Kafkas Univ Vet Fak Derg
20 (6): 857-862, 2014
DOI: 10.9775/kvfd.2014.11163

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

RESEARCH ARTICLE

The Effects of Dietary Oil Sources on Performance, Serum Corticosterone Level, Antibody Titers and IFN-γ Gene Expression in Broiler Chickens

Ali Asghar SADEGHI [1] Aida SAFAEI [1] Mehdi AMINAFSHAR [1]

[1] Department of Animal Science, Science and Research Branch, Islamic Azad University, Tehran - IRAN

Article Code: KVFD-2014-11163 Received: 06.03.2014 Accepted: 28.06.2014 Published Online: 05.08.2014

Abstract

The present study was performed to evaluate the effects of addition of fish oil, soybean oil and olive oil to diet on performance and immune response in broiler chickens. In a completely randomized design, 320 broiler chickens (Ross 308, 7 days old) were allocated to four dietary treatments (control and three vegetable oils), four replicates with 20 chicks per each. The results showed that body weight gain of chicks fed soybean oil was higher and feed conversion ratio was better than the other treatments in the total period (P<0.05). The gene expression of γ-interferon (IFN-γ) in spleen tissue was influenced by treatments. The mRNA level of IFN-γ was higher in chicks fed fish oil than the other groups, also antibody titers against Newcastle virus in chicks fed fish oil were higher than the other groups (P<0.05). For antibody titers against sheep red blood cell, there were no differences among treatments (P>0.05). Chicks fed fish oil had higher relative weights of bursa of Fabricius and there were no significant differences in relative weights of spleen among treatments. It was concluded that the source of oils in the diet may be affect the performance and immune response, the addition of fish oil and soybean oil to the diet resulted in significant improvement of performance and immune response in broiler chickens, respectively.

Keywords: *Oil sources, Gene expression, Immune response, Broiler chickens*

Broylerlerde Diyetteki Yağ Kaynaklarının Performans, Serum Kortikosteron Düzeyi, Antikor Titresi ve IFN-γ Gen Ekspresyonu Üzerine Etkileri

Özet

Bu çalışma diyete balık yağı, soya yağı ve zeytinyağı ilavesinin broyler tavuklarda performans ve bağışıklık cevabı üzerine etkisini araştırmak amacıyla yapılmıştır. Tamamıyla rastgele dizaynda 320 broyler tavuk (Ross 308, 7 günlük) her birinde 20 tavuk bulunan ve 4 tekrarlı olmak üzere 4 ayrı diyet grubuna (Kontrol ve üç ayrı yağ uygulaması) ayrıldı. Çalışmanın sonuçları soya yağı ile beslenen civcivlerin diğer gruplar ile karşılaştırıldığında vücut ağırlık artışlarının daha yüksek ve yem dönüşüm oranının tüm periyot içerisinde daha iyi olduğunu gösterdi (P<0.05). Dalakta γ-interferon (IFN-γ) gen ekspresyonu uygulamalar tarafından etkilenmiştir. IFN-γ mRNA düzeyi ile Newcastle virusa karşı antikor titresi balık yağı ile beslenen civcivlerde diğer gruptakilere oranla daha yüksekti (P<0.05). Koyun kırmızı kan hücrelerine karşı antikor titresi bakımından uygulama grupları arasında bir fark gözlemlenmedi (P>0.05). Balık yağı ile beslenen civcivlerin görece bursa Fabricius organ ağırlıkları daha yüksekti ve görece dalak ağırlıkları yönünden uygulama grupları arasında belirgin bir fark tespit edilmedi. Çalışmanın bulguları ışığında kullanılan yağ kaynağının performans ve bağışıklık cevabı üzerine etkisinin olabileceği ve diyete balık yağı ve soya yağı ilavesinin broyler tavuklarda sırasıyla performans ve bağışıklık cevabına önemli etkilerinin olabileceği sonucuna varılmıştır.

