



## Effect of Upstream Processing on Nutritional Value of Post-extraction Algal Residue for Beef Cattle

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### ABSTRACT

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Post-extraction algal residue (PEAR), a co-product from extracting algal oil for conversion to biofuel, has the potential to be marketed as livestock feed. However, the algal biofuel industry has not yet adopted standardized processing practices, which potentially causes nutrient fluctuations in the co-product, PEAR. Our objective was to determine the effect of various upstream processing methods on the nutritional value of PEAR for beef cattle production. To meet this objective, a batch of *Nannochloris oculata* was subjected to different methods of harvesting, drying, preparation for oil extraction, and oil extraction, yielding ten unique samples for nutritional analysis. Crude protein and lipid content of samples ranged 19-36 and 2-10 per cent, respectively, demonstrating wide macronutrient variation in accordance with different upstream processing methods. All PEAR contained S concentrations (>0.81%) which may limit the inclusion rate in beef cattle rations. For harvesting methods, flocculation increased Fe and Al >289% relative to centrifuged PEAR. Flocculated and centrifuged PEAR had Ca:P of 18:1 and 9:1, respectively, which would present additional formulation challenges. Flocculation decreased S and Na concentrations by 37 and 77%, respectively, relative to centrifuged PEAR; however, both minerals remained present in quantities, which would impact their level of inclusion in rations. Reducing mineral and increasing protein concentrations in PEAR will increase its value and utilization in beef cattle production, ultimately enhancing the success and viability of algal biofuel production. Mineral imbalances in PEAR can be partially attributed to upstream processing methods that are avoidable or manageable, underlining the need for upstream algal biofuel processors to be aware of the downstream (co-product) impacts of their practices.

**Keywords:** Algae, Beef cattle, Biofuel, Post-extraction algal residue, Protein supplementation

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### INTRODUCTION

Global energy demands are steadily increasing, emphasizing the need to identify renewable, sustainable alternatives to fossil fuels. Traditional first-generation biofuel

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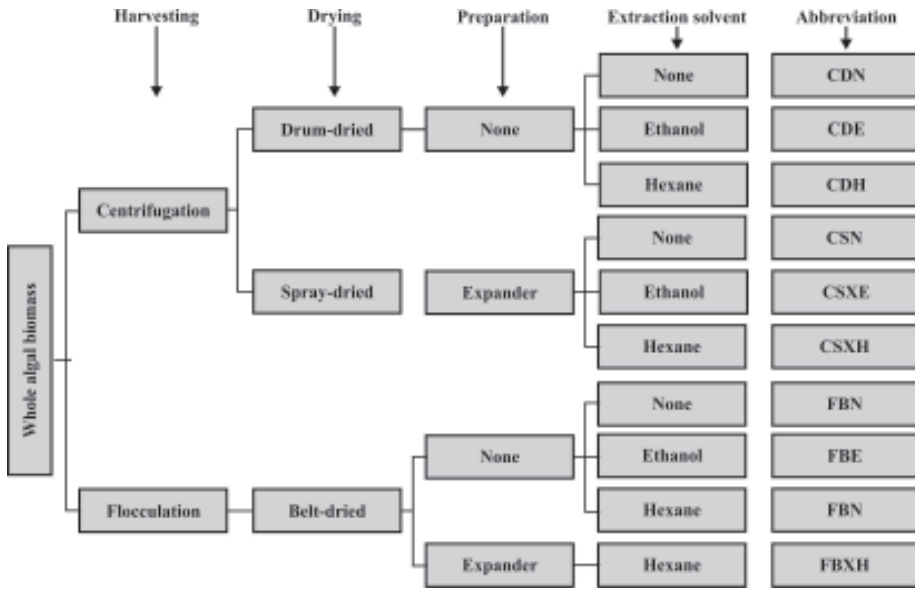
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feedstocks are limited in their production capacity, compete with food production, involve significant agricultural inputs, and require fresh water (Dragone *et al.*, 2010; Sander and Murthy, 2010). Second-generation biofuel feedstocks are an attractive alternative to first-generation feedstocks because they do not strain world food markets; however, conversion of cellulosic biomass to biofuel presents expensive technological challenges which have inhibited commercial scale-up (FAO, 2008). Algal biomass, a third-generation biofuel feedstock, is not associated with the challenges first- or second-generation biofuels face (Nigam and Singh, 2011) and, therefore, may serve as an economical and environmentally sustainable feedstock for biofuel. It has been suggested that algae species that are suitable for large-scale production have a lipid content of >22% (Becker, 2007), indicating the co-product (post-extraction algal residue; PEAR) will be produced in excess of oil yield. These observations were confirmed by Sander and Murthy (2010), who suggested a biodiesel to co-product ratio of 12:17. Thus, the economic feasibility of algal biofuel production is contingent on the placement and marketing of the co-product, PEAR.

