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EFFECT OF THE CONTENT OF FISH OIL, L- CARNITINE AND THEIR COMBINATION IN DIET ON IMMUNE RESPONSE AND SOME BLOOD PARAMETERS OF BROILERS

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ABSTRACT
An experiment was conducted in order to study the effect of fish oil, L- carnitine and their combination on immune response and some blood parameters of Broilers. One hundred fifty unsexed one day-old chicks (Ross 308) were randomly distributed into three equal groups, (50 birds per treatment) with two replicates as following: T1(control) / birds fed basal diet without supplemented while T2/birds fed basal diet supplemented daily 3% fish oil and T3 /birds fed basal diet supplemented daily 3% fish oil and L-carnitine (50mg /Kg). During the experiment period (35 days). Chicks were vaccinated against Newcastle disease (B1 strain) at the first day of age by spray. While, all others vaccines (Lasota strain) were administrated with drinking water at the age 10, 20, 30 days respectively. Blood samples were collected and then measured at the end of the experiment. Traits involved in this study were RBCs, WBCs, PCV, Hb, H/L Ratio, and antibody titers against Newcastle disease. The results indicate that T3 birds which fed basal diet supplemented daily 3% fish oil and L-carnitine (50mg/ Kg) have a significant ($p<0.05$) improving in RBCs, WBCs, PCV, Hb, H/L ratio and antibody titers against Newcastle disease at age 30 days. In conclusion fish oil with L-carnitine can be used during the breeding period with ration at a level (3% fish oil and L-carnitine (50mg /Kg) could enhance health status of broilers.

KEYWORDS: Broiler, Fish oil, L-Carnitine, Blood picture, Immune response.

INTRODUCTION

Most of the broiler diets include high level of n-6 fatty acids in their fat sources, which directly affects the omega-6: omega-3 fatty acids ratio (Dela Ossa, 2009). Dietary imbalance of omega-6: omega-3 may contribute to the acute inflammatory response and the prevalence of inflammatory-related disorders in broiler chicke Gonzalez, (2009). Polyunsaturated fatty acids are important constituents of the immune cell structure and eicosanoid formation (Stulnig, 2003). Eicosanoid activity depends on the ratio and content of omega-6 and omega-3 fatty acids (Calder, 1998). Eicosanoids play an essential role in modulating inflammatory response intensity and duration (Stulnig, 2003). They are involved in the increase in vascular permeability and vasodilation, which enhances the production of inflammatory cytokines. Cytokines produced by white blood cells serve as regulators to the whole body by exertion of different effects on lymphocytes and other immune cells in response to infection and injury. Omega-6 PUFAs exert pro-inflammatory properties that lead to increase inflammatory eicosanoid, cytokine production and immuno-suppression, while omega-3 PUFAs possess anti-inflammatory or less inflammatory properties by decreasing the release of pro-inflammatory eicosanoids and cytokines (Stulnig, 2003). Therefore, dietary supply of omega-3 PUFAs may affect the development of a strong immune system in birds, increase poultry productivity, reduce disease and thereby contributing to increase economic returns to poultry industry (Gonzales, 2009). Chekani-Azar *et al*. (2007) reported that fish oil contain omega-3 fatty acids Specifically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), as being an important factor in the diet for promoting of health in human and animals. Omega-3 (PUFAs) are essential for playing important role in the prevention of coronary heart disease, hypertension, inflammatory, autoimmune disorders and cancer (El-Yamany *et al*., 2008). From broilers health aspect, omega-3 PUFAs improve immunity, performance, lipid profile besides increasing in marketing weight (Jameel, 2013; Al-Zuhairy and Alasadi, 2013; Sahib, 2013; Jameel and Sahib, 2014; Al-Zuhairy and Jameel, 2014; Jameel, 2014). L-carnitine is a water-soluble quaternary amine that exists naturally in microorganisms, plants and animals (Bremer, 1983). In the presence of vitamin B6, ascorbic acid, nicotinic acid and folic acid, It is biosynthesized in the liver in vivo from two essential amino acids lysine and methionine (Rebouche & Paulson, 1986a). These vitamins are required as co-factors for the enzymes involved in the metabolic pathway of L-carnitine (Rebouche & Paulson, 1986b; Feller & Rudman, 1988; Rebouche, 1991; Baumgartner & Blum, 1993; Arslan, 2006). It has been reported that L-carnitine has two major functions. The best known is to facilitate the transport of long-chain fatty acids across the inner mitochondrial membrane. Thus, dietary L-carnitine supplementation promotes the β-oxidation of these fatty acids in order to generate adenosine triphosphate (ATP) energy and improve energy utilisation (Rabie *et al*., 1997; Neuman *et al*., 2002). L-carnitine is known to increase antioxidant status during aging. It is accepted that L-carnitine represents the second line of cell defence against reactive oxygen species and

