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Potential and Limitation of a New Defatted Diatom Microalgal Biomass in Replacing Soybean Meal and Corn in Diets for Broiler Chickens

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Supporting Information

ABSTRACT: Three experiments were conducted to determine if defatted diatom *Staurosira* sp. biomass (DFA) (Cellana, Kailua-Kona, HI, USA) from biofuel production could replace a portion of soybean meal (SBM) and (or) corn in diets for broiler chicks. In experiment 1, 2-day-old chicks were fed diets with DFA at 0% (control), 7.5% replacing SBM, or 7.5 and 10% replacing SBM and corn. Chicks fed the DFA-containing diets had lower body weight gain ($P < 0.05$) than the controls in the starter period. Two follow-up experiments, experiments 2 and 3, indicated that supplementing the 7.5% DFA diet (replacing SBM) with amino acids, but not exogenous protease or electrolytes, restored growth performance of chicks to the control levels. Responses of plasma and liver biomarkers and gross examination of digestive tract showed no toxicity of DFA. In conclusion, DFA could substitute for 7.5% of SBM alone, or in combination with corn, in diets for broiler chicks when appropriate amino acids are added.

KEYWORDS: *defatted microalgal biomass, soybean meal, amino acids, growth, broiler chickens*

INTRODUCTION

The global population is expected to reach 9 billion by the year 2050. Thus, the plant breeding community has been working toward doubling crop yields to keep up with future food demands.[1] This might be very challenging as agricultural land is shrinking, global water tables are depleting,[2] and crop inputs, especially chemical fertilizers, are reducing as a means of minimizing greenhouse gas emissions and the agricultural carbon footprint.[3] Meanwhile, food-producing animals rely heavily on soybean meal (SBM) and corn to meet their protein and energy requirements, creating a direct competition of these two foods for human consumption. This competition will only exacerbate the future food demand as meat consumption in developing countries increases. Therefore, alternative protein and energy sources are required to replace SBM and corn in animal feeds for sustainable animal agriculture.

Although single-cell protein sources such as microalgae are deemed suitable for animal or human consumption,[4,5] their inclusion in animal feed has regained attention mainly due its role as a promising source for biofuel production.[6] Diatoms comprise a large fraction of phytoplankton and are believed to be important contributors to the aquatic food web.[7] The diatom microalga *Staurosira* sp. is currently under investigation as a potential source of oil for biofuel production. The defatted biomass (DFA) contains 19% crude protein, compared to 47.5 and 8.5% found in SBM and corn, respectively.[8] Whereas the protein content of DFA makes it a suitable replacement of these two main ingredients in diets for swine and poultry, there is no report on such application or potential.[9] Instead, there has been a good amount of research on specialized products that can be derived from these algae,[10−12] because of their high contents of vitamins, minerals, antioxidants,[5] long-chain polyunsaturated omega-3 fatty acids,[13−16] and carotenoids.[17−21]

Broiler chicks are the fastest growing and most efficient food species that is consumed worldwide. Whereas the domestic broiler industry produces 36 billion pounds of meat with U.S. $22 billon value,[22] it also uses 13.5 and 30 million metric tons of SBM and corn per annum, respectively. Various algae have been tested as sources of protein for broiler chicks by replacing SBM or fish meal.[17,18,23−27] Dietary levels from 5 to 10% algae substituted safely in partial replacement of these conventional ingredients.[18,19,26] Similar results were seen in swine.[28] However, higher levels of inclusion (20%) led to adverse effects on performance in poultry,[25] probably due to relative deficiency in the sulfur-containing amino acids methionine and cysteine[5] and (or) low digestibility of microalgal protein. Likely, increasing the amount or availability of certain amino acids may alleviate the negative effects seen at higher levels of algae inclusion. Protease may be added to enhance amino acid availability and to improve growth performance.[29,30]

Chemically, DFA are uniquely different from other microalgae. They are supposed to contain high levels of ash and silicon (Si) in their cell membranes and have unique morphological structures known as frustules.[31] Like most algae, they exhibit considerably higher sodium contents than land-based plants.[32] Because high levels of ash[33] and sodium[34] and the balance of monovalent minerals[35,36] affect body metabolism and health status, it remains to be determined if the DFA inclusion into animal diets causes toxicity or side effects such as feed refusal. Therefore, the objective of the present study was to determine whether DFA of *Staurosira* sp.

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could be used for partial replacement of SBM and (or) corn in diets of broiler chicks and if additional supplementation of amino acids, electrolytes, and protease improved its feeding values.

MATERIALS AND METHODS

Animals, Diets, and Management. The protocols of all experiments were approved by the Institutional Animal Care and Use Committee of Cornell University. Hatchling Ross broiler chicks were obtained from a commercial hatchery and housed in a temperature-controlled room at the Cornell University Poultry Research Farm. The broiler chicks were housed in thermostatically controlled cage batteries for 3 weeks and were transferred to grower cages at room temperature from 3 to 6 weeks. Chicks had free access to feed and water and were provided with a lighting schedule of 22 h light, 2 h dark daily. Body weights were recorded at the beginning of experiments and were recorded weekly thereafter, along with feed intake. The DFA of *Staurosira* sp. (Table 1) (Cellana, Kailua-Kona, HI, USA) was included at a level of 7.5 or 10% of the diets in partial substitution for SBM or a combination of SBM and ground corn. Crystalline amino acids, minerals, and vitamins were added to satisfy nutrient requirements. Starter (0−3 weeks) and grower diets (4−6 weeks) were designed to meet the requirements for growth for each age group.[8] Diets containing the same amount of DFA used in different experiments were similar in the major ingredients but differed in amino acid concentrations. As examples, the control starter and grower diets and the diets containing alga substituting for SBM in experiment 1 are shown in Table 2. The complete formulas of all diets are presented in the Supporting Information. Dietary treatments and the durations of experiments differed, but the animal housing and feeding protocols were similar among experiments.

