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Supplemental Defatted Microalgae *Nannochloropsis oceanica* Affected Apparent Retention and Ileal Digestibility of Nutrients in a Corn-Soybean Meal Based Diet for Broiler Chickens

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Abstract

Objective: This study was to assess impacts of including 10% defatted microalgae (*Nannochloropsis oceanica*, 45% crude protein and 3.8% ether extract) into a corn-soybean meal basal diet for broiler chickens on apparent retention and ileal digestibility of nutrients.

Procedures: Day-old hatchling Cornish Giant cockerels were divided into two groups (6 cages/group, 6 chicks/cage) and fed either the basal (control) or the microalgae-supplemented (microalgae) diet for 6 weeks. Starting week 3, the two experimental diets were mixed with 0.2% chromium oxide as an indigestible marker. Total excreta of individual cages were collected daily for 3 consecutive days during week 6. At the end of week 6, chicks were euthanized to collect ileal digesta from 1 chick/cage. Energy and concentrations of dry matter, crude protein, ether extract, minerals, and amino acids in digesta, excreta, and diets were assayed. Apparent retentions and ileal digestibilities were calculated using the direct collection and indirect indicator methods. Data were analyzed by the Student's t-test and the significant level of difference was at $p < 0.05$.

Results: The microalgae diet had elevated (4.2% and 8.1% by the two methods, $p < 0.05$) apparent retention of ether extract, but similar apparent retention and ileal digestibility of dry matter and apparent metabolizable energy, compared with the control diet. Supplemental defatted microalgae decreased ($p < 0.05$) the apparent retention and ileal digestibility of dietary crude protein by 13% and 13%. The supplementation also decreased ($p < 0.05$) ileal digestibilities and apparent retentions of multiple amino acids ranging from 0.6% to 26%. Apparent retentions of sodium

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and total phosphorus were decreased ($p < 0.05$) by 13% and 71%, respectively, by the microalgae inclusion compared with the control.

Conclusion: The inclusion of 10% defatted *N. oceanica* in the corn-soybean meal basal diet for broiler chickens led moderate increases in apparent retention and ileal digestibility of ether extract, but decreased those of crude protein, amino acids, sodium, and phosphorus.

Introduction

More than 67 million and 30 million metric tons of corn and soybean meal were fed to broilers and their breeders in 2019 only in US [1]. Because the demand for animal-sourced protein is expected to rise for the predicated world population of 9 billion by 2030 [2], the need for corn and soybean meal as animal feed will be increased. Defatted microalgae have been studied as an alternative source of feed protein because of the high crude protein concentrations and excellent amino acid profiles [3]. Indeed, various types of microalgal biomass were supplemented into diets to replace corn and soybean meal without impairing growth performance in pigs [4], laying hens [5] and broiler chicks [6]. However, it remains largely unclear how the microalgal inclusions affect digestion and utilization of various nutrients in the diets.

Our laboratory has conducted several studies to determine such effects of supplemental defatted microalgae in diets for poultry and swine. In laying hens, feeding 25% defatted *Desmodesmus spp.* [crude protein (CP): 31%] in a corn-soybean meal diet improved ileal Amino Acid (AA) digestibility (91%) over the control (82%) [5]. Average amino acid digestibilities were improved from 78% (control) to 85% or 93% by feeding 7.5% or 15% defatted *Desmodesmus sp.* (CP: 38.2%) to weanling pigs [4]. While there was no difference in apparent nitrogen retention or digestibility in broiler chickens fed 0, 2, 4, 8 and 16% defatted *Nannochloropsis oceanica* (*N. oceanica*, CP: 38.2%) [7], the apparent retention of inorganic phosphorus was improved linearly with the increasing inclusion levels of defatted *N. oceanica* [6]. As none of our previous studies determined the effects of supplemental microalgae on digestion or retention of all nutrients simultaneously, our results or the discrepancies might been confounded with the microalgae type, species, or experimental procedure. Therefore, it is warranted to clarify effects of the microalgae supplementation on digestion and retention of all major nutrients important for diet formulation in a specially-designed study using the same microalgae and species.

Green microalgae *Nannochloropsis oceanica* have received increasing attention for biofuel production [8]. Supplementing the resultant defatted biomass did not impair the animal production performance [4,6], but helped enrich omega-3 fatty acids in chicken products [9-11]. Previously, we found that broiler chickens performed well and maintained good health when their corn soybean meal diets were supplemented defatted *N. oceanica* up to 8% [6]. Therefore, we considered a 10% inclusion of the defatted *N. oceanica* to be a safe upper and practically relevant level in the corn-soybean meal diets for broiler chickens. The present study was conducted to the systematic effects of that inclusion on apparent retention and ileal digestibility of energy, dry matter, crude protein, ether extract, amino acids, sodium, and total phosphorus.

