

A new type of defatted green microalgae exerts dose-dependent nutritional, metabolic, and environmental impacts in broiler chicks

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Primary Audience: Researchers and Nutritionists

SUMMARY

The potential of defatted microalgae from biofuel production in animal feeding remains unclear. The objective of this experiment was to determine the metabolic and environmental impacts of a defatted green microalgal biomass (DGA, *Nannochloropsis oceanica*, Cellana, Kailua-Kona, HI) in broiler chick diets. Day-old chicks (total = 180) were fed the control diet containing zero, 2, 4, 8, or 16% of DGA for 6 wk to determine the optimal level of DGA inclusion. Compared with chicks fed the control diet, only those fed the 16% DGA diet had lower ($P < 0.05$) ADG and feed use efficiency. The DGA feeding produced dose-dependent (linear) increases in starter period water intake ($P < 0.0001$, $R^2 = 0.76$) and in wk 6 relative weights of heart ($P < 0.0001$, $R^2 = 0.45$), liver ($P = 0.09$, $R^2 = 0.19$), and intestinal tract ($P = 0.02$, $R^2 = 0.19$), retentions of soluble inorganic phosphorus ($P = 0.001$, $R^2 = 0.39$) and DNA retention ($P = 0.001$, $R^2 = 0.46$), and ileal DNA content ($P < 0.0001$, $R^2 = 0.50$). Meanwhile, the DGA feeding led to weak linear decreases ($P < 0.05$, $R^2 = 0.14$ to 0.27) in wk 6 tibia weight and length and excretion of soluble inorganic phosphorus. In contrast, the DGA feeding did not affect relative weights of breast, proventriculus, or gizzard, tibial bone strength, or plasma DNA concentrations. In conclusion, this new type of DGA biomass may be supplemented into diets for broilers at 8% without adverse effects if the salt content is decreased.

Key words: bone strength, DNA, microalgae, phosphorus, water

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DESCRIPTION OF PROBLEM

Our laboratory has investigated the feasibility of incorporating various types of defatted diatom and green microalgal biomasses, the byproduct of biofuel research, into broiler [1, 2] and

laying hen [3] diets. These data conclude that moderate levels (~7.5%) of supplementation do not negatively affect growth or production performance. However, the biofuel research industry is constantly evolving to generate superior biofuel products and optimized residual byproducts. Currently, green microalgae is used for its promise for biofuel production and the superior nutrient content of its defatted biomass, so the question arose if broilers could tolerate higher levels of the nutritionally superior biomass. Importantly, the current biomass contains relatively high levels of sodium, phosphorus, and

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ash [4]. Additionally, being a single cell protein supplement, the defatted microalgae biomass also contains high levels of nucleic acids [5]. However, indicators of metabolic fate or excretion level of these nutrients have not been assessed. In particular, potential impacts of feeding the biomass on phosphorus excretion and water intake of broilers may be a major environmental concern [6].

Therefore, we conducted this experiment to determine effects of the dietary incorporation of a newly acquired defatted green microalgal biomass (**DGA**) on broiler growth performance, water intake, bone properties, and soluble inorganic phosphorus and DNA retentions and excretions.

MATERIALS AND METHODS

Experimental Diets and Animal Care

All animal protocols were approved by the Institutional Animal Care and Use Committee of Cornell University. The experiment was conducted at the Cornell University Poultry Research Farm. Male hatchling Ross broiler chicks (one d old) were obtained from a commercial hatchery and housed in temperature-controlled cage batteries. During the starter (wk zero to 3) and grower (wk 3 to 6) periods, chicks were housed in groups at 6 and 4 per cage, respectively. All birds had free access to feed and water and received a lighting schedule of 22 h light, 2 h dark daily. Body weights were recorded at the beginning of the experiment, and BW and feed consumption were recorded weekly thereafter. Water intakes were recorded daily. Water was provided in 500-mL chick waterers for wk one, and then in 3 L water pans for wk 2 and 3. Water was provided by water lines in the grower period and water intake was not assessed. Nutrient composition of the DGA biomass (*Nannochloropsis oceanica*, Cellana, Kailua-Kona, HI) is shown in Table 1. The corn-soybean meal basal diet (control) and all other experimental diets were designed to be isonitrogenous and isoenergetic and met all of the nutrient requirements for each stage of growth [7]. Feed was withheld for 6 h prior to recording weekly animal BW and/or taking blood and tissue samples.