Potential markets for PEAR include the livestock industry – specifically, beef cattle production – as ruminants are effective utilizers of co-products and have successfully been fed PEAR at up to 45% of dietary DM (Drewery *et al.*, 2014; Van Emon *et al.*, 2015; Morrill *et al.*, 2017). Developing processes that optimize biofuel production and co-product value is critical for the success and viability of algal biofuel production. Accordingly, our objective was to quantify the nutritional value of a batch of algae (*Nannochloris oculata*) subjected to various methods of upstream processes and determine factors which may limit the inclusion of PEAR in beef cattle rations.

## **MATERIALS AND METHODS**

Samples of algae (*Nannochloris oculata*) were grown in a field setting and then harvested by either centrifugation or with a commercially available flocculent. Three drying methods were used: steam-jacketed drum drying, forced-air belt drying, or spray-drying. Centrifuged samples were drum- or spray-dried, and partially dried flocculated samples were belt-dried. An expander was also used on the spray-dried samples and one of the belt-dried samples to observe the effect of preparation prior to oil extraction: an expander cooks and agglomerates samples into a denser material, known as collets. Hexane and ethanol were used as solvents for oil extraction. These processes yielded a total of three non-extracted (oil remained) and seven PEAR samples for nutritional analysis (Fig. 1): 1) centrifuged, drum-dried, non-extracted (CDN); 2) centrifuged, drum-dried, ethanol extracted (CDE); 3) centrifuged, drum-dried, hexane extracted (CDH); 4) flocculated, belt-dried, non-extracted (FBN); 5) flocculated, belt-dried, ethanol extracted (FBE); 6) flocculated, belt-dried, hexane extracted (FBH); 7) flocculated, belt-dried, expanded, hexane-extracted (FBXH); 8) centrifuged, spray-dried, non-extracted (CSN); 9) centrifuged, spray-dried, expanded, ethanol extracted (CSXE); and 10) centrifuged, spray-dried, expanded, hexane extracted (CSXH).



**Fig. 1.** Algal biomass processing methods to yield non-extracted ( $n = 3$ ) and post-extraction algal residue ( $n=7$ ) samples

[CDN, centrifuged, drum-dried, non-extracted; CDE, centrifuged, drum-dried, ethanol extracted; CDH, centrifuged, drum-dried, hexane extracted; FBN, flocculated, belt-dried, non-extracted; FBE, flocculated, belt-dried, ethanol extracted; FBH, flocculated, belt-dried, hexane extracted; FBXH, flocculated, belt-dried, expanded, hexane-extracted; CSN, centrifuged, spray-dried, non-extracted; CSXE, centrifuged, spray-dried, expanded, ethanol extracted; CSXH, centrifuged, spray-dried, expanded, hexane extracted]

Samples were ground with a Wiley mill to pass a 1-mm screen and dried in a forced-air oven for 24 h at 105°C for DM determination. Organic matter was determined as the loss in dry weight upon combustion for 8 h at 450°C. Nitrogen was measured with Dumas combustion (Rapid N Cube; Elementar, Hanua, Germany) and CP was calculated as  $N \times 6.25$ . Lipid content was determined by ether extract (EE) and acid-hydrolysis (AH). Starch, macro-, micro-mineral, and heavy metal analysis was performed by a commercial laboratory (SDK Laboratories, Hutchinson, KS).