Materials and methods

Animals, diets, and management

The detailed animal experimental design, diets, and management were previously described [10]. Briefly, day-old hatchling Cornish Giant broiler male chicks were purchased from a commercial hatchery, and birds were divided into the control and microalgae treatment groups (6 cages/treatment, 6 chicks/cage) and housed in cages (6 cages/treatments, 6 birds/cage) placed in a thermostatically-controlled room. Defatted microalgae *Nannochloropsis oceanica* (*N. oceanica*) biomass in powder form was generated from the biofuel production research (Cellana, Kailua-Kona, HI) and the nutrient composition of the biomass was shown previously [10]. The control chicks were fed a corn and soybean meal Basal Diet (BD), and the microalgae group were fed 10% defatted *N. oceanica* supplemented into the BD, shown as 4CO and 4CO + MA diet in the previous publication [10]. The nutritive values of starter and grower diets are shown in Supplemental Table 1. Chickens were given free access to water and feed for the whole period. Our experimental protocol was approved by the Institution of Animal Care and Use Committee of Cornell University.

Collection of excreta and digesta

Over the last 3 days of week 6, total excreta from each cage was weighed and sampled daily from multiple spots after the removal of feathers or debris. At the end of week 6, all animals were fasted for 8 hours before euthanization. Ileal digesta between Meckel's diverticulum and ileocecal junction on the intestine was collected [12]. Excreta and digesta samples were freeze-dried (Virtis freeze dryer, Model: 20 SRC-X, Gardiner, NY, USA). Resulting dried samples were ground to a fine powder and stored in -20°C until analysis.

Proximate analysis of nutrients

Dry matter, CP, ether extract, energy, amino acids and mineral profile from excreta, ileal digesta or diets were analyzed as described by AOAC [13]. Dry matter of experimental diets was determined by measuring weight loss as drying samples in 100°C oven for 24 hours. Concentrations of dry matter in the excreta and digesta were calculated by weight differences after the samples were freeze-dried. Nitrogen analyzer (2300 Kjeltec TM Analyzer, FOSS, Denmark) was used to determine CP (nitrogen times 6.25). Ether extract was determined by Soxhlet ether extraction method using petroleum ether. Energy was measured by Isoperibol Bomb Calorimeter (Model: Parr 126, Parr Instrument Company, Moline, IL, USA). Chromium oxide concentrations in the diets, excreta and ileal digesta were assayed using the method of Fenton et al. [14]. The amino acid concentrations (except for tryptophan) of the diets, excreta and ileal digesta were determined using the Shimadzu HPLC system (Nexera X2, Shimadzu, Kyoto, Japan) after acid hydrolysis [15]. Sulfur amino acids were measured through performic acid oxidation followed by acid hydrolysis [16]. Mineral concentrations were determined by Inductively Coupled Plasma (ICP) trace analyzer emission spectrometry (ICAP 6000 trace element analyzer, Thermo Fisher Scientific, Waltham, MA, USA).

Calculation and statistical analyses

Apparent nutrient retention was measured and calculated by indigestible marker (including 0.2% chromium oxide) and total excreta collection method to minimize variation. In addition, apparent ileal digestibility of nutrient was determined by

the indigestible marker method. Corresponding equations were presented as the following:

$$Apparent\ retention(\%) = \left(1 - \frac{Cr_{diet} \times Nutrient_{excreta}}{Cr_{excreta} \times Nutrient_{diet}}\right) \times 100\%$$

(or)

$$Apparent\ retention(\%) = \left(1 - \frac{Total\ collection_{excreta} \times Nutrient_{excreta}}{Total\ collection_{diet} \times Nutrient_{diet}}\right) \times 100\%$$

and

$$Apparent\ ileal\ digestibility(\%) = \left(1 - \frac{Cr_{diet} \times Nutrient_{ileal}}{Cr_{ileal} \times Nutrient_{diet}}\right) \times 100\%$$

Cr_{diet}, $Cr_{excreta}$ and Cr_{ileal} stand for chromium oxide concentration in diet, excreta and ileal digesta, while $Nutrient_{diet}$, $Nutrient_{excreta}$ and $Nutrient_{ileal}$ represent each nutrient concentration in the diet, excreta, and ileal digesta, respectively. Total $collection_{excreta}$ and Total $collection_{diet}$ are total amount of excreta and diet ingested during the collection period. Besides, the apparent metabolizable energy (AME/AMEn: nitrogen correction) of the control and microalgae diet were estimated according to Hill and Anderson [17].

$$AME_{diet} = GE_{diet} - GE_{excreta} \times \frac{Cr_{diet}}{Cr_{excreta}}$$

and

$$AMEn_{diet} = AME_{diet} - 8.22 \times N$$

as

$$N = N_{diet} - N_{excreta} \times \frac{Cr_{diet}}{Cr_{excreta}}$$

GE_{diet} and $GE_{excreta}$ stand for gross energy in diet and excreta, while N_{diet} and $N_{excreta}$ stand for nitrogen content in diet and excreta, respectively.

