



Application of Defatted *Scenedesmus Obliquus* Biomass for Broilers' Nutrition

■ Author(s)

- El-Baz FKⁱ  <https://orcid.org/0000-0002-4750-3559>
Abdoon SMⁱⁱ  <https://orcid.org/0000-0002-6416-3795>
El-Sayed DAAⁱⁱⁱ  <https://orcid.org/0000-0001-5995-4253>
Mostafa MA^{iv}  <https://orcid.org/0000-0003-2239-6096>
Elsherif HMR^v  <https://orcid.org/0000-0001-7106-9345>
Safaa HM^{vi}  <https://orcid.org/0000-0002-9192-6313>
Abdon ASⁱⁱ  <https://orcid.org/0000-0002-8430-9325>

- ⁱ Plant Biochemistry Department, National Research Centre, Cairo, Egypt.
ⁱⁱ Water Pollution Research Department, National Research Centre, Cairo, Egypt.
ⁱⁱⁱ Animal Production Research Institute, Agricultural Research Centre, Cairo, Egypt.
^{iv} Animal Production Department, Faculty of Agriculture, Cairo University, Giza 12613, Egypt.
^v Department of Biology, College of Science, University of Bisha, Bisha 67722, Kingdom of Saudi Arabia.
^{vi} Department of Animal Reproduction and Artificial Insemination, Veterinary Research Division, National Research Centre, Cairo, Egypt.

■ Mail Address

Corresponding author e-mail address
Hosam M. Safaa
Production Department of Agriculture, Cairo University, Egypt.
Phone: +201223475675
Email: hosam.safaa@agr.cu.edu.eg

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ABSTRACT

A total of 480 Arbor Acres Broiler chicks (mixed sex) from 1 to 35 days of age were divided randomly into 4 treatments to evaluate the impact of defatted *Scenedesmus obliquus* biomass (DAB) on broiler performance. The DAB was supplemented to broiler diets with levels of 0, 0.25, 0.50, 1.00% from one-day-old to 35 days of age. The DAB samples were analyzed in triplicates. The productive traits were measured weekly and the biochemical indices at the end of the trial. Data was statistically analyzed by One-way ANOVA. It was found that slaughter body weight, body weight gain and average daily feed intake were increased in birds fed diets supplemented with DAB compared to the control ones. However, feed conversion ratio was not affected by any of the treatments. Broilers' serum enzymes activities and markers indicated that liver function as indicated by aspartate transaminase (AST) and alanine transaminase (ALT) and kidney's function which indicated by serum creatine and urea were not affected by DAB supplementation. Furthermore, protein, glucose and cholesterol levels in broiler's serum were in normal levels and not affected by treatments. No significant changes were observed in relative organ weights (spleen, bursa, liver, gizzard, proventriculus and heart) or intestine length of broilers at 35 days of age in response to DAB supplementation in their diets. It could be concluded that DAB supplementation to broiler diets from 1 to 35 days of age up to 1% had no negative impacts and might have had a positive effect on broiler performance at 35 days of age.

INTRODUCTION

Recently, the use of algae as a source of biofuels and feed for poultry has received great attention. The quire for energy has become a must, and alongside the reduction of fossil fuels has been so rapid that it could lead to an energy emergency in the near future. Production of energy using photosynthetic organisms such as microalgae may be a feasible answer to some of these problems (El Baz *et al.*, 2016). The use of algae as a source of biofuels would be economically meaningful if defatted algal biomass (DAB) was reused. Since algal biomass is rich in proteins, carbohydrates, minerals and bioactive compounds it is ideal for feed applications (Population Reference Bureau, 2012). Therefore, it might be useful to be used as a source of protein feed for poultry.

The use of algae as a source of biofuels and feed for animals offers a double benefit since the use of algae as a source of fuel does not compete with agricultural crops and at the same time utilizes the remaining biomass of algae as feed for animals (Lum *et al.*, 2013; Vidyashankar *et al.*, 2013; El-Bahr *et al.*, 2020).

The DAB can be used in many fields such as bio-ethanol production through anaerobic digestion (Chandra *et al.*, 2014) or as a source of



feed supplement for animals since algae contain a large amount of carbohydrates. The feed application of the defatted biomass would create a new source of animal feed to alleviate the current competition with human food supply. In addition the biofuel production of microalgae will be economically feasible.