their derivatives as it breaks free-radical chain reactions (termination of peroxidation) and prevents undesirable oxidation reactions (Arenas *et al*., 1998). By reducing the amount of oxidative damage that occurs as a result of peroxidation of polyunsaturated fatty acids found in membrane phospholipids, L-carnitine plays a major role in stabilizing cell membranes and in regulating the function of ion channels (role in calcium transport) (Kalaiselvi and Panneerselvam 1998). Therefore, the present experiment was conducted to investigate the effects of ration that contained fish oil with or without L-Carnitine on RBCs, WBCs, PCV, Hb, H/L ratio and antibody titers against Newcastle disease of broilers.

MATERIAL & METHODS

Experimental design

One hundred fifty day-old unsexed broilers chicks (Ross-308) were bought from a commercial hatchery and divided randomly and equally into three treated groups of 50 birds, each treated group was subdivided into two replicates of twenty five birds per replicate. The first group T1(control) / birds fed basal diet without supplemented while T2/birds fed basal diet supplemented daily 0.3% fish oil and T3 /birds fed basal diet supplemented daily 0.3% fish oil and L-carnitine (50mg / Kg).

Rearing Program

The chicks were management according to (Aviagen, 2009). Feed and water provided in *ad-Libitum* during the experiment. A two-phase feeding program consists of offering a starter (1-21 days of age) and finisher (22-35 days of age) was provided to the broilers. Diets were formulated to meet or exceed requirements by the National Research Council (NRC, 1994) table (1). Light was provided the whole day long with only one hour cut off to get them used to the darkness.

Blood Samples Collection and Laboratory Analysis

On day 35th of age, blood samples from five broilers in each replicate randomly were collected from the bronchial vein in a test tube with EDTA anticoagulant. Hematological parameters such as RBCs, WBCs, PCV, Hb, as well as H/L ratio were determined by routine methods as previously described (Al-Daraji *et al*., 2008). Also on day 15th and 30th of age, blood samples were collected from five birds in each replicate from the bronchial vein in a test tube without anticoagulant. The blood allowed to clot and centrifuged for 10 minutes at 3000 rpm to obtain on serum which stored in deep freeze (-20) until analysis. Serum was performed according to the manufacturer's instructions listed in the Proflok ELISA Kit (Synbiotics–USA), which is a rapid serological test for the detection of antibody in chicken serum samples

Statistical analysis

Data generated from experiment was carried out in a complete randomized design (Steel and Torrie, 1980). These data were subjected to ANOVA according to general linear model procedure of SPSS software (SPSS, 2001). The significant differences among means were determined by Duncan's multiple range tests with ($p \leq 0.05$) level of significance.

TABLE 1: compositions of experimental diet according to (NRC, 1994)

Ingredient %	Starter diet			Finisher diet		
	T1	T2	T3	T1	T2	T3
Yellow corn	36	36	36	44	44	44
Soybean meal(48% protein)	30	30	30	26	26	26
Wheat	26	26	26	20	20	20
Protein concentrate	5	5	5	5	5	5
Sunflower oil	1.5	1.2	1.2	3.5	3.20	3.20
Fish oil"	-	0.3	0.3	-	0.3	0.3
L-Carnitine (mg\ Kg)	-	-	50	-	-	50
Premix*	0.1	0.1	0.1	0.1	0.1	0.1
Lime stone	1	1	1	1	1	1
Salt	0.3	0.3	0.3	0.3	0.3	0.3
Dicalcium phosphate	0.1	0.1	0.1	0.1	0.1	0.1
Total	100	100	100	100	100	100
Calculated chemical analysis						
Metabolize energy (kcal/kg)	2926	2926	2926	3097.8	3097.8	3097.8
Crude protein (%)	22.4	22.4	22.4	20.5	20.5	20.5
Calcium (%)	0.82	0.82	0.82	0.80	0.80	0.80
Available phosphorus (%)	0.61	0.61	0.61	0.58	0.58	0.58
Methionine (%)	0.61	0.61	0.61	0.58	0.58	0.58
Lysine (%)	1.74	1.74	1.74	1.63	1.63	1.63

