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Research Article
Improved Productivity and Health of Broiler Chicken by Micro Green Alga *Chlorella vulgaris*

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Abstract

Background and Objective: Algae provide an alternative food source for poultry. They are considered the most important food supplement of the 21st century as a source of proteins. The aim of study was to estimate the growth performance and health of commercial broiler chicks with watering alga culture and alga culture after freezing and thawing. **Materials and Methods:** A total number of 135 one week broilers (Cobb breed) chicks were randomly distributed into 3 treatments with 3 replicates of 15 chicks in each in a completely randomized design. Three experimental groups were fed this diet. The 1st group as a control, 2nd and 3rd groups were treated with 5 g of alga (*Chlorella vulgaris*) per L of water supplemented as freezing and thawing (T_1) and as fresh (T_2) and these two groups were not treated with vaccine against lasota disease at 21 days. The data obtained were analyzed statistically using the one-way ANOVA procedure of SPSS with Duncan's multiple range test. **Results:** The results indicated that control group has the best means of body weights but T_1 has best feed conversion ratio (FCR) as compared to (T_2) and the control group. Also, the results showed that birds watering freezing alga had significantly improved performance index than birds drank fresh alga and control groups. Abdominal fat was significantly ($p<0.05$) lower of treated algae groups (T_1 and T_2) than control. Means of the relative weights of lymphoid organs for groups showed that the bursa and thymus were significantly increased ($p<0.05$) for (T_1) and (T_2) groups compared with the control one while, spleen had insignificant increase. In the present study there were significant ($p<0.05$) decrease in blood serum concentration of cholesterol, triglycerides, creatinine, AST, ALT of (T_1) and (T_2). Total protein and globulin were significantly improved for levels of serum (T_1) and (T_2) compared with the control group. Economic efficiency of birds treated with *Chlorella* alga was superior to that of the control group. **Conclusion:** The result in this study demonstrated that *Chlorella* alga could be safely used in broiler watering without treated with vaccine against lasota disease at 21 days.

Key words: *Chlorella vulgaris*, water algal culture, lasota, blood serum, growth performance

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Poultry play a major role in developing countries. Poultry are most important in increasing income and gave high quality of proteins in the diets of rural people whose traditional foods are rich in carbohydrate[1]. Meat of chicken is healthier than others, it has high desirable monounsaturated fats[2] and containing low total fat. Poultry are important for increased protein production and incomes for smallholder farmers[3]. Consumption of poultry and fish has not been found to be associated with increased risk of cancers[4]. Feed poultry represent the major price of production. Another problem facing the poultry consumer is chemical residue derived from supplement component fed to broiler to fasten its growth that can trigger many diseases for consumer[5]. There have interest in evaluating alternative feed resources as substitutes for maize, soybean meal and animal proteins. Algae provide an alternative to the traditional sources[6]. Algae are valuable sources of food and trace elements. They are considered the most important food supplement of the 21st century as a source of proteins, lipids, polysaccharides, minerals, vitamins and enzymes[7]. Algae could have unlimited use in dried food and feed[8]. Becker[8] and David[9] reported that algae are a good source of fat and water soluble vitamins and pigments, such as chlorophyll. Algae can improve pigmentation of poultry products[8]. Algae upto a level of 5-10% can be used safely as partial substitution for conventional proteins in poultry feeding[10]. Components of *Chlorella vulgaris* affected animals' performance, health, reproduction and the egg quality[11]. *Chlorella vulgaris* is positively influenced in the laying performance, increasing the number and quality of laid eggs, as well as the hatching performance[12]. Selenium is a vital trace mineral for poultry that increase burse, thymus weight and increase immunity against coccidiosis in broilers[13]. *Chlorella* is reach in selenium[12] supplementation of *Chlorella* to poultry diet caused enhancement of immune system due to high content of selenium[14]. Supplementation of poultry diets with *Chlorella vulgaris* has been cleared to increase microbial diversity in the digestive tract[15]. The purpose of the experiment was to study the effect of *Chorella vulgaris* supplemented to the water of broiler as fresh alga (T_2) and freezing and thawing (T_1) on growth performance, health, some parameters of blood, carcass characteristics and economical efficiency of broiler chicks.

