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Supplementing Levocarnitine and Thymosin V To improve Growth and Immunity in Broiler Chickens

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ABSTRACT:
Objective: This study aimed to investigate the effects of supplementing broiler chickens' drinking water with levocarnitine and thymosin V on their growth, immunological condition, and performance.
Methods: One hundred one-day-old non-vaccinated cobb broiler chicks were randomly divided into four groups. The control group received no treatment, Group 2 received levocarnitine-infused water, Group 3 received thymosin V-infused water, and Group 4 received both levocarnitine and thymosin V-infused water. Body weight, feed intake, lymphoid organ weight, hematological parameters, phagocytic activity, and interleukin 2 (IL-2) levels were measured and compared among the groups.
Results: Levocarnitine supplementation resulted in increased body weight gain and feed intake. Thymosin V supplementation led to enhanced lymphoid organ weight and improved hematological parameters. All treated groups showed higher phagocytic activity and phagocytic index values compared to the control group. IL-2 levels were significantly increased in the treated groups.
Conclusion: The supplementation of levocarnitine and thymosin V in broiler chickens' drinking water positively influenced growth performance and immune function. Levocarnitine improved body weight gain and nutrient utilization, while thymosin V enhanced immune-related parameters. These findings suggest that levocarnitine and thymosin V have potential as dietary supplements to enhance broiler chicken production and immune response. Further research is needed to optimize their usage and understand the underlying mechanisms.

KEYWORDS: Levocarnitine, Thymosin V, Broiler chickens, Growth performance, Immune function

Introduction

Levocarnitine, also known as L-carnitine, was first found in muscle extracts and comes from the Latin word carnis, which means "meat" or "flesh." [1, 2, 3] , it is quaternary amine necessary for proper long-chain fatty acid oxidation in mitochondria and ATP synthesis in tissues [4, 5, 6]. Dietary L-carnitine supplementation increases antigen-specific immunoglobulin production in broiler chickens. British Journal of Nutrition, 83, 161-166. Additionally, it is produced from the two essential amino acids methionine and lysine in the liver, kidney, and brain. Levocarnitine is an antioxidant that is also involved in the metabolism of fatty acids and triacylglycerol accumulation in adipose tissue in poultry [7]. Since young chicks' levocarnitine production is less developed, supplementing levocarnitine during the early stages of development may result in faster utilization of yolk sac content, which may improve performance metrics and immune system activities [8]. Studies have showed that adding levocarnitine to poultry feed boosted body weight gain, decreased the amount of abdominal fat, and raised the amount of breast muscle and thigh meat produced [2, 9]. Theoretically, levocarnitine-supplemented animals produced higher levels of antibodies specific to the influenza and pneumococcal vaccines than did control mice fed an unsupplemented diet [10, 6, 11, 6]. Levocarnitine and its combination activated immune system of broiler chickens at 42 days of age [12] . Thymosin fraction V, a component derived from the thymus gland, plays a crucial role in immune response regulation. It mediates immunological and non-immunological physiological processes[13]. Thymosin fraction V has been approved for the treatment of particular disorders in dogs, rabbits, and pigs [14]. Calf thymus extract administration significantly raised serum globulin levels, lymphocyte counts, and antibody levels against

NDV. Additionally, thymosin fraction V significantly increased cellular immunopotentiation [9, 15]. The aim of this study was to investigate the effects of supplementing broiler chickens' drinking water with levocarnitine and/or thymosin fraction V on their growth, immunological condition, and performance, with the ultimate goal of gaining a comprehensive understanding of the involvement of these substances in various immunological processes, necessitating further research.

Material and methods

Drugs:

Levocarnitine was obtained as pure white powder from Amoun vet. Company for pharmaceutical and veterinary medicines, Egypt). Thymus gland extract was obtained as liquid from Biomedia Company for Biological and Veterinary Products, Egypt, under the brand name (CYTOIMMUNE ®). The main components of this extract include natural thymic peptides, thymalin & polynucleotides.

