tumor necrosis factor, IL2, IL-4, IL-6, vascular endothelial growth factor and insulin-like growth factor in serum.³⁶ This results may be attributed to a positive effect of L-carnitine on humoral immunity by increasing lymphocyte cells%, phagocytic activity, phagocytic index, globulins and interleukin 2.^{14,37,35}

Our results showed improvement in RBCs count, Hb concentration and PCV after oral administration of calf thymus extract in broilers. This agree with the previous findings reporting that calf thymus preparation (TFX-Polfa) was significantly increased in the hemoglobin concentration and erythrocyte counts.³⁸

In this study, thymus extract treated groups were shown significantly decreased in neutrophil / lymphocyte ratio that may be attributed to increase level of lymphocyte production. This results are similar to results that reported there was a very significant increase in number of lymphocytes in calf thymus extract treated groups, when compared to the control group, which is one of the probable reason for overall immunopotential and this could be due to the fact that thymic hormones activates T-cell and activates lymphocyte production.²² Relative spleen weights were significantly increased, this referred to increase lymphocyte cell%.³⁹ In CTE treated groups, there was considerable increase level of lymphocyte production, this could be due to the fact that thymic hormones activates T-cell rosettes and they enhance the differentiation, maturation and proliferation of lymphocytes.⁴⁰

In our study we documented a significant increase of phagocytic activity and phagocytic index. This agree with the previous findings reporting that thymic peptide mixtures (Thymosin fraction 5 thymulin) have been proved to stimulate the immune response and enhance phagocytosis.⁴¹ The increase in phagocytic activity of phagocytes in calf thymus extract treated group could be due to the ability of the thymus extract to act as an immunomodulator by exerting control on cytokine production by peripheral blood mononuclear cells.²²

The serum samples showed an increased level of total proteins in the CTE treated groups compared to the control group, the increased total serum proteins enhanced the general health status and immunity of the birds and also markedly without mortality of the birds during the entire experiment period. Thymus homogenate increases total blood proteins in calves and piglets.⁴² Our results were concurrent with recorded a significant increase in total proteins, particularly the serum globulins in thymus extract administered chickens.⁴³

In this study, thymus extract significantly increased globulin and significantly decreased A/G ratio. This agree with the previous findings reporting that thymus extract significantly increased globulin.²²

In this study, thymus extract significantly increased interleukin 2 producing from T-helper cells. Thymalin was increased production of interferon and interleukin 2 by lymphocytes as our results.¹⁶ In addition to thymus extract was increased production of interleukin 2.²⁰ This results may be attributed to a positive effect of intraperitoneal administration of calf thymus extract on humoral and cellular immunity by increasing lymphocyte cells%.²²

CONCLUSION

From this study, both L-carnitine and thymus extract have significantly improved the general health condition, performance, feed intake, feed conversion rate and total body weight of broilers, but the results were more obvious

in case of L-carnitine treatment. In addition, calf thymus extract improved not only the general body condition but also act has immunomodulatory effects which require further studies.

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Conflict of interest: None declared

Ethical approval: The study was approved by the institutional ethics committee

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