Table 1. Chemical Composition of the Defatted Diatom Microalgal Biomass (DFA)[a]

item	content	item	content
ME (kcal/g)	1.32	arginine	0.93
protein	19.1	lysine	0.83
fat	3.3	methionine	0.33
ND fiber[b]	14.0	cysteine	0.32
AD fiber[b]	0.7	glycine	0.96
ash	44.9	serine	0.76
moisture	6.9	histidine	0.3
calcium (Ca)	2.78	isoleucine	0.78
phosphorus (P)	0.76	leucine	1.33
sodium (Na)	3.94	phenylalanine	0.86
potassium (K)	1.66	tyrosine	0.57
magnesium (Mg)	0.79	threonine	0.88
chloride (Cl)	6.34	tryptophan	0.18
iron (Fe)	1820	valine	0.98
copper (Cu)	4	alanine	1.09
manganese (Mn)	101	aspartic acid	1.88
zinc (Zn)	25	glutamic acid	1.81
molybdenum (Mo)	2.2	proline	0.65
selenium (Se)	<0.01	hydroxyproline	0.04
taurine	0.08	hydroxylysine	0.3
lanthionine	0.00	ornithine	0.03

[a]All values are on an "as is" basis. Values other than ME and the trace elements (Fe, Cu, Mn, Zn, Mo, and Se) are expressed as % of biomass. These six trace elements are expressed in mg/kg of biomass. [b]ND, neutral detergent; AD, acid detergent.

Experiment 1. The objective was to determine whether DFA could replace a portion of SBM and (or) corn in diets for broiler chicks. A total of 80 2-day-old chicks were used. Duplicate cages of five chicks per gender were assigned to four diets in a 2 × 4 factorial arrangement of treatments. The four dietary treatments comprised chick starter and grower controls (diet 1), which were based on practical feed ingredients (Table 2), starter and grower diets containing 7.5% DFA substituting in part for SBM (diet 2, Table 2), and starter and grower diets containing 7.5% (diet 3) and 10% (diet 4) DFA substituting in part for SBM and corn; 1:3 and 1:4 SBM to corn mixtures were replaced with DFA for diet 3 and 4, respectively. All diets were formulated to be isoenergentic and to meet the requirements for all essential amino acids. The main differences in microalgal biomass inclusion and the calculated metabolizable energy, crude protein, and amino acid concentrations among diets of the three experiments are presented in Table 3. The calculations are based on chemical analyses of the DFA (Table 1) and published tables of the nutrient contents of conventional feedstuffs (NRC 1994).[8]

Table 2. Typical Composition of Control and 7.5% Algae-Containing Diets

	starter diets (0−3 weeks)		grower diets (4−6 weeks)	
	control	7.5% algae	control	7.5% algae
ingredient				
corn (yellow)	60.00	59.66	66.02	65.46
soybean meal (48.5% CP)	29.50	22.00	22.00	14.60
meat meal	5.00	5.00	5.00	5.00
corn gluten meal	2.00	2.00	2.00	2.00
corn oil	1.00	2.00	3.00	4.10
dicalcium phosphate	0.50	0.20	0.20	0.06
limestone	0.80	0.50	0.70	0.30
salt	0.50	0.00	0.50	0.00
vitamin mix[a]	0.25	0.25	0.25	0.25
mineral mix[b]	0.15	0.15	0.15	0.15
defatted diatom	0.00	7.50	0.00	7.50
DL-methionine	0.22	0.31	0.08	0.14
L-lysine hydrochloride	0.08	0.25	0.10	0.27
L-isoleucine	0.00	0.09	0.00	0.09
L-threonine	0.00	0.06	0.00	0.06
L-tryptophan	0.00	0.03	0.00	0.02
L-arginine (free base)	0.00	0.00	0.00	0.00
total ingredients	100.00	100.00	100.00	100.00
nutrient composition				
ME (kcal/g)	3.00	3.01	3.19	3.21
protein, %	23.1	21.3	20.0	18.2
fat, %	4.1	5.3	6.3	7.5
fiber, %	2.6	3.4	2.5	3.3
Ca, %	1.02	1.03	0.90	0.90
P, %	0.71	0.66	0.62	0.61
P, % (available)	0.46	0.45	0.40	0.41

[a]Provided (in IU/kg of diet): vitamin A, 6500; vitamin D_3, 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; thiamin hydrochloride, 7.0; pyridoxine hydrochloride, 25.0; menadione sodium bisulfite, 5.0; and ethoxyquin, 66. [b]Provided (in mg/kg of diet): $CuSO_4 \cdot 5H_2O$, 31.42; KI, 0.046; $FeSO_4 \cdot 7H_2O$, 224.0; $MnSO_4 \cdot H_2O$, 61.54; Na_2SeO_3, 0.13; ZnO, 43.56; $Na_2MoO_4 \cdot 2H_2O$, 1.26.

Experiment 2. This experiment was designed to determine if certain modifications of the diet containing 7.5% DFA replacing SBM would prevent the early growth depressions and lower body weight gain to feed intake ratio (G:F) that were observed in experiment 1. A total of 100 2-day-old chicks were used. Duplicate cages per gender of five chicks were assigned to five diets. Chicks received the control diet (diet 1) or 7.5% DFA diet (diet 2) with DL-methionine and L-lysine supplemented at 0.05% higher levels than in experiment 1 (Table 3). Diet 3 was diet 2 supplemented with arginine (Arg) and valine (Val), and the crude protein level was maintained similar to the control diet

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Table 3. Dietary Levels of Defatted Diatom and Selected Nutrients in Experiments 1, 2, and 3