Table 1. Nutrient composition of defatted green microalgal biomass.¹

Nutrient (%, "as is")		Amino acid (% "as is")	
DM	95.3	Pro	4.00
CP	38.2	Glu	3.34
Crude fat	3.60	Leu	2.90
ADF	7.40	Asp	2.80
NDF	24.2	Lys	2.27
Ash	19.6	Ala	2.22
Ca	0.28	Val	2.13
P	0.69	Arg	1.99
Na	4.73	Gly	1.92
K	1.20	Phe	1.57
Mg	0.63	Thr	1.54
Fe, mg/kg	2560	Ile	1.50
Cu, mg/kg	10.0	Ser	1.21
Mn, mg/kg	207	Tyr	1.20
Zn, mg/kg	39.0	His	0.64
Mo, mg/kg	1.50	Met	0.57
Se, mg/kg	0.01	Trp	0.49
		Cys	0.30

¹Proximate analysis was carried out by Dairy One Inc. (Ithaca, NY), and amino acids were determined by the Agricultural Experiment Station Chemical Laboratories at the University of Missouri (Columbia, MO).

Day-old chicks (total = 180) were divided into 5 treatment groups (n = 6 cages/treatment) and fed the control diet containing zero, 2, 4, 8 or 16% of DGA "as is" for 6 weeks. Starter diets were fed from wk zero to 3 (Table 2) and grower diets were fed from wk 3 to 6 (Table 3).

Blood Collection, Tissue Extraction, and Biochemical Analyses

Blood was collected from 2 randomly selected chicks per cage at wk 3 and wk 6. Blood was drawn from heart puncture, after the animal was anesthetized with CO₂, using heparinized needles. Blood was chilled on ice, centrifuged at 3,000 g for 15 min and the resulting plasma was stored at -20°C until analysis. Plasma inorganic phosphorus was determined by the spectrophotometric method of Gomori [8]. Plasma DNA was isolated using phenol:chloroform:isoamyl alcohol (25:24:1, Invitrogen, Grand Island, NY) and resulting DNA quality and quantity were detected spectrophotometrically (A_{260/280}).

Pectoralis major, liver, and heart were removed and weighed at wk 3 and 6. Additionally, total gastrointestinal tract including

Table 2. Formula and nutrient composition of diets used in the starter period.

Item	Diet				
	DGA (%)				
	0	2	4	8	16
Ingredient, %					
Corn (yellow, fine ground)	54.1	53.9	53.9	52.9	51.4
Soybean meal (48.5% CP)	36.8	35.3	33.7	30.6	24.5
Green algae	–	2.00	4.00	8.00	16.0
Corn oil	4.60	4.45	4.25	4.30	4.00
Dicalcium phosphate	1.95	1.95	1.95	1.95	1.9
Limestone	1.30	1.30	1.30	1.30	1.30
Sodium chloride	0.40	0.20	–	–	–
DL-methionine	0.35	0.35	0.35	0.35	0.35
L-threonine	0.08	0.08	0.08	0.08	0.08
L-lysine HCl	0.05	0.05	0.05	0.05	0.05
Vitamin mix ¹	0.10	0.10	0.10	0.10	0.10
Mineral mix ²	0.10	0.10	0.10	0.10	0.10
Nutritional composition					
ME, kcal/kg	3110	3110	3110	3120	3110
³ CP, % ³	22.0	22.4	22.3	22.2	21.8
³ Crude fat, % ³	6.80	6.80	6.70	6.90	7.60
³ Ash, % ³	5.43	5.63	5.61	6.13	7.65
³ Ca, % ³	0.77	0.77	0.78	0.80	0.89
³ P, % ³	0.72	0.73	0.72	0.76	0.79
³ Na, % ³	0.17	0.23	0.20	0.41	0.84

¹Provided (in mg/kg of diet): Copper sulfate, 31.42; potassium iodide, 0.046; iron sulfate, 224.0; manganese sulfate, 61.54; sodium selenite, 0.13; zinc oxide, 43.56; and sodium molybdate, 1.26.

²Provided (in IU/kg of diet): Vitamin A, 6500; vitamin D₃, 3500; vitamin E, 25 and (in mg/kg of diet): riboflavin, 25; d-calcium pantothenate, 25; nicotinic acid, 150; cyanocobalamin, 0.011; choline chloride, 1250; biotin, 1.0; folic acid, 2.5; thiamine hydrochloride, 7.0; pyridoxine hydrochloride, 25.0; and menadione sodium bisulfite, 5.0.

³Analyzed values.

gizzard and proventriculus was removed and washed with PBS for 3 times to measure weights and/or lengths of various segments at wk 3 and 6.

Tibia Characteristics

Tibias were obtained from one randomly selected chick per cage at wk 6. After removing the fibula and the surrounding connective tissue and muscle, the cleaned tibias were stored in closed plastic bags at 4°C until analysis. The length and weight of the bones were recorded and the mechanical properties were determined using a 3-point bending test using the Instron Universal Testing Instrument 5965 (Norwood, MA). Maximum extension, maximum slope, maximum load, and the extension at maximum load were collected.

