Short communication: Partial replacement of ground corn with algae meal in a dairy cow diet: Milk yield and composition, nutrient digestibility, and metabolic profile

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ABSTRACT

This study was undertaken to evaluate the effects of partially replacing dietary ground corn with a microalgae meal from *Prototheca moriformis* (composed of deoiled microalgae and soyhulls) on milk yield and composition, nutrient intake, total-tract apparent digestibility, and blood profile of lactating dairy cows. Twenty multiparous Holstein cows (57.7 ± 49.4 d in milk, 25.3 ± 5.3 of milk yield, and 590 ± 71 kg of live weight at the start of experiment, mean $\pm$ standard deviation) were used in a cross-over design experiment, with 21-d periods. Diets were no microalgae meal (CON) or 91.8 g/kg of microalgae meal partially replacing dietary ground corn (ALG). Cows showed similar milk yield and composition. The 3.5% fat-corrected milk production was 30.2 ± 1.34 kg/d for CON and 31.1 ± 1.42 kg/d for ALG. Despite cows having similar dry matter intake, ALG increased neutral detergent fiber and ether extract intake. In addition, cows fed ALG exhibited higher ether extract digestibility. No differences were detected in glucose, urea, amino-aspartate transferase, and gamma-glutamyl transferase blood concentrations. Feeding ALG increased the total cholesterol and high-density lipoprotein in blood compared with CON. The microalgae meal may partially replace ground corn in diets of lactating cows without impairing the animal's performance.

Key words: coproduct, energy source, feedstuffs, soyhulls

Short Communication

The large-scale production of heterotrophic microalgae (*Prototheca moriformis*) has been supported by recent advances in technology. These microalgae are genetically modified to produce various types of oil or

more oil (or both) than their wild-type counterparts, which are then used for food, personal care, and other animal nutrition applications. Previously, the deoiled extract (dry cell wall) was considered a waste product and burned. Now, a dry, deoiled, milled microalgae coproduct has been developed by researchers as a feed for ruminants. Based on the deoiled microalgae composition, Van Emon et al. (2015) suggested that a blend of microalgae and soyhulls could replace corn in diets for beef cattle. These authors combined these ingredients to form a microalgae meal (ALG) consisting of 570 g/kg of microalgae and then evaluated this product up to 450 g/kg of diet DM on performance of growing steers. Positive effects were found in DMI and in the ADG of animals, supporting the viability of ALG as a feedstuff for ruminants. This particular microalgae meal is a novel feedstuff, and to our knowledge no experiment has been published evaluating its inclusion in dairy cow diets, although there have been studies using meal derived from other algal species (Franklin et al., 1999; Moate et al., 2013). Because of the rising global population, the USDA estimated the demand for corn to climb to 327 million metric tonnes by 2022 (Westcott and Trostle, 2014). A large portion of the diets of lactating cows consists of cereals, especially ground corn. Because corn prices fluctuate throughout the year, replacing corn with by-products may reduce feed costs, decrease reliability on corn feedstocks, and increase dairy profitability. The aim of this study was to determine the effects of the partial replacement of ground corn with ALG on milk yield and composition, nutrient digestibility, and blood profile of lactating dairy cows. Our hypothesis was that ALG replacing ground corn would not affect the cow's performance and blood profile.

This experiment was carried out with permission of the Bioethics Committee of School of Veterinary Medicine and Animal Science, University of Sao Paulo. Twenty multiparous Holstein cows (57.7 ± 49.4 DIM, 25.3 ± 5.3 of milk yield, and 590 ± 71 kg of live weight at the start of experiment, mean $\pm$ SD) were selected

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from the Dairy Cattle Research Laboratory (Pirasununga, Brazil) herd for this experiment. Cows were housed in individual pens (17.5 m² of area), containing sand bedding, feed bunks, and forced ventilation. Animals were randomly assigned to the treatment sequence in a cross-over experimental design. Diets were formulated according to NRC (2001; Table 1), supplied twice daily (0700 and 1330 h) as a TMR, and consisted of a typical diet for lactating cow with no microalgae meal (CON) and inclusion of 91.7 g/kg of ALG. Microalgae meal (TerraVia Holdings Inc., South San Francisco, CA) is a combination of 570 g/kg of deoiled microalgae bodies (from *Prototheca moriformis*) and 430 g/kg of soyhulls and was used to partially replace 342 g/kg of ground corn from CON. During the production process, the microalgae undergoes 2 separate inactivation steps, heat treatment and drying; therefore, the ALG contains no viable microalgae. Trial periods lasted for 21 d, of which the first 14 d were to allow diet adaptation and d 15 to 21 were used for sampling and statistical analysis. Animals were individually fed daily to obtain refusals between 5 and 10% of feed offered. Wet content of corn silage was determined weekly and dietary adjustments were made when necessary.