There is no doubt that the nutritional value of algae has been studied many years ago by many researchers where, researches indicated that algae is a rich source of protein, sugars and antioxidants in addition to many dyes and has been used in many foods for humans and animals instead of synthetic materials (Yaakob *et al.*, 2014). Recently, microalgae are used as a feed ingredient for livestock production and to improve meat quality (Madeira *et al.*, 2017; Long *et al.*, 2018; 2020; Pestana *et al.*, 2020). On the same context, Park *et al.* (2018) indicated that *Spirulina* contains physiological active components. These components potentially work as antimicrobial, antioxidant, anti-inflammation and immune enhancer agents. It seems to be involved in metabolic actions related to growth performance in broiler chickens (Pestana *et al.*, 2020). Moreover, different species of microalgae are successfully used in poultry diets such as *Aurantiochy trium Chlorella*, *Dunaliella* and *Scenedesmus* (Abdo *et al.*, 2019; Kakhki *et al.*, 2020). In addition, previous studies have shown that DAB can be used as feed for swine and poultry instead of corn and soybeans (Wu *et al.*, 2018).

Therefore, the aim of this study was to investigate the possibility of supplementing defatted *Scenedesmus obliquus* biomass after extracting the oils from the biomass to broilers' diets from one-day-old to marketing age at 35 days of age on broilers' performance and physiological status, and evaluating the best level of supplementation.

MATERIALS AND METHODS

Experimental Design

This study was performed in accordance with the guidelines of the institutional Ethics of Animal Use in Research Committee (EAURC), Cairo University, Egypt. The experimental design is completely randomized with single effect of DAB supplementation levels in broilers' diets.

Birds' diets and husbandry

A total of 480 Arbor Acres Broiler chicks (mixed sex) at one-day old were obtained from a Cairo Poultry Company (10th of Ramadan city, Egypt). Chicks were divided randomly into 4 treatments (broiler basal diets supplemented (as percentage) with 0 (control),

0.25, 0.5 and 1 of defatted green alga *Scenedesmus obliquus* biomass).

At arrival in the farm, the birds were wing-banded and weighed. The chicks were housed under the same environmental conditions in battery cages. The birds were divided into 3 replicates each replicate consisting of 10 females and 10 males.

Productive traits

The BW (body weight) and feed consumption were recorded by replicate weekly and mortality was recorded daily. From these data BWG (body weight gained), average daily feed intake (ADFI) and feed conversion ratio (FCR) were calculated. The FCR was performed to evaluate the feed quality in the study and it was calculated as the ratio of weight gain over total feed intake during the experiment (Moreira *et al.*, 2013).

Serum biochemistry and Slaughter traits

At 14, 28, and 35 days of age 6 chicks were chosen at random per group from the battery cages. All birds were weighted, slaughtered and eviscerated carcass heart, liver, gizzard, spleen and bursa were weighed. Consequently, relative weight to live BW (g/100g of BW) were calculated.

Serum was separated by allowing the blood to stand for 2 to 4 h in an ice bath. Serum biochemical markers such as serum glutamate oxaloacetate transaminase which is called aspartate transaminase (SGOT=AST) and glutamate pyruvate transaminase which is called alanine transaminase (SGPT=ALT), cholesterol, glucose, albumin, total proteins, urea and creatinine were estimated using commercially available biochemical kits (Aspen Laboratories Pvt. Ltd, New Delhi, India) according to modified International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) method.

Extraction of lipid from chicken

Mixed parts of the thigh and chest were cut into pieces and then mixed well by vortex. Mixture of methanol and chloroform in the proportion of 2:1, was added and mixed well, then water was added and mixed again, then chloroform was applied again and centrifugation was done to get two phases. The chloroform layer is separated and the alcohol is evaporated. After that the fat is converted to fatty acid methyl ester (FAME) by 5 % methanolic HCl under reflux for 2 hrs. The samples were subjected to the GC analysis (Hewlett Packard HP 6890 series GC System Made in USA), each sample was analyzed in triplicate (Hulan *et al.*, 1988).



Microalgae Cultivation

The isolated microalgae species *Scenedesmus obliquus* was cultured on Bold's basal medium. The stock solutions were prepared from the chemicals *Bold's Nutrient Composition*: Urea, 0.3 g/L, K_2HPO_4 , 0.075 g/L, $KHPO_4$, 0.175 g/L, $MgSO_4(7H_2O)$, 0.075 g/L, NaCl, 0.025 g/L, $CaCl_2(2H_2O)$, 0.025 g/L, $ZnSO_4(7H_2O)$, 8.8 mg/L, $MnCl_2(4H_2O)$, 0.44 mg/L, MOO_3 , 0.071 mg/L, $CuSO_4(5H_2O)$, 1.57 mg/L, H_3BO_3 , 11.42 mg/L, EDTA, 50 mg/L, KOH, 31 mg/L, $FeSO_4(7H_2O)$, 4.98 mg/L, $Co(NO_3)_2 \cdot 6H_2O$, 0.49 mg/L, H_2SO_4 , 1 μ l/L.