## RESULTS AND DISCUSSION

Complete chemical, macromineral, micromineral, and heavy metal concentrations of the processed algal biomass are presented in Tables 1, 2, 3, and 4, respectively.

The goal of oil extraction is to minimize the residual oil content of PEAR. Samples harvested via centrifugation and either drum- or spray-dried (CDN and CSN, respectively) had a similar amount of lipid, approximately 10.1-10.2%, when analyzed by acid hydrolysis. The extracted drum-dried samples (CDE and CDH) had

lower residual lipid than extracted spray-dried samples (CSXE and CSXH), indicating drum-drying may be superior to spray-drying to maximize oil yield from centrifuged algae. Flocculated samples that were belt-dried and had not been extracted (FBN) had lower lipid content (2.39%) than CDN or CSN, which did not appear to be affected by either ethanol or hexane extraction.

The average OM of samples in our study was 54.4%, with a range of 42.4-75.8%. Comparatively, soybean meal and cottonseed meal, the conventional protein sources in beef cattle production, have an OM content of 93-94% (NRC, 2016). Thus, relative to the non-extracted and PEAR samples, these conventional protein sources provide greater concentrations of nutrients that drive animal production.

For PEAR samples, specifically, there was no discernible pattern linking OM content with processing methods, although ethanol-extracted samples had more OM than hexane-extracted in all instances within harvesting, drying, and preparation methods. The CSN sample had higher OM content (68.2%) than the other non-extracted samples (average of 48.2%). Accordingly, the respective processed samples (CSXE and CSXH) had higher concentrations of OM than any other sample except that of CDE. Our observation of CDE is, at this point, inexplicable. Further investigation and replication are warranted to establish methods which optimize OM content, thus increasing the market value of PEAR.

Due to the low OM content in our samples and the necessity of algal oil extraction for conversion to biofuel, PEAR would not be a viable energy source in cattle rations. Rather, the protein content of PEAR suggests its value would be optimized when marketed as a protein supplement to cow-calf operations or other

Table 1. Chemical composition of algae subjected to different processing methods

| Item                              | Processing method <sup>†</sup> |       |       |       |       |       |       |       |       |       |
|-----------------------------------|--------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
|                                   | CDN <sup>1</sup>               | CDE   | CDH   | FBN   | FBE   | FBH   | FBXH  | CSN   | CSXE  | CSXH  |
| DM, %                             | 93.74                          | 90.13 | 88.82 | 90.08 | 94.29 | 92.66 | 92.82 | 94.91 | 90.08 | 90.81 |
| <i>Composition, on % DM basis</i> |                                |       |       |       |       |       |       |       |       |       |
| OM                                | 46.39                          | 75.76 | 50.34 | 49.79 | 50.96 | 42.41 | 52.16 | 68.19 | 56.06 | 53.32 |
| CP                                | 26.66                          | 34.20 | 35.50 | 23.16 | 21.90 | 18.76 | 38.06 | 28.23 | 23.58 | 23.24 |
| Lipid (EE) <sup>‡</sup>           | 1.83                           | <0.20 | 0.66  | 1.08  | 0.31  | 0.68  | 1.90  | 0.42  | 2.96  | 2.93  |
| Lipid (AH) <sup>§</sup>           | 10.17                          | 2.44  | 5.76  | 2.39  | 2.60  | 3.61  | 3.63  | 10.12 | 9.60  | 8.40  |
| Starch                            | 4.10                           | 5.20  | 4.80  | 2.40  | 2.00  | 2.30  | 2.90  | 2.30  | 4.60  | 4.70  |