MATERIALS AND METHODS

This study was carried out in Department of Sustainable Development of Environment and its Projects Management, Environmental Studies and Research Institute (ESRI), University of Sadat City, Sadat City, Egypt and Department of Microbial Biotechnology, Genetic Engineering and Biotechnology Research Institute (GEBRI), University of Sadat city, Sadat city, Egypt during the period from 6/2016-8/2016 at the farm.

Alga: Green alga *Chlorella vulgaris* was taken from microbiology Lab., GEBRI, University of Sadat City. Alga was grown in Kuhl medium[16] for 15 days under light and dark natural days at 25±1°C.

Determination of algal growth: The growth of alga was measured by optical density using Unico UV-2000 spectrophotometer[17], Chlorophyll a, b and carotenoids pigments[18], total carbohydrate content[19], total soluble proteins[20] and lipids[21].

Determination of total phenolic content (TPC) and antioxidant activity: The total phenolic content (TPC) of green alga *Chlorella vulgaris* was determined by the Folin-Ciocalteu method[22], the antioxidant activity was determine according to Al-Saman *et al.*[23] as shown in Table 1.

Animals, diets and experimental design: A total number of 135 one week broilers (Cobb breed) chicks were randomly distributed into 3 treatments (Control, Treatment (T_1): Addition of 5 g freezing and thawing alga *Chlorella vulgaris* per L of water, without treated with vaccine against lasota disease at 21 days and treatment (T_2): Five gram fresh alga supplemented per L of water without treated with vaccine against lasota disease at 21 days with 3 replicates of 15 chicks in each in a completely randomized design. Housed in separated floor pens each with chopped wheat straw litter and provided with feeders and drinkers. Treated drinking water of algae was freely available. Diets were formulated to cover all recommended nutrient requirements according to broiler nutrition guide[24]. All chicks were vaccinated against Newcastle disease and against avian influenza at the 10th days of age. The chicks were also vaccinated against Gumboro disease (drinking water) on the 12 days of age.

Table 1: Various contents, total phenol content and antioxidant activity of green alga *Chlorella vulgaris* that was used for watering chickens

Chlorophyll a (mg mL^{-1})	13
Chlorophyll b (mg mL^{-1})	20
Carotenoids (mg mL^{-1})	56
Total carbohydrate mg sugar g^{-1} dry wt	369.5
Total soluble proteins (%)	30.4
Lipids (%)	12
TPC (mg g^{-1}) dry wt	41
Antioxidant activity (%)	29.29

Table 2: Composition and calculated analysis of the basal diets

Ingredients	Starter diets	Grower diets
Yellow corn	59	59
Soybean meal (44%)	30	25
Vegetable oil	2.0	6.0
Gluten meal (60%)	6.5	7.7
Sodium chloride	0.4	0.3
Vitamin and mineral premix[1]	0.3	0.3
Di calcium phosphate	1.0	1.0
Dl-methionine	0.45	0.4
Lysine	0.35	0.3
Total	100	100
Calculated analysis[2]		
Crude protein (%)	22.4	20.10
ME (kcal kg^{-1})	3000	3200
Methionine	1.02	1.01
Lysine	0.92	0.88
Available phosphorus (%)	0.48	0.44

[1]Vitamin and mineral premix. Each 3 kg of vitamin and minerals mixture contain: Vit A 1200 I.U., Vit. D3 2000 I.U., Vit E 40 mg, Vit K 34 mg, Vit B1 3 mg, Vit B2 6 mg, Vit B6 4 mg, Vit B12 0.03 mg, Niacin 30 mg, Pantothenic acid 12 mg and Folic acid 1.5 mg. Biotin 0.08 mg. Choline chloride 700 mg. Cu 10 mg. I 300 mg. Fe 40 mg. Mn 80 mg. Co 0.025mg., Zn 70 mg. and Se. 0.02 mg. [2]Calcaulated according to NRC[24]