Anahtar sözcükler: *Yağ kaynakları, Gen ekspresyonu, Bağışıklık cevabı, Broyler*

INTRODUCTION

Lipids are mainly included in poultry diets as energy and essential fatty acids, which they cannot be synthesized in body tissues. There are evidences that feeding the broilers with diets containing oils, such as fish oil, soybean

İletişim (Correspondence)
+98 91 9557 9663
a.sadeghi@srbiau.ac.ir

oil and olive oil have a many benefits [1,2]. In this regard, Dewitt et al.[3] reported that addition of fish oil, sunflower oil, and soybean oil improved feed conversion ratio. In another study, feeding the broilers with diets containing fish oil caused poorer feed conversion efficiency than the control [4]. Parmentier et al.[5] who reported that addition of soybean oil (as a source of omega-6 fatty acids) to diet increased antibody production in broilers, but other researchers [6-8] reported a decrease in antibody response against antigens. Another study [9] reported that the addition of soy oil to diet could increase serum corticosterone in broilers, which has been found to be immunosuppressive [10,11]. John et al.[12], Miles et al.[13] and Korver et al.[14] reported that addition of fish oil (as a source of omega-3 fatty acids) to diet could increase production of cytokines such as IFN-γ. In another study, Fritsche et al.[15] reported that spleen IFN- γ mRNA were lower in mice fed an omega-3 fatty acid-enriched diet compared with mice fed diets low in omega-3 fatty acid diet. Reports concerning the effects of oils on performance and immune responses are very contradictious. Moreover, in the literature, there are very few studies concerning the comparison between different sources of oils on antibody titers and gene expression of cytokines, especially IFN-γ. Therefore, this study was designed to evaluate the effects of different sources of oils on performance, serum corticosterone level, antibody titers and IFN-γ gene expression of broilers.

MATERIAL and METHODS

The study was approved by the Ethics Committee of Islamic Azad University, Science and Research Branch of Medical and Veterinary Sciences (approval date: 17.01.2013; no: 1292, AEC 3).

Animals and Diets

Three hundred and twenty broiler chicks (Ross 308, 7 days old) with the same weight (155±3 g) were separated and randomly allocated into four dietary treatments and four replicates in a completely randomized design. Birds were housed in deep litter pens (1×2 m). The relative humidity was controlled at 65% and temperature was set at 32°C on day 1 and lowered gradually to 24°C for the rest of the experiment period. Lighting schedule was 23 h light and 1 h dark. Water and feed were provided *ad libitum*. Birds were fed experimental diets from day 1 until day 42 of age in three periods: The starter (1-7 days), grower (8-28 days) and finisher (29-42 days). Diets were formulated based on the corn-soybean meal *(Table 1)*. Dietary treatments included of: 1) diet without oil as control; 2) diet with fish oil, as source of ω-3; 3) diet with soybean oil, as source of ω-6, and 4) diet with olive oil as source of ω-9. The fatty acids composition of used oils in this study was reported in *Table 2*. To reduce handling and weighting stress, average feed intake, body weight gain and feed conversion ratio of broilers in each pen were only measured at days 7 and 42 of age.

Humeral Immune Response

Blood samples were drawn from vein of two birds in each pen in day 13 of age. The blood samples were poured in tubes that had no anticoagulant and centrifuged at 1.500 × g for 15 min. Sera were collected for analysis and average antibody assessment. The titers of the anti-body against Newcastle disease were determined by hemagglutination inhibition test [16]. At day 27 of age, sheep red blood cell (SRBC) suspension (5% in sterile phosphate buffered saline) was injected in breast muscle of two birds in each pen. Seven days after each sensitization (day 34 of age), antibody titers against SRBC were measured according to Vander Zijpp and Leenstra [17] and expressed as the log 2 of the reciprocal of the highest serum dilution giving complete agglutination.

Quantification of Gene Expression by Real Time PCR

At the end of the period two birds per replicate were randomly selected, individually weighed, and killed by cervical dislocation. Their spleen were removed and immediately stored in liquid nitrogen for messenger RNA (mRNA) extraction. According to the kit Vivantis Company (Malaysia), total RNA was extracted. To convert mRNA into cDNA, Randon hexamer was used as a primer and after attachment of primer to RNA chain by reverse transcriptase cDNA synthesis according to the kit Vivantis Company (Malaysia) was done.