* Premix produced in Jordan (VAPCO®) which contains: vit A 8000000 IU; vit D3 1500000 IU; vit E 1000 IU; vit K3 2000 mg; vit B1 500 mg; vit B2 500 mg; vit B6 200 mg; vit B12 8 mg; ca pantothenate 400 mg; nicotinamide 6000 mg; folic acid 50 mg; methionine 13 mg; lysine 61 mg; aspartic acid 92 mg; glutamic acid 166 mg; cysteine 1 mg; valine 40 mg; tyrosine 9 mg; glycine 382 mg; arginine 117 mg; leucine 48 mg; phenylalanine 40 mg; Mn sulphate 0.40 gm; zinc sulphate 0.15 gm; iron sulphate 0.50 gm; copper sulphate 0.04 gm; cobalt chloride 0.01 gm.

RESULTS & DISCUSSION

The effects of dietary fish oil with or without L-carnitine on RBCs, WBCs, PCV, Hb, H/L Ratio, and antibody titers against Newcastle disease are shown in (Table 2 and 3). The result revealed that RBCs, WBCs, PCV, and Hb were increased, while H/L Ratio was improved significantly ($p>0.05$) in T3 (chicks fed basal diet supplemented with 0.3% fish oil and 50 mg/ Kg L-carnitine) as compared with T2 (chicks fed basal diet with 0.3% fish oil) and control group.

Data of antibody titers against Newcastle disease at 15th, and 30th days are presented in (Table 3) which are referred that improved significantly (p>0.05) in T3 (chicks fed basal diet supplemented with 0.3% fish oil and 50 mg/ Kg L-carnitine) as compared with T2 (chicks fed basal diet with 0.3% fish oil) and control group.

TABLE 2: Effect of different treatments on RBCs, WBCs, PCV, Hb, H/L Ratio. Mean ± SE

Parameters \ Treatments	T1	T2	T3
RBCs ($x10^6/mm^3$)	4.93±0.80 b	5.68±0.49 ab	6.3±0.54 A
PCV (%)	33±0.95 b	35±0.78 a	35.9±0.68 A
Hb (mg/dl)	11.01±1.32 b	11.63±2.21 b	12.33±1.12 A
WBCs ($x10^3/mm^3$)	21.2±0.09 b	23.33±1.09 ab	24.41±1.06 A
H/L ratio	0.32±0.01 b	0.29±0.03 ab	0.27±0.02 A

Different letters in the same raw denoted significant differences between treatments at a level ($p \leq 0.05$).

TABLE 3: Effect of different treatments on antibody titers against Newcastle disease (Mean ± SE)

Parameters \ Treatments	T1	T2	T3
15 days	2019±21.0 c	2641.05±31.44 b	2922.19±36.20 A
30 days	1001.13±14.33 c	1566±9.3 b	2172.53±42.49 A

Different letters in the same raw denoted significant differences between treatments at a level ($p \leq 0.05$).