	diet	algae, %	fat, %	ME kcal/g	CP, %	Arg, %	Met, %	Met + Cys, %	Lys, %	Ile, %	Thr, %	Trp, %	Val, %
					Experiment 1								
starter diets	(1) control	0.0	4.1	3.00	23.1	1.46	0.57	0.95	1.25	0.93	0.85	0.28	1.07
	(2) 7.5% algae[a]	7.5	5.3	3.01	21.3	1.26	0.62	0.97	1.22	0.91	0.84	0.26	0.97
	(3) 7.5% algae[b]	7.5	5.7	2.99	23.0	1.43	0.59	0.96	1.23	0.92	0.86	0.27	1.07
	(4) 10% algae[b]	10.0	6.3	3.01	23.0	1.42	0.59	0.96	1.22	0.92	0.86	0.27	1.07
grower diets	(1) control	0.0	6.3	3.19	20.0	1.22	0.40	0.73	1.05	0.78	0.73	0.22	0.93
	(2) 7.5% algae[a]	7.5	7.5	3.21	18.2	1.03	0.43	0.73	1.03	0.77	0.72	0.20	0.83
	(3) 7.5% algae[b]	7.5	8.0	3.19	20.0	1.20	0.40	0.73	1.05	0.78	0.74	0.22	0.93
	(4) 10% algae[b]	10.0	8.6	3.19	20.0	1.19	0.40	0.73	1.04	0.79	0.75	0.22	0.94
					Experiment 2								
starter diets	(1) control	0.0	4.1	3.00	23.1	1.45	0.62	1.00	1.30	0.93	0.85	0.27	1.07
	(2) 7.5% algae[a]	7.5	5.3	3.01	21.3	1.26	0.67	1.02	1.28	0.91	0.84	0.26	0.97
	(3) 7.5% algae[a] + aa[c]	7.5	5.1	2.99	23.6	1.44	0.67	1.01	1.29	0.91	0.85	0.27	1.06
	(4) 7.5% algae[a] + aa[c] + $KHCO_3$[d]	7.5	5.4	2.99	23.6	1.44	0.67	1.01	1.28	0.92	0.84	0.27	1.06
	(5) 7.5% algae[a] + aa[c] + minerals[e]	7.5	5.4	2.99	23.6	1.44	0.67	1.01	1.28	0.92	0.84	0.27	1.06
					Experiment 3								
starter diets	(1) control	0.0	3.7	2.97	23.4	1.47	0.68	1.06	1.43	0.93	0.94	0.28	1.08
	(3) 7.5% algae[a]	7.5	4.7	2.96	21.1	1.28	0.65	1.00	1.27	0.83	0.86	0.24	0.98
	(5) 7.5% algae[a] + aa[f]	7.5	4.4	2.94	21.7	1.45	0.69	1.04	1.40	0.92	0.91	0.27	1.06
grower diets	(1) control	0.0	6.1	3.18	20.6	1.26	0.45	0.79	1.15	0.81	0.81	0.23	0.95
	(3) 7.5% algae[a]	7.5	7.0	3.16	18.4	1.07	0.43	0.74	0.99	0.71	0.74	0.19	0.86
	(5) 7.5% algae[a] + aa[f]	7.5	6.9	3.15	18.9	1.24	0.46	0.77	1.13	0.80	0.78	0.22	0.93

[a]Defatted diatom replaced soybean meal with adjustments in dietary fat, salt, limestone, and dicalcium phosphate. [b]Defatted diatom replaced a combination of soybean meal and corn with adjustments in dietary fat, salt, limestone, and dicalcium phosphate. [c]The amino acids (aa; g/kg of diet) comprised Arg (free base), 1.9; DL-Met, 0.9; Lys·HCl, 2.0; Ile, 1.0; Thr, 0.8; Trp, 0.4; Val, 1.0; Asp + Glu, 3.7. [d]4.5% of the diet. [e]The minerals (mg/kg of diet) comprised $CuSO_4 \cdot 5H_2O$, 196; $MnSO_4 \cdot H_2O$, 154; ZnO, 62; $Na_2MoO_4 \cdot 2H_2O$, 126. [f]The amino acids (g/kg of diet) in the starter and grower diets comprised Arg (free base), 1.8; DL-Met, 0.4; Lys·HCl, 1.8; Ile, 0.9; Thr, 0.5; Trp, 0.3; Val, 0.8. See the Supporting Information for the compositions of all diets of experiments 1, 2, and 3.

Table 4. Effects of Dietary Inclusion of 7.5 or 10% of DFA on Growth Performance of Broiler Chicks in Experiment 1

									main effect, *P* value		
	weeks	gender	control	7.5% algae[a]	7.5% algae[b]	10% algae[b]	SEM	*P* value	diet	gender	diet × gender
body wt gain[c] (g/chick)	0–3	female	827 a	727 b	719 b	649 c	10	0.01	<0.0001	0.002	0.48
		male	893 a	736 b	827 a	734 b					
	4–6	female	1346	1211	1343	1170	78	0.75			
		male	1638	1346	1520	1388					
	0–6	female	2173	1938	2062	1819	68	0.48			
		male	2531	2082	2347	2122					
feed intake[c] (g/chick)	0–3	female	1180	1165	1080	1030	32	0.09	0.30	0.001	0.12
		male	1200	1120	1150	1170					
	4–6	female	2790 ab	2715 ab	2640 ab	2381 b	123	0.24			
		male	3050 ab	3045 ab	3245 a	3126 a					
	0–6	female	3970 ab	3880 ab	3720 ab	3411 b	131	0.12			
		male	4250 a	4165 a	4395 a	4296 a					
gain:feed[c] (g/kg)	0–3	female	701 abc	624 c	666 abc	655 bc	16	0.11	0.15	0.87	0.71
		male	744 a	657 abc	721 ab	627 c					
	4–6	female	482	448 a	509 a	492 a	23	0.33			
		male	536	442 a	469 a	444 a					
	0–6	female	547	500 a	554 a	539 a	19	0.16			
		male	595	500 a	535 a	494 a					

[a]Defatted diatom substituting for soybean meal with adjustments in fat, salt, limestone, and dicalcium phosphate. [b]Defatted diatom substituted for soybean meal and corn with adjustments in fat, salt, limestone, and dicalcium phosphate. [c]Means of two replicates of five chicks. Means within weeks not having a letter in common are different ($P < 0.05$).

