

EFFECT OF FISH OIL ON HUMORAL IMMUNITY OF BROILER CHICKS

Ali Abd Saham AL-Mayah

Department of Pathology and Poultry Diseases, College of Veterinary Medicine, University
of Basrah,Iraq

(Received 13 July 2009, Accepted 14 September 2009)

Keywords: Fish oil, Broiler, Vaccine,

ABSTRACT

Many clinical studies have reported that fish oil supplementation has beneficial effects supporting the idea that n-3 polyunsaturated fatty acids in fish oil are anti-inflammatory and immunomodulatory. Sixty one-day old broiler chicks were used in this study to determine the effect of fish oil on humoral immunity. They were divided into 3 equal groups (A, B &C) and raised under the same conditions until the end of the study at 49 day of age. The birds were vaccinated 3 times against Newcastle disease, at the 7th day they were vaccinated with Hitchner B1 and at 21st day and 35th day with LaSota type vaccine. Group A was fed fish oil supplemented diet for 14 days before the 3rd vaccination, whereas group B was fed after vaccination. Group C was served as control. Serum samples have been collected at 49th day from all groups. Antibody titer was detected by HI test; 2-mercaptoethanol-sensitive IgM and 2-mercaptoethanol-resistant IgG were also measured. Serum total protein, albumin and globulins were also estimated. Differential white blood cell count was performed to detect the number of heterophils and lymphocytes and their ratio. The results indicated that HI antibody titer was significantly ($p < 0.05$) increased in both group A and B. Two-mercaptoethanol-sensitive IgM and 2-mercaptoethanol-resistant IgG were also showed significant ($p < 0.05$) increase of IgG in both groups. The present study was also revealed a significant ($p < 0.05$) increase of serum globulins of group A and B in comparison to that of control group. Heterophils and lymphocytes percentage of both treated groups (A and B) significantly higher than that of untreated control group. These results showed that 50 gm/kg of diet fish oil accelerates antibody production and maintain proper immune function in chickens fed after vaccination against Newcastle disease with LaSota type vaccine at 35th day of age.

INTRODUCTION

Essential fatty acids are vital nutritional components that bodies need it for many functions. Fatty acids are considered essential if the body unable to synthesize it and the body can be obtained from the diet. In addition, it considered essential if its deficiency will cause a disease. Many researchers discovered that if an animal did not get essential fatty acids in the diet, it could cause symptoms such as: poor reproduction, lowered immunity, rough dry skin and slow growth (1).

Many clinical studies have reported that fish oil supplementation has beneficial effects in rheumatoid arthritis, asthmatics, supporting the idea that n-3 polyunsaturated fatty acids in fish oil are anti - inflammatory and immunomodulatory (2).

In animal species, dietary intake is the sole source of n-3 fatty acids, such as fish oil, due to lack of enzymes for synthesizing these fatty acids, therefore it is essential for maintenance of normal health (3; 4).

Omega-3 polyunsaturated fatty acids have very strong immunomodulatory activities and among those omega-3 polyunsaturated fatty acids, those from fish oil. Fish oil is the best single source of omega-3 fatty acid (5).

Due to easy accessibility of fish oil as n-3 fatty acid source, use of various levels of fish oil in poultry diets had been reported in the recent years (6).

Freidman (7) was examined the effect of increasing amounts of n-3 polyunsaturated fatty acids on antibodies production of growing turkeys after vaccination against Newcatle disease, specific antibody production was related quadratically to n-3 polyunsaturated fatty acids such as fish oil or linoleic acid. Therefore the aim of this study was to investigate the effect of fish oil on the humoral immunity before and after vaccination of chickens with Newcastle disease vaccine of LaSota type at 35th day of age.

MATERIALS AND METHODS

Sixty one- day old broiler chicks were used in this study. They were equally divided into three groups (A, B and C) and raised under the same conditions until the end of experiment at 49 days. The chicks were vaccinated via drinking water at the 7th day with Hitchner B1 of Newcastle disease vaccine (Ceve Sant Animale, Hungary) and LaSota strain (Ceva Sant Animale, Hungary) at 21st and 35th day respectively. Group A and B were fed diet

containing 50 gm/kg of fish oil (Shanghai – China), whereas group C was served as control. Fish oil supplementation has been used for 14 days before and after the third vaccination with LaSota in group A and B respectively as shown in the design of the study (8).