Nutrient Digestion and Retention

At wk 6, 2 birds were randomly selected from each cage for total excreta collection and indirect estimates of apparent phosphorus and apparent DNA digestibility and retention using chromium oxide as an indigestible marker (0.3% inclusion)[9]. After a 4-day acclimation period of feeding the chromium oxide-containing diets and 8 h fasting, fresh feed was weighed and fed to the birds to collect total excreta from each cage twice daily for 3 days. The collected excreta were stored at –20°C until drying. At the end of the 3-day collection period, all animals were fasted for 6 h to normalize the amount of excreta output and ileal content, and humanely euthanized via CO₂ to collect digesta samples from the ileum. Excreta and digesta were weighed and then dried at 80°C in a forced-air oven [10]. Excreta were pooled within cage and any feathers or debris were removed before the drying process.

Table 3. Formula and nutrient composition of diets used in the grower period.

Item	Diet				
	DGA (%)				
	0	2	4	8	16
Ingredient, %					
Corn (yellow)	61.6	61.3	61.3	60.5	58.8
Soybean meal (48.5% CP)	30.0	28.5	26.9	23.8	17.6
Green algae	–	2.00	4.00	8.00	16.0
Corn oil	4.60	4.55	4.35	4.25	4.10
Dicalcium phosphate	1.60	1.60	1.60	1.60	1.60
Limestone	1.20	1.20	1.20	1.20	1.20
Sodium chloride	0.30	0.20	–	–	–
DL-methionine	0.20	0.20	0.20	0.20	0.20
L-threonine	0.08	0.08	0.08	0.08	0.08
L-lysine HCl	0.05	0.05	0.05	0.05	0.05
Vitamin mix ¹	0.10	0.10	0.10	0.10	0.10
Mineral mix ²	0.10	0.10	0.10	0.10	0.10
Nutritional composition					
ME, kcal/kg	3200	3200	3200	3200	3200
³ CP, % ³	19.7	20.4	20.0	19.5	19.5
³ Crude fat, % ³	7.00	7.00	7.00	7.90	7.70
³ Ash, % ³	4.61	4.83	5.20	6.04	7.79
³ Ca, % ³	0.62	0.70	0.75	0.74	0.87
³ P, % ³	0.61	0.69	0.70	0.67	0.77
³ Na, % ³	0.12	0.15	0.17	0.39	0.93

¹Provided (in mg/kg of diet): Copper sulfate, 31.42; potassium iodide, 0.046; iron sulfate, 224.0; manganese sulfate, 61.54; sodium selenite, 0.13; zinc oxide, 43.56; and sodium molybdate, 1.26.

²Provided (in IU/kg of diet): Vitamin A, 6500; vitamin D₃, 3500; vitamin E, 25 and (in mg/kg of diet): riboflavin, 25; d-calcium pantothenate, 25; nicotinic acid, 150; cyanocobalamin, 0.011; choline chloride, 1250; biotin, 1.0; folic acid, 2.5; thiamine hydrochloride, 7.0; pyridoxine hydrochloride, 25.0; and menadione sodium bisulfite, 5.0.

³Analyzed values.

Resulting samples were then weighed, ground to a fine powder, and stored at -20°C for analysis. Chromium oxide in the ileal digesta and feed sample was determined by the method of Bolin and colleagues [11]. Soluble inorganic phosphorus and DNA were analyzed in both the ileal digesta and excreta for the estimates of apparent ileal digestibility and apparent retention, respectively. The same procedures as described above for the analysis of plasma inorganic phosphorus and DNA were applied to determine their concentrations in the dried ileal digesta and excreta.

Statistical Analyses

Data were analyzed using the GLM procedure of PC-SAS 8.1 (SAS Inst. Inc., Cary, NC). The overall main effects of dietary treatment were determined using one-way ANOVA. Mean comparisons were conducted using the Duncan's multiple range test. Data were also analyzed us-

ing the linear and quadratic regression models of SAS. Data are expressed as mean, $P < 0.05$ was considered statistically significant, and $P < 0.10$ was considered a trend.

RESULTS

Growth Performance and Water Intake

Body weight was decreased at wk 3 and wk 6 with increasing DGA. At wk 3 and wk 6, there were linear ($P < 0.05$, $R^2 = 0.15$ and $P < 0.01$, $R^2 = 0.37$, respectively) and quadratic ($P < 0.01$, $R^2 = 0.37$ and $P < 0.05$, $R^2 = 0.40$, respectively) effects of DGA consumption; however, only the 16% DGA-fed birds' weight displayed a reduction in body weight compared with the control diet-fed birds. There were linear ($P = 0.05$, $R^2 = 0.13$) and quadratic ($P < 0.01$, $R^2 = 0.38$) effects of increasing dietary DGA concentrations on ADG during wk zero to 3, but not wk 3 to

Table 4. Effects of increasing levels of supplemental defatted microalgae on growth performance and water intake of broiler chicks.