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Table 5. Effects of Dietary Inclusion of 7.5 or 10% DFA on Biomarkers in Plasma and Liver of Broiler Chicks at 6 Weeks of Age in Experiment 1

	control		7.5% algae[a]		7.5% algae[b]		10% algae[b]				main effect, $P <$		
	female	male	female	male	female	male	female	male	SEM	P value	diet	gender	diet × gender
Plasma													
alkaline phosphatase activity, (U/L)	271[c]	311	402	375	357	528	344	427	71	0.33	0.22	0.20	0.58
alanine transaminase activity, (U/L)	4.32 a	3.37 ab	3.16 ab	2.00 b	2.21 b	1.90 b	2.64 ab	2.00 b	0.6	0.09	0.03	0.09	0.90
uric acid (μmol/L)	451 ab	426 abc	482 a	444 ab	427 abc	420 bc	429 abc	377 c	17	0.02	0.02	0.02	0.60
cholesterol (mmol/L)	3.44 ab	3.36 ab	3.65 a	3.26 ab	2.72 b	2.72 b	3.00 ab	3.34 ab	0.3	0.18	0.80	0.87	0.001
triglycerides (μmol/L)	116 b	187 ab	252 a	231 ab	196 ab	198 ab	253 a	170 ab	41	0.25	0.16	0.06	0.06
NEFA[5] (μmol/L)	361 ab	151 c	388 a	219 c	240 bc	142 c	224 c	170 c	45	0.01	0.28	0.56	0.32
Liver													
cholesterol (mmol/g liver protein)	0.82b	0.92 ab	0.87 ab	1.05 a	0.95 ab	0.97 ab	0.80 b	0.94 ab	0.1	0.18	0.16	0.02	0.54
triglycerides (mmol/g liver protein)	592 a	502 ab	506 ab	607 a	845 a	268 ab	318 ab	156 b	29	0.08	0.05	0.32	0.25
NEFA[d] (μmol/g liver protein)	233 abc	222 abc	225 abc	356 a	344 ab	155 c	189 abc	158 bc	57	0.13	0.35	0.65	0.07

[a]DFA replaced soybean meal with adjustments in dietary fat, salt, limestone, and dicalcium phosphate. [b]DFA replaced soybean meal and corn with adjustments in dietary fat, salt, limestone, and dicalcium phosphate. [c]Means within a row without a letter in common differ ($P < 0.05$). [d]NEFA, nonesterified fatty acids.

Table 6. Growth Performance Responses of Broiler Chicks to the 7.5% DFA Diets with Manipulations of Various Nutrients in Experiment 2

										main effect, P value		
	week	gender	control	7.5% algae[b]	7.5% algae[b] + aa[c]	7.5% algae[b] + aa + $KHCO_3$[c]	7.5% algae[b] + aa + $KHCO_3$ + minerals[c]	SEM	P value	diet	gender	diet × gender
body weight[a] gain (g/chick)	0−3	female	789	721	714	700	676	32	0.66	0.02	0.57	0.50
		male	815	659	725	706	647					
feed intake[a] (g/chick)	0−3	female	1170	1150	1230	1195	1185	52	0.44	0.32	0.33	0.44
		male	1260	1050	1150	1140	1160					
gain:feed[a] (g/kg)	0−3	female	650	626	580	586	571	21	0.56	0.13	0.04	0.23
		male	647	630	631	619	559					

[a]Means of two replicates of five chicks per gender from 0 to 3 weeks of age. Means within a row not having a letter in common are different ($P < 0.05$). [b]Defatted diatom replaced soybean meal with adjustments in fat, salt, limestone, and dicalcium phosphate. [c]See footnotes *c*, *d*, and *e* in Table 3.

by supplementation with aspartic acid and glutamic acid in a 1.6:1 ratio. Diet 4 was diet 3 with the addition of potassium bicarbonate to adjust the dietary electrolyte balance (Na + K − Cl) from 173 mequiv/kg in diet 3 to 218 mequiv/kg in diet 4. Diet 5 was formulated to contain added copper (Cu), manganese (Mn), molybdenum (Mo), and zinc (Zn), but otherwise was similar to diet 4. The duration of the experiment was 3 weeks.

Experiment 3. This 6 week experiment involving 180 3-day-old male chicks was performed to confirm results of the previous experiments using 7.5% DFA replacing SBM and to determine whether the addition of proteases or amino acids improved broiler growth and G:F. Five treatments consisting of six replicates of six chicks were used. The control diet (diet 1) was similar to that of experiment 1 except that it contained added threonine. Diet 2 was the control diet plus the inclusion of 0.06% commercial protease (Ronozyme ProAct, DSM Nutritional Products, Inc., Parsippany, NJ, USA). Diet 3 contained 7.5% DFA and was similar to diet 2 of experiment 1, and diet 4 contained 7.5% DFA and 0.06% commercial protease. Diet 5 contained 7.5% DFA supplemented with Arg, Ile, Trp, and Val.

Blood Collection, Tissue Examination, and Biochemical Assays. After a 6 h fast, blood was drawn from the wing veins of two birds per pen at week 6 in experiment 1 and at 3 and 6 weeks in experiment 3. Blood was held in ice during collection, centrifuged at 3000*g* for 15 min, and stored at −20 °C until analyses. After blood sampling, birds were euthanized by cervical dislocation. In experiment 1, the proventriculus (true stomach), ventriculus (gizzard), small intestine, and large intestine and cecum from each of two randomly selected chicks per cage were opened and examined for evidence of gross pathology. The koilin layer of the ventriculus was examined and removed for observation of the underlying tissue. To assess liver health and function, plasma alanine transaminase (ALT) activities were determined spectrophotometrically with the Infinity Alt liquid stable reagent (Thermo Electron Corp.), and plasma alkaline phosphatase (AKP) activities were analyzed according to the method of Bowers and McComb.[37] Plasma glucose level was determined spectrophotometrically with glucose assay kit GAG020 (Sigma-Aldrich, Sigma Chemical Co., St. Louis, MO, USA). Plasma uric acid was analyzed with Infinity Uric Acid Liquid Stable Reagent (Thermo Scientific Corp.). Plasma and liver nonesterified fatty acid (NEFA), triglyceride (TG), and cholesterol (CHOL), indicators of lipid metabolism were analyzed using commercial enzymatic kits (Wako Pure Chemicals Industries, Ltd., Richmond, VA, USA). All samples were analyzed in duplicates.

Statistical Analyses. Data were analyzed by one-way or two-way ANOVA with or without time-repeated measurements for determining significance of the main effect using SPSS 17.0. Mean comparisons were conducted with Duncan's method. The significance level for differences was $P < 0.05$.