Data collection, sampling and analysis: Live performance measurements, for each feeding period, were measured and/or calculated in terms of live body weight (LBW), body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), growth rate (GR), performance index (PI) and mortality rate (MR). At 6 weeks of age, four birds from each treatment representing the average body weight of each treatment were slaughtered. After slaughtering and complete bleeding, the whole carcass was weighed. Giblets including liver, heart and gizzard were calculated in relation to respective live body weight. Blood samples were collected in dry clean centrifuge tubes from the slaughtered birds and un-heparinized tubes. Blood serum was then individually separated by centrifugation at 3000 rpm for 15 min, transferred into a clean Eppendorf vials and stored in a deep freezer at approximately -20°C until the time of chemical determination. Values of total protein and albumin were estimated by using commercial diagnosing kits. The Immunization response of broiler chicks will be determined by measuring globulin, A/G ratio and the differences in weights of lymphoid organs including spleen, bursa and thymus. To determine the economical efficiency (EEf) of the diets for meat production, the management factors (heating, lighting, vaccinations and medications) in all dietary treatments were stabilized. The price of the experimental diets were calculated according to the price of the used ingredients of the local market at the time of the study. So, the cost of feed consumed of each treatment was easy to be calculated. The EEf was calculated as the feed cost needed to obtain 1 kg of live body weight gain (LBWG) (Table 2).

Statistical analyses: The data obtained were analyzed statistically using the one-way ANOVA procedure of SPSS[25] (Windows Version of SPSS, release 22) with Duncan's multiple range test[26] to identify the significant differences between the means. All data were used to test mean differences at $p \leq 0.05$. The statistical model was as following:

$$Y_{ij} = \mu + T_i + E_{ij}$$

Where:

Y_{ij} = The individual observation
μ = The overall mean
T_i = Treatment effect
E_{ij} = The experimental error

RESULTS

Growth performance: Growth performance parameters, growth rate and mortality rate of broiler chicks affected by watering alga are shown in Table 3. Initial body weight of chicks was nearly similar between the dietary treatments. At the 1st period (1-3 weeks), body weight of chicks of (T_1) and (T_2) was significantly ($p<0.05$) lower than control chicks. Similar trend was observed in the 2nd period (4-6 weeks) and the total period (1-6 weeks). Body weight gain of broiler chicks at (T_1) and (T_2) significantly ($p<0.05$) decreased as compared to the control treatment during 1st period (1-3 weeks), 2nd period (4-6 weeks) and through total experimental periods. While, body weight chicks of (T_1) were heavier than (T_2). Also, data showed that (T_1) has higher significant ($p<0.05$) performance index. This related to lower feed conversion. However, data recorded that there was no significant differences among treatments of the total period (1-6 weeks) of growth rate affected by green alga *Chlorella vulgaris*. Also, there were significant differences among treatments affected by green alga *Chlorella vulgaris* on mortality rate.

Feed intake and feed conversion: Data of feed intake and feed conversion are presented in Table 3. Group (T_1) and (T_2) consumed significantly ($p<0.05$) less feed as compared to control group, at 1st period and 2nd period. Also total feed intake for all the experiment period was significantly ($p<0.05$) lower for chicks of (T_1) and (T_2). The feed intake of total period is 4890, 4200 and 4321 g. Feed conversion were 2.58, 2.27 and 2.47 with control (T_1) and (T_2), respectively. The results showed that there was no significant change in growth rate among groups. The performance index of (T_1) (chicks treated with freezing and thawing alga) was the best.