<sup>†</sup>CDN, centrifuged, drum-dried, non-extracted; CDE, centrifuged, drum-dried, ethanol extracted; CDH, centrifuged, drum-dried, hexane extracted; FBN, flocculated, belt-dried, non-extracted; FBE, flocculated, belt-dried, ethanol extracted; FBH, flocculated, belt-dried, hexane extracted; FBXH, flocculated, belt-dried, expanded, hexane-extracted; CSN, centrifuged, spray-dried, non-extracted; CSXE, centrifuged, spray-dried, expanded, ethanol extracted; CSXH, centrifuged, spray-dried, expanded, hexane extracted

<sup>‡</sup>Ether extract.

<sup>§</sup>Acid-hydrolysis.

production systems which rely heavily on forage that may be deficient in protein. Protein supplementation to cattle increases forage utilization (Köster *et al.*, 1996; Wickersham *et al.*, 2008; Drewery *et al.*, 2014) and is widely accepted as a cost-effective management practice to optimize animal performance.

Crude protein in PEAR samples averaged 26.5% (range 18.8-38.1%), which is less than that of soybean meal (53%) and cottonseed meal (45%; NRC, 2016), conventional protein sources used in livestock production. There was little difference in average CP for processed and non-extracted samples, averaging 27.9% and 26.0%, respectively. The highest CP content for an individual sample was for FBXH (38.1% CP), which was substantially more than other flocculated, belt-dried samples (average of 20.3% CP), although they were not expanded prior to extraction. The disparity in this observation is currently unexplained but may be related to the use of an expander, a hypothesis we cannot prove or disprove with our current data as the other samples that were expanded prior to extraction (CSXE, CSXH) do not have an unexpanded control sample with which to compare. When grouping processing methods, PEAR that was centrifuged, drum-dried, and extracted with either solvent had the most consistent CP concentrations, an average of 34.9%.

As stated above, the market value of PEAR in the beef cattle industry will likely be driven by CP content. Using a hedonic model, Bryant *et al.* (2012) demonstrated the market value of PEAR in beef cattle production would be 122 – 281 USD/metric ton, which was less than the market value of soybean meal (136 – 318 USD/metric ton). Discounts in value are associated with the minerals/ash (non-OM) content of PEAR, which effectively dilutes protein and other nutrients that drive animal production. On a per nutrient basis, the economic value of PEAR would likely be similar to that of conventional protein sources. The current data indicate centrifuged, drum-dried, ethanol extracted PEAR contains more desirable OM and CP concentrations while maintaining the lowest amount of residual lipid (<0.20% by EE; 0.66% by AH) for all processing methods analyzed. Accordingly, centrifuging, drum-drying, and extracting with ethanol may optimize oil yield, as well as the nutritional and market value of PEAR. Replication and further research are necessary to confirm these results and make recommendations for dually optimizing algal biofuel yield and co-product (PEAR) characteristics.

Minerals are necessary in cattle diets and are commonly provided as supplements designed to address specific deficiencies in basal rations. Feeds that cause mineral imbalances are included in rations on a limited basis to avoid challenges associated with toxicity. In our study, all samples contained an imbalanced Ca:P ratio and concentrations of S, Cu, Fe, and Al in excess of maximum tolerable concentrations for beef cattle (Table 2 and 3; NRC, 2016), suggesting PEAR inclusion level may be limited in cattle rations.

Calcium and P are implicated in bone growth and maintenance in beef cattle; the recommended feeding ratio is 1:1-7:1, provided each is provided in adequate

