

## THE EFFECTS OF DIFFERENT LEVELS OF DIETARY FISH OIL, SOYBEAN OIL, AND SUNFLOWER OIL ON PERFORMANCE AND IMMUNITY RELATED PARAMETERS OF BROILER CHICKEN

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### ABSTRACT

The study was designed to investigate the effects of different levels of dietary oil additives on the feeding performance and immunity related parameters of broiler chicken. In total, two hundred and eighty (280) day-old male broiler chicks (Ross 308) were randomly placed in 28 cages with dimensions of 1.0 × 1.0 meters based on a completely random design. There were two levels (1.0% vs. 2.0%) of additive soybean oil, sunflower oil, and fish oil with uniform vaccination programs and feed ingredients and nutrient treatments in the trials. The experimental results showed that there were no differences among the body weight indexes of broiler chicken (42nd day of age), while there were significant differences among the feed conversion ratio and feed intake indexes of these broiler chicken (from 1st to 42nd days of age). Particularly, the broiler chicken fed with 2.0% sunflower oil in diet had more significant lower performance indexes of feed conversion ratio and feed intake than the control and other dietary oil treatment panels (1.0% and 2.0%). Furthermore, all the dietary additives of two levels (1.0% and 2.0%) of fish oil, soybean oil, and sunflower oil brought about significant different immunity indexes of broiler chicken (42nd day of age). In conclusion, these results suggested that different dietary supplementation levels (especially high levels) of dietary oil additives can promote the feeding performance and alter immunity parameters of broiler chicken.

**Key words:** Feed Efficiency; Broiler Chick; Thymus; Humoral Antibody; Immune.

### INTRODUCTION

In Recent years, many agricultural and subsidiary agricultural products including specific oils derived from crops and herbs or other plant spices are frequently used as feed additives for functional immune enhancement and well developmental promotion in animal husbandry industry. These feed additives may have multiple physiological effects and nutritional actions. However, there were presently a few experiments carried out and reported on the effects of dietary supplementation oils or their combinations on the growth, development, and feeding performance of domesticated chicken. Ziaran *et al.* (2005) analyzed the effect of dietary oil extract of propolis on the immune system of broiler chicken. They found that both broiler humoral and cellular immune responses were modulated by different levels of oil extract of propolis. Ashan (2011) investigated the effect of dietary senna and anise oil medicinal plant on broiler performance and blood biochemical and immunity relevant parameters. They found that this medicinal plant oil had significant effects on the performance and carcass traits but no significant effect on the blood biochemical parameters and immune system of those broiler chickens. Kırkpınar *et al.* (2011) measured the possible effects of oregano and garlic essential oils on broiler growth and carcass indexes,

development characteristics, and blood parameters with four diets containing feed additives. They found that there were no significant effects in different feeding treatments. Hosseini-Mansoub and Bahrami (2011) studied the influence of dietary fish oil supplementation on humoral immune response and some blood biochemical parameters of broiler chicken. They found that the addition of 2% fish oil in the broiler diet might stimulate the development of the immune response and improve blood indices, while 4% additive fish oil should not be used because of probable off-flavors in the poultry products. Puzio *et al.* (2012) evaluated the feeding effect of dietary conjugated linoleic acid and false flax seed oil on bone quality in broiler chicken. They found that false flax oil and conjugated linoleic acid could replace sunflower oil. Maroufyan *et al.* (2013) investigated the dietary effects of methionine and fish oil on broiler immune indexes and blood parameters confronted with infectious bursal disease challenges. They found that the chicken fed with 5.5% fish oil acquired higher levels of total protein, white blood cell count, and IL-2 concentration than those of other groups after infectious bursal disease challenge, and a balance of moderate level of fish oil and methionine might enhance the immune response in the challenged broiler chicken. Vilarrasa *et al.* (2014) assessed the effects of re-esterified palm oils through fatty acid positional distribution within

acylglycerol molecules and the acylglycerol compositions on the evolution of postprandial lipemia and growth performance in broiler chicks. Khosravinia (2015) reported the dietary effects of *Satureja khuzistanica* essential oil on the mortality, production and carcass performance, water intake, and organ weight of Arian broiler chicks. Seidavi (2014) examined the feeding effects of dietary fish oil and green tea powder supplementation on broiler immunity. Seidavi and Simões (2015) evaluated again the feeding effect of dietary soybean oil substitution by fish oil plus low levels of green tea powder on the digestive tract microflora of 270 one-day old male broiler chicks. However, simultaneous comparisons of various oils in feeding diet are not studied well until now, and there exist only a few researches evaluating the growth and immunity and feeding parameters of commercial broilers fed diets differing in dietary oils. The objective of this study was designed to investigate and determine the possible effects of different levels of dietary fish oil, soybean oil and sunflower oil additives on the immunity and productivity characteristics of Ross 308 broiler chicken.