Data (n = 5 ~ 10) were analyzed with RStudio (RStudio, Version 1.1.447. Boston, MA, USA). The overall main effect of diets was determined by Student's t-test and the significant level for difference was at $p < 0.05$.

Result

Apparent retention and ileal digestibility of dry matter, crude protein, ether extract, and energy

The microalgae diet had 4.4% and 8.1% ($p < 0.05$) higher retention of ether extract than the control diet by the total collection and indigestible marker methods, respectively (**Table 1**). Meanwhile, no differences were found between the two diets in dry matter or energy retention by either method. The AME/AMEn from indigestible marker method didn't show difference between the two diets. However, retention of CP from the microalgae diet was decreased by 13.2% ($p < 0.05$) using total collection method, while there was no difference of CP by indigestible marker method. In addition, CP ileal digestibility in the microalgae diet was decreased ($p < 0.05$) by 13% over the control, whereas ileal digestibility of ether extract or energy was not different between the two diets.

Apparent retention and/or ileal digestibility of amino acids and minerals

The microalgae diet exhibited consistent lower amino acid retention and apparent ileal digestibility over the control (**Table 2**). Apparent ileal digestibilities of 7 essential amino acids (methionine, histidine, isoleucine, leucine, lysine, phenylalanine, and threonine) and 4 nonessential amino acids (asparagine, glutamine, glycine and serine) were 7.2% to 26% lower ($p < 0.05$) in the microalgae diet than the control. Meanwhile, apparent retentions of 6 essential amino acids (isoleucine, leucine, lysine, phenylalanine, threonine, and valine) and 5 nonessential amino acids (alanine, asparagine, glutamine, serine and tyrosine) were decreased ($p < 0.05$) by 0.6% to 16% in the microalgae diet. Apparent retentions of potassium, calcium, sodium, phosphorus and sulfur were shown in **Figure 1**. The microalgae diet had 13%, 71% and 26% lower ($p < 0.05$) apparent retentions of sodium, phosphorus, and sulfate, respectively, than the control. No major differences were found in potassium or calcium apparent retentions between the two diets.

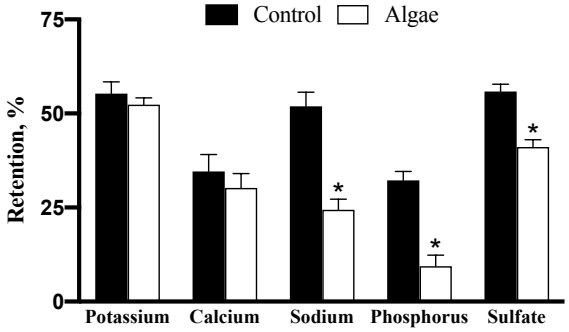

Figure 1: Effects of supplemental defatted microalgae on the apparent retentions of minerals. Apparent retention of potassium, calcium, sodium, phosphorus and sulfur in broiler chickens fed the basal diet (control) or microalgae diet (10% defatted microalgae Nannochloropsis Oceanica) for 6 weeks. Values are mean ± SE, n = 5-10. Main effects were analyzed by unpaired Student's t-test.

Table 1: Effects of supplemental defatted microalgae on the apparent retention and ileal digestibility of dietary nutrients in broiler chickens.