Table 2. Macromineral composition of algae subjected to different processing methods

| Item                   | Processing method <sup>†</sup> |      |      |       |       |       |       |      |      |      |
|------------------------|--------------------------------|------|------|-------|-------|-------|-------|------|------|------|
|                        | CDN                            | CDE  | CDH  | FBN   | FBE   | FBH   | FBXH  | CSN  | CSXE | CSXH |
| <i>Macromineral, %</i> |                                |      |      |       |       |       |       |      |      |      |
| Ca                     | 3.20                           | 3.72 | 2.99 | 10.02 | 10.76 | 11.28 | 10.21 | 5.41 | 5.70 | 5.28 |
| P                      | 0.67                           | 0.78 | 0.65 | 0.52  | 0.50  | 0.50  | 0.53  | 0.59 | 0.62 | 0.62 |
| K                      | 0.85                           | 0.63 | 0.84 | 0.67  | 0.70  | 0.73  | 0.69  | 0.81 | 0.85 | 0.84 |
| Mg                     | 0.26                           | 0.33 | 0.29 | 0.59  | 0.56  | 0.58  | 0.57  | 0.30 | 0.29 | 0.31 |
| Na                     | 5.06                           | 2.55 | 5.25 | 1.12  | 0.95  | 1.05  | 1.17  | 4.72 | 4.81 | 4.95 |
| S                      | 1.57                           | 1.20 | 1.51 | 0.86  | 0.82  | 0.81  | 0.87  | 1.36 | 1.28 | 1.28 |

<sup>†</sup>CDN, centrifuged, drum-dried, non-extracted; CDE, centrifuged, drum-dried, ethanol extracted; CDH, centrifuged, drum-dried, hexane extracted; FBN, flocculated, belt-dried, non-extracted; FBE, flocculated, belt-dried, ethanol extracted; FBH, flocculated, belt-dried, hexane extracted; FBXH, flocculated, belt-dried, expanded, hexane-extracted; CSN, centrifuged, spray-dried, non-extracted; CSXE, centrifuged, spray-dried, expanded, ethanol extracted; CSXH, centrifuged, spray-dried, expanded, hexane extracted

quantities to meet requirements (NRC, 2016). Forage P content decreases during winter, and P must be provided as a supplement in grazing operations. Cottonseed meal and distillers' grains supply more P than Ca (Ca:P of 0.25:1 and 0.17:1, respectively), complementing the Ca:P ratio of winter pastures while providing supplemental protein to grazing cattle (Drewery *et al.*, 2011; NRC, 2016). Calcium in non-extracted and PEAR samples appears to be related to the Ca content of the flocculent used because centrifuged samples contained 4.38% and flocculated samples contained 10.57% on average (Table 2). Upstream processing did not seem to impact P content, which was consistent across samples and averaged 0.61%. The Ca:P ratio of drum-dried samples was within feeding recommendations (5:1) but would provide an excess of Ca relative to P during winter. This imbalance would be exacerbated for flocculated PEAR (Ca:P ratio of 20:1 and 9:1, respectively). Feedlot rations typically provide a Ca:P ratio of 2.3-2.4:1 (Vasconcelos and Galyean, 2007; Samuelson *et al.*, 2016), suggesting drum-dried PEAR would not be limited in beef finishing rations.

The NRC (2016) lists the S requirement for growing and finishing beef cattle as 0.15% with a maximum tolerable concentration of 0.30-0.50%. Diets typical of beef cattle production provide sufficient S (NRC, 2016) and supplemental sources are generally not required. The samples in our study contained an average of 1.16% S (Table 2) which seemed to be related to harvesting method (e.g., flocculation versus centrifugation). Flocculated samples contained less than the average S content for all samples, but S concentrations were still higher than that of distillers' grains (0.62%) or cottonseed meal (0.43%; Drewery *et al.*, 2011; NRC, 2016). There was no apparent interaction between other upstream processes and S; thus, high S may be an inherent characteristic of PEAR. This is supported by the S content of PEAR utilized in related research published by our laboratory (0.94%; Drewery *et al.*, 2014).