## MATERIALS AND METHODS

**Animals and husbandry:** Two hundred and eighty (280) day-old Ross 308 (Aviagen, Newbridge, Scotland, UK 35805) male broiler chicken were purchased from a commercial hatchery. The broiler chicken were placed in 28 cages with dimensions of 1.0 × 1.0 meters, which provided a floor area of 0.1 m<sup>2</sup> per bird, in a thermostatically- controlled curtain side-wall poultry barn. The fowl cage floors were covered with paper roll litter, and the birds remained in the cages for the duration of the experiment, which ended at 42 days of age. Each cage of 10 chickens (42.0±1.6g) was assigned to a specific dietary treatment group.

**Temperature, humidity, light, and ventilation:** Ambient temperature within the poultry barn was maintained with supplemental heat from thermostatically controlled gasoline rocket heaters, and humidity was added to the barn atmosphere via a water spray to maintain relative humidity between 55-65%. Ambient temperature was controlled at 33°C at placement and decreased periodically to 23°C at 3 weeks of age and was maintained at 23°C until the termination of the investigation. Lighting was provided by 23 watt fluorescent tubes in ceiling fixtures. The constant light was provided on day 1, but since day 2, lighting was re-established at 21 hours per day until the end of the study. Air circulation within the poultry barn was facilitated by 3 wall-mounted 60 cm diameter fans on one end of the barn and 160 cm diameter wall-mounted fans on the other end of the barn to establish tunnel ventilation.

**Feed and treatments:** A three phase feeding program was used in this investigation and consisted of provision of starter feed from 1-14 days, grower feed from 15-28 days, and finisher feed from 29-42 days (Table 1). The diets met or exceeded Ross 308 catalogue recommendations (Table 1). The treatments were studied based on completely random design included 7 treatments. These studied treatments were as follows:  
Treatment 1: basal diet (the experimental control)  
Treatment 2: basal diet included 1.0% soybean oil  
Treatment 3: basal diet included 2.0% soybean oil  
Treatment 4: basal diet included 1.0% sunflower oil  
Treatment 5: basal diet included 2.0% sunflower oil  
Treatment 6: basal diet included 1.0% fish oil  
Treatment 7: basal diet included 2.0% fish oil

**Vaccination program:** Birds were vaccinated at the 10th (as Clone 30) and 23rd (as B1) days of age against Newcastle disease. Also birds were vaccinated at 1st day of age against Infectious bronchitis. Gumboro vaccination also was given at the 17th day of age. The influenza vaccination also was given at the 1st day of age of chicks.

**Performance and Immunity parameters:** Feed intake and weight gain were recorded. Feed conversion ratio calculated based on conventional protocol. At 42 days of age before blood collections were made, feed was removed from all the birds for a period of four hours in an attempt to allow stabilization of the various plasma constituents, and all blood sampling was done in the morning to further reduce the variability of the plasma constituents to be measured. A 5 mL volume of venous blood was collected from the ulnar vein in the wing of one bird taken from each replicate. Care was taken to choose the most representative male birds with respect to body weight compared to the group mean body weight. The whole blood sample was transferred from the syringe into a tube coated with 10 mg of the anticoagulant ethylene diamine tetra acetic acid (EDTA). Blood samples were centrifuged at 3000 rpm for 20 minute to assure separation of the blood cells from the plasma. Plasma was collected and stored at -20°C until subsequent plasma constituent analyses were made based on standard protocols.

**Statistical Analyses:** Using a completely random experimental design involving 7 treatments, all data were subjected to statistical analysis using the General Linear Model procedures of the Statistical Analysis System v8 (SPSS, 1997). Differences among main effect means (P 0.05) were assessed through Duncan's multiple range tests. Statements of significance were based on P 0.05.

