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Comparison of the Effect of Different Levels of *Scenedesmus sp.* Microalgae on Growth, Immune Response, Carcass Traits, and Some Blood Parameters of Broiler Chickens

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ABSTRACT

A study was conducted to compare the effects of different levels of a suspension of *Scenedesmus* Microalgae (SM) on growth, immune response, carcass traits, and some blood parameters of broilers. Two hundred 1-d-old broiler chicks were randomly assigned to each of 5 dietary treatments. Treatments consisted of: T1 (100% drinking water), T2 (50% drinking water+50% SM), T3 (25% drinking water+75% SM), T4 (100% SM) and T5 [(Basal diet (BD) + 2.5 mg Virginamycin kg^{-1} diet)] with 4 replicate cages of 10 birds each. The birds receiving T3 and T4 had higher feed intake compared with the control and antibiotic groups at 1-10 days (P< 0.001). Water replacement by 100% SM increased body weight (P< 0.001) and reduced feed conversion ratio (P< 0.05) in broilers. Inclusion of 100% SM increased SRBC antibody titer in primary and secondary responses compared to that of the control group. (P< 0.05). The birds receiving T4 had lower serum content of triglycerides and cholesterol compared with the control group (P< 0.05). Chickens at the T4 group showed higher relative breast weight compared with the control group (P< 0.05). It was concluded that SM at the levels of 75 and 100% can improve growth performance, immune responses, and some blood parameters of broiler chicks.

Keywords: Body weight, Feed intake, Triglycerides concentration, Virginiamycin.

INTRODUCTION

The addition of antibiotics for growth promotion in food animals is increasingly banned in order to reduce the development of resistant pathogenic organisms which compromise treatment of disease in humans and animals (Corpet, 1996; Williams and Heymann, 1998). Because consumers are very concern about this subject, it is of growing interest for academics and feed industry nutritionists to find appropriate alternatives to ensure the safety of animal products (Bach-Knudsen, 2001; Smith *et al*., 2002). With the elimination of antibiotic growth promoters from poultry diets in different regions of the world, it is important to investigate potential alternatives to sustain good growth performance and good intestinal microbial populations, especially to control the growth of harmful bacteria (Joerger, 2002). Many supplements are used or proposed as means to decrease or remove pathogens or enhance growth and feed conversion ratio (Joerger, 2002). The use of microalgae as a feed supplement could be one solution for problems associated with

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the use of antibiotics in feed. Specific algal species are appropriate for preparation of animal feed supplements. Algae species such as *Chlorella, Scenedesmus and Spirulina* have beneficial aspects, promoting enhanced immune response, improved fertility, better weight control, healthier skin and a lustrous coat (Pulz and Gross, 2004). Fatty acids are structural components of lipids, and the types and amounts of fatty acids vary considerably among algae. In recent years, fatty acid composition in large scale production of microalgae, including marine algae, have created considerable interest among researchers. This is mainly because of the health benefits of Mono- and PolyUnsaturated Fatty Acids (MUFAs and PUFAs) that can be found in microalgae. In addition, PolyUnsaturated Fatty Acids (PUFAs) play key roles in cellular and tissue metabolism, including the regulation of membrane fluidity, electron and oxygen transport, as well as thermal adaptation (Funk, 2001). Microalgae have other benefits also, such as their fast growth, non-competition with food crop production, and high efficiency in stabilizing CO_2 (Zhao *et al.,* 2013). The research has shown that application of microalgae has become popular to remove toxic heavy metals, due to its high absorbing capacity and low cost. Additionally, metals removed by absorption onto the cell surface may be successfully recovered, after desorption brought about by chemical agents (Bayramoglu and Arica, 2008; Doshi *et al.,* 2007). Costa and Franca (1998) reported that a 10 g L^{-1} EDTA solution could totally recover the Cd previously removed by adsorption onto the cell walls of the microalgae *Tetraselis chuii*. *Scenedesmus* microalga contains 22.4, 46.65, and 34.93% lipid, carbohydrates and CP, respectively (Toyub *et al.,* 2008). Many health supplement stores now sell preparation of microalgae such as *Spirulina* and *Chlorella* packed in capsule or caplets, or even in food and beverages known to have therapeutic values in hypercholesterolemia, hyperlipidemia, and atherosclerosis (Eussen *et al.,* 2010). There are results showing microalgae have various possible health promoting effects: the alleviation of hyperlipidemia, suppression of hypertension, protection against renal failure, growth promotion of intestinal *Lactobacillus*, and suppression of elevated serum glucose level (Liang *et al.,* 2004). Skrivan *et al.* (2010) showed that inclusion of selenium-*Scenedesmus* biomass in the diet increased oxidative stability of meat, presented as reduced malondialdehyde in breast meat after 10-day fridge storage. In one study on *Spirulina* alga, Mariey *et al.* (2014) showed that addition of 0.3mg of *Spirulina*/kg diet increased red and white blood cell counts in broiler chicks. Therefore, in view of the cited benefits of microalgae, the aim of the present study was to compare the effect of *Scenedesmus* microalgae and antibiotic (Virginiamycin) on growth performance, immune responses, carcass traits, and some blood parameters of broilers, suggesting the SM as a growth promoter instead of antibiotic.