Samples of corn silage and concentrate ingredients were collected weekly and analyzed for DM (method 930.15), CP (CP = N × 6.25; method 984.13), and ether extract (EE; method 920.39) according to AOAC International (2000). Ash was determined after 4 h at 600°C in a muffle furnace. Neutral detergent fiber, ADF, and lignin were determined in a fiber analyzer (TE-149 fiber analyzer, Tecnal Equipment for Laboratory Inc., Piracicaba, Brazil) according to Van Soest et al. (1991) using α amylase and no sodium sulfide. Nonfiber carbohydrate was estimated according to Hall (2000) as follows: NFC = 100 - [(CP - CP from urea + urea) + NDF + EE + ash], with all values expressed as a percentage.

Animals were milked twice daily (0600 and 1530 h) and milk production was electronically measured (Alpro, DeLaval, Tumba, Sweden). Milk sampling was performed on d 15, 16, and 17 of each period and analyzed for fat, protein, and lactose by infrared method (Lactoscan, Sao Paulo, Brazil). Fat-corrected milk yield was calculated according to Sklan et al. (1992), as follows: FCM (kg/d) = milk yield (kg/d) × (0.432 + 0.163 × milk fat percentage).

Feed intake was recorded daily and samples of refusals were collected during the 7-d sampling period and composited (on a wet basis) to form a representative sample for each cow and each period. Feces were collected directly from the rectum of cows on d 18, 19, and 20 of each period after each milking. Fecal samples were also composited (on wet-basis) to form representative

samples. Samples of dietary ingredients, refusals, and feces were dried in a forced-air oven at 55°C for 72 h and ground in a Wiley mill to pass through 2-mm and 1-mm screens. All samples were analyzed for DM, ash, CP, EE, and NDF, as previously described. Nutrient total apparent digestibility was determined based on daily fecal excretion using indigestible NDF (iNDF) as an internal marker. Ground samples (2-mm particle size) of ingredients, refusals, and feces were incubated using nonwoven textile bags (5 × 5 cm, 50 µm pore size; 20 mg of DM/cm²) in the rumen of 2 Holstein cows adapted to the CON diet of this experiment. After 288 h, bags were removed from the rumen and washed in running tap water and analyzed for NDF concentration (Casali et al., 2008).

Table 1. Ingredients and chemical composition of experimental diets (g/kg of DM, unless otherwise stated)

Item	Diet ¹	
	CON	ALG
Ingredient		
Corn silage	501	501
Ground corn	269	177
Soybean meal	113	113
Whole raw soybean	80.1	80.1
Microalgae meal ²	—	91.7
Minerals and vitamins ³	16.0	16.0
Sodium bicarbonate	9.00	9.00
Dicalcium phosphate	4.60	4.60
Urea	3.80	3.80
Limestone	1.40	1.40
Magnesium oxide	1.10	1.10
Salt	0.90	0.90
Ammonium sulfate	0.50	0.50
Chemical		
DM (g/kg)	598	602
OM	926	934
NFC	389	376
NDF ⁴	333	345
ADF	152	169
iNDF ⁵	80.7	84.4
Lignin	30.4	30.9
CP	166	163
Ether extract	37.6	39.5
Ash	74.1	76.3

¹CON = no microalgae meal; ALG = microalgae meal replacing 34.2% of ground corn.

²Contained 57% of deoiled microalgae bodies and 43% of soyhulls. Composition: 971 g/kg of DM, 975 g/kg of OM, 274 g/kg of NDF, 77.9 g/kg of CP, 55.7 g/kg of ether extract.

³Contained per kilogram of product: 88.0 g of Ca, 42.0 g of P, 18.0 g of S, 45.0 g of Mg, 123.0 g of Na, 14.0 mg of Co, 500.0 mg of Cu, 20.0 mg of Cr, 1,050.0 mg of Fe, 28.0 mg of I, 1,400.0 mg of Mn, 18.0 mg of Se, 2,800.0 mg of Zn, 80.0 mg of biotin, 200,000.00 IU of vitamin A, 40,000.00 IU of vitamin D, and 1,200.00 IU of vitamin E.

⁴Nonfiber carbohydrate estimated according to Hall (2000) in which NFC = 1,000 - [(CP - CP from urea + urea) + NDF + ether extract + ash]; all values expressed as grams per kilogram of DM.

⁵Indigestible NDF.