The increase of RBCs, WBCs and improved of antibody titers against Newcastle disease could be due to increase the ratio of omega-3: omega-6 PUFA to be more important in modulating eicosanoid synthesis. In addition, PUFAs have been associated with different effects on T-cell responses *in vivo* (Dewille *et al*., 1981; Stulnig *et al*., 2000). Enrichment of cell membrane with omega-3 PUFAs is associated with immune cell structure and eicosanoid formation. Omega-3 PUFAs possess anti-inflammatory or less inflammatory properties by decreasing the release of pro-inflammatory eicosanoids and cytokines (Stulnig, 2003). Our results are with agreement with (Bond *et al*., 1997; Radwan *et al*., 2012; Jameel, 2013; Al-Zuhairy and Alasadi, 2013; Jameel and Sahib, 2014; Al-Zuhairy and Jameel, 2014) who showed that omega-3 led to significant increase of WBCs, RBCs, and PCV and improving of antibody titer against Newcastle disease. This result may be accur due to physiological changes in metabolism due to the presence of omega-3 PUFA also the ratio of omega-3: omega-6 PUFA appears to be more important in modulating biosynthesis of eicosanoid than the absolute concentration of omega-3 PUFA in the diet (Boudreau *et al*., 1991). Also L-carnitine represents the second line of cell defence against reactive oxygen species and their derivatives as it breaks free-radical chain reactions (termination of peroxidation) and prevents undesirable oxidation reactions (Arenas *et al*., 1998). By reducing the amount of oxidative damage that occurs as a result of peroxidation of polyunsaturated fatty acids found in membrane phospholipids, L-carnitine plays a major role in stabilizing cell membranes and in regulating the function of ion channels (role in calcium transport) (Kalaiselvi and Panneerselvam 1998). Enrichment of cell membrane with omega-3 PUFAs could be decreased inflammatory response, improved of growth rate, erythropoiesis, leucopoiesis and increased specific immunity (Korever and Klasing, 1997). On the other time, early and daily fed bird with omega-3 may be led to rapidly develop their intestinal system and have a greater numbers of cells per crypt and number of crypts per villi (Uni *et al*., 1998).

CONCLUSION

It can be concluded from the results obtained in this study that RBCs, WBCs, PCV, Hb, H/L ratio and immune response against Newcastle disease were improved in broilers fed on ration containing (0.3% fish oil and 50 mg/ Kg L-carnitine).

REFERENCES

Al-zuhairy, M. A. & Alasadi, Y. j. (2013) Effect of in ovo injection with Newcastle disease vaccine, multivitamins AD3E, and omega-3 on performance and immune response of broiler. *International Journal of advanced Biological Research*, 3(2): 208-211.

Al-zuhairy, M. A. and Jameel Y. J. (2014) Effect of ND Vaccine, Multivitamins AD3E, and Omega-3 on Performance and Immune Response of Broilers. MRSVA 3 (1), 42-50.

Arenas J., Rubio J.C., Martin M.A., Camposy. (1998) Biological roles of L-carnitine in perinatal metabolism. Early Human Development, 53, 43–50.

Arslan C. (2006) L-carnitine and its use as a feed additive in poultry feeding a review. Revue de Médicine Véterinaire 157, 3, 134–142.

Aviagen (2009) Ross broiler management manual. Technical transfer and service teams. USA, pp: 1-112.

Baumgartner, M. & Blum, R. (1993) L-carnitine in animal nutrition. Pages 413-418 in vitamine und weitere zusatzstoffe bei mensch und tier (vitamins and other supplements for humans and animals). (G 5. Flachowsky and R Schubert, editors). Friedrich-Schiller Universitaet, Jena, Germany.

Bond, J. M., Julian, R.J. and Squires, E.J. (1997) Effect of dietary flaxseed on broiler growth, erythrocyte deformability, and fatty acid composition of erythrocyte membranes. *Canadian Journal of Animal Science*, 77:279-286.

Boudreau, M. D., Chanmugam, P.S., Hart, S.B., Lee, S.H. and Hwang, D.H. (1991) Lack of dose response by dietary n-3 fatty acids at a constant ratio of n-3 to n-6 fatty acids in suppressing eicosanoid biosynthesis from arachidonic acid. *American Journal of Clinical Nutrition*, 54: 111-117.

Bremer, J. (1983) Carnitine metabolism and functions. Physiol. Rev. 63, 1420-1480.

Calder, P. C. (1998) Immunoregulatory and anti-inflammatory effects of n-3 polyunsaturated fatty acids. *Brazilian Journal of Medical and Biological Research*, 31: 467-490.

Chekani-Azar, S., Maheri-Sis, N., Shahriar, H. A. and Ahmadzadeh, A. (2007) Effect of different substitution levels of fish oil and poultry fat on performance and parts of carcass on male broiler chicks. *Journal of Animal and Veterinary Advances*, 6(12): 1405-1408.

