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The effect of soy oil addition to the diet of broiler chicks on the immune response

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Abstract: The objective of this study was to evaluate antibody titers of challenged broiler chicks fed diets supplemented with soy oil. In a completely randomized design, 120 chicks were assigned to 3 treatments (isoenergetic and isonitrogenous diets based on corn-soybean meal containing 0%, 2%, or 4% soy oil), with 4 replicates for each treatment and 10 chicks per replicate. The challenging program included vaccination against Newcastle disease (B1 vaccine on day 12 by eye drop; Lasota vaccine on days 19 and 32 by drinking) and infectious bursal disease (Gumboro D78 vaccine on days 12 and 24 by eye drop). Blood samples were collected from 2 chicks per replicate at days 21 and 42 of age, and then total and differential white blood cells were counted and antibodies against Newcastle and infectious bursal diseases were measured. At days 21 and 42 of age, 2 chicks per replicate were killed and the bursa of Fabricius and spleen were removed and weighed. White blood cell count was the lowest in chicks fed soy oil-free diet. Inclusion of soy oil to diets resulted in significant increase in cell count. Heterophil-to-lymphocyte ratio, as an index of stress, was lower for chicks fed soy oil-free diet than those fed a diet containing soy oil. Relative weights of the bursa of Fabricius and spleen were the highest in chicks fed soy oil-free diet, but they decreased ($P < 0.05$) as soy oil levels increased in the diet. At days 21 and 42 of age, antibody titers against Newcastle disease and infectious bursal disease viruses were the highest in chicks fed the soy oil-free diet. Antibody titers decreased ($P < 0.05$) as soy oil levels increased in the diet. It was concluded that the addition of soy oil to the diet resulted in retardation of the immune organs weight, change in the immune cells profile, and consequently suppression of the antibody titers against Newcastle disease and infectious bursal disease viruses in broiler chicks.

Key words: Antibody, broiler chick, immune cells, immune organ, soybean oil

1. Introduction

Lipids are an important component of rations, both as an energy source and as the source of essential fatty acids, which poultry cannot synthesize but need for basic functions including the growth and maintenance of healthy tissues (1–3). Most Iranian poultry production systems under traditional management could not use lipid supplements because of their cost and poor air ventilation systems. However, industrial producers can use high amounts of lipids in broiler rations. Soy oil production in Iran is high and is available to the poultry industry as a cheap dietary lipid source. As the n-6 polyunsaturated fatty acid (n-6 PUFA) content of soy oil is high, it may affect some immune parameters in animals. In this regard, Parmentier et al. (4) found that n-6 PUFA resulted in an increase, but other researchers (5,6) reported that high levels of oils containing n-6 PUFA resulted in a decrease in antibody response against antigens or immunoglobulin production.

In the literature, reports on the effects of fatty acids (7–9) and different lipid sources (1,10,11) on the functionality of immune system are numerous. However, no study exists concerning the effects of addition of soy oil to the diet of broiler chicks on the antibody response against Newcastle and infectious bursal disease viruses as antigens. Therefore, the objective of the current study was to examine the effect of soy oil on the immune responses of vaccinated broiler chicks including white blood cell count, growth of immune tissue, and sera antibody titers against Newcastle disease and infectious bursal disease.

2. Materials and methods

2.1. Animals, diets, and experimental design

A total of 120 broiler chicks at 1 day of age from the Ross 308 strain were obtained from a commercial hatchery and raised in 12 pens (1.2 × 1.2 m). On day 7 of age, the number of birds per pen (10 chicks) and the average live weight per pen were balanced. In a completely randomized

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design, 4 pens were assigned to each of the 3 treatments. Starter (1–10 days), grower (11–28 days), and finisher (29–42 days) diets were formulated based on the corn-soybean meal presented in Table 1. Experimental diets were formulated to be isoenergetic and isonitrogenous as follows: 1) diet containing 0% soy oil with starch as the main energy source, 2) diet containing 2% soy oil, and 3) diet containing 4% soy oil. The soy oil used in this study contained 56.5% linoleic acid, 7.5% linolenic acid, and 35% other fatty acids. Celite (trademarked brand name of diatomaceous earth) was used in the soy oil diets as inert filler. Throughout the study, feed and water were provided for ad libitum consumption. Lighting schedule was 23 h light and 1 h dark while the temperature was gradually reduced by 3 °C from the initial temperature of 32 °C each week.