Table 7. Responses of Growth Performance of Male Broiler Chicks to 7.5% DFA Diets Containing Amino Acids and Protease in Experiment 3

									main effect, P value		
	week	control diet (C)	C + protease	7.5% algae[b]	7.5% algae[b] + protease	7.5% algae[b] + aa[c]	SEM	P value	diet	week	diet × week
body weight[a] gain (g/chick)	0−3	890	853	916	923	932	26.6	0.25	0.14	<0.001	0.94
	4−6	1870	1822	1876	1805	1894	37.2	0.41			
	0−6	2759	2675	2792	2728	2825	56.7	0.40			
feed intake[a] (g/chick)	0−3	1124 ab	1083 b	1186 a	1195 a	1184 a	29.7	0.05	<0.001	<0.001	0.23
	4−6	3269 b	3183 b	3480 a	3367 ab	3372 ab	66.4	0.04			
	0−6	4393 ab	4266 b	4666 a	4562 a	4556 a	86.9	0.03			
gain:feed[a] (g/kg)	0−3	791	787	773	772	787	9.7	0.49	<0.001	<0.001	0.19
	4−6	572 a	572 a	539 b	536 b	562 a	6.2	0.001			
	0−6	628 a	627 a	598 b	598 b	620 a	4.9	0.001			

[a]Means of six replicates of six chicks. Means within a row without a letter in common differ ($P < 0.05$). [b]Defatted diatom replaced soybean meal with adjustments in dietary fat, salt, limestone, and dicalcium phosphate. [c]See footnote *f* of Table 3 for the supplemental amino acids.

Table 8. Responses of Plasma Biomarkers of Male Broiler Chicks to 7.5% DFA Diets Containing Amino Acids and Protease at 3 and 6 Weeks of Age in Experiment 3

									main effect, P value		
	week	control diet (C)	C + protease	7.5% algae[b]	7.5% algae[b] + protease	7.5% algae + aa[c]	SEM	P value	diet	week	diet × week
total cholesterol (mmol/L)	3	2.77 c[a]	3.09 bc	3.33 ab	3.38 ab	3.47 a	0.12	0.001	0.01	0.55	0.09
	6	3.25	3.18	3.06	3.35	3.44	0.14	0.37			
total triglyceride (μmol/L)	3	264 b	357 a	349 ab	334 ab	383 a	29.1	0.1	0.44	0.05	0.11
	6	388	335	365	445	367	40.0	0.47			
NEFA[d] (μmol/L)	3	225	242	261	249	295	25.2	0.46	0.206	0.79	1.0
	6	225	246	258	259	310	38.1	0.59			
uric acid (μmol/L)	3	568	546	605	558	575	27.4	0.62	0.71	0.001	0.58
	6	511	486	479	470	444	31.4	0.65			
glucose (mmol/L)	3	11.3 b	21.0 a	17.4 a	19.8 a	16.7 a	1.7	0.01	0.01	0.02	0.03
	6	19.7	19.8	19.4	20.9	18.3	1.3	0.73			
alkaline phosphatase activity, (U/L)	3	939	964	1005	849	964	146.9	0.92	0.75	0.001	0.80
	6	377 ab	342 ab	466 a	367 ab	213 b	65.9	0.15			
alanine transaminase activity, (U/L)	3	2.94	2.10	2.13	2.13	2.59	0.292	0.19	0.03	0.002	0.71
	6	3.64	3.04	2.30	3.08	3.83	0.494	0.73			

[a]Mean of two chicks per replicate (total of 12 chicks per treatment). Means within a row without a letter in common differ ($P < 0.05$). [b]Defatted diatom replaced soybean meal with adjustments in dietary fat, salt, limestone, and dicalcium phosphate. [c]See footnote *f* of Table 3 for the added amino acids. [d]Nonesterified fatty acids.

RESULTS

Experiment 1. Body weight gain of chicks was affected by diet ($P < 0.0001$) and gender ($P < 0.002$) (Table 4). During the 0−3 week interval, all groups fed the DFA-containing diets except for those of males fed diet 3 had lower ($P < 0.01$) body weight gains than the control group. However, this adverse effect of DFA on body weight gain became statistically nonsignificant during the 4−6 week interval or for the cumulative 0−6 week period. Meanwhile, chicks fed the DFA-containing diets appeared to have lower feed intake ($P = 0.09$) and gain:feed ($P = 0.11$) than the control group during the 0−3 week interval. Gender affected feed intake ($P < 0.05$), but not gain:feed. There was no anomaly or gross pathology in the proventriculus, ventriculus, and intestinal tract.

Diet exerted an overall effect ($P < 0.05$) on plasma ALT activity and uric acid concentration (Table 5). Chicks fed DFA-containing diets tended to have lower activity of ALT than chicks fed diet 1, and the difference was significant ($P < 0.05$) between the female chicks fed diets 3 and 1. Plasma uric acid concentration was lower ($P < 0.05$) in male chicks fed diet 4 than diet 2. None of the plasma uric acid values of male or female chicks fed the DFA-containing diets differed from those fed the control diet. There was an overall effect of gender ($P < 0.02$) on plasma uric acid concentration, but no significant treatment mean difference was noted. There was on overall effect of gender ($P < 0.02$) on liver CHOL concentrations and also an overall effect of diet ($P < 0.05$) on plasma TG concentrations along with a significant difference between male chicks fed diets 4 and 2.

Experiment 2. There was an overall effect of diet ($P < 0.02$) on body weight gain (Table 6), although the decreases by any given DFA-containing diet over the control diet reached no statistically significant level. Gain:feed was affected by gender ($P < 0.04$) and showed a declining trend ($P = 0.13$) in response to the DFA-containing diets compared with the control diet. Supplementing the DFA-containing diet (diet 2) with amino acids, $KHCO_3$, and minerals (diets 3−5) produced no additional benefit to body weight gain, feed intake, or gain:feed. Three male chicks fed diet 2 in exhibited swollen hocks and difficulty walking at 3 weeks of age.