Table 1: experimental designe

GROUPS	NO. OF BIRDS	FISH OIL SUPPLEMENTATION			3 rd VACCINATION		TIME OF BLOOD COLLECTION
		AGE / DAY	QUANTITY/ MG/KG DIET	DURATION/ DAY	AGE / DAY	TYPE OF VACCINE	
A	20	21 st	50	14	35 th	LaSota	49 th day of age
B	20	35 th	50	14	35 th	LaSota	49 th day of age
C	20	CONTROL			35 th	LaSota	49 th day of age

Blood has been collected via wing veins from fifteen birds of each group. Serum samples have been collected at 49th day from all groups, as shown in Table 1. Blood samples were collected into a plastic test tubes containing EDTA. Serum samples were obtained by centrifuging the blood at 1500 rpm about 10 minutes. Ten serum samples of about 4 ml of until blood analysis (9). 10 each from each group were selected and kept at - 20 c

Hemagglutination inhibition (HI) test was applied for the detection of antibody level against Newcastle disease vaccine using the method designed by (10).

Two – mercaptoethanol resistant antibody (immunoglobulin IgG) was determined by incubating the serum samples with an equal volume (0.05 ml) of 2 molarity 2- for 30 minutes prior to HI 10 mercaptoethanol solution (BDH Limited Pool - England) at 37 c test. Two – mercaptoethanol sensitive antibody (IgM) was determined by subtracting (IgG) from total antibody (11).

Total serum protein, albumin and globulins were determined according to the method of or (12) and the directions of kits manufacturers (Biocon – Germany) and (Biomaghreb – Tunisia).

A thin smear of each blood sample was made on a glass slide and stained with Wright's stain and examined for differential white blood cells counting. Heterophils / lymphocyte ratio was determined through dividing the heterophils numbers by lymphocytes numbers (13,14).

All data were analyzed as a two way ANOVA with fish oil as main effect in a completely randomized design (15). Significance was declared at $p < 0.05$.

RESULTS AND DISCUSSION

Table (2): Effects of fish oil on HI antibody titers of group A & B

Groups	No. of examined Samples	Mean of HI titers (Log2) \pm SE
A	10	5.80 \pm 0.241 a
B	10	6.06 \pm 0.250 a
C	10	4.60 \pm 0.320 b

^{a, b} Mean figures with different superscripts in vertical column were significantly different at (p < 0.05) SE = standard error

Data presented in Table (2) indicated that the effect of fish oil on HI antibody titers in respectively vaccination after and chickens of groups A and B which fed fish oil before and vaccinated against

strain of vaccine at 35th day of age, resulted in a significant Newcastle disease with LaSota (p < 0.05) increase HI antibody titers in group A and B as compared with that of the control group.

Although, a numerical difference was present between the titer of group A and B but it was not statistically significant.

This result was in agreement with that of (16), who found that HI antibody titer in quail vaccinated with LaSota type vaccine at 21 day old and fed fish oil for two weeks was increased in comparison with that of control which vaccinated at the same age with the same type of vaccine and fed the same amount of soybean oil.

The result was also in agreement with that of (17) who reported that addition of fish oil to the chicken diet caused stimulation of antibody production.

(7) mentioned that turkey fed n-3 polyunsaturated fatty acids after vaccination with LaSota type vaccine resulted in an increase specific antibodies.

of chicken, in this study, was obviously enhanced. This humoral immunity that It is clear enhancement, as explained by (19,20)

(15), may be directly due to fish oil which considered to be a substrates for the generation of prostaglandin and leukotrene. The later two substances are known to be immunomodulators, fish oil also has the capacity to modulate cytokine production by lymphocyte and signal transduction in immune cell population. (21) was prove that broiler chicks vaccinated with Hitchner B1 and LaSota vaccines at 7th and 21st day of age respectively and supplemented with fish oil in a ratio of 50gm / kg of diet for 14 days before or after each vaccination were exhibited an obvious increase of HI antibody titers in comparison with those which were not fed fish oil.

On the other hand, the result of the present study was in disagreement with that of (22) who found that dietary supplementation of fish oil has been shown to suppress interleukin-1 decrease in (IL-1) production in various animal models. Suppression of (IL-1) causes and hampering the expression of cell adhesion molecules. proliferation lymphocyte

Table (3): Effect of fish oil on 2-mercaptoethanol – sensitive (IgM) and resistant (IgG) in group A and B

Groups	No. of tested Samples	Mean IgM titer (log2) \pm SE	Mean IgG titer (log2) \pm SE
A	10	0.9 \pm 0.143 a	4.9 \pm 0.195 a
B	10	0.66 \pm 0.081 b	5.4 \pm 0.282 a
C	10	0.8 \pm 0.132 a	3.8 \pm 0.119 b

^{a,b} Mean figures with different superscripts in the vertical column were significantly different at (p < 0.05).
SE = standard error

Table (3) showed that the effect of fish oil on 2-mercaptoethanol sensitive immunoglobulin IgM and 2-mercaptoethanol resistant immunoglobulin IgG in the chickens of group A and B which were vaccinated against Newcastle disease with LaSota vaccine strain at 35th day old, resulted in a significant increase ($p < 0.05$) IgG titer in both group A and B in comparison with that of group C.