2.2. Vaccination, serology, and hematology

The immunization program included vaccination against Newcastle disease (B1 vaccine on day 12 by eye drop; Lasota vaccine days 19 and 32 by drinking) and infectious bursal disease (D78 vaccine days 12 and 24 by eye drop). Blood samples (3 mL) of 8 birds per treatment (each bird being considered 1 replication) were collected from the wing veins, using sterile syringes, on days 21 and 42 of age. Immediately after collection, 900 µL of blood was transferred to a microtube containing 100-µL sodium citrate solutions (3.85 mg/100 µL) and immediately mixed. These tubes were transferred to Mabna Veterinary

Table 1. Ingredients and chemical composition of experimental rations.[a]

Ingredients (%)	Starter			Grower			Finisher		
	Soy oil-free	Soy oil 2%	Soy oil 4%	Soy oil-free	Soy oil 2%	Soy oil 4%	Soy oil-free	Soy oil 2%	Soy oil 4%
Yellow corn	48.36	57.21	51.04	39.51	51.08	59.28	40.95	58.44	63.75
Soy bean meal (48% CP)	34.86	32.98	35.46	31.47	30.66	30.00	29.53	26.27	25.20
Fish meal	3.00	3.00	2.00	4.00	3.00	2.36	2.50	2.50	2.50
Pure starch	10.14	–	–	22.1	10.11	–	24.06	7.83	–
Soy bean oil	–	2.00	4.00	–	2.00	4.00	–	2.00	4.00
DCP	1.56	1.54	1.70	1.22	1.33	1.39	1.20	1.15	1.13
$CaCO_3$	1.08	1.10	1.13	0.90	0.97	1.02	0.87	0.91	0.92
Salt	0.38	0.36	0.38	0.26	0.25	0.41	0.19	0.17	0.41
Mineral premix[b]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Vitamin premix[c]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	–	–	–	–	0.09	0.08	0.20	0.03	0.03
Lysine	0.17	0.17	0.18	0.09	0.06	0.09	0.05	0.25	0.30
Celite	–	1.19	3.66	–	–	0.92	–	–	1.31
Calculated chemical composition									
ME (kcal/kg)	2890	2890	2890	3050	3060	3050	3100	3100	3100
Protein (%)	21.1	21.0	21.0	19.5	19.5	19.5	18.00	18.00	18.00
Ether extract (%)	2.47	4.79	6.48	2.19	4.54	6.78	2.09	4.66	6.91
Linoleic acid (%)	1.21	2.41	3.31	1.00	2.27	3.47	1.02	2.38	3.55
Lysine (%)	1.29	1.25	1.26	1.22	1.21	1.21	1.24	1.23	1.24
Methionine (%)	0.55	0.55	0.55	0.44	0.44	0.43	0.37	0.36	0.36
Calcium (%)	1.00	1.01	1.00	0.90	0.90	0.90	0.80	0.80	0.80
Phosphorus (%)	0.50	0.50	0.50	0.45	0.45	0.45	0.40	0.40	0.40

[a] On an as-fed basis.

[b] The mineral mix composition was as follows (amounts in 10 g): 0.5 g Mg, 0.3 g S, 1.0 g Na, 1.6 g Cl, 6.0 mg Cu, 0.2 mg I, 45.0 mg Fe, 59 mg Mn, 0.2 mg Se, and 29 mg Zn.

[c] The vitamin mix composition was as follows (amounts in 10 g): 4000 IU vitamin A palmitate, 1000 IU cholecalciferol, 50 IU vitamin E acetate, 0.5 mg menadione sodium bisulfite, 0.2 mg biotin, 10 µg cyanocobalamin, 2 mg folic acid, 30 mg nicotinic acid, 16 mg calcium pantothenate, 7 mg pyridoxine-HCl, 6 mg riboflavin, and 6 mg thiamin HCl.

Laboratory (Karaj, Iran) for counting the total and differential white blood cells.

The remaining blood samples (2.1 mL) were transferred to clear glass tubes, kept at room temperature for 2 h, and then kept overnight at 4 °C in a refrigerator and centrifuged at 1500 × *g* for 15 min. Serum was obtained, inactivated at 56 °C for 30 min, and stored at –20 °C until analyses of the antibody titers. The titers of the antibody against Newcastle disease were determined by hemagglutination-inhibition test (12) and against infectious bursal disease by ELISA kit.