Primer Design and Real Time PCR

To design the primers, related studies [18,19] have reviewed and showed that all the consequences were compared to NCBI data center. Gene expression of this cytokine was analyzed by real time-PCR *(Table 3)*. In order to evaluate the samples by this method regarding the above mentioned Kit, cDNA and master mix for each sample done. The study fulfilled in 10- microliter tubs and beside each sample, a separate sample as β-actin primer prepared and were put in corbette, and according to number of cycles and temperature, diagram were drawn. Real time PCR was performed using Power SYBR Green PCR Master Mix (Applied Biosystems). Comparison of each gene expression with its control and stimulated states was determined with the delta-delta (ΔΔ) Ct, in this method a positive result reveals an increase in the expression of the gene of interest in stimulated conditions, whereas a negative result shows a decreased expression [19].

Corticosterone Measurement and Relative Weight of Immune Organs

At the end of the period, two birds per replicate were randomly selected, individually weighed, and their blood poured in tubes with no anticoagulant and were centrifuged at 1500 × g for 15 min, sera was collected for analysis, corticosterone hormone concentration was assessed by ELISA kit (Corticosterone ELISA RE52211, IBL

Table 1. *Ingredients and chemical composition of experimental rations*[1]
Tablo 1. *Deneysel rasyonların içerikleri ve kimyasal kompozisyonları*

Ingredients (as %)	**Starter**		**Grower**		**Finisher**	
Tretments	T_1	T_2, T_3, T_4	T_1	T_2, T_3, T_4	T_1	T_2, T_3, T_4
Corn	54.82	55.45	58.81	55.84	63.6	60.61
Soybean Meal (44%)	35.6	35.69	32.5	33.07	27.7	28.27
Starch	5	0	5	0	5	0
Oil	0	2.15	0	3	0	3
DCP	1.91	1.91	1.61	1.62	1.51	1.52
$CaCO_3$	1.18	1.18	0.96	0.95	0.93	0.92
DL- Methionine	0.29	0.29	0.19	0.2	0.16	0.16
L-Lysine HCl	0.21	0.20	0.04	0.03	0.02	0.01
L-Threonine	0.11	0.11	0.03	0.03	0.01	0.01
Salt	0.27	0.27	0.32	0.33	0.32	0.33
$NaHCO_3$	0.11	0.11	0.04	0.03	0.04	0.03
Zeolite	0	3.14	0	4.4	0.21	4.64
[2]Vitamin Premix	0.25	0.25	0.25	0.25	0.25	0.25
Mineral Premix	0.25	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100	100
Chemical composition						
Energy (kcal/kg)	2840		2890		2940	
Crude Protein (%)	20.57		19.26		17.5	
Methionine (%)	0.59		0.48		0.43	
Met+Cys (%)	0.88		0.76		0.69	
Lysine (%)	1.22		1.06		0.89	
Threonine (%)	0.78		0.66		0.59	
Tryptophan (%)	0.22		0.21		0.19	
Arginine (%)	1.30		1.20		1.1	
Valine (%)	0.90		0.90		0.8	
Isoleusine (%)	0.90		0.88		0.8	
Calcium (%)	0.98		0.82		0.78	
Av. Phos (%)	0.46		0.41		0.39	
Sodium (%)	0.16		0.16		0.16	
Chloride (%)	0.23		0.23		0.23	
Potassium (%)	0.86		0.81		0.73	