Item	Diet					SEM	P-value ²	
	DGA ¹ (%)						Linear	Quad
	0	2	4	8	16			
Body weight								
Wk 0, g	39.5	39.7	39.5	39.5	39.5	0.06	NS ³	NS
Wk 3, kg	0.96 ^{a,b}	1.00 ^a	0.99 ^a	1.02 ^a	0.92 ^b	0.01	0.03	0.004
Wk 6, kg	2.82 ^a	2.84 ^a	2.75 ^a	2.87 ^a	2.53 ^b	0.03	0.002	0.04
ADG, g								
Wk 0 to 3	44.1 ^{a,b}	46.0 ^a	45.1 ^a	46.9 ^a	41.7 ^b	0.51	0.05	0.003
Wk 3 to 6	96.1 ^a	95.1 ^a	90.8 ^a	95.7 ^a	82.4 ^b	1.47	NS ²	NS
Wk 0 to 6	71.2 ^a	71.8 ^a	69.5 ^a	72.6 ^a	63.8 ^b	0.85	NS	NS
ADFI, g								
Wk 0 to 3	57.0	58.5	59.5	59.4	56.1	0.78	NS	NS
Wk 3 to 6	184	178	183	188	176	1.75	NS	NS
Wk 0 to 6	120	118	121	124	116	1.04	NS	NS
Gain:Feed								
Wk 0 to 3	0.77 ^{a,b}	0.79 ^{a,b}	0.76 ^{a,b}	0.80 ^a	0.74 ^b	0.007	NS	NS
Wk 3 to 6	0.52 ^a	0.53 ^a	0.50 ^{a,b}	0.51 ^{a,b}	0.47 ^b	0.008	0.01	NS
Wk 0 to 6	0.65 ^a	0.66 ^a	0.63 ^{a,b}	0.65 ^a	0.61 ^b	0.006	0.007	NS
Daily water intake, mL/day								
Wk 1	97.2 ^b	95.3 ^b	99.6 ^b	102 ^b	112 ^a	1.83	0.001	NS
Wk 2	155 ^c	161 ^c	166 ^c	195 ^b	225 ^a	5.37	<0.0001	NS
Wk 3	235 ^c	247 ^c	250 ^c	285 ^b	375 ^a	10.3	<0.0001	0.11
Wk 0 to 3	162 ^c	168 ^c	172 ^c	197 ^b	237 ^a	5.61	<0.0001	NS

Data are expressed as mean (n = 6/treatment).

¹DGA = Defatted green microalgal biomass (*Nannochloropsis oceanica*, Cellana, Kailua-Kona, HI).

²Data were analyzed using linear and quadratic regression models of SAS.

^{a-c}Values with different superscripts in each row differ according to one-way ANOVA ($P < 0.05$).

³NS = not significant.

6 or wk zero to 6 (Table 4). At each time point, ADG of chicks fed the 16% DGA diet was lower ($P < 0.05$) than that of all other treatment groups. Whereas ADFI was not affected by any level of DGA inclusion, there was a linear reduction in feed use efficiency during the grower ($P < 0.01$) and the entire period ($P < 0.01$) with the increased DGA inclusions. Chicks fed the 16% DGA had lower ($P < 0.05$) feed use efficiency than that of the control and/or the other treatment groups. Water intakes were increased in a linear fashion in response to the increased DGA inclusions at wk one ($P < 0.01$, $R^2 = 0.29$), 2 ($P < 0.0001$, $R^2 = 0.82$), and 3 ($P < 0.0001$, $R^2 = 0.91$, Table 4). During the 3-week starter period, water intake increased linearly ($P < 0.0001$, $R^2 = 0.76$). Chicks fed the 8 and 16% DGA diets over the starter period consumed 16 to 39% ($P < 0.05$) more water, compared with the control diet-fed birds.

Organ Weights and Tibial Characteristics

Figure 1 shows linear increases in relative weights of liver at wk 3 ($P < 0.05$, $R^2 = 0.16$) and 6 ($P < 0.10$, $R^2 = 0.19$), of heart at wk 3 ($P = 0.001$, $R^2 = 0.33$) and 6 ($P < 0.0001$, $R^2 = 0.45$), and intestine at wk 6 ($P < 0.05$, $R^2 = 0.19$) with inclusion of DGA. However, there was no such effect on wk 6 relative weights of breast ($13.5 \pm 0.48\%$), gizzard ($1.24 \pm 0.15\%$), proventriculus ($0.24 \pm 0.04\%$), or intestinal weight per length (0.25 ± 0.02 g/cm, data not shown).

