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EFFECT OF DIETARY OMEGA-3 FATTY ACID ON ANTIBODY PRODUCTION AGAINST NEWCASTLE DISEASE IN BROILERS

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ABSTRACT
The objective of the present study was to analyze the effects of dietary omega-3 polyunsaturated fatty acids (flaxseed oil source) on immune response and antibody production against Newcastle disease. Three equally treated groups (50 birds per treatment) with two replicates (25 birds per replicate) of total 150 unsexed chicks (Ross 308) at age one day old chicks were randomly weighed and divided into three dietary treatments: T1 (as control group) birds fed basal diet without any supplemented. While, T2 and T3 fed basal diet supplemented daily with 0.25 and 0.5% flaxseed oil respectively to the end of experiment (35 days). ELISA antibody titers against Newcastle disease, and relative weights of spleen and bursa were determined. Blood samples were collected and then analysis, in addition, spleen index and bursa index were measured at the end of experiment. No significant difference in all parameters at a level 0.25% flaxseed oil While, antibody titers against Newcastle disease at age 15^{th} and 30 days, and spleen percentage and bursa of fabricius percentage, total protein, albumin, and globulin were significantly improved, while glucose, ALT, AST and ALP were decreasing by using 0.5 % flaxseed oil. In conclusion, dietary addition of 0.5% flaxseed oil may be stimulate the development of the immune response and antibody production against Newcastle disease and enhancement the blood biochemical parameters and health status of broilers.

KEY WORDS: Broilers, Nutrition, Omega-3, Antibody production, Newcastle disease.

INTRODUCTION

Supplementation of fats and oils in commercial broiler diets has become routine to achieve the recommended energy requirement, and realize optimum growth and development. One of the most efficient ways to induce the accumulation of polyunsaturated fatty acid (PUFA) in chicken meat has been achieved through the modification of dietary fatty acids. Fatty acids (FA) are important components of energy metabolism, cell membrane formation, and signaling processes (Jump *et al*., 2008). Linoleic acid (LA; C18:2; omega-6) and α-linolenic acid (ALA; C18:3; omega-3) are essential FA involved in many biological functions and must be supplemented in the diets of mammalian and avian species (Simopoulos, 2009). The consumption of high proportions of omega-6 fatty acids has been associated with a higher incidence of health problems, such as coronary artery diseases, obesity and type-2 diabetes (Simopoulos, 1999). High bioavailability of omega-6 fatty acids, leads to production of pro-inflammatory eicosanoids increasing the incidence of inflammatory related disorders in poultry (Calder, 1998); Therefore, a need exists to develop alternative food products with high levels of omega-3 fatty acids that could help in preventing or reducing the incidence of diseases. To prevent these adverse responses to excessive dietary omega-6 FA, a proper balance between omega-6 and omega-3 FA is required. However, an ideal omega-6: omega-3 ratio is difficult to maintain due to a high proportion of omega-6 FA in both animal and human diets. A typical ratio of omega-6 to omega-3 in western diets ranges from 10:1 to 25:1 (Simopoulos, 2000), and it is far from the ideal range of 3:1 to 6:1 (Simopoulos, 2000; Wijendran and Hayes, 2004; El-Badry *et al*., 2007). Omega-3 fatty acids possess anti-inflammatory or less inflammatory properties by decreasing the release of pro-inflammatory eicosanoids and cytokines (Stulnig, 2003). 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. From the human health aspect, omega-3 fatty acids are essential for playing important role in the prevention of coronary heart disease, hypertension, inflammatory, autoimmune disorders and cancer (El-Yamany *et al*., 2008). As well as, omega-3 fatty acids were improved immunity, performance, blood lipid profile besides increasing in market weight (Jameel, 2013; Al-zuhairy and Alasadi, 2013; Sahib, 2013; Jameel, 2014a, b; Al-zuhairy and Jameel, 2014; Jameel and Sahib, 2014). The objective of this study was to evaluate the effect of dietary omega-3 polyunsaturated fatty acids (flaxseed oil source) on immune response and ELISA antibody production against Newcastle disease also relative weights of spleen and bursa.

MATERIALS & METHODS

Birds, housing and 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 2 replicates of 25 birds per replicate. The first group (T1) was daily fed on basal diet without supplementation of flaxseed oil as a control group. While, second group (T2) and third group (T3) were fed daily on basal diet containing 0.25 and 0.5% flaxseed oil respectively. Birds were management according to (Aviagen, 2009). Feed and water were provided *ad libitum*. Two types of diets (starter and finisher) were used over the period of experiment (35 days) (Tables 1). Chicks were vaccinated against Newcastle disease (ND) (B1 strain) and infectious bronchitis at the first day of age by spray. While, all others vaccines were administrated with drinking water which includes: ND (Lasota strain) at age 10 days, Gumboro (IBD2) at age 14 days, ND (Lasota strain) at age 20 days and ND (Lasota strain) at age 30 days. Vitamin C was added at the rate of 1gm/liter for 3 days after each vaccination and for duration of 3 days.