Table 3: Growth performance as affected by green alga *Chlorella vulgaris*

Items	Treatments			Significant
	Control	T_1	T_2	
Initial body weight (g)	210.4±0.35	200.3±0.29	198.9±0.30	NS
Final body weight (g)	2100.56±35.4[a]	2050.13±32[a]	1932.35±33.4[b]	*
Body weight gain at				
1-3 weeks	709.7±1.90[a]	589.9±1.89[b]	562.7±1.92[b]	*
4-6 weeks	980.2±9.89[a]	849.7±8.95[b]	834.4±9.64[b]	*
1-6 weeks	1890.1±29.9[a]	1849.9±28.8[a]	1747.2±27.8[b]	*
Feed intake (g) at				
1-3 weeks	1030±5.90[a]	918±6.01[b]	941±5.85[b]	*
4-6 weeks	3860±4.65[a]	3100±4.71[bc]	3300±3.98[b]	*
1-6 weeks	4890±56.4[a]	4200±57.2[c]	4321±55.9[b]	*
Feed conversion at				
1-3 weeks	1.45±0.08	1.55±0.07	1.62±0.04	NS
4-6 weeks	3.93±0.01	3.64±0.02	3.95±0.01	NS
1-6 weeks	2.58±0.03	2.27±0.05	2.47±0.02	NS
Growth rate at				
1-3 weeks	126±0.80[a]	125±0.82[a]	123±0.65[b]	*
4-6 weeks	53±0.98[c]	54±0.79[a]	51±0.71[b]	*
1-6 weeks	200±0.15	199±0.13	198±0.14	NS
Performance index	81.39±0.66[b]	90.30±0.53[a]	78.7±0.64[c]	*
Mortality rate	7.5±0.01	7.7±.02	7.1±0.02	NS

[a-c]Means within the same row with different letters differ significantly ($p \leq 0.05$), T_1: Freezing and thawing alga, T_2: Fresh alga *Significant (Means±SE)

Table 4: Carcass traits of broiler chicks affected by green alga *Chlorella vulgaris*

Items (%)	Treatments			Significant
	Control	T_1	T_2	
Dressing	71.3±2.01[a]	68.4±2.54[c]	69.1±1.98[b]	*
Breast meat	25.5±0.82[c]	29.1±0.91[a]	27.1±0.90[b]	*
Liver	2.80±0.25	2.07±0.21	2.03±0.31	NS
Heart	0.47±0.07	0.48±0.02	0.47±0.03	NS
Gizzard	3.20±0.09	3.10±0.07	2.90±0.07	NS
Abdominal fat	3.09±0.33[a]	1.56±0.09[b]	1.89±0.06[b]	*
Lymphoid organs				
Spleen	0.23±0.05	0.24±0.03	0.24±0.08	NS
Bursa of fabricius	0.20±0.004[b]	0.006±0.22[a]	0.005±0.22[a]	*
Thymus	0.49±0.06[c]	0.53±0.08[a]	0.51±0.03[b]	*

[a-c]:Means within the same row with different letters differ significantly ($p \leq 0.05$), T_1: Freezing and thawing algae, T_2: Fresh alga, *Significant (Means±SE)

Carcass traits: The results obtained in Table 4 revealed that there were no significant effects among three groups in liver, heart, gizzard and spleen. Meanwhile there were significant effects of alga (*Chlorella vulgaris*) on percentage of dressings breast meat, abdominal fats, bursa of fabricius and thymus weight. The results indicate that breast meat was the highest in (T_1) following by (T_2) and control, respectively. The amount of abdominal fats in control (3.09%) was approximately double that present in (T_1) (1.56%) group. Alga had significantly effects in lymphoid organs (organs that are mostly responsible for the immunological response in chicks) and the best effects in (T_1) in comparison to (T_2).

Blood parameters: Effects of *Chlorella* alga on some blood parameters of broiler chicks are shown in Table 5. Chicks with watering *Chlorella* alga were significantly ($p<0.05$) higher than control group. Data showed that treatments watering *Chlorella* alga decreased the serum concentration of cholesterol, triglycerides, HDL and LDL. Also, ALT, AST and creatinine were significantly ($p<0.05$) better than control there is no significant effect on total protein, globulin and albumin.

