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In vitro digestion of microalgal biomass from freshwater species isolated in Alberta, Canada for monogastric and ruminant animal feed applications

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ABSTRACT

In vitro digestion studies were conducted to examine the potential nutritional value of whole (WAB) and lipid-extracted biomass (LEB) from freshwater microalgae from Alberta, Canada. For WAB, protein solubility (PS) was statistically highest and the same ($P = 0.109$) for *Chlorella vulgaris* at 84% and *Micractinium reisseri* at 78%, lowest ($P < 0.001$) for *Nannochloris bacillaris* at 64% and intermediate for *Tetracystis* sp. at 73%. Dilute pepsin digestibility (DPD) was highest ($P < 0.001$) for *C. vulgaris* at 80% and lowest ($P < 0.001$) for *N. bacillaris* and *Tetracystis* sp. at 60–64%, which were the same ($P = 0.075$) and *M. reisseri* was intermediate at 72%. Two-phase gastric/pancreatic digestibility of protein ($GPD_{Protein}$) and energy (GPD_{Energy}) were highest ($P < 0.001$) for *M. reisseri* at 78 and 57%, respectively, lowest ($P < 0.001$) for *N. bacillaris* and *Tetracystis* sp. at 49–52 and 41–43%, respectively, which were the same ($P = 0.101$ and 0.058, respectively) and *C. vulgaris* was intermediate at 69 and 52%, respectively. For LEB, PS was highest ($P < 0.001$) and the same ($P = 0.088$) for *C. vulgaris* and *M. reisseri* at 72–76%; which were higher ($P < 0.001$) than *N. bacillaris* and *Tetracystis* sp. at 60–62%, which were the same ($P = 0.405$). Similarly, DPD was highest ($P < 0.001$) and the same ($P = 1.000$) for *C. vulgaris* and *M. reisseri* both at 69%; which were higher ($P < 0.001$) than *N. bacillaris* and *Tetracystis* sp. at 58–62%, which were the same ($P = 0.083$). $GPD_{Protein}$ was highest ($P < 0.001$) and the same ($P = 0.944$) for *M. reisseri* and *C. vulgaris* at 79–80%, lowest ($P < 0.001$) for *N. bacillaris* at 50% and intermediate for *Tetracystis* sp. at 55%. GPD_{Energy} was highest ($P < 0.001$) for *C. vulgaris* at 69%, followed by *M. reisseri* at 61%, *Tetracystis* sp. at 48% and lowest ($P = 0.006$) for *N. bacillaris* at 45%. Organic matter digestibility (OMD) of a ruminant control diet was 45% and not significantly affected ($P \geq 0.071$) by dietary algal supplementation with an average OMD of 36% when incorporated at 50% forage replacement (equivalent to 25–43% of the diet); except *Tetracystis* sp. LEB which decreased ($P = 0.020$) OMD to 28%. Dietary inclusion of all biomass at 100% forage replacement (equivalent to 51–85% of the diet) decreased ($P \leq 0.026$) OMD to an average of 28%; except *M. reisseri* LEB which did not significantly affect ($P = 0.921$) OMD at 41%. Apparent metabolizable energy (aME) content of the control diet was 3.7 MJ kg^{-1} and was not affected ($P \geq 0.179$) by algal supplementation at an average of 3.1 MJ kg^{-1}, although a general trend of decreased aME with increased dietary levels was noted. Methane production from 48 h *in vitro* fermentation of diets with varying levels of WAB was 2.8–3.3 mol^{-10} and was the same ($P \geq 0.429$) as the control diet at 2.9 mol^{-10}. However, LEB at all levels decreased ($P < 0.001$) methane production by about 50% to 0.9–1.2 mol^{-10}, which suggests the potential for abating enteric methane emissions from ruminants by feeding microalgae, unrelated to its lipid content.