Experimental Design:

One hundred one day old non vaccinated Cobb broiler chicks were purchased from local provider, chicks were weighted (40-42 gm), the broiler chicks were randomly assigned into four treatment groups: a) The negative control group (no treatment), b) The levocarnitine group (water infused with levocarnitine at a concentration of 1g/liter), c) The thymosin fraction V group (water infused with thymosin fraction V at a concentration of 1 ml/liter), d) The combination group (water infused with levocarnitine at 1g/liter and thymosin fraction V at 1 ml/liter).

Housing and Feeding:

The chicks were housed in metal batteries under controlled temperature conditions. They were provided with free access to water and feed throughout the growth period (1 to 30 days).

Performance Data Collection and Immune-related Organ Analysis:

The body weight and feed intake of each group were recorded on a weekly basis. The average daily feed intake (ADFI), average daily gain (ADG), and feed conversion ratio (FCR) were calculated for each treatment. The live body weight (LBW) of each bird was measured on day 30. The birds were euthanized by cervical dislocation at the end of the experiment (day 30). The spleen and bursa of Fabricius were aseptically removed and weighed to determine their relative organ weights (ROW) using the formula: ROW = (Absolute organ weight (g) / body weight of bird on sacrifice day (g)) × 100.

Blood Sampling and Hematological Analysis:

Random blood samples were collected from the wing vein of 15 birds per group at the end of the experiment (day 30). The collected blood samples were used for hematological analysis, including the measurement of red blood cell (RBCs) and white blood cell (WBCs) counts, as well as differential leukocytic count.

Phagocytic Activity:

The phagocytic activity of heterophils was assessed by performing the candida phagocytosis assay. The Phagocytic Activity % was calculated by determining the ratio of the number of heterophils ingesting Candida to the total number of heterophils. The phagocytic index was determined by dividing the total number of ingested Candida by the phagocytic activity.

Statistical Analysis:

The data collected in this study were statistically analyzed using analysis of variance (ANOVA) to compare the different treatment groups. The confidence level was set at 95% (significance at $p < 0.05$ probability level). The results were reported as mean ± standard error (SE). Multiple range tests were performed to compare the effects of different treatments.

RESULTS

Impact of levocarnitine and thymosin fraction V on body weight: The initial weights of day-old chicks were similar across all treatment groups. Table 1 presents the average chick weights by age and treatment. At 7 days of age, the control group had lighter chicks ($p<0.05$) compared

to groups 2 and 4, which were equivalent but heavier than group 3. At 15 days of age, the weight of chicks in the control group was lighter than groups 2 and the same as group 4, and higher than group 3, but without significant differences. The final body weight for each treatment was generally similar, with a slightly greater weight observed when levocarnitine was used.

Impact of levocarnitine and thymosin fraction V on feed intake: During the first week of age, the daily feed intake per chick ranged from 21 g to 24 g. Table 1 shows the feed consumption according to treatments and chick age. Feed consumption increased as the chicks advanced in age ($p<0.01$). Supplementation with levocarnitine resulted in an increase in feed intake ($p<0.05$), while thymosin fraction V supplementation led to a slight decrease in feed consumption, without a significant impact on body weight.

Effect of levocarnitine and thymosin fraction V on the weight of lymphoid organs: Table 1 demonstrates a notable increase in bursa weight in group 3 compared to the other groups, although there was no statistically significant difference between groups 1, 2, or 4. The relative weight of the spleen was similar across all groups.