MATERIALS AND METHODS

Animals and Diets

A total of 200, one-day-old Ross-308 male broiler chicks (Average body weight: 42±2 g) were gained from a commercial hatchery. The chicks were allocated randomly to five dietary treatments with 4 cages (replicates, n=10 birds, 1×1 m^2) per treatment. The rearing house had windows and was equipped with ventilation and heating systems set by thermostats. During the first 3 weeks, recommended brooding temperatures were used, so that the temperature was decreased progressively from 33 to 23.9°C by the end of the 3rd week of age. The lighting schedule was 23 hours of light/1 hour dark cycle with an average light intensity of 15 lux was kept until the end of the experiment. The birds were fed with the starter diet (22% protein, 3,026.18 kcal ME kg^{-1}) for 10 days, followed by a grower diet (21% protein, 3,150.38 kcal ME

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kg^{-1}), from days 11 to 24, and finishing diet (19% protein, 3,200.54 kcal ME kg^{-1}), from days 25 to 42. The ingredients and chemical composition of diets are shown in Table 1. Treatments consisted of T1 (100% drinking water), T2 (50% drinking water+50% SM), T3 (25% drinking water+75% SM), and T4 (100% SM) and T5 (BD+2.5 mg Virginamycin kg^{-1} diet), with 4 replicate cages of 10 birds each. A pure stock solution of SM (concentration of 25×10^6 cell mL^{-1} solution) was purchased from Caspian Research Group of Fisheries and Water Pollutants (CRFWP), Sari, Iran, and this solution was then added to drinking water to prepare the abovementioned treatments. The cells numbers of SM in each mL of solutions were zero, (12.5×10^6), (18.75×10^6), and (25×10^6), respectively, for abovementioned treatments. Every 2 to 3 hours, the SM solution was stirred to prevent settling of the solution. The nutrients compositions of *Scenedesmus* microalga were 22.4, 46.65, and 34.93% lipid, carbohydrates, and CP, respectively. Body Weight (BW) and Feed Intake (FI) were recorded at d 10, 24, and 42 and Feed Conversion Ratio (FCR) was calculated accordingly. Daily water intake for each pen was measured. Every day, a known volume of water was placed in each cup water trough, and the volume of water remaining the following morning was measured as refusal. Water intakes were subsequently adjusted according to measured daily evaporative losses from similar troughs at appropriate locations within the building to calculate water intake.

Humoral Immune Response

Sheep Red Blood Cells (SRBCs) were applied as a test antigen to evaluate the humoral immune response. The same birds (day 28) were then immunized intravenously via the right wing with 0.5 mL of 0.9% SRBC suspension. Seven days after injection, blood samples were collected by

Table 1. Composition of the basal diet.

Ingredients (%)	Starter	Grower	Finisher
Corn	53.00	54.81	60.63
Soybean meal	38.74	36.24	30.80
Oil	3.70	5.00	4.85
Limestone	1.40	1.35	1.30
Dicalcium phosphate	1.80	1.29	1.21
Vitamin premix[a]	0.25	0.25	0.25
Mineral premix[b]	0.25	0.25	0.25
Salt	0.30	0.29	0.29
l-Lysine hydrochloride	0.32	0.23	0.13
dl-Methionine	0.24	0.29	0.29
Nutrient analysis			
Metabolizable energy (Kcal kg^{-1})	3026.18	3150.38	3200.54
Crude protein (%)	22	21	19
Lysine (%)	1.43	1.30	1.09
Methionine+Cystine (%)	1.07	0.95	0.86
Threonine (%)	0.94	0.90	0.82
Calcium (%)	1.05	0.90	0.85
Phosphorus (%)	0.52	0.45	0.42

[a] Vitamin premix provided the following per kilogram of diet: Vitamin A, 9,000 IU; vitamin D3, 2,100 IU; vitamin E, 30 mg; nicotinic acid, 30mg; vitamin B 12, 0.12 mg; calciumpantothenate,10 mg; vitamin K 3, 5 mg; thiamin, 1.1 mg; riboflavin, 4.5 mg; vitamin 6, 2.0 mg; folic acid, 0.5 mg; biotin. [b]Mineral premix provided the following per kilogram of diet: 5 mg; Fe, 50 mg; Cu, 10 mg; Mn, 70 mg; Zn, 50 mg; Co, 0.2 mg; I, 1.0 mg; Se, 0.3 mg; butylated hydroxytoluene (BHT), 150 mg; monensin, 100 mg.

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wing venipuncture and 6 mL blood was collected. The samples were incubated at 37°C for 1 hour to aid clotting, and then centrifuged at 3,000×g for 10 minutes and the serum collected and stored at -4°C until assay for assessment of the primary antibody response to SRBC. Seven days after the first challenge, birds were intravenously administered a booster injection of 0.5 mL 0.9% SRBC, and blood samples were collected 3 days post-injection using the same procedures as for the first sample collection. Serum samples were tested for total antibody as previously explained by Lepage *et al.* (1996).

Blood Parameters and Carcass Traits

At 42 days of age, after a 12-hour fast, blood samples (2.5 mL per bird) were collected from two birds in each cage by puncturing the brachial vein. Samples were collected into non-heparinized tubes and centrifuged at 2,500×g for 15 minutes to obtain serum (SIGMA 4–15 laboratory centrifuge, Osterode am Harz, Germany). Individual serum samples were analyzed for glucose, High Density Lipoprotein (HDL), Low-Density Lipoprotein (LDL), cholesterol, and triglycerides with a spectrophotometer using the kit package (Pars Azmoon; Tehran, Iran).

At 42 days of age, further blood samples were collected from two birds in each cage into vials containing EDTA to avoid blood clot formation. The Red Blood Cell (RBC) and White Blood Cell (WBC) counts were examined by a hemocytometer method using Natt–Herrick solution; hematocrit values and hemoglobin amounts were assessed by microhematocrit and cyanmethaemoglobin methods, respectively, as described by Kececi *et al.* (1998).

Also, at 42 days of age, two birds were chosen randomly from each replicate, weighed and slaughtered, and their breast, thigh, liver, spleen, pancreas, bursa of Fabricius, empty duodenum, jejunum, ileum and caecum were weighed and expressed as a percentage of live body mass.