The culture temperature was $22 \pm 3^\circ C$. Fluorescent light was used to supply constant light intensity for the culture ≈ 2500 lx. The microalgae were grown in 5 Liters flasks, the culture was grown for three days then harvested and the whole biomass was transferred to a photo-bioreactor containing 4000 liters capacity. The cultures were supplied with air using an air pump to generate large, slow bubbles to mix the culture and increase the contact of the culture with air and the medium. The microalgae cells were harvested by settling, and then the settled biomass was subjected to centrifugation at 2000 rpm for 10 min. The collected biomass was dried using sun dryer.

Oil Extraction

The biomass of microalgae was dried in solar dryer and ground into homogenous fine powder. The dry cells were mixed with *n*-hexane and Isopropanol with the ratio of 3:2 (v/v) as co solvent. The homogenate mixture was subjected to a magnetic stirrer at $30^\circ C$ for 2hr. Cell residue was removed by filtering. The filtrate material was transferred into a separating funnel and sufficient water was added to induce biphasic layering. After settling, the solvent mixture was partitioned into two distinct phases, top dark green hexane layer containing most of the extracted lipids and bottom light green layer containing most of the co-extracted non lipids. The hexane layer was collected in a pre-weighted flask and evaporated using a rotary evaporator (Halim *et al.*, 2011; El Baz *et al.*, 2016). The residual solvent in the defatted biomass was removed by drying at constant temperature $30^\circ C$ until constant weight.

Chemical analysis and functional properties of defatted algal biomass

The moisture content was determined by oven drying at a known quantity of algal biomass at $110^\circ C$ to a constant weight. Total ash content was determined by muffle preheated to $600^\circ C$. Samples were heated at this temperature overnight (AOAC, 2005). The

protein content was estimated by quantifying nitrogen content using Kjeldahl apparatus according to (Chapman and Pratt, 1978). Total carbohydrates content was determined by the phenol sulphuric acid method (Dubois, 1956).

Determination of Mineral Elements

To 0.25 g ashes specimens was digested with 10 ml of boiling nitric acid (1N HNO_3) and heated to dryness. The residue was then dissolved in 10 ml 0.1 N HNO_3 filtered and stored in polyethylene flasks for analysis as described by Lacerda *et al.* (1985).

Statistical analysis

The DAB samples were analyzed in triplicates for proximate composition. Haematology profile and serum biochemical indices were measured from 6 birds per each group. Results are expressed as mean \pm standard deviation. Data on weekly body weight, feed intake, relative organs' weights and biochemical traits were statistically analyzed using one-way ANOVA. Tukey–Kramer multiple comparison test was performed to analyze the significant differences among means. $p < 0.05$ was considered significant. The statistical analyses were carried out using general linear model (GLM), SAS software (SAS, 2004).

RESULTS AND DISCUSSION

Composition of defatted algal biomass

Using *n*-hexane for algal biomass extraction resulted in a reduction in oil content (Table 1) up to 80% in the DAB. The total carbohydrate content was 16 g/100g. In addition the DAB has 12.8% ash content. Referring to moisture content the DAB has 7.55% moisture content.

Table 1 – Proximate composition of defatted algal biomass.

Proximate components (%)	g 100 g ⁻¹ biomass
	Defatted algal biomass
Protein	15.30 \pm 0.14
Carbohydrates	16.00 \pm 0.04
Total ash	12.80 \pm 0.60
Moisture	07.55 \pm 0.50
Total lipid as oil content	02.00 \pm 0.51

Values are average of triplicates and represented as mean \pm standard deviation.

Fatty acids composition of *Scenedesmus obliquus*

Table (2) showed that DAB of *Scenedesmus obliquus* contains palmitic acid (C: 16:0) with a high percentage (30.2%), stearic acid (C: 18:0) with a percentage of



(24.2%). Oleic and Linoleic acids (C: 18:1, C: 18:2) has the percentage (11.8, 26%), respectively. In addition, Linolenic and ω -Linolenic were detected in smaller amounts.

Table 2 – Fatty acids profile of defatted green microalgae *Scenedesmus obliquus*.

Fatty acids	Common name	Defatted algal biomass (%)
C:14:0	Myristic	1.4
C:16:0	Palmitic	30.2
C:17:0	Margaric acid	1.3
C:18:0	Stearic acid	24.2
C:16:1	Palmitoleic	2.1
C:18:1	Oleic	11.8
C:18:2	Linoleic	26.0
C:18:3	Linolenic	0.9
C:18:3	ω -Linolenic	1.4

Mineral content of *Scenedesmus obliquus* DAB

Elemental composition such as sodium, potassium, calcium, magnesium, Iron, zinc and copper in addition to ash content were measured in the DAB of *Scenedesmus obliquus*. Potassium was the highest among the elements followed by calcium (Table 3).