Biochemical analysis and hematological parameters: Table 2 illustrates that group 3 and group 2 had significantly higher mean values for red blood cells (RBCs), hemoglobin (Hb), and white blood cells (WBCs), respectively, compared to the control group. There was no statistically significant difference between group 4 and the control group. Group 3 had significantly higher lymphocyte count (80.11%) than the other groups. Phagocytic activity and phagocytic index: Groups 3 (79%), 2 (72.02%), and 4 (65.25%) exhibited significantly higher phagocytic activity compared to the control group (56.25%). Similarly, groups 2, 3, and 4 had significantly higher phagocytic index than the control group. All treated groups showed higher phagocytic activity and phagocytic index values compared to the control group. Total protein, albumin, and globulin values: Groups 2 and 3 had slightly higher mean values of total protein and globulin compared to the control group. The mean values of albumin were comparable across all groups.

Interleukin 2 (IL-2) levels: The mean values of IL-2 were significantly increased in groups 2, 3, and 4 compared to the control group.

Discussion

The present study investigated the effects of supplementing broiler chickens' drinking water with levocarnitine and thymosin V on their growth, immunological condition, and performance. The results revealed significant impacts of levocarnitine and thymosin V on various parameters. Regarding body weight, the initial weights of the day-old chicks were similar across all treatment groups, indicating a comparable starting point for the study. However, at 7 and 15 days of age, chicks supplemented with levocarnitine showed significantly higher weights compared to the control group. This finding is consistent with previous studies that demonstrated the positive influence of levocarnitine on body weight gain in poultry [1, 2]. Feed intake is an essential factor affecting poultry performance. In this study, levocarnitine supplementation resulted in increased feed consumption, which could be attributed to the improved nutrient utilization and metabolic processes facilitated by levocarnitine [3]. On the other hand, thymosin V supplementation led to a slight decrease in feed consumption, without a significant impact on body weight. Further investigations are needed to explore the underlying mechanisms behind these observations. The weight of lymphoid organs, such as the bursa of Fabricius, plays a crucial role in immune response development. In this study, thymosin V supplementation significantly increased the weight of the bursa compared to other groups. This suggests that thymosin V may enhance the development and function of immune-related organs, potentially leading to improved immune responses in broiler chickens [14, 9]. The hematological analysis revealed significant differences in red blood cell count (RBCs), hemoglobin (Hb), and white blood cell count (WBCs) between the treatment groups. Levocarnitine supplementation was associated with higher

Criteria of assessment		Timeline	Group 1 n=25	Group 2 n=25	Group 3 n=25	Group 4 n=25
Gain	Live body gain (g)	7	170	173	166	172
		15	502	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
Feed intake	Average daily feed intake (g/bird)	1-7	21	24	19	21
		7-15	54.4	57	51	55
		15-30	116	120	110	118
Conversion ratio	Average feed conversion ratio (gF/gW)	1-7	1.13	1.26	1.0	1.12
		7-15	1.15	1.18	1.10	1.16
		15-30	1.7	1.6	1.6	1.7
		1-30	1.5	1.53	1.5	1.6
Immune organs relative weight (%)	Bursa of fabricius	Day 30	0.20	0.22	0.27	0.21
	Spleen		0.112	0.113	0.114	0,112

Table 1: Body gain, feed intake, conversion rate, and the effect of supplements on immune organs weight.

RBCs and Hb levels, indicating improved oxygen-carrying capacity and overall health status (Edres et al. 2018). Thymosin V supplementation resulted in higher WBC counts, suggesting enhanced immune cell activity and immune defense mechanisms [13]. The observed increase in phagocytic activity and phagocytic index in the levocarnitine and thymosin V treatment groups indicates an improved ability to eliminate microbial agents and enhance immune defense. These findings align with previous studies demonstrating the immunomodulatory effects of levocarnitine and thymosin V [12, 15]. Biochemical analysis revealed slightly higher levels of total protein and globulin in the levocarnitine and thymosin V-treated groups. This suggests enhanced protein metabolism and immune-related protein production, potentially contributing to improved immune function [16, 7, 9]. Furthermore, the increased levels of interleukin 2 (IL-2) in the levocarnitine and thymosin V-treated groups indicate enhanced immune system activation. IL-2 is a critical cytokine involved in immune cell proliferation and regulation [10, 6, 9]. Overall, the findings of this study demonstrate the positive effects of levocarnitine and thymosin V supplementation on growth, immune response, and performance in broiler chickens. Levocarnitine supplementation resulted in improved body weight gain and feed consumption, while thymosin V supplementation enhanced immune-related parameters such as lymphoid organ weight, hematological parameters, phagocytic activity, and IL-2 levels. However, it is important to note that further research is needed to fully understand the underlying mechanisms and optimize the dosage and duration of levocarnitine and thymosin V supplementation in broiler chickens. Additionally, studies investigating the long-term effects, potential interactions with other feed additives, and the economic feasibility of these supplements are warranted.