2.3. Weighing of immune system organs

On days 21 and 42 of age, a total of 24 birds (8 per treatment; 2 per pen) were randomly selected, individually weighed, stunned, killed by cervical dislocation, and plucked in a slaughterhouse. Their spleens and bursae of Fabricius were removed and weighed. The bursae of Fabricius were then fixed in 10% neutral buffered formalin for histology. Samples were dehydrated, cleared, and paraffin-embedded. Tissue samples were sectioned at 6 μm thickness, placed on glass slides, and processed by hematoxylin and eosin stain for examination by light microscopy.

2.4. Statistical analysis

The chicks (8 determinations per diet) were the experimental unit for organ weight, white blood cells, and antibody titers. All values were analyzed by one-way ANOVA using the GLM procedure of SAS for Windows version 9.1 (SAS Institute Inc., Cary, NC, USA). The F-test for the treatments was significant at $P \leq 0.05$ in the ANOVA table; means were compared for significant differences using the Tukey test of SAS.

3. Results

3.1. Immune tissue weights

As shown in Table 2, the data indicated that the relative spleen weight at days 21 and 42 of age was affected by the diets. At day 21 of age, inclusion of 2% and 4% soy oil to the diet decreased spleen weight of chicks by 17% and 26%, compared to those fed a soy oil-free diet. At this period, no significant difference was observed; however, at day 42 of age, differences appeared between relative spleen weights of chicks fed a diet containing 2% and 4% soy oil. At day 21 of age, there was no difference for relative bursal weights among treatments, but numerically the chicks fed the soy oil free diet had the highest bursal weight and those fed 4% soy oil had the lowest. At day 42 of age, the differences in the percentage of bursa of Fabricius to body weight among the chicks fed diets containing soy oil appeared ($P < 0.05$). At this period, inclusion of 2% soy oil to the diet decreased numerically and inclusion of 4% decreased ($P < 0.05$) relative weights of the bursa of Fabricius by 5% and 10%, respectively, compared to the oil-free diet. Figure 1 shows histological status of the bursa of Fabricius related to chicks fed the soy oil-free diet and the diet containing 4% soy oil. Higher stored fat as lines was observed among lymphocytes in chicks fed soy oil compared to those fed the oil-free diet.

3.2. Effects on immune cells

As shown in Figure 2, there were differences in total white blood cells among chicks fed dietary treatments ($P < 0.05$) on day 21, but not on day 42 of age. On day 21 of age, total white blood cell count in the chicks fed a diet containing 4% soy oil was 11% higher than in the chicks fed a diet containing 2% soy oil and 22% higher than in the chicks fed an oil-free diet. As shown in Figure 3, there were no differences ($P > 0.05$) for percentages of heterophils and lymphocytes between chicks fed the oil-free diet and 2% soy oil in diet. Inclusion of 4% soy oil to the diet resulted in an increase ($P < 0.05$) in heterophil and a decrease ($P < 0.05$) in lymphocyte percentages. Differential white blood cell count of chicks on day 42 of age is shown in Figure 4. At this period, significant differences ($P < 0.05$)

Table 2. Relative spleen and bursal weights of chicks fed soy oil free or rich diets (as g/kg body weight).

	Dietary treatments			SEM*
	Soy oil-free	Soy oil 2%	Soy oil 4%	
Spleen				
Day 21	1.20[a]	1.02[b]	0.95[b]	0.103
Day 42	1.01[a]	0.98[a]	0.87[b]	0.060
Bursa of Fabricius				
Day 21	1.62	1.57	1.41	0.126
Day 42	1.48[a]	1.41[ab]	1.35[b]	0.062

*SEM: standard error of the means

[a,b]: Means in the same row with different superscripts are significantly different ($P < 0.05$).