Statistical Analysis

The data were analyzed using the general linear model procedure of Minitab software as a completely randomized design. Differences among treatment means were calculated using the Tukey test. The $\log_2$ transformations were done on antibody titers before statistical analysis. All parameters were examined as follows:

$Yij = \mu + Ti + eij$

Where, Yij= Individual observation, μ= Overall mean, Ti= Effect of treatment and eij represents the random error.

RESULTS

Performance

Effects of dietary inclusion of microalgae suspension and virginiamycin on broiler performance are presented in Table 2. Microalgae level and dietary supplementing by virginiamycin had no significant effect on feed intake at grower and finisher periods. SM at high levels (75 or 100%) had significant effect on feed intake at the starter period ($P< 0.05$) and the birds receiving SM at levels of 75 and 100% consumed more feed compared with other groups. SM at high levels (T3 and T4) had positive effects ($P< 0.05$) on Body Weight Gain (BWG) at finisher periods, so that birds receiving T4 produced higher body weight gain than that of other birds. The broilers receiving T3 showed higher BWG at starter and grower periods, respectively, when compared with other birds. In overall (1-42 days), birds receiving T4 were heavier ($P< 0.001$) than those that received other treatments. As expected, birds treated with SM at level of 100% showed lower *FCR* ($P< 0.05$) compared with other groups at 25-42 days and also overall the experiment ($P< 0.023$). Inclusion of microalgae in water had

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Table 2. The effect of *Scendesemus* microalgae (SM) and virginiamycin on body weight (BW), feed intake (FI), food conversion ratio (FCR) and water intake (WI) of broiler chicks.

	Treatments [a]						
Measurement	T1	T2	T3	T4	T5	*SEM*	*P*-value
FI (g)							
1-10 days	285^{b}	286^{b}	324^{a}	309^{a}	271^{b}	5.64	0.001
11-24 days	1227	1291	1432	1339	1252	45.53	0.079
25-42 days	3373	3301	3190	3175	3201	155.4	0.907
1-42 days	4885	4878	4946	4823	4724	206.6	0.365
BWG (g)							
1-10 days	210^{b}	211.5^{b}	231^{a}	227^{a}	193.5^{c}	3.957	0.001
11-24 days	728.5^{b}	729.5^{b}	867.9^{a}	762.1^{b}	780.0^{b}	21.73	0.018
25-42 days	1636^{c}	1656^{c}	1608^{c}	2001^{a}	1770^{b}	25.94	0.001
1-42 days	2574.5^{c}	2597^{c}	2706^{b}	2990^{a}	2743^{b}	51.63	0.001
FCR (g g^{-1})							
1-10 days	1.34^{b}	1.35^{b}	1.36^{b}	1.36^{b}	1.41^{a}	0.013	0.034
11-24 days	1.72^{a}	1.77^{a}	1.67^{b}	1.76^{a}	1.60^{b}	0.036	0.032
25-42 days	2.06^{a}	1.89^{a}	1.98^{a}	1.59^{b}	1.81^{a}	0.135	0.050
1-42 days	1.89^{a}	1.87^{a}	1.83^{ab}	1.76^{bc}	1.72^{c}	0.039	0.023
WI (ml)							
1-10 days	420.5^{c}	452.5^{b}	442.4^{b}	478.7^{a}	350.5^{c}	7.30	0.001
11-24 days	1520^{b}	1708^{a}	1513^{b}	1784^{a}	1498^{b}	81.3	0.049
25-42 days	7282^{a}	7221^{a}	7535^{a}	6328^{b}	7258^{a}	315.5	0.049
1-42 days	9222.5^{a}	9381.5^{a}	9490.4^{a}	8590.7^{b}	9106.3^{ab}	198.4	0.006

[a] Means in rows with different superscripts were significantly differ (P< 0.05). SEM: Standard Error of Means. T1 refers to (100% drinking water), T2 refers to (50% drinking water+50% SM); T3 refers to (25% drinking water+75% SM); T4 refers to (100% SM) and T5 refers to BD+2.5 mg antibiotic.

significant effects (P< 0.05) on water intake, so that birds receiving T4 had higher water intake during 1-10 and 11-24 days and lower water intake during 25-42 days (P< 0.05) compared to those that received T1 and T5. In overall (1-42 days), birds receiving T4 had significantly (P< 0.006) lower *WI* compared to those receiving T1, T2, and T3.

Immune Response

Antibody titer for primary response was significantly elevated by T4 (P< 0.05; Table 3). Chicks given supplementary T4 had significantly higher titer of antibodies for primary and secondary responses compared to those fed the control diet or those receiving the T2 (P< 0.05).

Blood Parameters

The effect of microalgae suspension and virginiamycin on some blood parameters are presented at Table 4. SM and dietary supplementation with virginiamycin had no

Table 3. The effect of *Scendesemus* Microalgae (SM) and virginiamycin on the anti-SRBC antibody response (primary and secondary; $\log_2$) of broiler chicks.

	Antibody titers	
Treatments[a]	Primary	Secondary
T1	5.13^{b}	5.50^{b}
T2	4.16^{b}	5.80^{b}
T3	6.25^{a}	6.50^{ab}
T4	6.73^{a}	7.00^{a}
T5	7.00^{a}	7.70^{a}
SEM	0.36	0.44
P-value	0.001	0.025

[a] Means in each column with different superscripts were significantly differ (P< 0.05). SEM: Standard Error of Means. [a] T1 refers to (100% drinking water), T2 refers to (50% drinking water+50% SM); T3 refers to (25% drinking water+ 75% SM); T4 refers to (100% SM) and T5 refers to BD+2.5 mg antibiotic

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Table 4. The effect of *Scenedesmus* microalgae (SM) and virginiamycin on glucose (mg dL^{-1}), HDL (%), LDL (%), cholesterol (mg dL^{-1}), triglycerides (mg dL^{-1}), Red Blood Cells (RBCs; 10^6 μL^{-1}), White Blood Cells (WBCs; 10^3 μL^{-1}), hemoglobin (g dL^{-1}) and hematocrit (%) of broiler chicks.