The IgG titer of group B which fed fish oil after vaccination was higher than that of group A but it has no significant value, whereas IgM titer of group A which fed fish oil before vaccination was more than that of group B. These differences may be due to the time of fish oil supplementation.

The significant increment of IgG in both treated groups in comparison to the control group in this study was in agreement with that of Weng and Denbow who found that IgG titer 0.05) in quail fed fish oil when compared with that of other <was significantly higher at (p groups which fed hydrogenated soybean oil or chicken fat.

The result of the present study was also in agreement with that of (23) who reported that chicks which fed fish oil had higher ($p < 0.05$) serum IgG than that which fed sunflower oil or linseed oil. This result was also in agreement with that of (21) who reported that broiler chickens fed fish oil before and after vaccination with LaSota vaccine at 21st day of age revealed significant ($p < 0.05$) increase in IgG titer as compared with those of control birds which were only vaccinated.

Table (3) also showed that IgG titer was higher than IgM titer. This may be explained by the fact that IgG production is larger in quantity and more prolonged in the secondary immune response compared to the primary immune response.

The increase IgG production in this study was in disagreement with that of. (24) who found that fish oil decrease antibody IgG production.

Table (4): Effect of fish oil on serum total protein, albumin and globulins of chickens in group A and B

Groups	*Mean of total Protein \pm SE	Mean value of Albumin \pm SE	Mean value of Globulins \pm SE
A	4.97 \pm 0.091 a	1.90 \pm 0.031 a	3.07 \pm 0.032 a
B	5.15 \pm 0.087 a	1.83 \pm 0.052 a	3.32 \pm 0.043 a
C	4.16 \pm 0.081 a	2.23 \pm 0.030 b	1.93 \pm 0.011 b

^{a,b} Mean figures with different superscripts in the vertical column were significantly different at $p < 0.05$. SE = standard error

*Data are means of ten samples for each parameter analyzed.

Table (4) expressed that the effect of fish oil on serum total protein, albumin and globulins in chickens of group A and B which fed fish oil before and after vaccination with Lasota at 35th day of age, resulted in a significant increase ($p < 0.05$) of serum globulins in both group A and B in comparison with that of control.

This increment is an indication of immune status improvement which related to fish oil supplementation. There was no significant difference between group A and B, which indicated that fish oil feeding before or after vaccination has no important effect on immunoglobulins production.

The results presented in Table (4) were in agreement with that of (21) who found that chicks fed fish oil before or after vaccination with Hitchner B1 at 7th day or with LaSota at 21st day of age resulted in a significant ($p < 0.05$) increase of globulins at both ages.

(25) found that quail fed fish oil had a significant ($p < 0.05$) increase of globulins in comparison to that which fed the same amount of chicken fat or soybean oil.

The result of the present study was in line with that of. (22) who mentioned that birds which fed a high level of n-3 poly unsaturated fatty acids such as fish oil had a significantly higher antibody production ($p < 0.05$) than that fed animal fat.

On the other hand this result was in disagreement with that of. (25) who reported that n-3 poly unsaturated 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. (23) mentioned that fish oil impaired IgG antibody production in mice infected with influenza virus.

Table (5): Effect of fish oil on heterophils and lymphocytes and Heterophil / lymphocyte (H/L) ratio in group A and B

Groups	*Mean of heterophils %\pmSE	Mean of lymphocytes %\pmSE	H / L \pm SE
A	28.09 \pm 0.784 a	68.84 \pm 0.664 a	0.40 \pm 0.752 a
B	23.4 \pm 0.860 b	66.00 \pm 0.477 b	0.49 \pm 0.954 b
C	23.06 \pm 0.442 ab	60.30 \pm 0.336ab	0.38 \pm 6.124 ab

^{a,b,ab} Means figures with different superscripts in the vertical column were statistically different at ($p < 0.05$) . SE = standard error

*Data are means of ten samples for each parameter analyzed of mixed sex of birds.