Economic efficiency: Economic efficiency of birds treated with *Chlorella* alga presented in Table 6. Economic efficiency was superior to that of the control group. Birds were watered alga (T_1) and (T_2) had the higher economic efficiency than that of control group. The birds feed intake, feed cost and economic efficiency were low in treated groups (T_1), (T_2) and the (T_1) group was more efficient than (T_2) group. The percentage relative economic efficiency of chickens was watered freezing and thawing alga was higher than control by 38%.

Table 5: Blood parameters of broiler chicks affected by green alga *Chlorella vulgaris*

	Treatments			
Items	Control	T_1	T_2	Significant
Total protein (g dL^{-1})	2.90±0.10[b]	3.30[a]±0.09[a]	3.10[a]±0.11[a]	*
Cholesterol (mg dL^{-1})	143±6.5[a]	131±5.98[c]	133±5.65[b]	*
Triglyceride (mg dL^{-1})	193±8.1[a]	86±6.01[b]	82±6.20[c]	*
Albumin (g dL^{-1})	1.35±0.09	1.50±0.08	1.40±0.07	NS
Globulin (g dL^{-1})	1.55±0.05[b]	1.80±0.05[b]	2.20±0.11[a]	*
LDL (mg dL^{-1})	8.2±0.91[b]	10.1±0.81[a]	5.2±0.25[c]	*
HDL (mg dL^{-1})	56±3.65[a]	54±4.0[b]	46±4.01[c]	*
Creatinine (mg dL^{-1})	0.63±0.04[a]	0.56±0.02[b]	0.52±0.01[c]	*
AST (u L^{-1})	60.6±2.9[a]	51.8±3.4[c]	59.3±3.2[b]	*
ALT (u L^{-1})	25±0.11[a]	19±1.09[c]	23.2±2.05[b]	*

[a-c]Means within the same row with different letters differ significantly ($p \leq 0.05$), T_1: Freezing and thawing algae, T_2: Fresh alga, *Significant (Means±SE)

Table 6: The economic efficiency of the experimental diets

Items	Control	T_1	T_2
Final body weight (g)	2100	2050	1932
Body weight gain (g)	1890	1849	1747
Revenue from gain (L.E/bird)	37.8	36.9	34.9
Feed intake (kg)	4.89	4.2	4.321
Feed cost (L.E.)	24.45	21	21.6
[1]Net revenue (L.E.)	13.35	15.9	13.3
[2]Economic efficiency (%)	54.6	75.7	61.5
Relative economic efficiency (%)	100	138	111.7

[1]Net revenue = revenue from gain-feed cost, [2]Economic efficiency = (net revenue/feed cost)×100, Price of Kg live body weight was 20 L.E., Price of one kg diet was 5 L.E.

DISCUSSION

The results of study show that the alga did not effect on body weight, feed intake or FCR. Ross *et al.*[27], found that no negative effect of dietary *Spirulina* on final body weight. While, Ross and Dominy[28] cleared that chicks fed dietary *Spirulina* has benefit effects on productive performance. However, Kaoud[29] found that body weight gain and body weight had increased significant ($p<0.05$) by the diet provided with *Spirulina platensis*. Also, birds fed 0.3 and 0.2 g *Spirulina* diets showed higher means of body weights and body weight gain that obtained by Mariey *et al.*[30]. A significant increase in feed conversion ratio was achieved by chicks drink alga may due to partially the improvement of viability percentage. These results are similar to the obtained by Kaoud[29] and Kharde *et al.*[31], who reported that feed conversion ratio significantly ($p<0.05$) improved by dietary inclusion of *Spirulina platensis* as compared to control. They also reported that *Spirulina platensis* supplementation significantly decreased mortality rate of broilers. Gruzauskas *et al.*[32] cleared that *Spirulina* has improved absorption of minerals.