	Treatments[a]						
Measurement	T1	T2	T3	T4	T5	*SEM*	*P*-value
Glucose	218.5	192	215.5	205	205	7.43	0.161
Triglycerides	100.75[a]	64.75[c]	84.5[b]	46[d]	70.75[c]	5.29	0.002
Cholesterol	130.80[a]	113.2[b]	111.3[b]	106[b]	104.5[b]	4.12	0.005
HDL	74.75	60.75	70.75	74.75	72.00	2.76	0.778
LDL	24.50	24.25	26.00	18.50	15.29	2.76	0.078
WBC	22.03	22.74	22.88	23.44	22.75	4.20	0.490
RBC	2.15	2.15	2.49	2.67	2.37	0.29	0.07
Hemoglobin	9.87	11.32	11.82	11.35	10.77	0.68	0.361
Hematocrit	28.95	33.32	35.52	33.42	31.45	1.96	0.249

[abcd] Means in rows with different superscripts are significantly different (P< 0.05). SEM: Standard Error of Means. [a] Treatments are defined under Table2.

significant effects on glucose, HDL, LDL, hematocrit, hemoglobin and red and white blood cell. SM at high levels (100%; T4) had a significant impact on serum contents of cholesterol and triglycerides (P< 0.05) so that birds receiving SM at a level of 100% had lower serum contents of cholesterol and triglycerides concentrations compared with the control group.

Carcass Traits

The effects of dietary treatments of microalgae suspension and virginiamycin on relative weights of organs are presented at Table 5. Dietary treatments had significant effects (P< 0.05) on relative weight of some organs, so that birds supplemented by microalgae (T4) had higher breast weight compared with other birds. The birds treated with antibiotic had higher liver weights compared with birds supplemented with microalgae. In addition, birds treated with microalgae had lower weights for pancreas and ileum compared with other groups. Also, birds supplemented with microalgae at a level of 75% produced higher spleen weight compared with those receiving other levels of microalgae.

Table 5. The effect of *Scenedesmus* microalgae (SM) and virginiamycin on relative weight (%) of organs of broiler chicks.

	Treatments*						
Measurement	T1	T2	T3	T4	T5	SEM	P-value
Breast	24.3[b]	23.9[b]	24.5[b]	27.1[a]	23.5[b]	1.20	0.011
Thigh	22.2	21.1	22.1	21.9	20.7	0.61	0.103
Liver	2.25[b]	1.95[b]	1.99[b]	1.92[b]	3.01[a]	0.608	0.001
Spleen	0.189[a]	0.116[c]	0.176[a]	0.137[bc]	0.171[ab]	0.012	0.006
Bursa	0.079	0.080	0.075	0.074	0.107	0.008	0.076
Pancreas	0.325[a]	0.290[b]	0.290[b]	0.284[b]	0.340[a]	0.007	0.001
Abdominal fat	1.63	0.77	1.32	1.34	1.11	0.181	0.147
Duodenum	0.998	0.862	0.972	0.917	0.923	0.077	0.791
Jejunum	2.78	1.85	2.34	2.30	2.72	0.220	0.271
Ileum	2.13[a]	1.34[b]	1.45[b]	1.50[b]	2.39[a]	0.091	0.001
Caecum	0.730	0.807	0.673	0.791	0.740	0.116	0.929

[abc] Means in rows with different superscripts are significantly different (p<0.05). SEM: Standard Error of Means. Treatments are defined under Table 1.

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DISCUSSION

In the current study, chicks treated with SM at high levels showed better performance during growth periods when compared with virginiamycin antibiotic. There is no previous study in the literature documenting effects of SM on performance of broiler chicks. In similar studies on other microalgae in broiler chicks, Ross and Dominy (1990) and Nikodemusz *et al.* (2010) reported that birds fed with *Spirulina* microalgae showed better performance. Zahroojian *et al.* (2013) reported that addition of different levels of *Spirulina* microalgae had no significant effect on performance of laying hens, Recently, Shanmugapriya *et al.* (2015) reported a significant improvement in body weight gain and *FCR* in broilers fed a diet containing *Spirulina* microalgae. Improvement in growth may associate with microalgae components because of their high content of protein and unsaturated fatty acids (e.g., EicosaPentaenoic Acid [EPA], Arachidonic Acid [AA] and DocosaHexaenoic Acid [DHA]) (Reitan *et al.*, 1997). El-Sayed (2010) believed that an increase in growth associated with feeding of green algae *Scenedesmus* sp. may be attributed to the initial content of some macro and micro-nutrients. SM caused a significant increase in water intake during starter and grower periods. We believe that SM can improve water quality and, consequently, leading to an increase in water intake(WI). However, the birds receiving T4 had exceptionally the lowest *WI* during finisher and overall period of the experiment. Lower *WI* in this group could be attributed to the lower FI during finisher and overall periods of the experiment.