Although there were linear reductions in tibial weight ($P = 0.01$, $R^2 = 0.27$) and length ($P < 0.05$, $R^2 = 0.21$) with increasing DGA inclusions, only tibia weight, but not length, of chicks fed the 16% DGA diet was lower ($P < 0.05$) than that of chicks fed the control diet (Table 5). There were no linear or quadratic

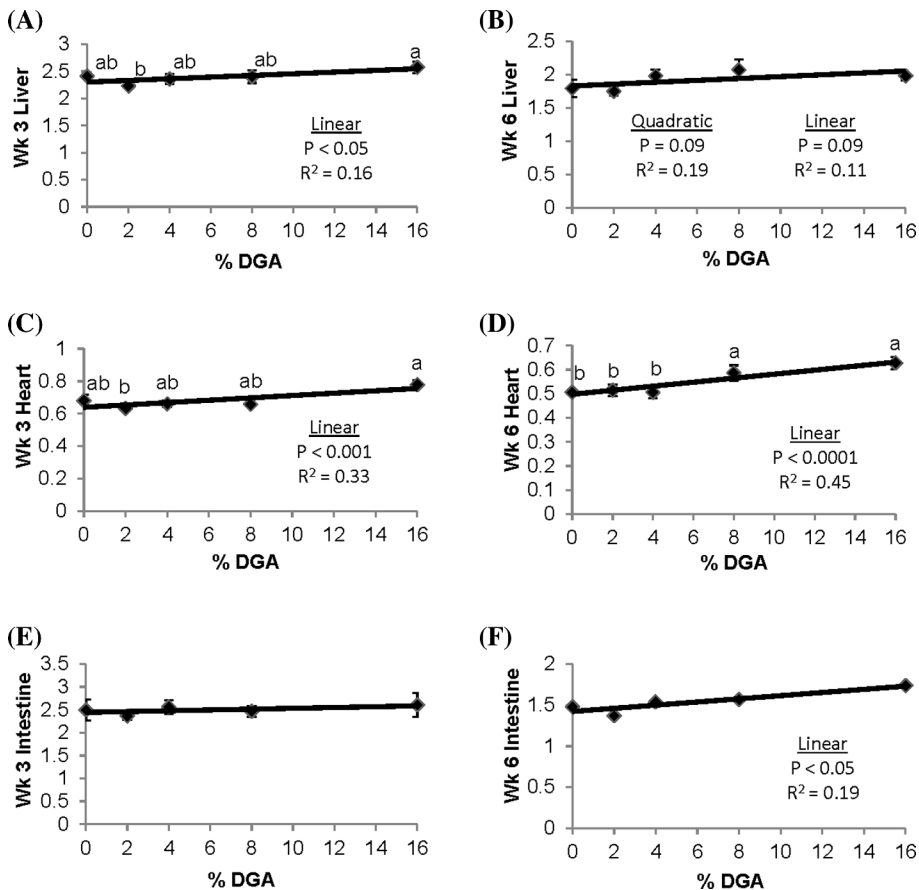


Figure 1. The effect of increasing levels of dietary microalgal biomass on wk 3 and wk 6 relative organ weights in broiler chicks. Data are expressed as mean \pm SEM ($n = 6/\text{treatment}$). Values with different superscripts in each group differ significantly according to one-way ANOVA ($P < 0.05$). Linear and quadratic regression analyses also were deemed significant at $P < 0.05$. DGA = defatted green microalgal biomass (*Nannochloropsis oceanica*, Cellana, Kailua-Kona, HI).

effects of DGA inclusion on max slope or energy to and extension at maximum load. However, chicks fed the 8% DGA diet had higher ($P = 0.10$) max slope than those fed the 4 or 16% DGA diet and lower ($P < 0.10$) extension at maximum load than that of chicks fed the control, 2 and 16% diets.

Phosphorus and DNA Excretions

Plasma inorganic phosphorus concentrations at wk 6 were not affected by the DGA inclusion. There was a linear ($P < 0.01$, $R^2 = 0.26$) decrease in ileal soluble inorganic phosphorus digestibility, but linear ($P < 0.001$, $R^2 = 0.39$) increase in soluble inorganic phosphorus retention with the increased DGA inclusions. The

daily excretion of soluble inorganic phosphorus showed a declining trend ($P = 0.10$) with the increased DGA inclusions. Neither plasma nor excreta concentrations of DNA were affected by DGA inclusion (Table 6). However, there were linear increases in ileal DNA concentration ($P < 0.0001$, $R^2 = 0.50$) and DNA retention ($P < 0.001$, $R^2 = 0.46$) in response to different levels of DGA supplementation.