The algae groups led to improved efficiency of feed utilization. This is agreed with Mariey *et al.*[30], who showed that during the starter period, chicks fed 0.0 and 0.1 g kg^{-1} *Spirulina* diet consumed significantly ($p<0.05$) less feed as compared to other 2 treatment groups, which consumed approximately equal amount of diets. During finisher period, treatments of diets had no significant influence of feed intake of chicks. The results indicated that breast muscle weight significantly ($p<0.05$) increased of (T_1) and (T_2) as compared with the control group and feed conversion ratio were also significantly ($p>0.05$) increased. These results agree with Ross and Dominy[28], Kaoud[29] and Kharde *et al.*[31], who reported dietary inclusion of *Spirulina platensis* increased feed conversion ratio as compared to the control diet. The results revealed that increased thymus weight in chickens treated with alga may be related to alga enhancement T-cells and improves thymus gland function. That was in agreement with Qureshi *et al.*[33], who reported that dietary *Spirulina* could increase size of thymus glands for greater T-cell production of broiler chicks. It is of amazing to note that relative abdominal fat weight was significantly ($p<0.05$) reduced by watering alga treatment. This reduction may be attributed to the reduction in plasma total lipids and cholesterol. In this respect, Khan *et al.*[34] reported that *Spirulina* have shown regulatory role on lipid and carbohydrate metabolism by exhibiting glucose and lipid profile correcting activity in animals. Kaoud[29] reported that the absolute and relative weights of bursa and thymus were increased for all groups fed dietary *Spirulina* compared with the control group. The chicks drank watering algae perhaps has tolerate immunology due to be good health groups. That is congruent to Bennett and Stephens[35] reported

that functions of bursa are half of the birds immune system and the size of the bursa reflects the birds overall health status. Sick or stressed birds have small bursa while, healthy productive birds have large bursa. Bursa size is a biological measure of how well flocks are managed and protected from disease. The bursa of Fabricius is a lymphoid organ which plays an important role in developing immunity against mainly Gumboro in chickens. The results indicate that watering chickens with alga caused reduction in triglyceride, HDL, LDL, creatinine, AST and ALT. Moradi Kor *et al.*[36] showed that *Chlorella* microalgae at high levels had positive effects on the serum contents of triglycerides, cholesterol, LDL and the serum content of HDL. It seems that *Chlorella* alga had hypolipidemic impacts and related to lipid metabolism. May be most important substance in *Chlorella* is β-1, 3-glucan, which is an active free radical scavenger and a reducer of blood lipids[37]. An improvement in lipid profile may be a result of chemical composition of *Chlorella* microalgae. As mentioned before, biochemical and physiological events correlated with hyperthermia can potentially stimulate reactive oxygen species (ROS) production[38]. Harsini *et al.*[39] showed that antioxidants play main action in protecting cells from ROS by decreasing free radicals and inhibiting the peroxidation of lipids. In human study Tsuchida *et al.*[40], indicated that a hypolipidemic effect of *Chlorella* in hypertensive patients. Baojiang[41] reported that *Spirulina* polysaccharide (as a type of algae) acts similarly to phycocayanin. It improves the immune system's ability to detect and destroy foreign microbes or eliminate toxins.

CONCLUSION

The results of this study have revealed that broiler chicken watered with culture of *Chlorella vulgaris* (freezing and thawing) had less consumption of food, highest percentage of performance index, relative economic efficiency and breast meat. There was also reduction in abdominal fat, triglyceride, creatinine, AST and AIT. The present study showed the ability to produce a healthy broiler for consumer by using *Chlorella* alga in watering of broiler with no vaccine against lasota disease at 21 days.

SIGNIFICANCE STATEMENT

This study shows that *Chlorella vulgaris* positively influenced in the broiler performance and contributes to the effective role of aquatic environment and produce healthy broiler for consumer.

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