Inclusion of SM (high levels) in water improved primary and secondary immune responses to SRBC injection. It is very important to enhance immunity as a means to avert infectious diseases in poultry production. Application of immunostimulants is one solution for improvement in the animals' immunity and to reduce their susceptibility to infectious disease (Liu, 1999). In contrast to our findings, Yakhkeshi *et al.* (2011) showed that the primary immune response against SRBC was not affected by virginiamycin, but the lowest secondary immune responses against SRBC were observed in virginiamycin treatments. Hosseini and Mohammad Nezhady (2011) showed that diet supplementation with antibiotic and yogurt treatment had no significant effect on the titre of SRBC. Gharib *et al.* (2012 reported that inclusion of diet with probiotic improved immune response against SRBC test. Previous studies showed that antibiotics reduce the microbial competition for nutrients in the host and raise the availability of nutrients (Vukic Vramjes and Wenk, 1995) by decreasing pathogenic bacteria (Miles *et al.,* 2006) and their toxins in the intestine. We could not find any report showing the effect of microalgae on immune responses of boiler chicks to compare with the present study. In one human study on *Chlorella* microalga, Nakano *et al.* (2007) showed that *Chlorella* supplementation had a positive effect on the reduction of dioxin levels in breast milk and it may also have beneficial effects on nursing infants by an increase in IgA levels in breast milk. Khan *et al.* (2005) showed that *Spirulina* microalga has immuno-stimulatory effects and antiviral activity. Also, *Spirulina* sp. caused greater levels of cytotoxic lymphocytes that were critical to innate immunity, and had elevated phagocytic activity and antibody production (Luescher-Mattli, 2003).The idea is confirmed by Table 4. Result showed that birds treated by SM (100%) produced higher white blood cell than that of the other birds. In addition, microalgae's have positive effects on *Lactobacillus* (profitable bacteria) population in intestine (Liang *et al.,* 2004) and may help immunity system.

In the current study, diet supplementation with antibiotic and *Scenedesmus* microalgae had no significant impacts on glucose, hemoglobin, and hematocrit. In similar

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studies, Mariey *et al.* (2012) showed that dietary supplementation with *Spirulina platensis* algae at levels of 15% and 20% increased the serum content of glucose in laying hens. Parallel with our findings, Howe *et al.* (2011) showed that diet supplementing with *Nannochloropsis oculata* in adolescent male rabbits had no significant effect on the serum content of glucose. Microalgae carbohydrates can be found in the form of starch, glucose, sugars, and other polysaccharides. Their overall digestibility is high, which is why there is no limitation to using dried whole microalgae in foods or feeds (Becker, 2004). Hashemzadeh *et al.* (2013) reported that birds receiving virginiamycin antibiotic produced higher blood glucose content compared with those fed with basal diet. Rahimi and Khaksefidi (2006) reported that diet supplementation with virginiamycin antibiotic failed to have any significant impact on hemoglobin in heat-stressed birds when compared with a control diet. We have been unable to find any report showing the effect of microalgae on hemoglobin and hematocrit of animals or humans to compare with the present study.

Results indicated that addition of *Scenedesmus* microalgae to water reduced the serum content of triglycerides compared with birds receiving T1. Also, the serum concentration of cholesterol was reduced in birds receiving *Scenedesmus* microalgae compared with those fed basal diet. The HDL-C and LDL-C were not affected by dietary treatments. Parallel to our findings, Ginzberg *et al.* (2000) showed that inclusion of 10% *Porphyridium sp.* red algal biomass lowered egg yolk cholesterol levels by 24%. In one study on mice, Fong *et al.* (2000) showed a significant decrease of triglycerides and cholesterol content in mice fed with *Spirulina*. It was concluded that *Scenedesmus* microalgae have positive effects on lipid profile, although the mechanism of this association are not known. We believe that *Scenedesmus* microalgae inhibit lipid peroxidation and, therefore, help the lipid profile. Skrivan *et al.* (2010) documented that usage of selenium-enriched *Scenedesmus* in chicken diets caused an elevation in selenium concentration in breast muscle, enhancement of glutathione peroxidase activity, and a decrease of lipid oxidation in meat without any toxic effect on the experimental animals. On the other hand, Iwamoto (2004) showed that the cell wall of some microalgae contained high levels of β-1, 3-glucan, a bio-active free-radical scavenger and help to decrease triglycerides content. In addition, improvement in some parameters of lipid profile may associate with microalgae components which reduce cholesterol and triglycerides, such as unsaturated fatty acids ([EPA], [AA] and [DHA]) (Reitan *et al.*, 1997). In the present study, diet supplementation with antibiotic had no significant effect on LDL-C and HDL-C. In contrast to our results, Hashemzadeh *et al.* (2013) reported that virginiamycin antibiotic reduced the serum content of HDL-C but had no significant effect on cholesterol and triglyceride contents compared with chickens fed with basal diet. It seems that the levels of microalgae in the present study were not adequate to meet the requirements for measurable changes in LDL-C and HDL-C.

White blood cell counts numerically increased in birds supplemented with T4 (microalgae at high level) compared with broiler chicks receiving other levels, antibiotic, and basal diet. In another study on microalgae, Mariey *et al.* (2014) indicated that addition of 0.3mg *Spirulina*/kg diet elevated white blood cell counts. In contrast, Yan and Kim (2013) reported that broiler chicks treated with *Schizochytrium* at 0.1 or 0.2% had no significant differences with other birds for white cell blood counts. In agreement with our observations, Rahimi and Khaksefidi (2006) reported that diet supplementation with virginiamycin antibiotic failed to have any significant impact on WBC count of heat-stressed birds when compared with a control diet. As mentioned before, Khan *et al.* (2005) showed that *Spirulina* microalga has

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immuno-stimulatory effects and antiviral activity. Also, *Spirulina* sp. increased cytotoxic lymphocytes that were critical to innate immunity, and elevated phagocytic activity and antibody production (Luescher-Mattli, 2003).