Table 3 – Mineral Composition of *Scenedesmus obliquus* biomass (mg/Kg).

Mineral	Defatted algal biomass
Sodium	1000
Potassium	6250
Calcium	6070
Magnesium	5550
Iron	1230
Zinc	100
Copper	50.5

The DAB of microalgal species, after the biofuel production research, has only recently been used as supplementary in diets for poultry, swine, and cattle (Isaacs *et al.*, 2011; Lum *et al.*, 2012; Austic *et al.*, 2013). Moreover, the study of Swiatkiewicz *et al.* (2016) proved that the use of DAB improved the performance of birds, health and the quality of meat and eggs.

Table 4 – Effect of defatted algal biomass on the productivity of broiler chicks.

Scenedesmus supplementation (g/100 g diet)	Body Weight BW at 35 day (g)	Average Daily Body Weight Gain BWG (g)	Average Daily Feed Intake ADFI (g)	Feed Conversion Ratio FCR (g:g)
0.00	1780 ^c	49.65 ^c	93.2 ^c	1.88
0.25	1834 ^b	51.20 ^b	94.1 ^c	1.84
0.50	1837 ^b	51.26 ^b	95.8 ^b	1.87
1.00	1929 ^a	53.84 ^a	98.6 ^a	1.83
SEM (n=6) ¹	20.6	0.533	0.48	0.065
Standard deviation	50.5	1.306	1.16	0.148
p value	0.0005	0.0130	0.0201	0.1113

¹Standard error of the mean (n=6 replicates of 10 birds per each).

^{a,b,c}Means with different superscript significantly differ at $p \leq 0.05$.

Boilers' performance

Dietary treatments increased body weight (BW), BW gain (BWG), and average daily feed intake (ADFI) of the birds fed diets supplemented with DAB compared to the control ones (Table 4). However, the feed conversion ratio (FCR) did not differ among the groups. These results are in agreement with El-Bahr *et al.* (2020) who indicated that the inclusion of microalgae in broiler diet up to 36 days of age enhanced growth performance. In the same context, these results are in consistence with the results obtained by Park *et al.* (2018) and Pestana *et al.* (2020) with Spirulina supplementation in broilers' diets. In addition, Long *et al.* (2020) reported that *Forsythia suspensa* extract improved Arbor Acre broilers' BWG and ADFI from 1 to 42 days of age in comparison with the control ones. On the other hand, Martinez *et al.* (2019) noted that broiler diets supplemented with up to 0.30% red algae powder from 1 to 32 days of age did not affect BW at 32 days of age and FCR but, feed intake was increased. However, Abdo *et al.* (2010) detected no significant effect of BW, and ADFI in broilers fed diets supplemented with 1% dried microalgal biomass harvested from high rate algal ponds. Moreover, Leng *et al.* (2014) showed that feeding with 7.5% defatted *Staurosira* spp has no adverse effect of feeding layers when used for partial replacement of soybean meal. However, feeding with 15% decreased egg performance, feed intake and FCR. Sodium chloride concentration and ash content may be the cause in such a decrease in performance. Although, Ekmay *et al.* (2015) demonstrated that using defatted *Desmodesmus* and *Staurosira* spp up to 25% in the diet could be used in laying hen diets as a source of well-digested dietary protein, without any negative effect on egg production.

Serum biochemistry

The toxicity of a test material can be determined by the biochemical study of the serum (Schilter *et al.*,



2003). Table (5) showed the serum biochemical profile of broilers fed diets supplemented with DAB. Birds fed diets supplemented with DAB had no effects on liver functions as indicated by the levels of glutamate oxaloacetate transaminase (SGOT) and glutamate pyruvate transaminase (SGPT). Consequently, activities of serum enzymes in broilers were not affected by DAB supplementation in broilers' diets. The clinical studies of kidney function indicated by creatinine and urea levels in the serum were at the same level among the various groups. Total cholesterol which is the indicator of lipid metabolism was in the normal range and did not vary among the DAB fed and control groups. Moreover, total serum albumin levels were not changed also in DAB-fed groups compared with the

control one. In general, the serum biochemical profile of broiler suggests the non-toxic nature of DAB at tested levels. In the same context, Austic *et al.* (2013) and Gatrell *et al.* (2014) reported no differences in plasma total cholesterol, triglycerides, NEFA, glucose, alkaline phosphatase and or alanine transaminase activity when broiler chicks fed diets containing 7.5% defatted algal for 6 weeks in comparison with the control birds. However, it was found that the volume and wetness of excreta was increased compared with the control group when fed with diets containing DFA (Grau & Klein 1957; Austic *et al.*, 2013). In addition, Vidyashankar *et al.* (2014) found that liver and kidney functions were not affected when broiler was fed with DAB.