1- *T_1: diet without oil, T_2: diet with fish oil, T_3: diet with soybean oil and T_4: diet with olive oil*
2- *Vitamin-mineral premix (each kg contained): calcium, 195 g; potassium, 70 g; sodium,18 g; magnesium, 6 g; zinc, 1.200 mg; iron, 2.000 mg; copper, 400 mg; manganese, 1.200 mg; selenium, 8 mg; cobalt, 20 mg; iodine, 40 mg; vitamin A, 200.000 IU; vitamin D_3, 80.000 IU; vitamin E,1.072 IU; vitamin K_3, 34 mg; ascorbic acid, 1.300 mg; thiamine, 35 mg; riboflavin, 135 mg; niacin, 1.340 mg; vitamin B_6, 100 mg; folic acid, 34 mg; vitamin B_{12}, 670 µg; biotin, 3.350 µg*

Gesellschaft fur Immunchemie und Immunbiologie MBH, Hamburg, Germany). Then, the birds were killed by cervical dislocation, thereafter their bursa of Fabricius, spleen and thymus were removed and their relative weights (organ weight/total weight×100) were calculated.

Statistical Analysis

All values were analyzed by one-way ANOVA using the GLM procedure of SAS software [20]. When the F-test for treatments was significant at P≤0.05 in the ANOVA table, means were compared for significant differences using the Duncan's multiple range tests [21].

RESULTS

Effects on Performance

The effects of different sources of oil on feed intake, body weight gain and feed conversion ratio of the birds are shown in *Table 4*. There were significant effects on

Table 2. *The fatty acids composition of oils (as percent)*
Tablo 2. *Yağların yağ asidi kompozisyonları (yüzde olarak)*

Fatty Acid	Fish Oil	Soybean Oil	Olive Oil
C14:0	1.94	0.48	0.02
C16:0	19.17	10.27	18.91
C18:0	4.82	3.95	4.9
C18:1n-9	22.5	22.73	72.02
C18:2n-6	3.92	56.69	3.13
C18:3n-3	1.37	5.17	0.61
C20:1n-9	2.84	0.71	0.41
C20:3n-6	4.58	-	-
C20:5n-3	13.26	-	-
C22:5n-3	3.7	-	-
C22:6n-3	21.9	-	-

Table 3. *Real-time PCR primers*
Tablo 3. *Real-time PCR primerleri*

Amplified Product		Sequence (5´- 3´)	Base	Tm (°C)	Vol
IFN-γ	F	ACACTGACAAGTCAAAGCCGC	21	61.2	281
	R	AGTCGTTCATCGGGAGCTTG	20	51.27	328
β-Actin	F	CAACACAGTGCTGTCTGGTGGTA	23	60.18	24.0
	R	ATCGTACTCCTGCTTGCTGATTCC	23	60.49	27.0

Table 4. *Effect of different sources of oil on performance of broilers at day 42 of age*
Tablo 4. *42. günde broylerlerin performansı üzerine değişik yağların etkileri*

Treatment	Feed Intake	Gain (g)	FCR
Control	3363	1558^{d}	2.15^{a}
Fish oil	3234	1713^{c}	1.88^{b}
Soybean oil	3327	1888^{a}	1.76^{c}
Olive oil	3343	1858^{b}	1.79^{b}
SEM	46.24	37.33	0.02
P-value	NS	*	*

Value in the same column with no common superscript are differ (P<0.05);
** Significant at P<0.05;* ***NS:*** *Not significant (P>0.05)*

body weight gain and feed conversion ratio among treatments at 42 of age (P<0.05). Body weight gain and feed conversion ratio were affected by the supplemented fat sources (P<0.05). Broilers fed with soybean oil had the highest body weight and the lowest level of feed conversion ratio, and these differences were significant in comparison to the other groups. By contrast there were no feed intake differences among the treatments. Although the highest feed intake observed in the birds fed control, but this difference was not significant among treatments. Also, there were no significant effect on feed conversion ratio between treatments fed fish oil and olive oil (P>0.05). Totally those birds fed soybean oil performed better than the other groups.