Table 2. Effects on milk yield and composition by partially replacing ground corn with microalgae meal in the dairy cow diet

Item	Diet ¹		Mean	SEM	<i>P</i> -value
	CON	ALG			
Yield (kg/d)					
Milk	27.3	27.7	27.5	0.922	0.492
3.5% FCM ²	30.2	31.1	30.6	0.970	0.255
Fat	1.13	1.18	1.15	0.040	0.237
Protein	0.81	0.82	0.81	0.026	0.669
Lactose	1.21	1.22	1.22	0.038	0.739
Composition (g/kg)					
Fat	42.1	43.2	42.63	1.007	0.246
Protein	29.9	29.7	29.84	0.239	0.192
Lactose	44.8	44.6	44.71	0.356	0.221

¹CON = no microalgae meal; ALG = microalgae meal replacing 34.2% of ground corn.

²Calculated according to Sklan et al. (1992), in which FCM (kg/d) = milk yield (kg/d) × (0.432 + 0.163 × milk fat percentage).

Digestibility of DM

$$= 100 - \left[100 \times \left(\frac{\% \text{ iNDF intake}}{\% \text{ iNDF in feces}} \right) \right].$$

Digestibility of nutrient = 100 –

$$\left[100 \times \left(\frac{\% \text{ iNDF intake}}{\% \text{ iNDF in feces}} \right) \times \left(\frac{\% \text{ nutrient in feces}}{\% \text{ nutrient intake}} \right) \right].$$

Blood samples were collected on d 21 of each period from coccygeal vessels before the morning feeding, and analyzed for glucose, total protein, albumin, urea, triglycerides, total cholesterol, high-density lipoprotein (**HDL**), and hepatic enzymes by colorimetric method using commercial kits (Bioclin, Belo Horizonte, Brazil) and reading performed by a semi-automatic biochemistry analyzer (SBA-200, CELM, Sao Caetano do Sul, Brazil).

Data were submitted to ANOVA using the PROC MIXED SAS 9.4 (Statistical Analysis for Windows, SAS Institute Inc., Cary, NC) according to the following model:

$$y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + e_{ijk},$$

where y_{ijk} represents the observation on animal k given treatment i at period j ; μ is the overall mean; α_i represents the fixed effect of the i th treatment ($i = 1$ to 2); β_j represents the fixed effect of the j th period ($j = 1$ to 2); γ_k represents the random effect of animal ($k = 1$ to 20); and e_{ijk} represents the random error associated with each observation. Means were adjusted by LSMEANS, and degrees of freedom were calculated by the Kenward and Roger (1997) method. The significance level was set at $P \leq 0.05$.

Partially replacing ground corn by ALG did not ($P \geq 0.192$) affect milk yield and composition (Table 2). The 3.5% FCM production did not differ between diets and was 30.2 ± 1.34 kg/d for CON and 31.1 ± 1.42 kg/d for ALG. Despite no observed differences for milk production, cows fed ALG showed higher ($P \leq 0.012$) NDF, iNDF, and EE intake (kg/d) compared with those fed CON (Table 3). The NDF intake relative to the live weight (g/kg of live weight) also increased ($P = 0.010$) when cows were supplied ALG. Ether extract digestibility was higher ($P = 0.002$) in cows fed ALG compared with those fed CON. Cows fed ALG had higher total cholesterol and HDL blood concentrations compared with CON (Table 4). Hepatic enzymes were not affected ($P \geq 0.107$) by ALG supply.

Although the DMI was not influenced by experimental diets, NDF and EE intake were increased when ALG was fed to cows. These effects can be related to the microalgae meal composition, which slightly increased dietary NDF and EE of ALG. In contrast with our findings, Van Emon et al. (2015) found a linear increase of DMI when growing beef steers were fed with microalgae meal up to 450 g/kg of DM partially replacing wet corn gluten feed. Stokes et al. (2015) evaluated the addition of microalgae meal up to 450 g/kg DM in place of corn on DMI and nutrient digestibility of wethers and also reported a linear increase of DMI. The differences among results are related to the inclusion of microalgae meal and NFC content of diets. Whereas in our study the microalgae meal inclusion was 91.7 g/kg of diet DM, the minimum inclusion of this coproduct in the other trials was 150 g/kg, and the dietary NFC content in the current experiment was decreased by 11.3 g/kg of DM when adding microalgae meal, whereas the other trials exhibited a decrease in dietary NFC content up to 143.3 g/kg. Changes in NFC

Table 3. Effects on nutrient intake and total-tract apparent digestibility by partially replacing ground corn with microalgae meal in the dairy cow diet

Item	Diet ¹		Mean	SEM	P-value
	CON	ALG			
Intake (kg/d)					
DM	22.0	22.6	22.3	0.687	0.275
OM	20.4	20.8	20.6	0.635	0.338
NFC ²	10.2	10.3	10.3	0.315	0.515
NDF	7.18	7.69	7.44	0.237	0.012
CP	3.76	3.78	3.77	0.114	0.794
Ether extract	0.86	0.93	0.90	0.027	0.002
Intake (g/kg of live weight)					
DM	37.3	38.2	37.76	1.071	0.292
NDF	12.2	12.9	12.59	0.363	0.010
Digestibility coefficient					
DM	0.737	0.736	0.737	0.0060	0.941
OM	0.760	0.758	0.759	0.0071	0.842
NDF	0.668	0.666	0.667	0.0070	0.835
CP	0.737	0.723	0.730	0.0084	0.336
Ether extract	0.830	0.884	0.857	0.0090	0.002

¹CON = no microalgae meal; ALG = microalgae meal replacing 34.2% of ground corn.