DISCUSSION

The most significant finding from this experiment was the substantial increases in daily water intakes by chicks fed diets containing 8% or higher levels of DGA, compared with the

Table 5. Effects of increasing levels of defatted microalgae on tibia characteristics and soluble inorganic phosphorus digestion and retention.

Item	Diet					SEM	P-Value ²	
	DGA ¹ (%)						Linear	Quad
	0	2	4	8	16			
Tibia characteristics								
Weight, g	113 ^a	112 ^{a,b}	116 ^a	113 ^a	105 ^b	1.08	0.01	NS ³
Length, cm	23.8 ^{a,b}	26.3 ^a	25.7 ^{a,b}	23.7 ^{a,b}	19.4 ^b	0.76	0.03	NS
Max slope, N/mm	152 ^{a,b}	161 ^{a,b}	132 ^b	181 ^a	126 ^b	6.95	NS	NS
Extension at max load, mm	3.27 ^a	3.25 ^a	3.02 ^{a,b}	2.53 ^b	3.48 ^a	0.11	NS	0.01
Energy to max load, J	0.51	0.62	0.42	0.44	0.42	0.03	NS	NS
Soluble inorganic phosphorus								
Plasma, mg/dL	1.50	1.48	1.47	1.50	1.55	0.03	NS	NS
Digestibility ⁴ , g/kg	958 ^a	961 ^a	949 ^{a,b}	920 ^{a,b}	911 ^b	0.70	0.004	NS
Retention ⁵ , g/kg	841 ^c	903 ^b	929 ^a	919 ^{a,b}	932 ^a	0.72	0.0004	<0.0001
Excretion ⁵ , mg/chick*day	76.8	74.1	66.9	76.2	58.3	3.70	0.10	NS

Data are expressed as mean (n = 6/treatment).

¹DGA = Defatted green microalgal biomass (*Nannochloropsis oceanica*, Cellana, Kailua-Kona, HI).

²Data were analyzed using linear and quadratic regression models of SAS.

^{a-c}Values with different superscripts in each row differ according to one-way ANOVA ($P < 0.05$).

³NS = not significant.

⁴Estimated at wk 6 using the indirect method of chromium oxide as an indigestible marker.

⁵Estimated at wk 6 using the total collection data.

Table 6. Effects of increasing levels of supplemental defatted microalgae on phosphorus and DNA concentrations in plasma, ileal digesta, and excreta of chicks at wk 6.

Item	Diet					SEM	P-Value ²	
	DGA ¹ (%)						Linear	Quad
	0	2	4	8	16			
DNA								
Diet, mg/kg	181	184	226	292	486			
Plasma, mg/dL	304	490	479	608	776	111	NS ³	NS
Ileum, mg/kg	297 ^b	388 ^b	300 ^b	626 ^a	639 ^a	35.7	<0.0001	NS
Excreta, mg/kg	716	727	778	846	841	33.1	NS	NS
Retention ⁴ , g/kg	385 ^b	357 ^b	426 ^b	458 ^b	661 ^a	3.31	0.0003	NS

Data are expressed as mean (n = 5/treatment).

¹DGA = Defatted green microalgal biomass (*Nannochloropsis oceanica*, Cellana, Kailua-Kona, HI).

²Data were analyzed using linear and quadratic regression models of SAS.

^{a-b}Values with different superscripts in each row differ according to one-way ANOVA ($P < 0.05$).

³NS = not significant.

⁴Estimated at wk 6 based on data from the total excreta collection study.

controls. While high salt concentrations of the defatted marine microalgal biomass did lead us to previous observations of bulky excreta by the experimental chicks [1, 3], the present study represents the first direct measurement of the actual water intake while feeding the biomass. Although the increased water intake associated with the 8% DGA diet did not depress growth performance, the extra water usage will lead to

not only higher demand for the agricultural water needs but also a larger amount of litter. The latter is a major concern in modern poultry production [12]. Chicks consuming the 16% DGA diet also displayed incidences of water regurgitation when feeding after a bout of drinking. That was probably due to pressure exerted on the crop and could lead to feed loss. Concurrently, the increased consumption of the DGA diets, mainly due

to the high salt intake, produced heavier heart and/or liver weights, although the correlation coefficient was rather moderate. Mirsalimi and others [13] reported increased relative weights of right and total ventricle and total blood volume in broiler chicks consuming 0.5% salt water compared with untreated controls. Metabolically, high sodium intake leads to increases in blood volume and flow, ultimately causing right ventricular hypertrophy and pulmonary hypertension [14]. Apparently, additional processing steps must be taken to remove the extra salt present in the DGA biomass for the full potential of its high protein and other nutrients in animal feeding.