TABLE 1: Composition of experimental diets (starter and finisher) according to (NRC, 1994).

	Starter diet (1- 21day)			Finisher diet(22-35day)		
Ingredient %	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	1.25	1	3.5	3.25	3
Flaxseed oil	-	0.25	0.5	-	0.25	0.5
Premix	0.1	0.1	0.1	0.1	0.1	0.1
Limestone	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	397.8
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

Blood samples collection and laboratory analysis

At day 15th and 30th of age, blood samples from six birds in each treatment randomly were collected from the bronchial vein in a test tube without anticoagulant. The blood was allowed to clot and centrifuged for 10 minutes at 3000 rpm to obtain on serum which stored in a deep freeze (-20Cº) (Al-Daraji *et al*., 2008). Antibody titers against Newcastle disease were determined by using of diagnostic kit and ELISA.

Calculation of relative weights of spleen and bursa

At day 35th of age, three birds were taken from each replicate randomly and weighted individually using digital balance and then slaughtered. The head, feather, viscera, and legs were removed and washed. The spleen and bursa were separated from other organs and tissues and then weighted using an electrical balance, to get the weight of spleen and bursa as percentage.

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 L.S.D. multiple range tests with $p \leq 0.05$ level of significance.

RESULTS & DISCUSSION

The effect of supplementation ration with flaxseed oil on antibody titers against ND virus were presented in (Table 2) and result revealed that antibody titers were increased significantly ($p \leq 0.05$), in T3 (chicks fed basal diet supplemented with 0.5% flaxseed oil) as compared with T2 (fed basal diet with 0.25% flaxseed oil) and control group. Spleen and bursa percentage were presented in (Table 3) and result revealed that increased significantly ($p \leq 0.05$) in T3 (chicks fed basal diet supplemented with 0.5% flaxseed oil) as compared with T2 (fed basal diet with 0.25% flaxseed oil) and control group.

Total protein, albumin, globulin and glucose were presented in (Table 4). Result revealed that total protein, albumin, and globulin were increased significantly ($p \leq 0.05$). While, glucose was decreased significantly ($p \leq 0.05$) in T3 (chicks fed basal diet supplemented with 0.5% flaxseed oil) as compared with T2 (chicks fed basal diet with 0.25% flaxseed oil) and control group. Data of ALT, AST and ALP were presented in (Table 5). The highest significant ($p \leq 0.05$) decrease of liver enzymes were found in T3 (chicks fed basal diet supplemented with 0.5% flaxseed oil) as compared with T2 (chicks fed basal diet with 0.25% flaxseed oil) and control group.

TABLE 2: Antibody titers against ND of broiler chicks during the experimental period. Mean ± standard error

Age \ Treatment	T1(control)	T2 (flaxseed oil 0.25%)	T3 (flaxseed oil 0.5%)
15 days	2352±9.41b	2557.28±15.45 ab	2969.61±31.12 a
30 days	1351.33±9.11b	1950.16±9.42a	2020.33±12.14 a

Different letters in the same raw denoted that significant differences between treatments at a level (p≤0.05).

TABLE 3: Spleen and bursa of fabricius percentage of broiler chicks. Mean ± standard error

Parameters \ Treatment	T1(control)	T2 (flaxseed oil 0.25%)	T3 (flaxseed oil 0.5%)
Spleen	0.06±0.002b	0.07±0.003ab	0.10±0.002 a
Bursa of fabricius	0.08±0.003b	0.08±0.007b	0.10±0.005 a

Different letters in the same raw denoted that significant differences between treatments at a level (p≤0.05).

TABLE 4: Serum total proteins, albumins, globulins and glucose concentration at 35 day old chicks. Mean ± standard error

Parameters \ Treatment	T1(control)	T2 (flaxseed oil 0.25%)	T3 (flaxseed oil 0.5%)
Total protein (g/l)	35±1.82 c	40.36±1.42 b	45.53±1.27 a
Albumin (g/l)	17.60±0.48 a	17.10±0.48 a	17.10±0.46 a
Globulin (g/l)	17.40±2.20 c	23.26±1.40 b	28.43±1.53 a
glucose (mg/dl)	186±2.41 c	172.03±1.34 b	158.08±2.40 a

Different letters in the same raw denoted that significant differences between treatments at a level (p≤0.05).

TABLE 5: AST, ALT and ALP enzyme activity (IU/L) at 35day old chicks. Mean ± standard error

Parameters \ Treatment	T1(control)	T2 (flaxseed oil 0.25%)	T3 (flaxseed oil 0.5%)
ALT	26.60±2.60 b	21.83±2.12 ab	16.08±2.018 a
AST	69.93±1.92 c	59.93±3.21 b	49.91±3.43 a
ALP	248.71±14.41 b	193.40±18.65 ab	138.15±21.40 a

Different letters in the same raw denoted that significant differences between treatments at a level (p≤0.05).