DeLa Ossa, T. I. P. (2009) Omega-3 enrichment and oxidative stability of broiler chicken meat. Msc. Thesis in food science and technology. Alberta University.

Dewille, J. W., Fraker, P. J. and Romsos, D. R. (1981) Effects of dietary fatty acids on delayed-type hypersensitivity in mice. *Journal of Nutrition*, 111: 2039-2043.

El-Yamany, A. T., El-Allawy, H. M. H., El-Samee, L. D. A. and EL-Ghamry, A. A. (2008) Evaluation of using different levels and sources of oil in growing Japanese quail diets. *American-Eurasian Journal of Agricultural & Environmental Sciences*, 3(4): 577-582.

Feller, A.G. & Rudman, D. (1988) Role of carnitine in human nutrition. J. Nutr. 118, 541-547.

Gonzalez, D. (2009) Effect of dietary fatty acids, Time of feeding and immune response in poultry. M.Sc. Thesis. Oregon State University.

Jameel, Y. J. (2014) Effect of Supplementation Ration with Fish Oil, L- Carnitine and their combination on Broilers Performance. International journal of science and nature (IJSN). Vol., 5(3).

Jameel, Y. J. (2013) The effect of in-ovo injection with Newcastle disease vaccine, multivitamins AD3E and omega-3 oil on subsequence productive performance and some physiological parameters of broiler chicks. Ph.D. Dissertation in science of poultry hygiene. College of Veterinary Medicine. University of Baghdad. Iraq.

Jameel, Y.J. and Sahib A.M. (2014) Study of some Blood Parameters of Broilers Fed on Ration Containing Fish Oil. Journal of Biology, Agriculture and Healthcare. ISSN 2224-3208 (Paper) ISSN 2225-093X (Online). Vol.4, No.7, pp:67-71.

Kalaiselvi C.J. & Panneerselvam C. (1998) Effect of L-carnitine on the status of lipid peroxidation and antioxidants in aging rats. Journal of Nutritional Biochemistry 9, 575–581.

National Research Council (NRC) (1994) Nutrient requirements of poultry. 9th ed. National Academy Press. Washington. D. C. USA.

Neuman, S.L., Lin, T.L. & Hester, P.Y. (2002) The effect of dietary carnitine on semen traits of white leghorn roosters. Poult. Sci. 81, 495-503.

Rabie, M.H., Szilagyi, M., Gippert, T., Votisky, E. & Gerendai, D. (1997) Influence of dietary L-carnitine on performance and carcass quality of broiler chickens. Acta Biolog. Hung. 48 (2), 241-252.

Radwan, N. L., Abd El-Samad, M. H. and Sherin, A. (2012) Effects of different dietary rations of linoleic acid to alinolenic acid on productive performance, immunity of laying hens and egg yolk fatty acid composition. *Egypt Poultry Sciences*, 32: 163-188.

Rebouche, C.J. (1991) Ascorbic acid and carnitine biosynthesis. Amer. J. Clin. Nutr. 54, 1147-1152.

Rebouche, C.J. & Paulson D.J. (1986a) Carnitine metabolism and functions in humans. Ann. Rev. Nutr. 6, 41-66.

Rebouche, C.J. & Paulson, D.J. (1986b) Carnitine. Ann. Rev. Biochem. 57, 261-283.

Sahib, A. M. (2013) Effect of ration supplementation with different levels of fish oil and flaxseed oil on productive traits in broilers. M.Sc. Thesis in science of public health. College of Veterinary Medicine. University of Baghdad. Iraq.

Statistical Packages for the Social Sciences (2001) Statistical software for windows version 11.Microsoft. Chicago. I. L. USA.

Steel, R. G. and Torrie, J. H. (1980) Principle and procedures of statistics.2nd ed. McGraw-Hill Book Co. Inc. New York. USA, pp: 183-193.

Stulnig, T. M. (2003) Immunomodulation by polyunsaturated fatty acids: Mechanisms and effects. *International Archives of Allergy and Immunology*, 132: 310-321.

Stulnig, T. M., Berger, M., Roden, M., Stingl, H., Raederstorff, D. and Waldhäusl, W. (2000) Elevated serum free fatty acid concentrations inhibit T lymphocyte signaling. *FASEB Journal*, 14: 939-947.