²Nonfiber carbohydrate estimated according to Hall (2000), in which NFC = 1,000 - [(CP - CP from urea + urea) + NDF + ether extract + ash]; all values expressed as g/kg of DM.

content results in differences of dietary energy density, altering DMI of animals. In addition, other authors reported linear decrease of DMI when soyhulls replaced corn in lactating dairy cow diets (Ipharraguerre et al., 2002). However, our findings agree with several experiments as described by Ipharraguerre and Clark (2003) which analyzed data from 15 studies that evaluated diets containing soyhulls, and 13 of these studies did not report differences on DMI between control diets and those containing soyhulls.

In the current experiment, microalgae meal increased the EE digestibility compared with CON. Stokes et al.

(2015) reported a linear increase of DMI and of nutrient digestibility, including DM, NDF, EE, nitrogen, and NFC, when wethers were fed microalgae meal. Although the aim of algae production is to extract oil, residual oil remains in the deoiled microalgae, increasing the EE of diets and consequently increasing EE digestibility. Wu et al. (1991) have described improvement on fat digestibility when increasing 30 g/kg of dietary EE. None of the experiments evaluating microalgae meal cited above evaluated blood metabolites. In the current experiment, we observed an increase of total cholesterol and HDL cholesterol concentration in blood. The

Table 4. Effects on blood profile (mg/dL, unless otherwise stated) by partially replacing ground corn with microalgae meal in dairy cow diet

Item	Diet ¹		Mean	SEM	P-value
	CON	ALG			
Glucose	78.7	78.4	78.6	1.658	0.912
Total protein	8.39	8.57	8.48	0.125	0.221
Albumin	3.19	3.22	3.20	0.067	0.646
Creatinine	1.27	1.31	1.29	0.021	0.184
Urea	39.3	36.2	37.7	0.903	0.056
Triglycerides	15.6	15.8	15.7	0.455	0.787
Cholesterol					
Total ²	152	175	163	5.828	<0.001
HDL	123	134	129	3.806	0.037
Hepatic enzymes ³ (IU/L)					
AST	74.40	76.25	75.33	3.095	0.644
GGT	27.95	24.70	26.33	1.991	0.107

¹CON = no microalgae meal; ALG = microalgae meal replacing 34.2% of ground corn.

²Total cholesterol is composed of high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low density lipoprotein (VLDL).

³Hepatic enzymes consist of aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT).

increase of total cholesterol is related to the increased EE digestibility when feeding ALG. The absorption of fat increases the lipoprotein cholesterol production by the intestine, which is the major site of cholesterol synthesis in ruminants (Noble, 1981). High-density lipoprotein comprises almost 80% of total lipoproteins in blood (Grummer and Carroll, 1988), and thus, the increased total cholesterol in blood appears to be due to the HDL cholesterol (Puppione et al., 1982).

Certain algal species can accumulate heavy metals at high concentrations, and currently no regulation for heavy metals concentration in microalgal products exists (Becker, 2004). The microalgae meal used in the current experiment does not appear to have any toxic effect because the concentration of hepatic enzymes in blood, specifically aspartate-amino transferase and gamma-glutamyl transferase that are associated with liver damage caused by toxic substances, were within the standard ranges described for lactating cows (Kaneko et al., 1997). Although *Prototheca moriformis* has been associated with udder infection in cows (Buzzini et al., 2004), it is not expected that ALG supplementation will cause mastitis because the microalgae is completely inactivated. As part of the production process, the microalgae is heat treated then dried and pelletized, thus the ALG contains no live organisms. Additionally, the actual risk of *P. moriformis* as a causative agent for bovine mastitis was assessed by bovine mastitis experts. Their determination was that protothecal mastitis is a disease of poor farm management and not of environmental presence on dairy farms (R. J. Erskine, Michigan State University, East Lansing, personal communication). In conclusion, addition of microalgae meal in a dairy cow diet at 91.7 g/kg of DM in place of ground corn does not alter milk yield and composition, increases NDF and EE intake, has minimal effects on nutrient digestibility, and increases blood cholesterol.

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