**Ethic rules:** The procedures have been approved by the Authors' Institution's Ethic Committee, and care was taken to minimize the number of animals used.

## RESULTS AND DISCUSSION

The experimental trial results of different supplementation levels of dietary fish oil, soybean oil, and sunflower oil on broiler feeding performance and immunity indexes are obtained and the data are summarized in Tables 2 and 3.

**1. The feeding performance and characteristics of Ross 308 broiler chicken:** The feeding performance and indexes of Ross 308 broilers at the 1st-6th weeks of age were summarized in Tables 2. There were three categories of feeding characteristics measured in the experiment, i.e. feed intake, body weight and feed conversion ratio. The different treatment panels of Ross 308 broilers were fed with two levels (1.0% vs. 2.0%) of dietary fish oil, soybean oil, and sunflower oil during the periods of postnatal 1st-42nd days (Table 2). Provided different levels of dietary fish oil, soybean oil, and sunflower oil, the experimental results showed that there was almost no significant difference between the two records of broiler body weight fed with three additive oils in treatment panels compared to the control panels ( $P>0.05$ ) during the experimental periods. However, there were significant differences observed in the performance of the broiler feed conversion ratio and feed intake indexes parameters during the 1st to 42nd days of age ( $P<0.01$ ). In particular, those broiler chicken fed with 2.0% sunflower oil in diet had significant lower performance indexes of feed conversion ratio and feed intake than the control and other dietary oil treatment panels (1.0% and/or 2.0%;  $P<0.05$ ), and there were obvious differences between those two levels (1.0% vs. 2.0%) of treatment panels of dietary soybean oil, and fish oil too ( $P<0.05$ ). These results partly agreed with Vilarrasa *et al.* (2014) and Khosravinia (2015). Vilarrasa *et al.* (2014) found the supplementation of re-esterified palm oils by fatty acid positional distribution within acylglycerol molecules resulted in a lower gross-energy content and an increased solid-fat index at the chicken's body temperature, but re-esterified palm oils did not alter the fat absorption, postprandial lipemia, or growth performance of broiler chicken, compared to native palm oil, so they were regarded to be used as alternative broiler fat sources in chick diets. Khosravinia (2015) found that additive satureja khuzistanica essential oil (400 mg/L) in drinking water for heat-stressed broiler chickens could improve the economic efficiency. However, Seidavi and Simões (2015) examined and evaluated the effect of soybean oil substitution by fish oil plus low levels of green tea powder on the immunity performance of broiler chicks, and reported that no significant differences between trial groups were observed from the gizzard and ileal contents. Their results also revealed that dietary fish oil and green tea supplementations did not affect

negatively the gizzard, ileum and cecum microflora balance in healthy broilers.

**2. The immunity parameters of Ross 308 broiler chicken:** The immunity parameters of Ross 308 broilers fed with different additive levels of dietary fish oil, soybean oil, and sunflower oil on some blood elements at the 42nd day of age was presented in Table 3. It should be noted that animal and plant oils are particularly rich sources of agricultural and/or subsidiary agricultural products in developing countries. The modulatory effects of two additive levels of dietary fish oil, soybean oil, and sunflower oil were obvious and significant on all the immunity parameters of broiler chicken in the experiment. According to these results in Table 2-3, the dietary supplementations of two levels (1.0% and 2.0%) of fish oil, soybean oil, and sunflower oil had significant dietary effects on the different immunity indexes (i.e. the secretion levels of total protein, albumin, beta globulin, gamma globulin, alpha 2 globulin, and alpha 1 globulin) of Ross 308 broiler chicken (42nd day of age), although there were little significances between the immunity indexes of the two levels (1.0% vs. 2.0%) of treatment panels of dietary sunflower oil. The dietary supplementations of two levels (1.0% and 2.0%) of fish oil, soybean oil, and sunflower oil brought about significant different indexes of secretion levels of total protein ( $P=0.02$ ), albumin ( $P=0.01$ ), beta globulin ( $P=0.003$ ), gamma globulin ( $P=0.002$ ), alpha 2 globulin ( $P=0.001$ ), and alpha 1 globulin ( $P=0.001$ ) of Ross 308 broiler chicken (42nd day of age), although there were low significances between the immunity indexes of the two levels (1.0% vs. 2.0%) of treatment panels of dietary sunflower oil ( $P<0.05$ ). These results partly agreed with those of Ziaran *et al.* (2005), Ashan (2011), Hosseini-Mansoub and Bahrami (2011), Maroufyan *et al.* (2013), Seidavi (2014), and Seidavi and Simões (2015). Particularly, Ziaran *et al.* (2005) reported the positive modulatory roles of different levels of oil extract of propolis on immune system of broiler chicken. Ashan (2011) revealed the positive modulatory effects of dietary senna and anise oil medicinal plant on broiler performance, blood biochemical and immunity parameters. Hosseini-Mansoub and Bahrami (2011) made an investigation and showed a positive influence of dietary fish oil supplementation on humoral immune response and some selected biochemical parameters of broiler chicken as well as some blood parameters. Their results suggested that the addition of 2% fish oil in broiler diet might stimulate the development of immune responses and improve blood indices, while 4% additive fish oil was not recommended because of probable off-flavors in the poultry product (Hosseini-Mansoub and Bahrami, 2011). Maroufyan *et al.* (2013) carried out an experiment on the effects of dietary methionine and fish oil in broiler chickens confronted with infectious bursal