Polioencephalomalacia (PEM) in cattle is associated with over-ingestion of dietary S (Sager *et al.*, 1990; Beke and Hironaka, 1991; McAllister *et al.*, 1997). Ruminant microbes convert S to hydrogen sulfide, potentially causing necrosis of the cerebral cortex (Gould, 1998; Mayland and Shemaker, 2001). Distillers' grains have high S content (0.62%; Drewery *et al.*, 2011) due to the addition of sulfuric acid to optimize fermentation during ethanol production (Han and Liu, 2010). Distillers' grains have been linked to PEM (Buckner *et al.*, 2007) when fed to cattle at a high inclusion rate (50% of ration). Despite this, distillers' grains are nearly ubiquitous in feedlot rations, with 87.5% of feedlot nutritionists reporting the use of either wet or dried distillers' grains in their finishing rations (Samuelson *et al.*, 2016). Feedlot rations incorporate wet or dried distillers' grains as the primary grain co-product at an average 16.5% inclusion rate (Vasconcelos and Galyean, 2007). Assuming a feedlot ration contains 0.22% S (Vasconcelos and Galyean, 2007), and distillers' grains are utilized as the primary grain co-product, the S from distillers' grains would be 47% of the ration S content. If centrifuged or flocculated PEAR replaced distillers' grains completely (16.5% overall inclusion rate), the ration would provide 0.34% S (66% of S from PEAR) or 0.26% S (54% of S from PEAR), respectively. The S content of forages utilized in cow-calf operations is similar to that of feedlot rations (Vasconcelos and Galyean, 2007; NRC, 2016); thus, if we calculate the inclusion rate of PEAR in grazing operations without causing concerns over S toxicity, the maximum rate would be 30%. It should be noted; however, this calculation greatly depends on the harvesting method of PEAR and S content of basal forage and drinking water.

The recommended concentration of Cu for beef cattle is 10 ppm (NRC, 2000). Copper is present in higher concentrations in concentrates than forages; thus, feedlot diets are typically adequate in Cu (Vasconcelos and Galyean, 2007; NRC, 2016). Our samples averaged 1924 ppm Cu, which is clearly in excess of the recommended feeding concentration (Table 3). Ruminants store Cu hepatically; upon reaching the maximum storage level, the liver undergoes hemolytic crisis and releases Cu into circulation, potentially resulting in anorexia, jaundice, and death (Church *et al.*, 1971; Bradley, 1993). As with S, Cu accumulation in PEAR seemed to be related to harvesting method. Copper content was lowest in flocculated PEAR but still averaged 1550 ppm. Between groups of samples that were both harvested via centrifugation, it seems as though drum-drying further increases Cu content, as centrifuged, spray-dried samples averaged 1983 ppm Cu and centrifuged, drum-dried samples averaged 2423 ppm. Assuming Cu is not provided through other dietary means, a typical characteristic of grazing operations, PEAR inclusion rate would be limited to 4.5-6.5% of the diet depending on harvesting method. Feedlot rations typically contain 17-93 ppm Cu (Vasconcelos and Galyean, 2007; Samuelson *et al.*, 2016). Distillers' grains, the component of a finishing ration PEAR may replace, contain 4.9 ppm Cu (Drewery *et al.*, 2011). Therefore, incorporation of PEAR in an existing feedlot ration would not be an option due to toxicity concerns. The Cu

Table 3. Micromineral composition of algae subjected to different processing methods

| Item                     | Processing method <sup>†</sup> |       |       |      |      |      |      |       |      |       |
|--------------------------|--------------------------------|-------|-------|------|------|------|------|-------|------|-------|
|                          | CDN                            | CDE   | CDH   | FBN  | FBE  | FBH  | FBXH | CSN   | CSXE | CSXH  |
| <i>Micromineral, ppm</i> |                                |       |       |      |      |      |      |       |      |       |
| Co                       | 0.61                           | 0.96  | 0.70  | 1.83 | 1.74 | 2.06 | 1.82 | 0.77  | 0.79 | 0.83  |
| Cu                       | 2110                           | 2920  | 2240  | 1610 | 1550 | 1560 | 1540 | 1870  | 2070 | 2010  |
| Fe                       | 1740                           | 2260  | 1740  | 7400 | 7240 | 8160 | 6890 | 1780  | 1950 | 1980  |
| Mn                       | 43.90                          | 60.30 | 46.30 | 126  | 132  | 142  | 121  | 51.50 | 57.5 | 55.70 |
| Se                       | <1.5                           | <1.5  | <1.5  | <1.5 | <1.5 | <1.5 | <1.5 | <1.5  | <1.5 | <1.5  |
| Zn                       | 150                            | 230   | 159   | 130  | 131  | 142  | 124  | 140   | 158  | 152   |