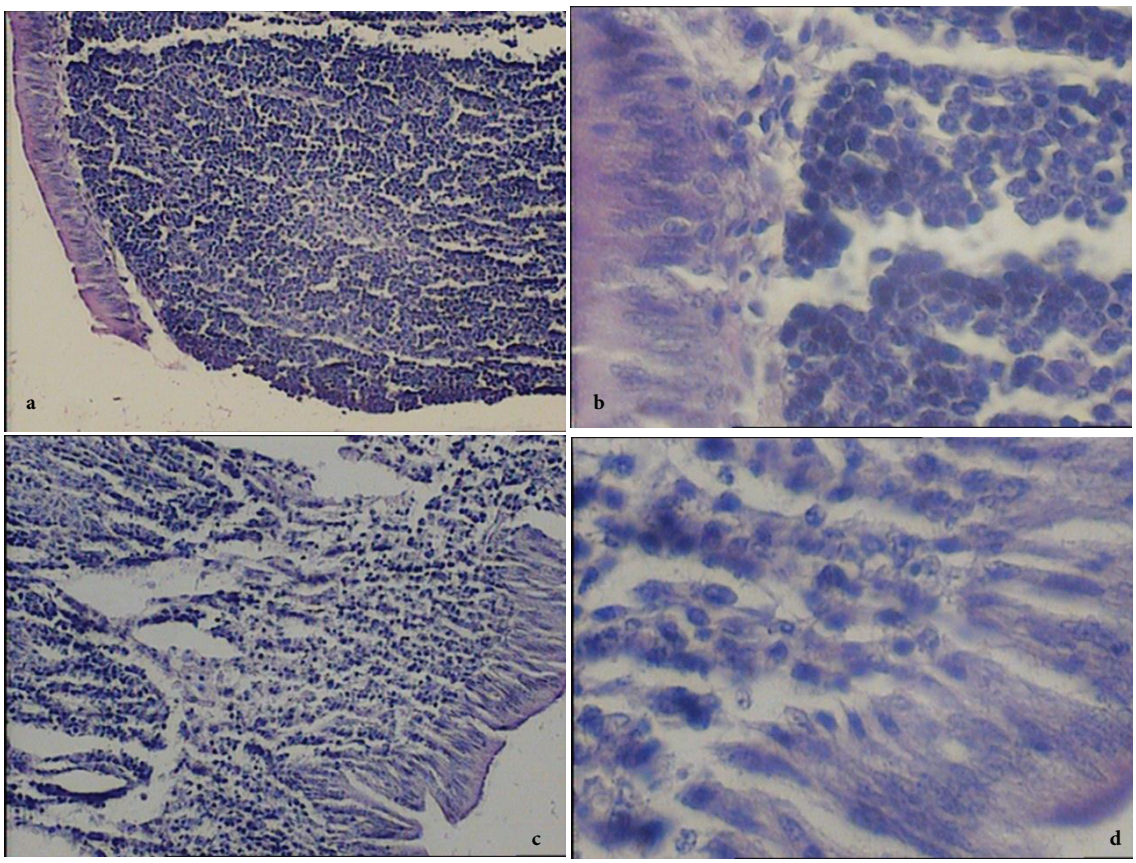

Figure 1. a (10×) and b (40×) show the hematoxylin and eosin staining of the bursa of Fabricius related to chicks fed a soy oil-free diet. Lymphoids are in the follicles with mild fat deposited around them (arrow). c (10×) and d (40×) show the hematoxylin and eosin staining of the bursa of Fabricius related to chicks fed a diet containing 4% soy oil. Lymphoid depletion is seen in the follicles with more fat deposited within and around them (arrow).

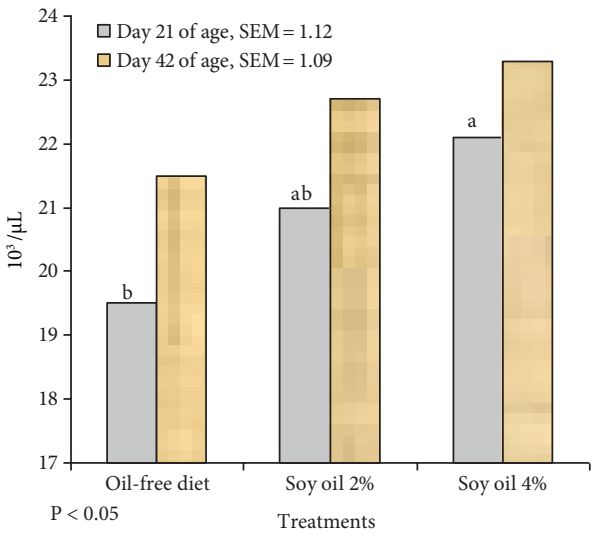

Figure 2. Total white blood cell count of chicks fed experimental diets.