Experiment 3. Diet affected ($P < 0.001$) feed intake and gain:feed, but not body weight gain (Table 7). The feed intakes

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of chicks fed the three DFA-containing diets were greater ($P < 0.05$) than those fed diet 2 during the 0−3 week interval and the 0−6 week period. There was no difference among dietary groups in gain:feed during the 0−3 week interval, the ratios during the 4−6 and 0−6 week periods were lower ($P < 0.05$) in chicks fed 7.5% DFA diet (diet 3) and the 7.5% DFA plus protease (diet 4) than in those fed the diets (1 and 2) without DFA and the DFA-containing diet supplemented with amino acids (diet 5). Chicks fed diet 5 had gain:feed ratios similar to those fed diets 1 and 2.

Three of the biochemical measures of plasma, CHOL, glucose, and ALT, were affected by diet ($P < 0.05$) (Table 8). There were effects ($P < 0.05$) of time (3 or 6 weeks) on plasma total triglyceride, uric acid, glucose, AKP, and ALT ($P < 0.05$). Total plasma CHOL was higher at 3 weeks in the chicks fed diets containing DFA than in chicks fed the control diet ($P < 0.05$). Plasma glucose was higher ($P < 0.05$) at 3 weeks in chicks fed the DFA-containing diets than in those fed the control diet. Despite an overall effect of diet ($P < 0.03$) on plasma ALT activity, there was no significant difference between any treatment means. Plasma uric acid concentration and AKP activity of chicks were lower ($P < 0.05$) at 6 weeks than at 3 weeks.

DISCUSSION

The overall finding of experiment 1 indicated that inclusion of the defatted diatom microalgal *Staurosira* sp. biomass, from biofuel production, at 7.5% of diet in replacing the same amount of SBM and corn had an adverse effect on growth in the 0−3 week interval, but did not significantly affect body weights by the end of the experiment. In contrast, the 10% of inclusion or the 7.5% of inclusion in replacing SBM alone decreased body weight gain, feed intake, and gain:feed, especially during the 0−3 week period. The DFA used in the present study contained 19% crude protein (CP) on an "as fed" basis and had no major limitations of amino acids as a source of feed protein. However, it was expected that its substitution for SBM would require a supplementation of the chick diets with Met and Lys. Because the CP content of the DFA was lower than the CP (47.5%) of SBM, but was greater than the CP (8.5%) of corn,[8] substitution of DFA for a mixture of SBM and corn had little effect on the dietary CP level. However, the supplementation of the DFA for SBM resulted in a lower level of CP in the diet compared to the control diet. Thus, this depressed growth performance of chicks might be associated with the lower protein level, an insufficient dietary level of one or more indispensable amino acids, lower protein digestibility, or a combination of these factors.

The notion of amino acid limitation in the DFA-containing diets was supported in part by the results of experiment 3 in which the decreased gain:feed due to the 7.5% DFA inclusion was prevented by the addition of Met, Lys, Ile, Thr, Trp, and Val to the diet. Apparently, one or more of these amino acids must have been limiting in the DFA-containing diets, contributing to the reduction in feed use efficiency compared with the control diet. Meanwhile, the dietary levels of Met and Lys were raised by 0.05 percentage points in experiment 2 as compared to experiment 1 to ensure that the levels would be adequate for rapidly growing broilers. Sulfur-containing amino acids (Met or Cys) and Lys are generally the first and second most limiting amino acids in practical diets based on corn and soybean for broilers.[38] The inability to ascertain the specific effects of amino acid supplementation in experiment 2 might have been due to the increased dietary levels of Met and Lys. The increase in supplementation could have been sufficient to mitigate a mild deficiency of one or both amino acids. The further addition of Arg and Val to raise the levels of these indispensable amino acids and the addition of glutamic acid and aspartic acid to raise the CP level to 23.5% did not enhance growth rate or feed efficiency. These results suggest that Arg, Val, and CP were not limiting. However, the addition of protease to the control diet or the DFA-containing diet in experiment 3 did not improve broiler growth rate or gain:feed. The failure of the protease to improve performance, whereas amino acid supplements were effective, may indicate that proteins in the DFA were not hydrolyzed by the protease and (or) did not require additional proteolysis. Algaenan, a class of proteins in some algae, is reported to be resistant to enzymatic hydrolysis.[39] Proteins of this class have been detected in many, but not all, algae.[40] It is unknown whether they are present in the DFA used in the present study.

The dietary balance of the monovalent minerals sodium (Na), potassium (K), and chlorine (Cl) influences the growth and skeletal development of broiler chickens.[35,36] The optimum dietary electrolyte balance (Na + K − Cl) for broilers is near 200 mequiv/kg of feed for maximum growth and minimum incidence and severity of tibial dyschondroplasia.[41] Raising the electrolyte balance from 173 to 218 mequiv/kg, in the DFA-containing diet, did not improve broiler growth or feed utilization. Therefore, electrolyte balance probably was not a limiting factor for the adverse effect of the DFA-containing diet. Because diatoms contain amorphous Si, and this element interacts in biological systems with divalent minerals such as iron (Fe), molybdenum (Mo), copper (Cu), and zinc (Zn),[42,43] the dietary levels of trace minerals were increased in diet 5 of experiment 2. Likewise, these elevations of trace minerals were ineffective in restoring growth or feed utilization.

Although some minor differences in plasma uric acid, glucose, and lipid concentrations were observed, the plasma biomarkers did not reveal any indication of adverse effects of the DFA on metabolism. Plasma AKP and ALT, indicators of liver health and function, were not increased by the inclusion of the DFA in the diet. In contrast, the 10% level of DFA tended to decrease the uric acid concentration in the males at 6 weeks in experiment 1. The males tended to grow more rapidly and ingested more feed than the females. These results were consistent with known gender differences in growth rate, efficiency of feed utilization for body weight gain, and nitrogen retention.[44] To determine if algal inclusion affected nutrient metabolism, measures of plasma and liver lipid and carbohydrate metabolism were examined. Plasma glucose concentrations were elevated at 3 weeks in chicks fed the DFA-containing diets in experiment 3, perhaps indicating a higher availability of carbohydrates in the DFA than in the replaced SBM. Chicks fed the DFA-containing diets tended to have higher plasma total CHOL and TG concentrations at 3 weeks than chicks fed the control diet. This increase might also reflect increased carbohydrate availability from DFA and the fact that the DFA-containing diets had higher crude fat contents than the control diets. The metabolizable energy value that was used in the dietary formulations in these experiments was estimated from the fat and protein content of the DFA. It is possible that it was an underestimate, resulting in higher dietary energy concentration in the DFA-containing diets than what was targeted.