<sup>†</sup>CDN, centrifuged, drum-dried, non-extracted; CDE, centrifuged, drum-dried, ethanol extracted; CDH, centrifuged, drum-dried, hexane extracted; FBN, flocculated, belt-dried, non-extracted; FBE, flocculated, belt-dried, ethanol extracted; FBH, flocculated, belt-dried, hexane extracted; FBXH, flocculated, belt-dried, expanded, hexane-extracted; CSN, centrifuged, spray-dried, non-extracted; CSXE, centrifuged, spray-dried, expanded, ethanol extracted; CSXH, centrifuged, spray-dried, expanded, hexane extracted

content of PEAR we have utilized in related research was 31 ppm (Drewery *et al.*, 2014), indicating high Cu is not an intrinsic characteristic of PEAR and can be controlled. Indeed, we discovered a Cu-based product was used to control an invasive pond species during algae cultivation in the present study; this Cu was clearly retained in the algal biomass and, thus, the co-product. This observation emphasizes the need for upstream operators to be aware of the impact their actions have on the nutritional value of downstream co-products and, ultimately, the ability of those co-products to positively contribute to the success of algal biofuel production.

Beef cattle ingest adequate Fe through consumption of water, soil, and feed; accordingly, deficiencies are not a concern (Spears, 2003). The requirement of Fe for growing and finishing beef cattle is 50 ppm with a maximum tolerable concentration of 500 ppm (NRC, 2016). Iron toxicity is characterized by reduced animal performance, the onset of diarrhoea, and metabolic acidosis (Standish *et al.*, 1971; NRC, 2016). Flocculated samples were high in Fe (average 7423 ppm), while centrifuged samples had lower Fe concentrations (average 1908 ppm). It is apparent the flocculant used contained Fe, which was, consequently, retained in the algal biomass and co-product, PEAR. The Fe content of cottonseed meal (150 ppm) and dried distillers' grains plus solubles (103 ppm) does not pose toxicity concerns or limit inclusion rate in feedlots or grazing operations (NRC, 2016). The iron content of grazing pastures ranges from 52-218 ppm (NRC, 2016); assuming the average forage contains 135 ppm Fe, the inclusion rate of centrifuged PEAR would be  $\leq 19\%$ , while flocculated PEAR would be limited to approximately 5% maximum inclusion rate. A similar limitation in inclusion rate is expected in feedlot rations; thus, PEAR could not completely replace distillers' grains in beef cattle production. Iron content in PEAR can be controlled by utilizing a harvesting method other than flocculation or using a flocculant not concentrated in Fe.



Table 4. Heavy metal concentrations of algae subjected to different processing methods