Another novel finding from the present study is the linear increases in retention and linear decreases in excretion of total soluble inorganic phosphorus in chicks fed the graded levels of DGA. Remarkably, chicks fed the 16% DGA diet decreased their total soluble phosphorus excretion by 24% compared with the controls. This decrease is an unanticipated benefit of feeding this new type of DGA biomass because minimizing environmental pollution of manure phosphorus excretion is currently a major interest of animal agriculture [6]. Apparently, more extensive research is needed to follow up this finding for fully understanding the mechanism and environmental impact of this decreased phosphorus excretion associated with the DGA feeding. Interestingly, ileal total soluble phosphorus digestibility displayed a linear decrease with increases in dietary DGA inclusion. Opposite responses between digestion and retention of nutrients to microalgal feeding were previously reported. Weanling pigs fed the microalgae *Spirulina maxima* displayed reduced apparent nutrient digestibility with a simultaneous increase in the metabolic utilization of the absorbed nutrient, compensating for the low digestibility [15]. In the present study, ileal phosphorus digestibility was fairly high, irrespective of DGA inclusion levels.

An additional concern with increased dietary DGA inclusion is the subsequent increase in dietary iron. Dietary iron metabolism is known to be linked to the metabolism of other key nutrients including zinc [16], copper [17], calcium, and phosphorus [18]. Although the effects of excess iron on copper and zinc need to be assessed,

the elevated iron in the DGA-containing diets did not affect bone development or Ca/P nutrition status. While the DGA inclusions caused dose-dependent linear decreases in tibial weights and lengths, the treatments did not affect tibial bone strength or other functional indices. In fact, the decreased tibial weight or length in chicks fed the 16% DGA diet can potentially be explained by the corresponding decrease in body size. Altogether, phosphorus from the DGA biomass was as bioavailable as, if not more than, that from the ingredients of the control diet for maintaining body phosphorus status and bone function. This feature of DGA is a requisite for its application in broiler feeding because selections for rapid growth in broilers may render them prone to skeletal deformities or bone breakage [19]. These problems lead to mortality, low productivity, and carcass condemnations and are associated with a loss of several hundred millions of dollars annually [20].

The present study signifies our first effort to determine fate and retention of DNA in the DGA. One noted concern for the use of single-cell protein sources such as microalgae is the high content of nucleic acids [21, 22]. Nucleic acids and other non-protein nitrogen contribute 10% of the total nitrogen found in microalgae [23]. Excess nucleic acid consumption results in high production of uric acid that may cause gout and kidney stone formation in animals lacking the uricase enzyme [24]. Since uric acid is the end product of protein metabolism in chicks, they may be evolved for consuming feeds high in nucleic acid content [25]. However, the metabolic fate and excretion of the nucleic acids in DGA have not been examined. Our preliminary findings from the present study demonstrated that an increased consumption of DGA did not alter plasma DNA concentrations. When ileal DNA concentrations were increased linearly with DGA inclusion in the 6-week-old chicks, their excreta DNA remained similar among treatment groups, potentially due to gut microbe contribution. Notably, DNA retention was increased with DGA supplementation. Retained nucleotides may be used for nucleic acid synthesis, resulting in increased DNA and RNA contents in organs and muscles [21]. Therefore, a more detailed profile of nucleic acid metabolism and deposition due to DGA consumption is warranted.

CONCLUSIONS AND APPLICATIONS

1. Overall, multiple measures in this experiment show that broiler chicks tolerated the 4% inclusion of DGA throughout the starter and grower periods.
2. Despite no adverse effect on growth performance or various biochemical and metabolic measures, the 8% DGA diet resulted in elevated water consumption and relative weights of vital organs.
3. Meanwhile, the 16% DGA diet caused many, if not all, responses inferior to the control. The most limiting factor of the tested DGA biomass is likely its high salt concentration, the direct adverse effects of which included elevated water intake and hypertrophy of heart and/or other organs. Clearly, the extra salt in the DGA must be removed using additional processing steps for exploring its full nutritional, metabolic, and environmental potentials.