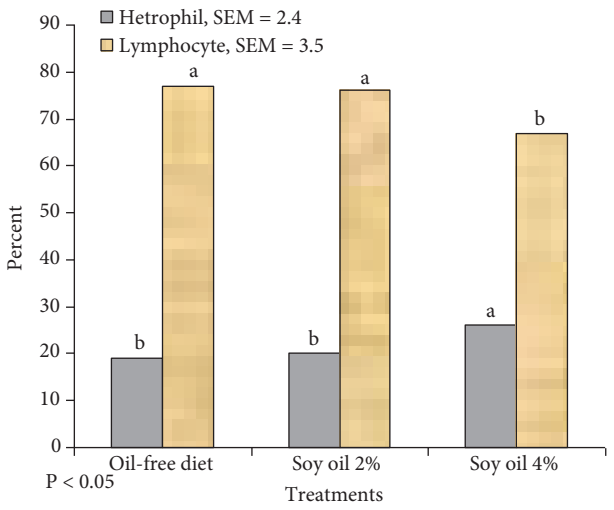

Figure 3. Differential white blood cell count of chicks at 21 days of age.

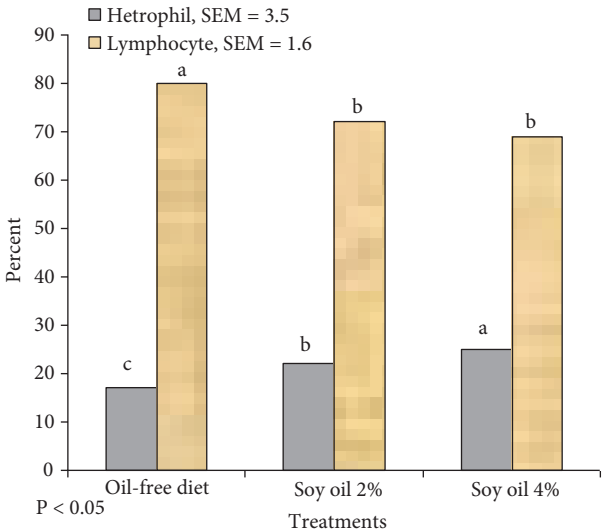

Figure 4. Differential white blood cell count of chicks at 42 days of age.

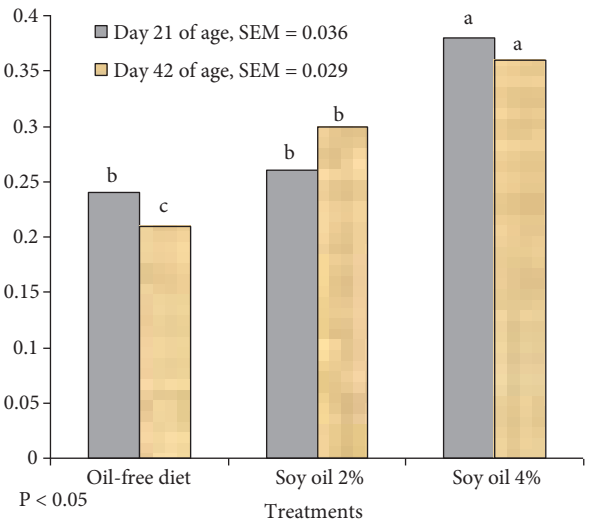

Figure 5. Heterophil-to-lymphocyte ratio in blood samples of chicks at days 21 and 42 of age.

in percentages of heterophils and lymphocytes appeared between chicks fed diets containing no oil and 2% soy oil. Increasing soy oil in the diet resulted in significant increase of heterophil percentage and significant decrease of lymphocyte percentage. Consequently, at days 21 and 42 of age, chicks fed the diet containing 4% soy oil had the highest heterophil-to-lymphocyte ratio, and those fed the oil-free diet had the lowest (Figure 5). There was no difference ($P > 0.05$) for this parameter between chicks fed the oil-free diet and those fed diets containing 2% at day 21, but significant differences appeared at day 42 of age.

3.3. Effects on antibody titration

Antibody titers against Newcastle and infectious bursal viruses in chicks fed with the soy oil-free diet were higher ($P < 0.05$) than those fed diets containing soy oil on days 21 and 42 of age (Table 3). At day 21 of age, antibody titers against Newcastle disease decreased with the addition of soy oil to the diet, but at day 42 of age there was no difference ($P > 0.05$) between the chicks fed the diet containing 2% and 4% soy oil. On day 42 of age, chicks fed the diet containing 2% soy oil had numerically higher antibody titers of Newcastle than those fed the diet containing 4% soy oil. Similar trends were observed for antibody titers against infectious bursal virus; however, effects of the experimental diets on them were more evident than with Newcastle disease.