Effect of fish oil supplementation on heterophils and lymphocytes of chicken in group A and B which has been shown in table (5) indicated a significant increase ($p < 0.05$) of both heterophils and lymphocytes of group A and B.

These results were in agreement with that of (16,24) who reported that recruiting of polymorphonuclear cells relies on n-3 fatty acids, prostaglandins are required for activation of cell adhesion molecules, and leukotrienes are potent chemo-attractive. Fish oil has the ability for supplementation of these substances. Moreover, movement of heterophils and monocytes of avian species to site of invasion is greatly dependent on cell adhesion molecules. On the contrary, this result was in disagreement with that of (19) who stated that consumption of fish oil diminish T- cell – mediated cytotoxicity, macrophage-mediated cytotoxicity, monocytes and neutrophils chemotaxis in human being.

The present study expressed that birds which fed fish oil after vaccination exhibited better immune response than those fed the same amount before vaccination. This finding is not so easy to be explained, but it may be due to inability of fish oil to provoke immune system directly by itself without the aid of vaccine.

In conclusion, fish oil considered to be a humoral immune modulator, and it is also has the ability to stimulate heterophils and lymphocytes production when given after Newcastle disease vaccination.

Acknowledgement

I will remain grateful and highly indebted to my colleague Dr. Walled Majeed Sakr who painstakingly carried out the laboratory analyses.

تأثير زيت السمك على المناعة الخلطية في أفراخ فروج اللحم

علي عبد سهم المياح

فرع الأمراض وأمراض الدواجن ، كلية الطب البيطري ، جامعة البصرة ، البصرة ، العراق

الخلاصة

تشير الدراسات إلى وجود تأثيرات نافعة لزيت السمك كمضاد للالتهابات ومنظم للمناعة. ولمعرفة هذه التأثيرات الايجابية على المناعة الخلطية صمم هذا البحث حيث تم استخدام ستون فرخا من فروج اللحم بعمر يوم واحد تم تقسيمها إلى ثلاث مجاميع متساوية وتمت تربيتها تحت نفس الظروف ولمدة 49 يوما. لقحت جميع الأفراخ بلقاح النيوكاسل ثلاث مرات ، المرة الأولى بعمر 7 أيام حيث استخدم لقاح نوع Hitchner B1 أما المرة الثانية والثالثة فقد

استخدم لقاح نوع LaSota واستخدم بعمر 21 ، 35 يوما من عمر الأفراخ . تم استخدام زيت السمك بنسبة 50 غم / كغم من العليقة قبل وبعد 14 يوما من التلقيح الثالث للمجموعتين الأولى والثانية على التوالي واستخدمت المجموعة الثالثة كمجموعة سيطرة . جمعت عينات من الدم بواقع 15 عينة لكل مجموعة. تم اختيار عشر عينات من المصل لكل مجموعة وتم قياس الأضداد المثبطة لتلازن الدم فيها بالإضافة إلى حساب الأضداد الحساسة لمادة mercaptoethanol وهي أضداد IgM وكذلك الأضداد المقاومة لنفس المادة وهي أضداد IgG . كما تم كذلك قياس البروتين الكلي والألبومين لمعرفة الكلوبولينات في مصل الدم . وقد تم حساب أعداد Heterophils و Lymphocytes وقد أظهرت النتائج حصول ارتفاع معنوي في عدد الأضداد المثبطة لتلازن الدم وارتفاع معنوي في الـ IgG المقاومة لمادة mercaptoethanol-2. كذلك أظهرت النتائج ارتفاع معنوي في عدد كريات الدم البيضاء من نوع Heterophils و Lymphocytes . في ضوء النتائج المذكورة يمكن الاستنتاج إن استخدام 50 غم / كغم علف من زيت السمك قبل وبعد التلقيح ولمدة 14 يوما ضد مرض النيوكاسل عند استخدام لقاح عترة LaSota بعمر 35 يوما أي في موعد التلقيح الثالث سيسرع في إنتاج الأضداد بالإضافة إلى إدامة وظائف الجهاز المناعي في أفراخ فروج اللحم .