Treatment	Control	Microalgae	SEM	*p* value
Apparent retention				
Total collection				
Dry matter, %	71.5	68.1	1.24	0.06
Crude protein, %	66.1	57.4*	2.15	0.02
Ether extract, %	82.7	86.3*	1.17	0.04
Energy, %	76.4	73.4	1.01	0.05
Indigestible marker				
Dry matter, %	71.2	72.4	0.44	0.07
Crude protein, %	62.2	64.7	1.43	0.25
Ether extract, %	83.8	90.6*	0.91	<0.01
Energy, %	73.8	74.9	0.42	0.07
AME (kcal/kg)[1]	2903	2929	12.2	0.20
AMEn (kcal/kg)[2]	2887	2912	12.1	0.21
Apparent ileal digestibility[3]				
Dry matter, %	61.0	58.1	3.21	0.60
Crude protein, %	77.7	67.4*	2.17	0.03
Ether extract, %	81.5	81.4	2.37	0.98
Energy, %	66.4	63.2	3.48	0.56

[1-2] AME: Apparent metabolizable energy; AMEn: Nitrogen-corrected AME.
[3] Apparent ileal digestibility of nutrients were only measured by indigestible marker.
Data are expressed as mean (n=5-10). Main effects were analyzed by unpaired Student's t-test, * P < 0.05.

Table 2: Effects of supplemental defatted microalgae on the apparent retention and ileal digestibility of amino acids in broiler chickens.

Treatment	Control	Microalgae	SEM	*p* value
Apparent retention[1], %				
Isoleucine	89.3	81.9*	0.56	<0.001
Leucine	98.7	98.1*	0.10	0.03
Lysine	91.8	89.8*	0.47	0.03
Phenylalanine	93.6	84.7*	0.79	0.004
Threonine	90.4	75.9*	0.52	<0.001
Valine	85.0	78.5*	0.69	<0.001
Alanine	94.1	93.2*	0.12	0.01
Asparagine	96.7	90.3*	0.22	<0.001
Glutamine	97.3	95.3*	0.15	<0.001
Serine	96.1	92.1*	0.20	<0.001
Tyrosine	97.2	95.4*	0.13	<0.001
Apparent ileal digestibility[2], %				
Methionine	83.3	69.9*	3.32	0.02
Cysteine	67.0	67.9	5.00	0.90
Arginine	86.0	82.3	1.54	0.12
Histidine	85.6	68.8*	3.56	0.01
Isoleucine	79.0	54.2*	4.87	0.01
Leucine	95.6	88.1*	1.61	0.02
Lysine	88.9	79.2*	2.49	0.04
Phenylalanine	87.0	64.0*	1.34	<0.001
Threonine	92.3	74.4*	1.82	0.001
Valine	93.1	83.3	3.03	0.07
Alanine	87.0	77.7	2.62	0.05
Asparagine	94.1	77.9*	1.25	<0.001
Glutamine	95.1	88.3*	0.98	0.002
Glycine	82.0	62.1*	3.19	0.005
Serine	93.1	79.6*	1.40	0.001
Tyrosine	95.6	91.4	1.25	0.06

[1-2] Apparent retention and ileal digestibility were only measured by indigestible marker.

Data are expressed as mean (n=5-10). Main effects were analyzed by unpaired Student's t-test, * P < 0.05.

Discussion

The present study showed that the apparent metabolizable energy (AMEn: nitrogen corrected) of the control and the microalgae diets were 2887 and 2912 kcal/kg, respectively. Since we had only one microalgae inclusion level, it was difficult to estimate AME of the defatted *N. oceanica* accurately through linear regression. Nevertheless, we indirectly calculated and estimated the AME of the microalgae biomass. Because total energy was mainly from corn, soybean meal, corn oil and/or microalgae in the two diets, we could fraction their AMEn using the following equation (AME_{corn} + $AME_{soybean\ meal}$ + $AME_{corn\ oil}$ + $AME_{microalgae}$ = AME_{diet}). The AME values of corn and soybean meal could be estimated by using the AME prediction equations: AMEn = 4021.8-227.55 Ash (for corn); AMEn = -822.33+69.54 CP-45.26 ADF+90.81 EE (for soybean meal), confirmed by Alvarenga et al. [18]. Therefore, the calculated AMEn values of corn and soybean meal based on our proximate analysis would be 3618 kcal/kg and 2128 kcal/kg, respectively. Thus, the simple estimation of the AME from defatted *N. Oceanica* (CP: 45.1%) by using the predicted corn and soybean meal AMEn from above equation would be 2706 kcal/kg (as fed base). Since defatted *N. Oceanica* (CP: 45.1%) has close CP content as soybean meal (CP: ~48%), the estimated value of the microalgae by using the soybean meal equation would be 2519 kcal/kg that is close to the calculation using the corn equation (2706 kcal/kg). Previous studies have reported TMEn (2839 kcal/kg) of *Spirulina* (CP: 76%) fed to Single Comb Leghorn rooster [19] and AME (3220 kcal/kg) of *Spirulina platensis* (CP: 52%) fed to broiler chicks [20]. It seemed that the estimated AME (2706 kcal/kg) of defatted *N. oceanica* was fairly comparable with that of *Spirulina*.