Table 5. *Effect of different sources of oil on relative weight of lymphoid organs at day 42 of age*
Tablo 5. *42. günde görece lenfoid organ ağırlıkları üzerine değişik yağların etkileri*

Treatment	Bursa	Thymus	Spleen
Control	0.10^{b}	0.34^{c}	0.10
Fish oil	0.19^{a}	0.52^{b}	0.15
Soybean oil	0.12^{b}	0.61^{b}	0.13
Olive oil	0.12^{b}	0.90^{a}	0.11
SEM	0.01	0.09	0.02
P-value	*	*	NS

Value in the same column with no common superscript are differ (P<0.05);
** Significant at P<0.05;* ***NS:*** *Not significant (P>0.05)*

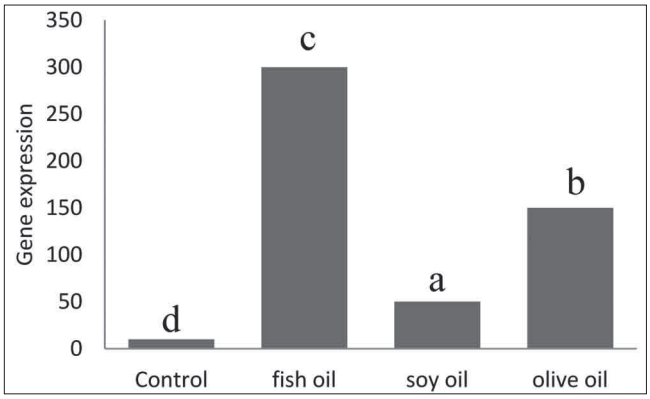

Fig 1. The effects of different sources of oil on IFN-γ gene expression
Şekil 1. IFN-γ gen ekspresyonu üzerine değişik yağların etkileri

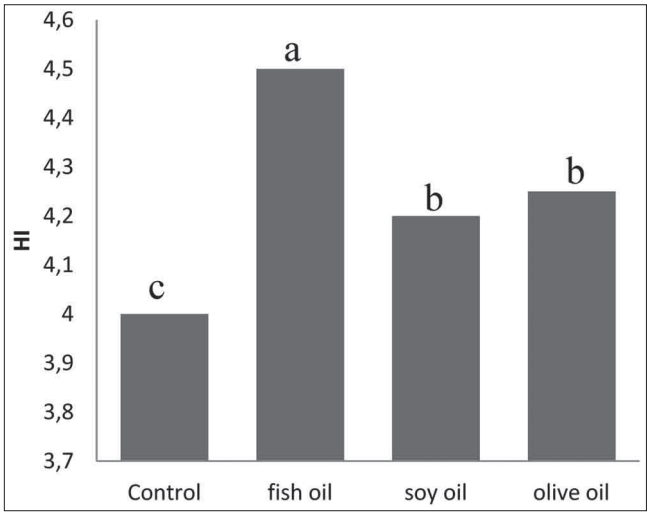

Fig 2. The effects of different sources of oil on antibody titer against hemagglutination inhibition (HI, log 2)
Şekil 2. Hemaglutinasyon inhibisyona (HI, log 2) karşı antikor titresi üzerine değişik yağların etkileri

Analysis of IFN-γ mRNA Expression

The results in *Fig. 1* show IFN-γ mRNA expression. It was influenced by oil sources in spleen tissue (P<0.05). The mean of mRNA levels of IFN-γ in birds fed fish oil increased

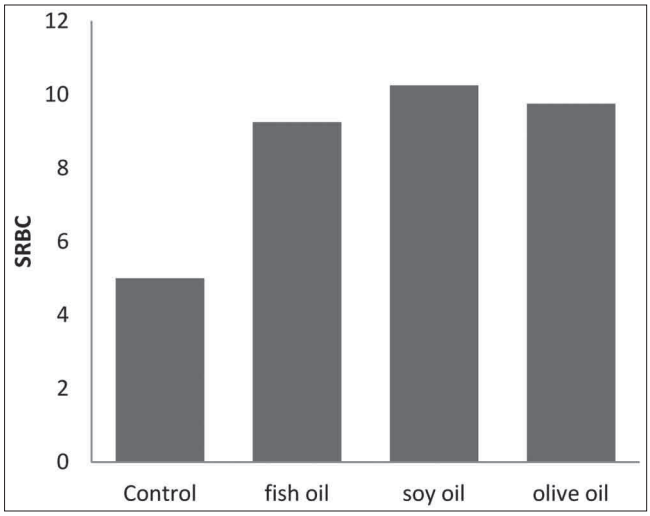

Fig 3. The effects of different sources of oil on antibody titer against sheep red blood cell (SRBC, log 2)