4. Discussion

The main purpose of the present study was to examine the effect of dietary supplementation of soy oil on the immune responses of broiler chicks challenged with Newcastle and bursal infectious viruses as antigens. Effects of these dietary treatments on white blood cells and relative weights of immune organs were also measured. We hypothesized that supplementation of soy oil (as a source of n-6 PUFA) to the diet results in a change (negative effect) of the antibody response. The results showed that supplementation of soy oil to the diet suppressed antibody titers against Newcastle and infectious bursal diseases. Our result was inconsistent with the findings of some authors (4), who reported that n-6 PUFA increased antibody production. These discrepant findings might be associated with the types and doses of n-6 PUFA used and the classes of the antigen to which the antibodies were directed.

Our findings are consistent with studies (5,7) that found that high levels of oils containing n-6 PUFA decreased antibody response. In agreement with our results, antibody response to sheep red blood cells in the study of van Heugten et al. (6) was greater than in pigs fed a diet supplemented with starch. However, antibody response in pair-fed pigs was decreased significantly when fat was included in the diet.

At least 4 modes of action have been proposed to explain the potential action of fatty acids on the modulation of immune systems in both animals and humans. Accordingly, immune system modulation by dietary lipids may be attributed to changes in the composition of membrane phospholipids, lipid peroxidation, alteration of gene expression, or production of eicosanoids, cytokines, and arachidonic acid (1,7,10,13–17).

Moreover, an in vitro study (17) showed that the addition of arachidonic acid can directly reduce lymphocyte proliferation and is toxic to these cells. As shown in Figure 1, lymphoid depletion occurred in the bursa of Fabricius of chicks fed the diet containing 4% soy oil. Fat deposition was observed around follicles of the bursa of Fabricius of

Table 3. Serum antibody titers (log_2) of chicks against Newcastle and infectious bursal diseases at 12 and 42 days of age.

	Dietary treatments			SEM
	Soy oil-free	Soy oil 2%	Soy oil 4%	
Day 21				
Newcastle disease	4.50[a]	3.75[ab]	3.25[b]	0.527
Infectious bursal disease	434[a]	396[b]	324[c]	13.54
Day 42				
Newcastle disease	6.75[a]	5.50[b]	4.75[b]	0.707
Infectious bursal disease	3157[a]	3060[b]	2854[c]	43.21

[a,b,c]: Means in the same row with different superscripts are significantly different ($P < 0.05$).

chicks fed an oil-free diet; however, more fat was observed within and around follicles in chicks fed a diet containing 4% soy oil.

In the present study, white blood cell counts increased with the inclusion of soy oil in the diet at days 21 and 42 of age. Corticotropin-releasing factor is known to increase the adrenocorticotropic hormone from the pituitary gland; the adrenocorticotropic hormone then stimulates corticosterone production from the adrenal gland. An interesting study (18) revealed that inclusion of soy oil in the diet could induce significant increases in serum corticosterone concentration. Corticosterone has been found to be immunosuppressive (19,20), inhibiting the production and actions of antibodies, lymphocyte function, and leukocyte population (21,22). Based on the results in Figures 2–4, chicks fed a diet containing soy oil may experience physiological stress. The heterophil-to-lymphocyte ratio has been accepted as a reliable index for determining stress in poultry (21). The increases in heterophil-to-lymphocyte ratio in chicks fed a diet containing soy oil may be attributed to increased corticosterone secretion, which resulted in a decrease of the antibody titers (23).

Immune tissue development is the basis of immune system functionality. Relative organ weights of the bursa of Fabricius and spleen were influenced negatively by the addition of soy oil to the diet, particularly on day 42 of the experiment. The increase of soy oil in chick diets significantly reduced the growth of the spleen and bursa of Fabricius. Low bursal weight could be interpreted as an indicator of low immune activity because it is a major lymphoid organ in poultry. The decrease of immune tissue weight produces an effect on immune cell phenotypes, immune cell proliferation, and antibody production. One likely reason for reducing immune tissue growth by inclusion of soy oil to the diet may be an increase of serum cortisol in chicks. As stated previously (18), soy oil could induce significant increases in serum corticosterone concentrations. Under this condition, retardation of immune organs occurs (24).

The results of this study indicated that the addition of soy oil to the diet has a negative effect on the immune response of broiler chicks through retarding the immune organs growth, changing the immune cells profile, and decreasing antibody production.

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