Breast weight in birds supplemented with microalgae at high levels was higher when compared with other birds, and liver weight increased in birds that received diet supplemented with antibiotic compared to those fed with microalgae. Also, birds treated with microalgae had lower weights for pancreas and ileum compared with other groups. Yan and Kim (2013) reported that broiler chicks treated with *Schizochytrium* at 0.1 or 0.2% had no significant differences from other birds in relative weight of breast meat. Toyomizu *et al.* (2001) showed that *Spirulina* did not increase the relative weight of organs. Mariey *et al.* (2014) showed an increase in liver weight of birds nourished with *spirulina platensis* algae. We believe that an increase in liver weight in the birds supplemented with antibiotic may be a result of the liver trying for antibiotic detoxification. The treatments had no effect on abdominal fat.

CONCLUSIONS

On the basis of our findings, it could be concluded that SM at high levels (75 and 100%) had beneficial effects on growth promotion, immune response, and some blood parameters. This study also indicated that SM (T4) increased breast weight. The results of the present study suggested that *Scenedesmus microalgae* at high levels could be a viable alternative to antibiotics in broiler diets. The benefits of feeding microalgae merit much more attention.

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REFERENCES

1. Bach-Knudsen, K. E. 2001. Development of Antibiotic Resistance and Options to Replace Antimicrobials in Animal Diets. *Proc. Nat. Soc.*, **60**: 291–299
2. Bayramoglu, G. and Arica, M.Y. 2008. Removal of Heavy Mercury (II), Cadmium (II) and Zinc (II) Metal Ions by Live and Heat Inactivated Lentinus Edodes Pellets. *Chem. Eng. J.*, **143**:133–140.
3. Becker, W. 2004. Microalgae in Human and Animal Nutrition. In: "*Handbook of Microalgal Culture*", (Ed.): Richmond, A. Blackwell, Oxford, PP. 312– 351.
4. Corpet, D. E. 1996. Microbiological Hazards for Humans of Antimicrobial Growth Promoter Use in Animal Production. *Rev. Med. Vet.*, **147:** 851.
5. Costa, A. C. A. and Franca, F. P. 1998. The Behavior of the Microalgae *Tetraselmis chuii* in Cadmium-Contaminated Solutions. *Aqua. Int.*, **6**: 57–66.
6. Doshi, H., Ray, A. and Kothari, I. L. 2007. Bioremediation Potential of Live and Dead *Spirulina*: Spectroscopic, Kinetics and SEM Studies. *Biotechnol. Bioeng.*, **96**:1051–1063.
7. El-Sayed, A. B. 2010. Carotenoids Accumulation in the Green Alga *Scenedesmus* sp. Incubated with Industrial Citrate Waste and Different Induction Stresses. *Nat. Sci.*, **8 (10):** 34-40.
8. Eussen, S., Klunge, L.O., Garssen, J., Verhagen, H., Van Kranen, H., Van Loveren, H. and Rompelberg, C. 2010. Support of Drug Therapy Using Functional Foods and Dietary Supplements: Focus on Statin Therapy. *British J. Nutr.*, **103 (9)**: 1260-1277.
9. Fong, B., Cheung, M. and Lee, M. 2000. Effect of Dietary Spirulina on Plasma Cholesterol and Triglyceride Levels in Mice. In: Abstracts. *4th Asia-Pacific Conference on Algal Biotechnology*, Hong-Kong. 150 PP.
10. Funk, C. D. 2001. Prostaglandins and Leukotrienes: Advances in Eicosanoids Biology. *Sci.*, **294:** 1871–1875.
11. Gharib Naseri, K., Rahimi, S. and Khaki, P. 2012. Comparison of the Effects of

[DOR: 20.1001.1.16807073.2018.20.6.16.8]

Probiotic, Organic Acid and Medicinal Plant on *Campylobacter jejuni* Challenged Broiler Chickens. *J. Agr. Sci. Tech.,* **14**: 1485-1496.