Two observations may lead to further research on the use of the DFA in broiler feeds. First, the chicks fed the DFA-containing diets had, by visual observation, an increased volume and wetness of excreta as compared to the volume and moisture of excreta from chicks fed the control diet. The ash fraction of the DFA accounted for nearly 45% of the weight of the biomass "as fed" and contained substantial amounts of Na, K, Mg, Fe, and Cl. The increased wet droppings undoubtedly were a consequence of the ash content of the defatted diatom. Excessive output of excreta is a potential liability of the diatom feeding. Further processing to reduce the ash content of the DFA would improve this coproduct as a feed ingredient for poultry and other animals. The second observation of potential concern was the incidence of hock disorder in males fed the basic 7.5% DFA diet in experiment 2. The disorder was not observed in experiments 1 and 3. Given the propensity of broiler chicks to develop leg abnormalities,[45] however, an investigation of skeletal development in chicks fed diets containing the DFA is advisable.

In summary, the results of the present study indicate that the DFA of *Staurosira* sp. could be used as a protein and energy source in broiler diets. The inclusion level of 7.5% for replacing a mixture of corn and SBM was well tolerated by broilers. The same inclusion level for replacing SBM alone was also feasible when certain amino acids were supplemented. Strikingly, the latter inclusion of 7.5% DFA in broiler diets to replace SBM could potentially spare over 2.4 million metric tons of soybean for human consumption annually. Ultimately, further research into the bioavailability and interaction of the amino acids and other nutrients in the DFA will be needed to provide the quantitative information necessary for optimal diet formulation and cost analysis.

■ ASSOCIATED CONTENT

Supporting Information

Additional tables. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) World Population Prospects, 2007. *World Population Prospects, The 2006 Revision, Highlights*, Working Paper ESA/P/WP.202; United Nations, Department of Economic and Social Affairs, Population Division: New York, 2007.

(2) Brown, L. *Outgrowing the Earth, a Food Security Challenge in Age of Falling Water Tables and Rising Temperatures*; W. W. Norton: New York, 2004.

(3) Food and Agricultural Organization. In *World Agriculture: Towards 2015/2030. Summary Report. Prospects for the Environment: Agriculture and the Environment*; Rome, Italy, 2002.

(4) Giesecke, D.; Tiemeyer, W. Availability and metabolism of purines of single-cell proteins in monogastric animals. *Proc. Nutr. Soc.* **1982**, *41*, 319−327.

(5) Becker, W. In *Handbook of Microalgal Culture: Biotechnology and Applied Phycology*; Microalgae in Human and Animal Nutrition; Richmond, A., Ed.; Blackwell Science: Oxford, UK, 2004; pp 312−351.

(6) Goldemberg, J. *World Energy Assessment, Preface*; United Nations Development Program: New York, 2000.

(7) Norton, T. A.; Melkonian, M.; Andersen, R. A. Algal biodiversity. *Phycologia* **1996**, *35* (4), 308−326.

(8) National Research Council. *Nutrient Requirements of Poultry*, 9th revised ed.; National Academy Press: Washington, DC, 1994.

(9) Pulz, O.; Gross, W. Valuable products from biotechnology of microalgae. *Appl. Microbiol. Biotechnol.* **2004**, *65*, 635−648.

(10) Lebeau, T.; Robert, J.-M. Diatom cultivation and biotechnologically relevant products. Part II. Current and putative products. *Appl. Microbiol. Biotechnol.* **2003**, *60*, 624−632.

(11) Spolaore, P.; Joannis-Cassan, C.; Duran, E.; Isambert, A. Commercial applications of microalgae. *J. Biosci. Bioeng.* **2006**, *101* (2), 87−96.

(12) Kroth, P. Transgenic microalgae as green cell factories. In *Molecular Biology and the Biotechnological Potential of Diatoms*; Leόn, R., Galván, A., Fernández, E., Eds.; Landes Bioscience and Springer Science, Business Media: Austin, TX, 2007; pp 23−33.

(13) Herber, S. M.; Van Elswyk, M. E. Dietary marine algae promotes efficient deposition of n-3 fatty acids for the production of enriched shell eggs. *Poult. Sci.* **1996**, *75*, 1501−1507.

(14) Abril, R.; Barclay, W. Production of docosahexaenoic acid-enriched eggs and meat using an algae-based feed ingredient. In *The Return of ω3 Fatty Acids into the Food Supply. I. Land-Based Animal Food Products and Their Health Effects*; Simopoulos, A. P., Ed.; World Rev. Nutr. Diet; Karger: Basel, Switzerland, 1998; Vol. 83, pp 77−88.

(15) Barclay, W.; Abril, R.; Abril, P.; Weaver, C.; Ashford, A. Production of docosahexaenoic acid from microalgae and its benefits for use in animal feeds. In *The Return of ω3 Fatty Acids into the Food Supply. I. Land-Based Animal Food Products and Their Health EffectsWorld Rev. Nutr. Diet.*; Simopoulos, A. P., Ed.; World Rev. Nutr. Diet; Karger: Basel, Switzerland, 1998; Vol. 83, pp 61−76.

(16) Nitzan, Z.; Mokady, S.; Sukenik, A. Enrichment of poultry products with ω3 fatty acids by dietary supplementation with the alga *Nannochloropsis* and Mantur oil. *J. Agric. Food Chem.* **1999**, *47*, 5127−5132.

(17) Grau, C. R.; Klein, N. W. Sewage-grown algae as a feedstuff for chicks. *Poult. Sci.* **1957**, *36*, 1046−1051.

(18) Lipstein, B.; Hurwitz, S. The nutritional value of sewage-grown, alum flocculated Micractinium algae in broiler and layer diets. *Poult. Sci.* **1981**, *60*, 2628−2638.

(19) Ross, E.; Dominy, W. The nutritional value of dehydrated, blue-green algae (*Spirulina plantensis*) for poultry. *Poult. Sci.* **1990**, *69*, 794−800.