Our results suggest that apparent retentions and ileal digestibilities of CP and amino acids were lower in the 10% *N. oceanica* diet than the control diet. However, our lab previously fed 0, 2, 4, 8 and 16% defatted *N. oceanica* (CP: 38.2%, same species but lower CP) diet to broilers and found no significant differences in either nitrogen retention or digestibility between the control and microalgae diets [7]. Since we followed the same procedures as using the same broiler strain and microalgae species, the plausible reason to the discrepancy might be microalgae difference (e.g., CP: 45.1% vs. 38.2%) between those two studies. Ekmay et al. [5] found that feeding 25% defatted *Desmodesmus spp.* (CP: 25%) and 11.7% full-fatted *Staurosira spp.* (CP: 11.7%) to 26-week old laying hens increased apparent retentions and ileal digestibilities of amino acids. Manor et al. [4] found that feeding 15% *Desmodesmus sp.* (CP: 38.2%) to weanling pigs enhanced apparent amino acid digestibilities. Evans et al. [19] found that feeding 0, 6, 11, and 16% but not 21% full-fatted *Spirulina* (CP: 76%) to broiler improved the apparent ileal amino acid digestibility. Other researchers also reported that 34% full-fatted *N. oceanica* inclusion in seabass fish had the similar nitrogen retention efficiency to the control [21]. However, Tavernari et al. [20] fed 20% *Spirulina platensis* (CP 51.5%) to broiler chicks and observed lower nitrogen metabolizability coefficient in the test diet over the basal diet. Because microalgae species, culture conditions, harvesting procedures, and tested animal species affect microalgae digestibility, the negative impacts on dietary CP/amino acid digestibility or retention need further research.

The present study showed a lower retention of total phosphorus in the microalgae diet than the control diet. As intrinsic phosphorus in corn and soybean meal is largely presented in the form of phytate [22], solubility or bioavailability is low [23]. Thus, the lower retention of total phosphorus in the microalgae diet implied an unavailable form of phosphorus in the biomass and (or) other inhibitors that decreased the digestion, absorption or utilization of phosphorus from other ingredients. However, Gatrell et al. [6] found a higher retention of soluble inorganic phosphorus by feeding 8% defatted *N. oceanica* (CP: 38.2%) microalgae diet to broiler chicks compared with the control. This discrepancy may be explained by the analysis differences in total phosphorus (present study) and soluble inorganic phosphorus, highlighting the need for a complete characterization of different forms of phosphorus for an accurate evaluation. The lower retention of sodium in the microalgae diet reflects the need for excretion of the excess sodium intake from the marine *N. oceanica*. High salt intakes could increase animal water intake [24], and cause water regurgitation [6] and environmental concern over the elevated litter discharge [25]. Therefore, it is useful to remove extra salt in microalgae prior

to feeding.

Apparent retentions of amino acids were higher than their ileal digestibilities in both diets. This type of differences was reported in some feed ingredients [26] and was explained as the hindgut microflora could catabolize amino acids at rates exceeding the microbial synthesis of protein or amino acids from the digesta [27]. This notion might also help explain the higher energy and dry matter retention than their digestibilities. However, the average retention of CP was lower than average ileal digestibility of CP, which matches with the nitrogen retention and digestibility differences reported by Gatrell et al. [7] and reflects the additional nitrogen loss as uric acid in the excreta [28].

Both total collection and indigestible marker (chromium oxide) methods predicted similar apparent retentions of dry matter, ether extract, and energy in a given diet. In fact, these two methods are commonly used to evaluate amino acid and energy digestion and utilization by poultry. As ileal amino acid digestibility is more accurate than apparent amino acid retention due to the microbial fermentation of amino acids in the hindgut of broiler chickens [29], the indigestible marker method would be an appropriate option for estimating amino acid digestibility in broiler chickens without conducting cecectomy surgery. In addition, both methods are used to determine AME [29], but produced controversial results [20, 30]. Thus, Tavernari et al. [20] recommended that the AME values of different ingredients should be derived the same method for an accurate diet formulation.

Conclusions

The present study illustrated that the 10% defatted *N. oceania* microalgae biomass inclusion in the corn-soybean meal basal diet enhanced apparent retention and ileal digestibility of ether extract, but decreased that of CP, AA, sodium, and total phosphorus. The biomass inclusion did not affect digestion or retention of dry matter or energy. Based on the determined AMEn values in the two diets and previous estimate equations of corn and soybean meal, we predicted the approximate AMEn to be 2706 kcal/kg for the defatted *N. oceanica* biomass under the current testing conditions.

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