Şekil 3. Koyun kırmızı kan hücrelerine karşı antikor titresi (SRBC, Log 2) üzerine değişik yağların etkileri

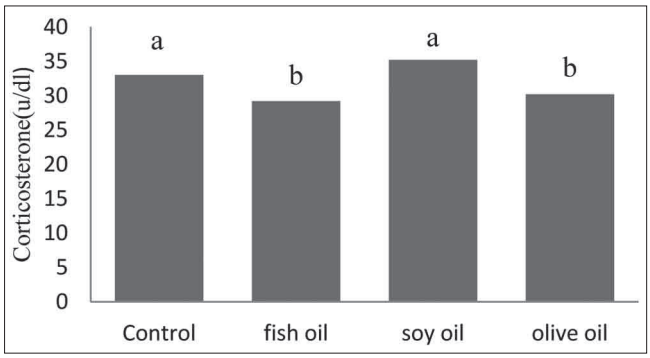

Fig 4. The effects of different sources of oil on corticosterone level

Şekil 4. Kortikostreol düzeyi üzerine değişik yağların etkileri

in comparison to other treatments. The mean of mRNA levels are notably greater in the birds fed olive oil and soybean oil compared to the control diet.

Effects on Antibody Titer Against Newcastle and SRBC

The effects of different sources of oil on antibody titer against HI and SRBC are presented in *Fig. 2* and *Fig. 3*. Antibody titers against Newcastle in chicks fed with fish oil were higher than the other groups (P<0.05). There were no significant effect on antibody titer against Newcastle between treatments fed soybean oil and olive oil (P>0.05). For antibody titers against SRBC, there were no remarkable differences among treatments (P>0.05).

Relative Lymphoid Organs Weight and Corticosterone Concentration

The effects of different sources of oil on relative lymphoid organs weight are shown in *Table 5*. The data indicate that the chicks fed fish and olive oil had the highest relative bursal and thymus weight, respectively (P<0.05). There was no significant effect among control treatment with broilers fed soybean oil and olive oil in the relative bursal weight (P>0.05). Moreover, there was no significant difference in weight of relative spleen weight among treatments (P>0.05). There were significant differences for corticosterone concentration among treatments (P<0.05). The highest corticosteroid hormone concentration was in broilers fed soybean oil and control treatment. Whereas there was no significant effect of fish oil and olive oil on corticosterone level *(Fig. 4)*.

DISCUSSION

The main purpose of the present study was to examine the effects of different sources of oil on performance, serum cortisol level, antibody titers and IFN-γ gene expression in broilers. The addition of soybean oil (as a source of omega-6) to the diet resulted in a positive effect on performance. The results showed that chicks fed diets containing soybean oil showed better performance than the other groups. Our result was consistent with the finding of some others studies that found that soybean oil improves performance [3,8]. The improved performance of broilers fed soybean oil were probably because of fatty acid composition of this oil; a long-chain n-6 fatty acid that makes it possible to increase diet digestibility and to enhance growth.

According to the results of the IFN-γ gene expression and poorly expressed genes of control treatment compared with other treatments can be argued that addition of polyunsaturated fatty acids (PUFA) in the diet can enhance IFN-γ mRNA expression significantly. These results are compatible with several other studies [14,22] that showed feeding birds with PUFA can affect lymphocyte proliferation. Among different treatments the fish oil treatments showed the highest level of gene expression, This result was agree with a study [23] that found diets enriched with fish oil increase the activity of T-helper-1. In addition, an *in vitro* study [12,24] showed that fish oil would be expected to increase production of cytokines such as interferon-gamma by decreasing production of prostaglandin E_2 (by peripheral blood mononuclear cells). Prostaglandin E_2 inhibits activity of lymphocytes. While these results were inconsistent with studies, that reported splenic IFN-γ mRNA were lower in mice fed a n-3-PUFA-enriched diet compared with low n-3-PUFA diet, indicating shift from T-helper-1 to T-helper-2 of immune response [15]. Based on these studies, we concluded that the increase of IFN-γ mRNA expression in the chickens fed fish oil diet may be attributed to enhance of innate immune cells such as T-helper-1 with reducing production of eicosanoids such as prostaglandin E_2 by peripheral macrophages [7]. Effects of oils on antibody titer against sheep red blood cells were not significant. These results were inconsistent