12. Ginzberg, A., Cohen, M., Sod-Moriah, U., Shany, S., Rosenshtrauch, A. and Arad, S. 2000. Chickens Fed with Biomass of the Red Microalga *Porphyridium sp* Have Reduced Blood Cholesterol Level and Modified Fatty Acid Composition in Egg Yolk. *J. Appl. Phycol.,* **12:** 325–330.
13. Hashemzadeh, F., Rahimi, S., Karimi Torshizi, M. A.and Masoudi, A. A. 2013. Effects of Probiotics and Antibiotic Supplementation on Serum Biochemistry and Intestinal Microflora in Broiler Chicks. *Int. J. Agric. Crop Sci.,* **5(20)**: 2394-2398.
14. Hosseini, M. N. and Mohammad Nezhady, M. A. 2011. Effect of Garlic, Thyme and Yogurt Compared to Antibiotics on Pperformance, Immunity and Some Blood Parameters of Broiler Chickens. *Indian J. Anim. Sci.,* **81(12):** 1197–1200.
15. Howe, B. A., Roman-Muniz, I. N., Willson, B. D. and Archibeque, S. L. 2011. *Nannochloropsis oculata* Meal Did Not Alter Nutrient Usage and Had No Adverse Health Effects When Fed to Rabbits as a Protein Source. *J. Anim. Sci.,* **89 (Suppl. 2)**: 724. (Abstrac)
16. Iwamoto, H. 2004. Industrial Production of Microalgal Cell-Mass and Secondary Products-Major Industrial Species: Chlorella. In: "*Handbook of Microalgal Culture: Biotechnology and Applied Phycology"*, (Ed.): Richmond, A. Willey-Blackwell Publishing, New Jersey, PP. 255–263.
17. Joerger, R. D. 2002. Alternatives to Antibiotics: Bacteriocins, Antimicrobial Peptides and Bacteriophages. *Poult. Sci.,* **82:** 640–647.
18. Kececi, O., Oguz, H. and Kurtoglu, V. 1998. Effects of Polyvinyl Poly Pyrrolidone, Synthetic Zeolite and Bentonite on Serum Biochemical and Hematological Characters of Broiler Chickens during Aflatoxicosis. *British Poult. Sci.,* **39:** 452–458.
19. Khan, M., Shobha, J. C., Mohan, I. K., Naidu, M. U. R., Sundaram, C., Singh, P. K. and Kutala, V. K. 2005. Protective Effect of *Spirulina* against Doxorubicin-Induced Cardiotoxicity. *Phyto. Res.,* **19(12):** 1030-7.
20. Lepage, K. T., Bloom, S. E. and Taylor Jr, R. L. 1996. Antibody Response to Sheep Red Blood Cells in a Major Histocompatibility (B) Complex Aneuploid Line of Chickens. *Poult. Sci*., **75:** 346–350.
21. Liang, S., Xueming, L., Chen, F. and Chen, Z. 2004. Current Microalga Health Food R and D activities in China. *Hydrobiol.,* **512:** 45-48.
22. Liu, X. Y. 1999. Stress and Immunity. In: "*Poultry Immunology*", (Ed.): Yin, T. B. China Agriculture Press, Beijing, China, PP. 230–252.
23. Luescher-Mattli, M. 2003. Algae, a Possible Source for New Drugs in the Treatment of HIV and Other Viral Diseases. *Curr. Med. Chem. Anti Infection Agents*, **7:** 219–225.
24. Mariey, Y. A. and Samak, H. R. 2014. Effect of Using *Spirulina platensis* Algae as Feed Additives for Poultry Diets. 2. Productive Performance of Broiler. *Egypt Poult. Sci.,* **34:** 245-258
25. Mariey, Y. A., Samak, H. R. and Ibrahem, M. A. 2012. Effect of Using *Spirulina platensis* Algae as a Feed Additive for Poultry Diets. 1. Productive and Reproductive Performances of Local Laying Hens. *Egypt Poult. Sci.,* **32:** 201-215.
26. Miles, R. D., Butcher, G. D., Henry, P. R. and Littell, R. C. 2006. Effect of Antibiotic Growth Promoters on Broiler Performance, Intestinal Growth Parameters and Qualitative Morphology. *Poult. Sci.,* **85:** 476-485.
27. Nakano, M., Shiro, M., Takekoshi, N., Hideo, A., Nakano, B. and Masuo, A. 2007. *Chlorella* (*Chlorella pyrenoidosa*) Supplementation Decreases Dioxin and Increases Immunoglobulin a Concentrations in Breast Milk. *J. Med. Food.,* **10(1):** 134-142.
28. Nikodemusz, E., Paskai, P., Toth, L. and Kozak, J. 2010. Effect of Dietary *Spirulina* Supplementation on the Reproductive Performance of FarmedPheasants. *Technical Articles-Poultry Indust*., PP. 1-2, Institute of Animal Husbandry, Szent István University Gödöllő, Hungary. Available at: https://en.engormix.com/poultry-industry/articles/effect-dietary-spirulina-supplementation-t34615.htm

[DOR: 20.1001.1.16807073.2018.20.6.16.8]

29. Pulz, O. and Gross, W. 2004. Valuable Products from Biotechnology of Microalgae. *Appl. Microbiol. Biotechnol*., **65(6):** 635–48.
30. Rahimi, S. and Khaksefidi, A. 2006. A Comparison between the Effects of a Probiotic (Bioplus 2B) and an Antibiotic (Virginiamycin) on the Performance of Broiler Chickens under Heat Stress Condition. *Iran. J. Vet. Res.*, **7:** 23-28.
31. Reitan, K. I., Rainuzzo, J. R., Øie, G. and Olsen, Y. 1997. A Review of the Nutritional Effects of Algae in Marine Fish Larvae. *Aqua.*, **155:** 207–221.
32. Ross, E. and Dominy, W. 1990. The Nutritional Value of Dehydrated, Blue-Green Algae (*Spirulina platensis*) for Poultry. *Poult. Sci*., **69:** 794-800.
33. Shanmugapriya, B., Babu, S. S., Hariharan, T., Sivaneswaran, S. and Anusha, M. B. 2015. Dietary Administration of *Spirulina platensis* as Probiotics on Growth Performance and Histopathology in Broiler Chicks. *Int. J. Recent Sci. Res.*, **6:** 2650-2653.
34. Skrivan, M., Skřivanova, V., Dlouha, G., Branyikova, I., Zachleder, V. and Vitova, M. 2010. The Use of Selenium-Enriched Alga *Scenedesmus quadricauda* in a Chicken Diet. *Czech J. Anim. Sci.*, **55 (12):** 565–571.
35. Smith, D. L., Harris, A. D., Johnson, J. A., Silbergeld, E. K. and Morris, J. G. 2002. Animal Antibiotic Use Hhas an Early but Important Impact on the Emergence of Antibiotic Resistance in Human Commensal Bacteria. *Proc. Nat. Acad. Sci. USA*, **99:** 6434–6439.
36. Toyomizu, M., Sato, K., Taroda, H., Kato, T. and Akiba, Y. 2001. Effects of Dietary *Spirulina* on Meat Color in Muscle of Broiler Chickens. *British Poult. Sci*., **42:** 197-202.
37. Toyub, M. A., Miah, M. I., Habib, M. A. B. and Rahman, M. M. 2008. Growth Performance and Nutritional Value of *Scenedesmus obliquus* Cultured in Different Concentrations of Sweetmeat Factory Waste Media. *Bang. J. Anim. Sci.*, **37(1)**:86-93.
38. Vukic Vramjes, M. and Wenk, C. 1995. Influence of Dietary Enzyme Complex on the Performance of Broilers Fed on Diets with and without Antibiotic Supplementation. *British Poult. Sci.*, **36:** 265- 275.
39. Williams, R. J. and Heymann, D. L. 1998. Containment of Antibiotic Resistance. *Sci.*, **279:** 1153.
40. Yakhkeshi, S., Rahimi, S. and Hemati Matin, H. R. 2012. Effects of Yarrow (*Achillea millefolium* L.), Antibiotic and Probiotic on Performance, Immune Response, Serum Lipids and Microbial Population of Broilers. *J. Agr. Sci. Tech.*, **14**: 799-810.
41. Yakhkeshi, S., Rahimi, S. and Gharib Naseri, K. 2011. The Effects of Comparison of Herbal Extracts, Antibiotic, Probiotic and Organic Acid on Serum Lipids, Immune Response, Gut Microbial Population, Intestinal Morphology and Performance of Broilers. *J. Med. Plants*, **10(37):** 80-95.
42. Yan, L. and Kim, I. H. 2013. Effects of Dietary ω-3 Fatty Acid-Enriched Microalgae Supplementation on Growth Performance, Blood Profiles, Meat Quality, and Fatty Acid Composition of Meat in Broilers. *J. Appl. Anim. Res*., **41:** 392-397.
43. Zahroojian, N., Moravej, H. and Shivazad, M. 2013. Effects of Dietary Marine Algae (*Spirulina platensis*) on Egg Quality and Production Performance of Laying Hens. *J. Agr. Sci. Tech.*, **15:** 1353-1360.
44. Zhao, G., Chen, X., Wang, L., Zhou, S., Feng, H. and Chen, W. N. 2013. Ultrasound Assisted Extraction of Carbohydrates from Microalgae as Feedstock for Yeast Fermentation. *Bio. Resour. Technol.*, **128:** 337-344.