| Item             | Processing method <sup>†</sup> |       |       |       |       |       |       |       |       |       |
|------------------|--------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
|                  | CDN                            | CDE   | CDH   | FBN   | FBE   | FBH   | FBXH  | CSN   | CSXE  | CSXH  |
| Heavy metal, ppm |                                |       |       |       |       |       |       |       |       |       |
| Ag               | 16.90                          | 24.30 | 19.20 | 16.30 | 15.90 | 16.10 | 14.60 | 15.80 | 17.30 | 16.80 |
| Al               | 1580                           | 2330  | 1820  | 11000 | 9800  | 11600 | 9500  | 2020  | 2150  | 2160  |
| As               | 2.03                           | <1.5  | <1.5  | 3.46  | 3.19  | 2.78  | 2.65  | 1.87  | <1.5  | <1.5  |
| Ba               | 15.40                          | 21.60 | 16.00 | 67.80 | 64.60 | 77.20 | 63.40 | 20.70 | 22.80 | 22.30 |
| Be               | 0.48                           | 0.48  | 0.48  | 0.56  | 0.69  | 0.66  | 0.63  | 0.45  | 0.48  | 0.48  |
| Cd               | 0.28                           | 0.38  | 0.37  | 0.56  | 0.57  | 0.57  | 0.51  | 0.23  | 0.30  | 0.28  |
| Cr               | 5.68                           | 9.65  | 6.55  | 17.60 | 17.40 | 23.00 | 26.60 | 8.03  | 12.20 | 12.90 |
| Hg               | <0.5                           | <0.5  | <0.5  | <0.5  | <0.5  | <0.5  | <0.5  | <0.5  | <0.5  | <0.5  |
| Pb               | 2.02                           | 2.83  | 2.30  | 5.55  | 5.86  | 6.28  | 5.38  | 2.73  | 2.89  | 2.87  |
| Ni               | 2.72                           | 3.58  | 2.86  | 8.60  | 8.75  | 11.40 | <0.5  | 3.76  | 5.76  | 5.90  |

<sup>†</sup>CDN, centrifuged, drum-dried, non-extracted; CDE, centrifuged, drum-dried, ethanol extracted; CDH, centrifuged, drum-dried, hexane extracted; FBN, flocculated, belt-dried, non-extracted; FBE, flocculated, belt-dried, ethanol extracted; FBH, flocculated, belt-dried, hexane extracted; FBXH, flocculated, belt-dried, expanded, hexane-extracted; CSN, centrifuged, spray-dried, non-extracted; CSXE, centrifuged, spray-dried, expanded, ethanol extracted; CSXH, centrifuged, spray-dried, expanded, hexane extracted

Aluminium is either not required or required in minute amounts in cattle diets, and the maximum tolerable concentration is 1000 ppm (NRC, 2016). Low dietary concentrations of Al (<1000 ppm) minimally impact mineral metabolism in cattle; however, increasing concentrations affect the utilization of certain minerals by forming insoluble complexes (Allen, 1984). Aluminium concentrations were consistent across centrifuged samples, averaging 2010 ppm, and would not practically limit PEAR inclusion for animals in grazing operations or feedlots. However, flocculated samples contained greater Al, averaging 10,475 ppm, which would limit the inclusion rate of PEAR to 9.5% of a ration, assuming Al was not provided through other dietary means. These observations, combined with the increases in Fe associated with flocculation, suggest the utilization of flocculants containing substantial amounts of Al or Fe may not be viable if PEAR is marketed as a feed ingredient for beef cattle production.

In applying our data to the nutritional requirements and practical diets of beef cattle, it is clear that Cu concentrations will be the first-limiting factor for PEAR in beef cattle production; however, this is controllable during algal biomass cultivation. Iron and Al especially limit dietary inclusion of flocculated PEAR but are manageable during harvesting. High S is likely a characteristic of PEAR that cannot be altered by upstream processes but is least constraining to dietary inclusion rate. A Ca:P ratio aligned to nutrient requirements and complementing that of basal forage would increase acceptability and incorporation of PEAR in grazing operations, suggesting another drawback of flocculation as a harvesting method.

## CONCLUSION

Market value and incorporation of PEAR in the beef industry will be driven by OM and CP concentrations; thus, efforts should be made to maximize OM, which would minimize mineral toxicity concerns and increase CP. Further research is necessary to understand the interactions and consequences of upstream processes (algal harvesting, drying, preparation for oil extraction, and solvent used for oil extraction) on downstream co-products (PEAR). While this study demonstrated that upstream processing affects characteristics of PEAR, it should not be assumed that the initial source of the algae does not factor into the oil yield and co-product value. Instead, we suggest that both the source and processing methods should be selected for and standardized to maximize oil yield for biofuel conversion as well as the nutritional value of PEAR for beef cattle production.

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