with studies [25,26] that reported that fish oil was shown to enhance the antibody response of chicks to sheep red blood cells than the birds that were not treated with fish oil. In antibody titer against Newcastle, we observed significant differences among treatments by adding the different sources of oil. It is concluded that by adding fish oil, immune response improved, probably because of the effects on long chain n-3 PUFA of fish oil on eicosanoid levels [13,26]. Also this result was inconsistent with the findings of Parmentier et al.[5], who reported that n-6 PUFA increased antibody production. It seems that, these discrepant resulting might be associated with the types and dose oil used. It was concluded that the addition of fish oil in the diet may be resulted to enhance antibody titer against Newcastle due to long chain n-3 PUFA metabolical function (eicosapentaenoic acid and docosahexaenoic acid).

Increasing of bursal weight could be interpreted as an indicator of increase immune activity [27]. The results of this study indicated that the addition of fish oil to the diet has a positive impact on the immune response of broilers. Previous studies have shown that decrease in the relative weight of lymphoid organs, are associated with blood corticosterone levels [9,28]. Corticosterone has been found to be immunosuppressive. Another study revealed that inclusion of soybean oil in the diet could induce significant increases in serum corticosterone level. These results agree with studies that found that on weight of bursa Fabricius [29], but it was not consistent with its effect on the weight of other organs (30). The results of this study indicated that the addition of that the addition of fish oil and soybean oil to the diet may be resulted in better improvement of immune response and performance in broiler chickens.

REFERENCES

1. Schwalfenberg G: Omega-3 fatty acids: Their beneficial role in cardiovascular health. *Can Fam Physician,* 52, 734-740, 2006.

2. Leeson S: Utilization of fats and fatty acids by turkey poult. *Poult Sci*, 74, 2003-2010, 1995.

3. Dewitt J, Copeland C, Strynar M: Perfluorooctanoic acid-induce immunomodulatin in adult femal chicks. *Environ Health Perspect,* 116, 645-650, 2009.

4. Hulan H, Ackman R, Ratnayake W: Omega-3 fatty acid levels and performance of broilers chickens fed red fish meal or red fish oil. *Anim Sci,* 68, 533-547, 1998.

5. Parmentier H, Nieuwland M, Barwegen M, Kwakkel R, Schrama J: Dietary unsaturated fatty acids affect antibody responses and growth of chickens divergently selected for humoral responses to sheep red blood cells. *Poult Sci,* 76, 1164-1171, 1997.

6. Mossab A, Hallouis J, lessire M: Utilization of soybean oil and tallow in young turkeys compared with young chickens. *Poult Sci,* 79, 1326-1331, 2002.

7. Sijben J, Groot H, Nieuwland M, Schrama J, Parmentier H: Dietary linoleic acid divergently affects immune responsiveness of growing layer hens. *Poult Sci,* 79, 1106-1115, 2000.

8. Friedman A, Sklan D: Effect of dietary fatty acids on antibody production and fatty acid composition of lymphoid organs in broiler chicks. *Poult Sci,* 74, 1463-1469, 1995.

9. Song C, Horrobin D: Omega-3 fatty acid ethyl-eicosapentaenoate, but not soybean oil, attenuates memory impairment induced by central IL-1β administration. *J Lipid Res,* 45, 1112-1121, 2004.