1. Introduction

Microalgae are one of the most efficient organisms at converting solar energy, carbon dioxide and inorganic elements into nutrient-rich biomass [1], which represents a potential source of renewable fuel and animal feed. Although algal oil for third generation biodiesel production has been the subject of much research and a major driver for technological innovations in recent years, by most assessments it is still not economically viable [2–4]. Consequently, algal products/co-products resulting from biofuel applications have been identified in Canada and elsewhere as a priority for investigation as valuable commodities for revenue generation and sustainable replacement of terrestrial livestock and aquaculture feed inputs [4–6]. Four freshwater species isolated in Northern Alberta, Canada have been identified as promising candidates for industrial carbon conversion in Northern climates; including *Chlorella vulgaris*, *Nannochloris bacillaris*, *Tetracystis* sp. and *Micractinium reisseri* [7] and have been mass cultivated in

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enclosed photobioreactors [8]. The first in a series of studies with this algal biomass showed them to have similar growth characteristics, proximate compositions, favorable essential amino acid and fatty acid profiles, attractive minerals and trace element compositions and were devoid of contaminating heavy metals [9]. However, there were notable differences in their carbohydrate compositions with respect to starch and fiber, which could greatly affect their extent of digestion and ultimately their overall nutritional value when fed to target animal species. Bioavailability of nutrients from novel ingredients varies between animal species due to the differences in feeding habits and digestive physiologies found in the livestock sectors broadly classified as monogastric (e.g., fish, poultry, swine) or ruminant (e.g., cattle, sheep, goats). The differences in their digestion, absorption and metabolism can be vast and, in particular, when associated with high levels of dietary fiber, which is utilized at varying degrees by farmed animals species. As a result, nutritional evaluation methods for monogastric animals can generate highly valuable data for the broad class of monogastric livestock but are likely of little value for ruminant livestock and *vice versa*. Beyond biochemical composition, digestibility is generally one of the most important aspects defining the nutritional quality of novel feed ingredients and is largely dependent upon their solubility and the extent of their chemical hydrolysis and enzymatic digestion in the gut; which can be affected by processing treatment(s) [10–13].

Evaluation of nutritional quality *in vivo* is time-consuming and costly, while *in vitro* assays that involve simulated digestion of test ingredient suspensions with purified digestive enzymes or ruminal fluid allow screening of large numbers of samples with minimal use of animals [14]. While not fully definitive of whole animal response, these methods can complement biochemical composition data as they are relatively inexpensive, results are rapidly obtained using small sample sizes, they side-step animal palatability issues and they are generally regarded as effective tools for making predictions of potential nutritional quality for research and industrial use [15]. Due to the difficulties in extrapolating nutritional value results between monogastric and ruminant animals, separate *in vitro* digestion techniques are required, however, both can provide valuable data on bioavailability of dietary protein and energy from novel feed ingredients and may also provide a preliminary selection of treatments prior to undertaking costly *in vivo* feeding trials. The present study is the second in a series of projects designed to evaluate the nutritional value of four freshwater chlorophytic microalgae species isolated in Northern Alberta, Canada for animal feed applications [9]. The main objective was to generate novel *in vitro* digestion data of whole algal biomass (WAB) and lipid-extracted biomass (LEB) for both monogastric and ruminant livestock including protein solubility (PS), dilute pepsin digestibility (DPD), two-phase gastric/pancreatic protein digestibility ($GPD_{Protein}$) and energy digestibility (GPD_{Energy}), ruminal organic matter digestibility (OMD), apparent metabolizable energy (aME) content and methane production.

2. Materials and methods

2.1. Microalgal biomass

Microalgae species used in this study included *C. vulgaris* (AB02-C-U-BBM), *N. bacillaris* (AB03-C-F-PLM), *Tetracystis* sp. (AB04-C-F-PLM02) and *M. reisseri* (AB05-C-U-BBM02). Isolation conditions, 18S gene sequence identification, screening criteria, mass cultivation, harvesting and processing and biochemical characterization are fully described in Tibbetts et al. [9]. Proximate and caloric content of whole and lipid-extracted biomass are presented in Table 1.

Table 1
Proximate composition and caloric content of whole algal biomass (WAB) and lipid-extracted biomass (LEB) used for *in vitro* digestion studies (DW basis)[a].

