Functional characterisation of *Euglena gracilis* following growth medium enrichment

Davide Lanzoni[a], Ingrid Horn[a], Federica Cheli[a], Antonella Baldi[a], Leonardo Rebuzzi[c], Giacomo Ghilardi[c], Raffaele Cavaliere[c], Carlotta Giromini[a,b]

[a]*Department of Veterinary and Animal Science (DIVAS), Università degli Studi di Milano, Via dell'Università 6, 29600 Lodi, Italy.* [b]CRC, Innovation for Well-Being and Environment, Università degli Studi di Milano, 20122 Milano, Italy. BGreen Technologies S.r.l., Via Stezzano 87, 24126, Bergamo, Italy.

In recent years, microalgae, in particular *Euglena gracilis*, has been a candidate in the food/feed industry due to its high nutritional (high protein and lipid content) and functional properties due to its ability to produce vitamin C, E and paramylon, a high molecular weight β-1,3 glucan with immunomodulatory properties. However, the inclusion of *E. gracilis* in the diets of farm animals is little investigated, especially its antioxidant activity. Moreover, microalgae are known for their variability and adaptability in their composition and nutritional properties, mainly due to their cultivation's conditions. For these reasons, the aim of the present work is to study the total phenolic content (TPC, Folin-Ciocalteu assay) and antioxidant activity (ABTS and FRAP assays) of *E. gracilis* grown under three different nutritional conditions. In particular, the algae growth media are characterised as follows: HEgM (yeast extract, soy peptones and casein broth); ETX (animal derived amino-acidic (AA) extract;) DOE-ETX (animal derived AA extract, MgSO4, KH2PO4, and a mixture of microelements). The samples undergo green chemical extraction and ex vivo digestion. For green extractions, 150 mg of Euglena HEgM, ETX, DOE-ETX were incubated for 1h, at room temperature, under shaking, with different concentrations of water:ethanol (W:E) (100:0, 75:25, 50:50, 25:75, 0:100). At the end of the incubation, the samples were centrifuged for 5 min at 4000 rpm and subjected to multiple extraction process (n=3). Considering ex vivo digestion, after collecting gastric and intestinal fluids from swine (50-110 d, n=24 at slaughter house), they were pooled and kept at 4°C until use. Then, 500 mg of Euglena HEgM, ETX, DOE-ETX were subjected to gastric digestion (39°C *x* 2h) in gastric fluid, and further exposed to intestinal fluid (39°C *x* 2h). Aliquots were taken at the beginning/end of gastric phase and at the end of the intestinal phase, to assess TPC and antioxidant activities. Data are expressed as mean±SEM. Considering green extraction, DOE-ETX showed a high TPC with statistically significant differences compared to ETX and HEgM, especially following the 25:75 W:E extraction, with values of 1104.4±68.2; 310.9±7.0 and 117.2±6.2 mg Tannic Acid Equivalent (TAE)/100g respectively ($p<0.05$). These values were also confirmed for ABTS (630.8±19.0; 41.8±7.0; 21.5±2.3 mg Trolox Equivalent (TE)/100g). Following ex vivo digestion, DOE-ETX showed good results for TPC (0h:501.0±12.0; 2h:584.9±6.7; 4h:465.2±37.4 mg TAE/100g). Although the functional profile was improved following medium enrichment, the production yields showed an inverse trend (0.67; 0.81; 1 g/l*day) for DOET-ETX, ETX and HEgM, respectively. These results confirmed the potential of *E. gracilis* as a valuable source of functional ingredients for feed application. Further investigations will be of paramount

importance to optimise growth medium formulation to obtain high algae yield with improved functional characteristics.