REFERENCES

1. Deborah, Lee (1997). Essential fatty acids (good) fats, all about fat Functions, deficiencies, benefits. J.Nutr.160.(Abstract)
2. Calder, P.C. (2004). Effect of n -3 polyunsaturated fatty acids on Chronic disease (asthma , arthritis) and on immunity. J. Clin. Nutr. 6: 66 – 78.
3. Watkins, B.A. (1991). Importance of essential fatty acids and their Derivatives in poultry. J. Nutr.121 (Abstract). Weng,B.C and D.Denbow (2000).Dietary lipids fish oil,soybean and chicken fat affected both cell - mediated immunity and humoral immunity in vivo and vitro of quail .An . Sci 160:170-180.
4. Calder,P.C.(1997). N-3 polyunsaturated fatty acids and immune cell function. Adv. Enzy. Regul. 37:197 – 237.
5. Richell,B.D. (2001). Fish oil, fish consumption, omega -3 fatty acids And cardiovascular disease. J. Nutr.160: 1050 -1055.
6. Marshall,L.H. (1994) .The essential omega -6 and omega -3 fatty acids In food. J.Lip.Resea.20:40 – 45.
7. Freidman, A.C.(1997) . Effect of dietary fatty acids on humoral Immune response of turkeys.Poult. Scie.38.(abstract).

8. Abdukalykova,S. and A. Ruiz.Feria.(2006). Arginine and vitamin E improve the cellular and humoral immune response of broiler chickens. Int.J.Poult.Sci. 5(2): 121-127.
9. Rahman,M.M; A.S.M.Bari; M.Giasuddin; M.R.Islam; J.Alam; G.C.sil .(2002). Evaluation of maternal and humoral immunity against Newcastle disease virus in chickens. int. J. Poult.Sci. 1(5):161-163.
10. Anon(1971). Methods for examining poultry biologic and for Identification and quantifying avian pathogens, New - castle disease, p.66. National Academy of Science, Washington , D.C.
11. Tsiagbe,M.E; A.E.Cook and M.L.Harber.(1987). Enhanced immune Response in broiler chicks fed methionine - supple- Mented diet. Poul.Sci. 66: 1147 – 1154.
12. Fasuyi, A.O. and V.A.Aletor. (2005). Protein replacement value of Cassava leaf protein concentrate in broiler starter:
13. Cross,W.B. and P.B.(1983).Evaluation of the heterophil-lymphocyte ratio as a measure of stress in chicken. Av. Dis .27 : 972 – 979.
14. Robert,B.D. and D.V.Altman. (1995). Avian medicine and surgery. 1st Ed. Philadelphia – Pennsylvania 19106, USA.
15. Steel,B.C.and R.G.D. and J.H.Torrie(1980). principles &procedures of statistics : A biometrical approach. 2nd ed McGraw Hill Book Co., Inc., New York. USA .
16. Wang,Y.W.; C.J.,Field and J.S.,Sim.(2000).Dietary polyunsaturated Fatty acids alter lymphocyte subset proportion and proliferation, serum immunoglobulin G concentration, and immune tissue development in chicks. Poult.Sci 79. (Abstract).
17. Fritsche,K.L. ; N.A., Cossity and S.C., Huang(1991). Effect of dietary fat source on antibody production and lymphocyte proliferation in chickens. Poul. Sci. 70 (Abstract).
18. Betz, M.A. and B.S., Fox(1991). Immunology: a short course, Wiley - Liss, New York,0158 USA.(www.immunology.com).
19. Calder,P.C.(1998). Immunoregulatory and anti - inflammatory effect. Of n-3 polyunsaturated fatty acids.Brazl. J.Med.Bio. 31 (Abstract).
20. Sakr,W.M. (2007). Enhancement of humoral and cellular immunity of broiler chicks vaccinated with Newcastle disease by the dietary supplementation of fish oil.Msc. thesis, Univ. Basrah, Basrah, Iraq.

21. Tobarek, M.;Y.W.,Lee; R.Garrido; S.Kaiser and B.Henning. (2002). Unsaturated fatty acids selectively induce an inflamm- atory environment in human endothelial cells.Amer.J. Clin. Nut. 75 : 119 – 125.
22. Wang,Y.W;C.J.Field and J.S.Sim.(2001).Effect of dietary fatty acids, fish oil, soybean oil and chicken fat on humoral immunity of birds. Poult.Sci. 90. (Abstract).
23. Paul,M.;T.,Gerald;C.,Pang;L.,Robert and C.K.,David.(1999).Fish oil feeding delays influenza virus specific IgG in mice. J. Nut. 129. (Abstract).
24. Michel, D . (2002) . Immunomodulation by dietary lipids : Fish oil, soybean oil, chicken fat and hydrogenated soybean oil. Anim.Sci. 195 : 199 – 218.
25. Parmentier, H.K.;M.G.,Nieuwland;M.W.,Barwegen & R.P.Scharma. (1997).dietary unsaturated fatty acids affect antibody Response and growth of chickens. Poult. Sci. 76. (Abstract).