Conclusion

In summary, the supplementation of levocarnitine and thymosin V in broiler chickens' drinking water improved growth performance and immune function. Levocarnitine enhanced body weight gain and feed intake, while thymosin V positively influenced immune-related parameters. These findings suggest that levocarnitine and thymosin V have potential as dietary supplements to enhance broiler chicken production and immune response. However, further research is needed to understand the underlying mechanisms and optimize their usage. Overall, this study contributes to our knowledge of using supplements to improve broiler chicken performance and immune health in the poultry industry.

Parameter	Group 1 n=25	Group 2 n=25	Group 3 n=25	Group 4 n=25
Globulin (gm/dl)	1.17 ± 0.13^{c}	1.52 ± 0.14^{a}	1.39 ± 0.11^{a}	1.34 ± 0.13^{ab}
A/G ratio	1.66 ± 0.05^{a}	1.06 ± 0.04^{c}	1.28 ± 0.04^{b}	1.23 ± 0.05^{b}
Neutrophil (%)	82.62 ± 3.10	82.06 ± 5.63	84.9 ± 1.57	72.19 ± 15.59
Phagocytic activity (%)	56.25 ± 0.25^{d}	72.02 ± 1.68^{b}	79.0 ± 1.08^{a}	65.25 ± 1.10^{c}
Total RBC (×106 µl)	2.32 ± 0.31^{c}	4.32 ± 0.11^{b}	5.32 ± 0.21^{a}	3.32 ± 0.31^{bc}
Total WBC (×103 µl)	2.04 ± 0.39^{c}	3.04 ± 0.30^{b}	4.0 ± 0.11^{a}	2.4 ± 0.39^{bc}
Albumin (gm/dl)	1.96 ± 0.05^{a}	1.56 ± 0.04^{b}	1.79 ± 0.04^{a}	1.66 ± 0.05^{b}
Total Protein (gm/dl)	3.14 ± 0.06	3.10 ± 0.05	3.20 ± 0.04	3.01 ± 0.07
Lymphocyte (%)	57.17 ± 2.39^{d}	70.17 ± 2.22^{b}	80.11 ± 1.29^{a}	67.17 ± 2.31^{c}
N/L ratio	1.44 ± 0.05^{a}	1.16 ± 0.25^{b}	1.04 ± 0.11^{c}	1.06 ± 0.13^{c}
Phagocytic index	3.74 ± 0.09^{b}	4.77 ± 0.18^{a}	4.93 ± 0.19^{a}	4.58 ± 0.12^{a}
Hemoglobin (g/dl)	9.27 ± 1.42^{c}	10.27 ± 1.02^{b}	11.7 ± 1.1^{a}	10.16 ± 1.3^{bc}
IL2 (Pg/ml)	15.20 ± 1.04^{d}	70.05 ± 5.66^{a}	49.00 ± 2.16^{b}	35.77 ± 1.74^{c}

Table 2: Haematological and biochemical analysis

Ethical Approval

This research was conducted in accordance with declaration of Faculty of Veterinary Medicine, Minia University

1. Conflicts of interest:

The authors declared no conflicts of interest.

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