Table 5 – Effect of defatted algal biomass on broilers' serum biochemistry.

<i>Scenedesmus</i> supplementation (g/100 g diet)	AST (U/dL)	ALT (U/dL)	Creatine (mg/dL)	Urea mg/dL	T.protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	Glucose (mg/dL)	Cholesterol (mg/dL)
0.00	269.3	19.6	0.48	5.7	3.60	1.91	1.69	234.8	102.6
0.25	251.0	20.4	0.48	6.5	3.57	1.82	1.75	250.3	114.2
0.50	250.8	21.2	0.53	6.3	3.39	1.87	1.53	244.2	115.5
1.00	241.9	18.9	0.44	5.9	3.62	2.04	1.58	242.0	128.6
SEM (n=6)	6.82	1.12	0.041	0.26	0.243	0.139	0.149	8.99	11.78
Standard deviation	16.71	2.74	0.100	0.64	0.595	0.341	0.365	22.02	28.86
<i>p</i> value	0.0530	0.4851	0.4093	0.1430	0.9016	0.6801	0.6982	0.6817	0.4668

¹standard error of the mean (n=6 replicates of 10 birds per each).

No significant differences were detected for all traits at $p \leq 0.05$.

Feeding broiler for 5 weeks with DAB showed no variations on the body weight among the control and test groups (Table 6). Results indicated no difference between the control and DAB supplementation groups. The DAB proved to be non-toxic during repeated feeding. No significant changes were observed in relative organ weights such as spleen, bursa, liver and gizzard. Vidyashankar *et al.* (2014) were in agreement with the results obtained, where they stated that the relative organs weights indicated no unwanted effects on birds upon DAB feeding. In the same context, Martinez *et al.* (2019) reported that broiler diets

supplemented with up to 0.30% algae powder did not affect the relative weights of spleen, bursa of fabricius, liver, gizzard and heart.

It has been reported that protein metabolites in blood and the bursa and spleen relative weights as immune organs, indicate the immunity status of the birds (Safaa *et al.*, 2011; Safaa, 2013). In this respect, the results of serum globulin, total protein and albumin (Table 5) and spleen and bursa relative weights (table 6) might indicate that no impaired impact of DAB supplementation up to 1% to broilers' diets affects broilers' immunity.

Table 6 – Effect of defatted algal biomass on Broilers' Slaughter traits.

<i>Scenedesmus</i> supplementation (g/100 g diet)	Body weight BW (g)	Slaughter weight (g/100 g BW)	Spleen (mg/100 g BW)	Bursa (mg/100 g BW)	Liver (g/100 g BW)	Gizzard (g/100 g BW)	Intestine Length (cm/100 g BW)	Proventriculus (g/100 g BW)	Heart (g/100 g BW)
0.00	1645 ^c	91.3 ^b	117.0	95.7	2.04	1.88	11.3	0.52	0.51
0.25	1773 ^b	93.5 ^a	123.6	160.2	2.18	1.80	9.6	0.44	0.52
0.50	1809 ^b	93.1 ^a	135.2	135.4	1.89	1.80	8.2	0.50	0.45
1.00	1915 ^a	93.8 ^a	138.8	172.0	2.30	1.62	9.2	0.43	0.53
SEM (n=6)	18.7	0.48	16.72	17.82	0.246	0.129	1.47	0.045	0.049
Standard deviation	45.8	1.18	40.96	43.65	0.603	0.316	3.60	0.110	0.120
<i>p</i> value	0.0162	0.0188	0.0834	0.0991	0.5874	0.7641	0.6333	0.4824	0.6563

¹Standard error of the mean (n=6 replicates of 10 birds per each).

^{a,b,c}Means with different superscript significantly differ at $p \leq 0.05$.



CONCLUSION

Implications of DAB generating value-added in poultry production were proved to be applicable in broiler production. The use of algae in poultry feed will not only provide a safe solution for human and animal nutrition, but will also facilitate the cost-effective of biofuel production and reduces greenhouse gas production of agriculture. In addition, it has a positive impact on broiler productivity at market age (35 days of age).

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COMPETING INTERESTS

"The authors declare that they have no competing interests."

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