10. Gross WB: Effect of short-term exposure of chickens to corticosterone on resistance to challenge exposure with *Escherichia coli* and antibody response to sheep erythrocytes. *Am J Vet Res,* 27, 972-979, 1992.

11. El-Lethey H, Huber-Eicher B, Jungi TW: Exploration of stress-induced immunosuppression in chickens reveals both stress-resistant and stress-susceptible antigen responses. *Vet Immunol Immunopathol,* 95, 91-101, 2003.

12. John W, Kirk C, John S, Henk K, Jan V, Huub S, Pete K: Early *in vivo* cytokine genes expression in chickens after challeng with *Salmonella typhimurium* lipopolysaccharide and modulation by dietary n-3 poly-unsaturated fatty acids. *Develop Comp Immunol,* 27 611-619, 2003.

13. Miles E, Calder P: Modulation of immune function by dietary fatty acids. *Proc Nutr Sci,* 57, 277-292, 1998.

14. Korver D, Klasing K: Dietary fish oil alters specific and inflammatory immune responses in chicks. *Nurt,* 127, 2039-2049, 1997.

15. Fritsche K, Feng C, Berg N: Dietary fish oil enhances circulating interferon-gamma in mice during listeriosis without altering *in vitro* production of this cytokine. *Interferon Cytokine Res,* 17, 271-277, 1997.

16. Allan WH, Gough LE: A standard hemagglutination inhibition test for Newcastle disease: A comparison of macro and micro-methods. *Vet Record,* 95, 120-123, 1974.

17. Vander ZAJ, Leenstra FR: Genetic analysis of the humoral immune response of white Leghorn chicks. *Poult Sci,* 59, 1363-1369, 1980.

18. Jennifer T, Brisbin G, Payvand SS: Effect of lactobacilli on cytokine expression by chicken spleen and cecal tonsil cells. *Clin Vaccine Immunol,* 17, 1337-1343, 2010.

19. Livak Kj, Schmittengent TD: Methods analysis of relative gene expression data using real time quantitative PCR and the 2(-Delta Delta C(T)). *Methods,* 25, 402-408, 2001.

20. SAS Institute: SAS/Stat Users Guid: Statistics. Release 8.2, SAS Institute Inc. Cary, NC., USA, 2002.

21. Duncan DB: Multiple rang and multiple F test. *Biometrics,* 11, 1-42, 1995.

22. Fritsche K, Cassity N, Hung S: Effect of dietary fat source on antibody production and lymphocyte proliferation in chickens. *Poult Sci,* 70, 611-617, 1991.

23. Timothy T, Stephen W, Elizabeth M, Mark M, Nigel A, Anne B, Mike S, Graham B, Philip C: Prostaglandin E2 production and T cell function after fish oil supplementation: Response to antioxidant cosupplementation. *Am J Clin Nutr,* 78, 376-382, 2003.

24. Betz M, Fox B: Prostaglandin E2 inhibit production of th1 lymphokines but not of th_2 lymphokines. *Immunol,* 146, 108-113, 1991.

25. Torki M, Golian A, Tavakkoli J: Effect of dietary fat source and fatty acid composition on immune response of male growing broiler chicks. *Poult Sci,* 79, 105, 2002.

26. Kidd M: Nutritional modulation of immune function in broilers. *Poult Sci,* 83, 650-657, 2004.

27. Hecker T, Estevez R, Russek C, Pettit: Effects of density and perch availability on the immune status of broilers. *Poult Sci,* 81, 451-457, 2002.

28. Khansari,D, Murgo A, Faith R: Effects of stress on the immune system. *Immunol Today,* 11, 170-175, 1990.

29. Wang Y, Fied C, Sim J: Dietary polyunsaturated fatty acids alter lymphocyte subset proportion and proliferation, serum immunoglobulin G, and immune tissue development in chicks. *Poult Sci,* 79, 1741-1748, 2000.

30. Khalifa H, Givens D, Rymer C, Yaqoob P: Effect of n-3 fatty acids on immune function in broiler chickens. *Poult Sci,* 91, 74-88, 2012.