	C. vulgaris	*M. reisseri*	*N. bacillaris*	*Tetracystis* sp.
WAB				
Ash (%)	2.4	2.4	1.9	1.8
Crude protein (%)	14.8	14.8	14.9	14.7
Esterifiable lipid (%)	34.8	32.3	35.4	36.1
Carbohydrate (%)	29.8	30.0	27.2	27.7
Starch (%)	15.4	19.3	1.3	1.5
Fiber (%)	14.4	10.7	25.9	26.2
Gross energy (MJ kg^{-1})	26.9	26.3	28.0	28.3
LEB				
Ash (%)	2.7	2.6	2.8	2.7
Crude protein (%)	18.8	18.2	23.3	24.3
Esterifiable lipid (%)	31.8	27.7	6.1	9.4
Carbohydrate (%)	33.6	35.7	43.9	43.2
Starch (%)	20.1	24.3	2.5	3.0
Fiber (%)	13.5	11.4	41.3	40.2
Gross energy (MJ kg^{-1})	23.9	24.5	20.7	21.2

[a] From Tibbetts et al. [9].

2.2. In vitro digestion

2.2.1. Monogastric assays

Protein solubility was measured by incubation of 250 mg of WAB or LEB in 0.2% potassium hydroxide (0.036 N KOH, pH 13) for 20 min at 22 °C according to Araba and Dale [16] and Parsons et al. [17]. Dilute pepsin digestibility was measured by incubation of 200 mg of WAB or LEB in 0.0002% porcine pepsin (P7000, Sigma-Aldrich) enzyme solution (1:10,000 *w/v* in 0.075 N HCl; pH 1.5) for 16 h at 39 °C according to AOAC [18] and Komaki et al. [19]. Two-phase gastric/pancreatic digestibility was measured by incubation of 250 mg of WAB or LEB in porcine pepsin (P7000, Sigma-Aldrich) enzyme solution (25 mg mL^{-1} *w/v* in 0.2 N HCl, pH 1) for 2 h at 39 °C (gastric phase) and then subsequent incubation in porcine pancreatin, containing amylase, lipase and protease (P1750, Sigma-Aldrich) enzyme solution (100 mg mL^{-1} *w/v* in 0.05 M Tris, 0.0115 M $CaCl_2$ buffer; pH 7) for 4 h at the same temperature (pancreatic phase) according to Yegani et al. [20]. For all *in vitro* assays, three 5 mm glass beads (Cat. 11-312C, Thermo Fisher Scientific, Waltham, MA, USA) were included to aid sample dispersion. Due to the small particle size of microalgae (generally 2–20 μm) all *in vitro* assays had a minor modification with regard to filtering. After termination of chemical/enzymatic digestion, hydrolyzed contents were passed through a Whatman GF/A filter (1.6 μm porosity) rather than a Whatman no. 54 filter (20–25 μm porosity). Additionally, a microalgae-appropriate nitrogen-to-protein conversion factor (N × 4.78) [21] was used instead of the conventional N × 6.25. All reagents and enzyme cocktails were prepared fresh on a weekly basis and kept refrigerated (4 °C); with the exception of KOH, which was kept at room temperature. *In vitro* digestibility (IVD) of protein and energy were calculated as: IVD (%) = ([{Protein or Energy in initial sample − Protein or Energy in dry residue} ÷ {Protein or Energy in initial sample}] × 100%). All *in vitro* digestion assays were conducted with five replicates and procedural blanks were run in parallel to correct final IVD calculations.

2.2.2. Ruminant assays

Organic matter digestibility and apparent metabolizable energy content of diets containing varying levels of WAB and LEB were estimated using a modified batch-culture *in vitro* ruminal fermentation system with total gas capture [22]. Twenty-five isonitrogenous (12.4% crude protein; CP, DM basis) dietary treatments (Table 2) were formulated using a constant inclusion level of medium grind corn (15% of diet; 10% of total CP) and three inclusion levels of WAB or LEB (Low, 23% of total CP; Medium, 45% of total CP; High, 90% of total CP) replacing grass/legume forage; 1 mm grind (Low, 67% of total CP; Medium, 45% of total CP; High, 0% of total CP) and nitrogen-free cellulose. These levels represented dietary *as-fed* ratios of forage and algae corresponding to Control (100Forage:0Algae), Low (75Forage:25Algae), Medium (50Forage:50Algae) and High (0Forage:100Algae). Mixed rumen fluid was obtained from two ruminally-fistulated mid-lactation Holstein-Friesian dairy cows that were housed and cared for in accordance

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