(20) Sauveur, B.; Zybko, A.; Colas, B. Protéines alimentaires et qualité de l'œuf. I. Effet de quelques protéines sur la qualité interne de l'œuf et les proprieies fonctionnelles. *Ann. Zootechnol.* **1979**, *28* (3), 271−295.

(21) Herber-McNeill, S. M.; Van Elswyk, M. E. Dietary marine algae maintains egg consumer acceptability while enhancing yolk color. *Poult. Sci.* **1998**, *77*, 493−496.

(22) USDA National Agriculture Statistics Service. Poultry − Production and Value, 2012 Summary (April 2013); ISSN 1949-1573.

(23) Combs, G. F. Algae (*Chlorella*) as a source of nutrients for the chick. *Science* **1952**, *116*, 453−454.

(24) Blum, J.-C.; Calet, C. Valeur alimentaire des algues spirulines pour la croissance du poulet de chair. *Ann. Nutr. Aliment.* **1976**, *29*, 551−574.

(25) Mokady, S.; Yannai, S.; Einav, P.; Berk, Z. Algae grown on wastewater as a source of protein for young chickens and rats. *Nutr. Rep. Int.* **1979**, *19*, 383−390.

(26) Lipstein, B.; Hurwitz, S. The nutritional value of sewage-grown samples of *Chlorella* and *Micractinium* in broiler diets. *Poult. Sci.* **1983**, *62*, 1254−1260.
(27) vankatarman, L. V.; T. Somasekaran, T.; Becker, E. W. Replacement value of blue-green alga (*Spirulina platensis*) for fishmeal and a vitamin-mineral premix for broiler chick. *Br. Poult. Sci.* **1994**, *35*, 373−38.
(28) Hintz, H. F.; Heitmann, H. Sewage-grown algae as a protein supplement for swine. *Anim. Prod.* **1967**, *9*, 135−140.
(29) Simbaya, J.; Slominski, B. A.; Guenter, W.; Morgan, A.; Campbell, L. D. The effects of protease and carbohydrase supplementation on the nutritive value of canola meal for poultry: In vitro and in vivo studies. *Anim. Feed Sci. Technol.* **1996**, *61*, 219−234.
(30) Ghazi, S.; Rooke, J. A.; Galbraith, H.; Bedford, M. R. The potential for the improvement of the nutritive value of soya-bean meal by different proteases in broiler chicks and broiler cockerels. *Br. Poult. Sci.* **2002**, *43*, 70−77.
(31) Martin-Jézéquel, V.; Hildebrand, M.; Brzezinski, M. A. Silicon metabolism in diatoms: implications for growth. *J. Phycol.* **2000**, *36*, 821−840.
(32) Rupérez, P. Mineral content of edible marine seaweeds. *Food Chem.* **2002**, *79*, 23−26.
(33) Keegan, T. P.; DeRouchey, J. M.; Nelssen, J. L.; Tokach, M. D.; Goodband, R. D.; Dritz, S. S. The effects of poultry meal source and ash level on nursery pig performance. *J. Anim. Sci.* **2004**, *82*, 2750−2756.
(34) Gal-Garber, O.; Mabjeesh, S. J.; Sklan, D.; Un, Z. Nutrient transport in the small intestine: Na^+,K^+-ATPase expression and activity in the small intestine of the chicken as influenced by dietary sodium. *Poult. Sci.* **2003**, *82*, 1127−1133.
(35) Leach, R. M., Jr.; Nesheim, M. C. Further studies on tibial dyschondroplasia (cartilage abnormality) in young chicks. *J. Nutr.* **1972**, *102*, 1673−1680.
(36) Sauveur, B.; Mongin, P. Interrelationship between dietary concentrations of sodium, potassium and chloride in laying hens. *Br. Poult. Sci.* **1978**, *19*, 475−485.
(37) Bowers, G. N., Jr.; McComb, R. B. A continuous spectrophotometric method for measuring the activity of serum alkaline phosphatase. *Clin. Chem.* **1966**, *12*, 70−89.
(38) Emmert, J. L.; Baker, D. H. Use of the ideal protein concept for precision formulation of amino acid levels in broiler diets. *J. Appl. Poult. Res.* **1997**, *91*, 683−692.
(39) Nguyen, R. T.; Harvey, H. R.; Zang, X.; van Heemst, J. D. H.; Hetényi, M.; Hatcher, P. G. Preservation of algaenan and proteinaceous material during the oxic decay of *Botryococcus braunii* as revealed by pyrolysis-gas chromatography/mass spectroscopy and ^{13}C NMR spectroscopy. *Org. Geochem.* **2003**, *34*, 483−497.
(40) Kodner, R. B.; Summons, R. E.; Knoll, A. H. Phylogenetic investigation of the aliphatic, non-hydrolyzable, biopolymer algaenan, with a focus on the green algae. *Org. Geochem.* **2009**, *40* (8), 854−862.
(41) Mongin, P.; Sauveur, B. Growth and poultry meat production. In *Interrelationships Between Mineral Nutrition, Acid-Base Balance, Growth and Cartilage Abnormalities*; Boorman, K. N., Wilson, B. J., Eds.; British Poultry Science: Edinburgh, Scotland, 1977; pp 235−247.
(42) Emerick, R. J.; Kayongo-Male, H. Interactive effects of dietary silicon, copper, and zinc in the rat. *J. Nutr. Biochem.* **1990**, *1*, 35−40.
(43) National Research Council. *Mineral Tolerance of Animals*, 2nd revised ed.; National Academy Press: Washington, DC, 2005; Chapter 26, Silicon, pp 348−354.
(44) Hernández, F.; López, M.; Martínez, S.; Megías, M. D.; Catalá, P.; Madrid, J. Effect of low protein diets and single sex on production performance, plasma metabolites, digestibility, and nitrogen excretion in 1- to 48-day-old broilers. *Poult. Sci.* **2012**, *91*, 683−692.
(45) Waldenstedt, L. Nutritional factors of importance for optimal leg health in broilers; a review. *Anim. Feed Sci. Technol.* **2006**, *126* (3−4), 291−307.