disease challenges. Their results suggested that the broilers fed with 5.5% fish oil had relatively higher levels of total protein, white blood cell count, and IL-2 concentration than those of other groups after the infectious bursal disease challenge, and an inclusion of

fish oil in diet had no effect on IFN- concentration. Moreover, the fish oil supplementation did not affect the liver enzymes concentration in the challenged broiler chicken.

**Table 1. Feed ingredients and nutrient analysis of used diets during the experiment**

Ingredients (%)	starter (1 -14 days)	Grower (15 - 28 days)	Finisher (29 - 42 days)
Corn	59.6	63.0	67.17
Soybean meal (43% CP)	36.12	32.72	28.5
DCP	1.6	1.6	1.6
Oyster shell	1.1	1.1	1.2
DL-methionine	0.18	0.18	0.13
Vitamin premix*	0.5	0.5	0.5
Mineral premix**	0.5	0.5	0.5
Salt	0.3	0.3	0.3
Bicarbonate	0.1	0.1	0.1
<b>Nutritional content</b>			
ME (KJ/kg)	2910	2938	2970
Crude protein (N×6.25) (%)	20.05	18.77	17.35
Calcium (%)	0.95	0.9	0.9
Total phosphorus (%)	1.23	1.06	1.06
Available phosphorus (%)	0.45	0/36	0/36
Metthionine (%)	0.551	0.535	0.463
Lysine (%)	1.364	1.174	1.135
Met + Cys (%)	0.9	0.86	0.759
Tryptophan (%)	0.277	0.26	0.239
Threonine (%)	0.89	0.843	0.783

Note: The marker \* stands for the constitution of vitamin A, 3600000 IU; D3, 800000 IU; vitamin E, 7200 IU; vitamin B1, 710 mg; vitamin B2, 2640 mg; vitamin B6, 1176 mg; vitamin B9, 400 mg; vitamin B12, 6 mg; vitamin k3, 800 mg; pantothenic acid, 3920 mg; vitamin Biotin, 40 mg; vitamin Niacin, 12000 mg and choline chloride, 200000 mg.

The marker \*\* includes Mn, 40000 mg; Fe, 20000 mg; Zn, 33900 mg; Cu, 4000 mg; I, 400 mg and Se, 80 mg.

**Table 2. Feed intake, body weight and feed conversion ratio ( $\pm$  standard error of means= SEM) of Ross 308 broilers at the 1st-6th weeks of age fed diets containing the different levels of oils<sup>1</sup>.**