The increase of antibody titer against ND vaccine could be due to supplementation diets with omega-3fatty acids which is an important constituent of the immune cell structure and eicosanoid formation (Stulnig, 2003). 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 during the early post hatch may impact the development of a strong immune system in birds may increase antibody production. Also, competition between LA and LNA in conversion to long-chain FA and eicosanoids in immune tissues most likely contributed to improve antibody production in response to vaccines (Wang *et al*., 2002; Puthpongsiriporn and Scheideler, 2005). Furthermore, Wang *et al*. (2004) reported that, LA to LNA ratio may influence in the binding activity of IgG-receptor on the yolk sac membrane and thus it affects the maternal-embryo transfer of yolk IgG. The increased total IgG and specific antibody IgG in the embryo circulating system during the incubation with the increased LNA or decreased LA to LNA ratio could benefit young chicks by improving the capability of immune defense (Radwan *et al*., 2012). Our results are agreement with (Tobarek *et al*., 2002; AL-Mayah, 2009) showed that diet with fish oil increased significantly antibody titer after vaccination against Newcastle disease with LaSota type vaccine at 35th day of age, these results could be due to omega-3 which considered to be a substrates for the generation of prostaglandin and leukotriene, the two substances were known immunomodulators, fish oil also has the capacity to modulate cytokine production by lymphocyte and signal transduction in immune cell population. Recently, Ebeid *et al*. (2008) indicated that dietary FO levels below 3.5% increased the antibody titre in laying hens. This response is similar to the observations of Yuming *et al*. (2004), who proved that antibody levels were higher in hens fed oils rich in n-3 PUFA (FO or LO) than in hens fed maize oil rich in n-6 PUFA.

The highest significant increase of total proteins may be due to improving immunity and immunoglobulin and increasing of WBCs count because total protein is composed of antibodies and albumin. Al-Mayah (2009) showed that 50 gm/kg of diet fish oil accelerates antibody production (IgG and IgM) then increased serum globulins and maintain proper immune function in chickens fed after vaccination against ND with LaSota type vaccine at day 35th of age as compared with the control group. Tobarek *et al*. (2002) found that chicks fed fish oil before or after vaccination with Hitchner B1 at 7th day or with LaSota at 21th day of age resulted in a significant increase of globulins at both ages. Michel (2002) found that quail fed fish oil had a significant increase of globulins in comparison to that fed the same amount of chicken fat or soybean oil. Wang *et al*. (2000) who mentioned that birds which fed a high level of omega-3 fatty acids such as fish oil had a significantly higher antibody production than that fed animal fat. On the other hand, the results in the experiment are disagreement with (Parmentier *et al*., 1997) who reported that omega-3 fatty acids have a decreasing effect on antibody response in bovine serum albumin injected chickens therefore, total protein increasing has not been noticed because total protein is composed of antibodies and albumin. Adverse effect between glucose and total protein concentration in serum were showed in this study, the highest significant increase of total protein in T3 could be due to improving immunity and immunoglobulin and increasing of WBCs count, on the other hand, decrease in glucose concentration in the same treatment. These results may occur due to a decrease of corticosteroid hormones because there is a relationship between glucose and total protein concentration in serum and corticosteroid hormone levels (Al-Daraji, 1998; Al-Daraji and Al-Hasani, 2000). The increment of corticosteroid hormones leading to increase of glucose levels in plasma due to catabolism of protein by gluconeogenesis process then decrease of serum total protein (Williams, 1984; Freeman, 1988).The reduction of ALT, AST, and ALP activity could be due to the benefit effect of dietary supply of omega-3 PUFAs during early post hatch may affect the development of a strong immune system that quickly and efficiently adapts to the different immune challenges. Omega-3 PUFAs, such as EPA and DHA, have been associated with immuno-modulatory and anti-inflammatory effects by interfering with eicosanoid production derived from archidonic acid (Calder, 1998). Al-Daraji *et al*. (2010) reported that supplemented diet of quails with fish oil and flaxseed oil significant decrease of ALT and AST as compared with treatments fed sunflower and corn oil also with control group.

CONCLUSION

It can be concluded from this study that supplemented diets with flaxseed oil at a level 0.5% better than 0.25% for enhancement antibody titers against Newcastle disease at age 15th and 30 days, spleen percentage and bursa of fabricius percentage, and enhancement of blood total protein, albumin, globulin, glucose, ALT, AST, ALP concentration.

RECOMMENDATIONS

Ration that daily supplementation 0.5% flaxseed oil may be stimulate the development of the immune response against Newcastle disease at the age 15 and 30 days, lymphoid organs weight, finally enhance health status and thereby contributing to increase economic returns to the broilers industry.

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