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مقایسه اثرات سطوح مختلف میکروجلبک سندسموس بر رشد، پاسخ ایمنی، صفات لاشه و بعضی فراسنجه های خونی جوجه های گوشتی

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چکیده

این تحقیق به منظور بررسی اثرات سطوح مختلف سوسپانسیون میکروجلبک سندسموس و مقایسه اثر آن با آنتی‌بیوتیک ویرجینامایسین بر عملکرد، برخی فراسنجه‌های خونی ،وزن اندام‌های داخلی ،سامانه ایمنی ومصرف آب جوجه‌های گوشتی اجرا شد. در این آزمایش از ۲۰۰ قطعه جوجه نر سویه راس ۳۰۸ در قالب یک طرح کاملا" تصادفی با ۵ تیمار و ۴ تکرار و تعداد ۱۰ پرنده در هر تکرار که همگی جیره یکسان دریافت کرده بودند، از ۱تا ۴۲ روزگی انجام شد. تیمارهای آزمایشی شامل: تیمار ۱(۱۰۰٪ آب آشامیدنی)، تیمار ۲ (۵۰٪ آب آشامیدنی + ۵۰٪ سوسپانسیون میکروجلبک سندسموس)، تیمار ۳ (۲۵٪ آب آشامیدنی + ۷۵ ٪ سوسپانسیون میکروجلبک سندسموس)، تیمار ۴ (۱۰۰ ٪ سوسپانسیون میکروجلبک سندسموس) و تیمار ۵ (جیره پایه + ۲/۵ میلی گرم در کیلوگرم ویرجینامایسین) بودند. آب و خوراک نیز تا پایان دوره آزمایش به صورت آزاد در اختیار جوجه‌ها قرار گرفت. در پایان هر دوره آزمایش میزان مصرف خوراک و اضافه وزن اندازه گیری و ضریب تبدیل محاسبه شد.در روزهای ۲۸ و۳۵ آزمایش تزریق جهت تست SRBC انجام شد وهفت روز پس ازهر تزریق، خونگیری جهت بررسی تیترآنتی بادی انجام شد. علاوه بر این در پایان آزمایش از هر تکرار یک پرنده انتخاب و سه میلی لیتر خون جهت اندازه‌گیری فراسنجه‌های خونی از هر پرنده گرفته شد. نتایج این آزمایش نشان داد که تیمارهای مختلف اثر معنی داری بر مصرف خوراک در طول دوره آغازین داشت ($P\leq$۰/۰۵). جوجه هایی که تیمار های ۳ و یا ۴ را دریافت کرده بودند، خوراک بیشتری نسبت به گروه کنترل و گروه آنتی بیوتیک مصرف کردند. پرندگانی که تیمار ۴ را دریافت کرده بودند افزایش وزن بیشتر و ضریب تبدیل غذایی بهتری نسبت به گروه کنترل داشتند ($P\leq$۰/۰۵). در تست SRBC تیمار۴ تفاوت معنی‌داری را نسبت به گروه شاهد نشان داد ($P\leq$۰/۰۵). استفاده از تیمار ۴ به طور معنی‌داری باعث کاهش غلظت تری گلیسرید و کلسترول خون نسبت به گروه شاهد شد ($P\leq$۰/۰۵). جوجه هایی که تیمار ۴ را دریافت کردند وزن نسبی سینه بیشتری نسبت به گروه کنترل داشتند($P\leq$۰/۰۵). نتایج این آزمایش نشان داد که استفاده از سطوح بالای سوسپانسیون میکرو جلبک سندسموس(۷۵ و یا ۱۰۰ درصد) می تواند موجب بهبود عملکرد، افزایش سیستم ایمنی و برخی فراسنجه های خونی در جوجه های گوشتی شود.

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