Treatments	Feed intake (g) (1st-42nd days of age) (g/chick/duration)	Body weight (g) (42nd day of age) (g/chick)	Feed conversion ratio (1st-42nd days of age)
<b>Control</b>	4645.66 <sup>c</sup>	2233.17 <sup>a</sup>	2.08 <sup>c</sup>
<b>Soybean oil,0.01</b>	3778.74 <sup>b</sup>	2262.71 <sup>a</sup>	1.67 <sup>ab</sup>
<b>Soybean oil,0.02</b>	4149.59 <sup>b</sup>	2172.50 <sup>a</sup>	1.91 <sup>abc</sup>
<b>fish oil,0.01</b>	3976.57 <sup>bc</sup>	2137.90 <sup>a</sup>	1.86 <sup>abc</sup>
<b>fish oil,0.02</b>	3919.34 <sup>bc</sup>	2020.27 <sup>a</sup>	1.94 <sup>bc</sup>
<b>Sunflower oil,0.01</b>	3904.21 <sup>bc</sup>	2218.30 <sup>a</sup>	1.76 <sup>ab</sup>
<b>Sunflower oil,0.02</b>	3467.17 <sup>a</sup>	2088.65 <sup>a</sup>	1.66 <sup>a</sup>
<b>P-Value</b>	*	Ns	*
<b>SEM</b>	7.1	5.7	0.05

Note: The marker \* means the significance at P<0.01, while the marker *ns* means non significance;

The foot note <sup>1</sup> means within each column of dietary treatments with no common superscript differ significantly at P<0.05.

**Table 3. Immunological parameters ( $\pm$  standard error of means = SEM) of Ross 308 broilers at the 42nd day of age fed diets containing the different levels of oils (g/dl)<sup>1</sup>**

Treatments	Total protein	Albumin	Alpha 1 globulin	Alpha 2 globulin	Gama globulin	Beta globulin بتاگلوبولين g/dl)
Control	4.6 <sup>abc</sup>	2.71 <sup>abc</sup>	0.70 <sup>abc</sup>	0.56 <sup>abc</sup>	0.37 <sup>bc</sup>	0.32 <sup>abc</sup>
Soybean oil,0.01	5.0 <sup>c</sup>	2.94 <sup>c</sup>	0.76 <sup>c</sup>	0.61 <sup>c</sup>	0.40 <sup>c</sup>	0.35 <sup>c</sup>
Soybean oil,0.02	3.8 <sup>a</sup>	2.22 <sup>a</sup>	0.57 <sup>a</sup>	0.46 <sup>a</sup>	0.30 <sup>a</sup>	0.26 <sup>a</sup>
fish oil,0.01	4.0 <sup>ab</sup>	2.36 <sup>ab</sup>	0.6 <sup>ab</sup>	0.48 <sup>ab</sup>	0.32 <sup>ab</sup>	0.28 <sup>ab</sup>
fish oil,0.02	5.9 <sup>d</sup>	3.44 <sup>d</sup>	0.88 <sup>d</sup>	0.71 <sup>d</sup>	0.47 <sup>a</sup>	0.41 <sup>d</sup>
Sunflower oil,0.01	4.6 <sup>abc</sup>	2.69 <sup>abc</sup>	0.69 <sup>abc</sup>	0.55 <sup>abc</sup>	0.38 <sup>bc</sup>	0.32 <sup>abc</sup>
Sunflower oil,0.02	4.8 <sup>bc</sup>	2.81 <sup>bc</sup>	0.72 <sup>abc</sup>	0.58 <sup>bc</sup>	0.38 <sup>bc</sup>	0.34 <sup>bc</sup>
P-Value	*	*	*	*	*	*
SEM	0.02	0.01	0.003	0.002	0.001	0.001

Note: The marker \* means the significance at P<0.01;

The foot note <sup>1</sup> means within each column of dietary treatments with no common superscript differ significantly at P<0.05.

**Conclusion:** The study was designed to investigate the effects of two levels (1.0% vs. 2.0%) of dietary fish oil, soybean oil and sunflower oil supplementations on the immunity and productivity characteristics of broiler chicken. In summary, the experimental trial results suggested that different dietary supplementation levels of dietary fish oil, soybean oil, and sunflower oil can promote the feeding performance and alter the immunity parameters of Ross 308 broiler chicken. Based on the results stated above, the utilization of high levels (i.e. 2%) of additive fish oil, soybean oil and sunflower oil in diets may have significant positive effects of immune enhancement and growth promotion of broiler chicken. It was also observed that the small samples of treatments used might lead to some bias. Therefore, more studies are still needed to assess the dietary effects of these supplements as broiler feed resources in the future.

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