REFERENCES AND NOTES

1. Austic, R. E., A. Mustafa, B. Jung, S. Gatrell, and X. G. Lei. 2013. Potential and limitation of a new defatted diatom microalgal biomass in replacing soybean meal and corn in diets for broiler chickens. *J. Agric. Food Chem.* 61:7341–7348.
2. Ekmay, R., S. Gatrell, K. Lum, J. Kim, and X. G. Lei. 2014. Nutritional and metabolic impacts of a defatted green marine microalgal (*Desmodesmus* sp.) Biomass in Diets for Weanling Pigs and Broiler Chickens. *J. Agric. Food Chem.* 62:9783–9791.
3. Leng, X., K. N. Hsu, R. E. Austic, and X. G. Lei. 2014. Effect of dietary defatted diatom biomass on egg production and quality of laying hens. *J. Anim. Sci. Biotechnol.* 5:3-1891-5-3.
4. Gatrell, S., K. Lum, J. Kim, and X. G. Lei. 2014. Nonruminant Nutrition Symposium: Potential of defatted microalgae from the biofuel industry as an ingredient to replace corn and soybean meal in swine and poultry diets. *J. Anim. Sci.* 92:1306–1314.
5. Becker, E. W. 2004. Microalgae in human and animal nutrition. Pages 312 in *Handbook of Microalgal Culture: Biotechnology and Applied Phycology*. Richmond, A. ed. Blackwell Science Ltd, Oxford.
6. Bourgeois, L. 2012. A discounted threat: Environmental impacts of the livestock industry. *Earth Common. J.* Vol. 2, No. 1.
7. NRC. 1994. *Nutrient Requirements of Poultry: Ninth Revised Edition*. The National Academies Press, Washington, DC.
8. Gomori, G. 1942. A modification of the colorimetric phosphorus determination for use with the photoelectric colorimeter. *J. Lab. Clin. Med.* 27:1941–1942.
9. Ekmay, R., K. Chou, A. Magnuson, and X. Lei. 2015. Continual feeding of two types of microalgal biomass affected protein digestion and metabolism in laying hens. *J. Anim. Sci.* 93:287–297.
10. Ravindran, V., L. Hew, G. Ravindran, and W. Bryden. 1999. A comparison of ileal digesta and excreta analysis for the determination of amino acid digestibility in food ingredients for poultry. *Br. Poult. Sci.* 40: 266–274.
11. Bolin, D. W., R. P. King, and E. W. Klosterman. 1952. A simplified method for the determination of chromic oxide (Cr₂O₃) when used as an index substance. *Science.* 116:634–635.
12. Francesch, M., and J. Brufau. 2004. Nutritional factors affecting excreta/litter moisture and quality. *Worlds Poult. Sci. J.* 60:64–75.
13. Mirsalimi, S. M., P. J. O'Brien, and R. J. Julian. 1993. Blood volume increase in salt-induced pulmonary hypertension, heart failure and ascites in broiler and White Leghorn chickens. *Can. J. Vet. Res.* 57:110–113.
14. Julian, R. J., L. J. Caston, and S. Leeson. 1992. The effect of dietary sodium on right ventricular failure-induced ascites, gain and fat deposition in meat-type chickens. *Can. J. Vet. Res.* 56:214–219.
15. Fevrier, C., and B. Seve. 1975. Incorporation of a spiruline (*Spirulina maxima*) in swine food. *Ann. Nutr. Aliment.* 29:625–650.
16. Yadrick, M. K., M. A. Kenney, and E. A. Winterfeldt. 1989. Iron, copper, and zinc status: Response to supplementation with zinc or zinc and iron in adult females. *Am. J. Clin. Nutr.* 49:145–150.
17. Arredondo, M., and M. T. Núñez. 2005. Iron and copper metabolism. *Mol. Aspects Med.* 26:313–327.
18. Sell, J. L. 1965. Utilization of iron by the chick as influenced by dietary calcium and phosphorus. *Poult. Sci.* 44:550–561.
19. Julian, R. 1998. Rapid growth problems: Ascites and skeletal deformities in broilers. *Poult. Sci.* 77: 1773–1780.
20. Rath, N., G. Huff, W. Huff, and J. Balog. 2000. Factors regulating bone maturity and strength in poultry. *Poult. Sci.* 79:1024–1032.
21. Schulz, E., and H. Oslage. 1976. Composition and nutritive value of single-cell protein (SCP). *Anim. Feed Sci. Technol.* 1:9–24.
22. Giesecke, D., and W. Tiemeyer. 1982. Availability and metabolism of purines of single-cell proteins in monogastric animals. *Proc. Nutr. Soc.* 41:319–327.
23. Becker, E. W. 2007. Micro-algae as a source of protein. *Biotechnol. Adv.* 25:207–210.
24. Ravindra, P. 2000. Value-added food: Single cell protein. *Biotechnol. Adv.* 18:459–479.
25. Shannon, D., and J. McNab. 1972. The effect of different dietary levels of an-paraffin-grown yeast on the growth and food intake of broiler chicks. *Br. Poult. Sci.* 13: 267–272.

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