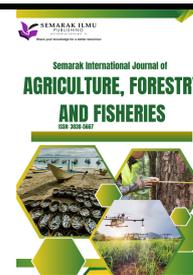




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# Evaluating the Nutritional and Antioxidant Potential of *Chlorella Vulgaris* in Comparison to Conventional Feed Ingredients

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### ABSTRACT

*Chlorella vulgaris*, a protein-rich unicellular microalgae, is increasingly recognized as a sustainable and functional alternative to conventional feed ingredients in ruminant diets. This study evaluated the proximate composition, antioxidant capacity, and in vitro digestibility of *C. vulgaris* (CV) compared to soybean meal (SBM) and black soldier fly (BSF). Proximate analysis was conducted to determine the nutritional profile, while antioxidant activity was assessed through the 2,2-diphenyl-1-picrylhydrazyl assay (DPPH), the ferric reducing ability of plasma assay (FRAP), and the total polyphenol content (TPC) assays. At similar crude protein level, both CV and BSF exhibited significantly ( $p < 0.05$ ) higher dry matter (DM), and ash content than SBM, whereas BSF yielded the highest crude fiber content (30.83%). Fermentation kinetic profile indicated that SBM and CV share the similar fermentation potential (a+b), in vitro organic matter digestibility (IVOMD), and acetate-to-propionate ratios, all significantly ( $p < 0.05$ ) higher than BSF. Yet, BSF exhibited higher volatile fatty acid (VFA) concentration (90.11mM) and the greatest fractional degradation rate ( $c = 0.08$  mL/h). Antioxidant assays demonstrated that CV possessed significantly ( $p < 0.05$ ) greater DPPH radical scavenging activity (81.86%), FRAP value (0.15 mg AAE/ml), and TPC (291.23 mg GAE/g) compared to SBM and BSF. Collectively, these findings highlight the strong nutritional value and antioxidant capacity of CV, supporting its potential as a partial substitute for conventional protein sources such as soybean meal in ruminant feeding strategies.

## 1. Introduction

Sustainable intensification of animal production requires nutritionally balanced with protein sources being critical to livestock growth and productivity. Soybean meal (SBM), the predominant plant-based protein, is valued for its favourable amino acid composition and low anti-nutritional

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factors (ANFs) [20]. However, its sustainability is increasingly challenged by market volatility [9] and ecological concerns such as deforestation and greenhouse gas emissions [22]. These challenges highlight the urgent need for alternative protein sources that can support animal performance while reducing environmental burdens.

Insects and microalgae have emerged as promising candidates for sustainable livestock nutrition. The Black Soldier Fly (BSF; *Hermetia illucens*) efficiently converts organic waste into protein-rich biomass [5,6] and exhibits a low carbon and water footprint, underscoring its ecological potential. Similarly, *Chlorella vulgaris* (CV), a unicellular green alga, is notable for its high protein content (50–60%) and bioactive compounds, including peptides, polyunsaturated fatty acids (PUFAs), and pigments [2,12,17] that may enhance animal health and productivity.

Despite the beneficial advantages, information on comprehensive comparative assessments of BSF, CV, and SBM in nutrient availability and antioxidant properties remains scarce in the supplementation of the dietary supply in animal diets. Therefore, this study aimed to assess the nutritional composition and antioxidant potential of CV and BSF in relative to SBM, which may provide insights into their suitability as sustainable protein alternatives in ruminant diets.

## 2. Methodology

Commercial powder of *Hermetia illucens* (black soldier fly; BSF) and soybean meal (SBM) were obtained from the local producer, while *Chlorella vulgaris* (CV) was gained from an e-commerce supplier (Alibaba.com). The samples were ground by using a hammer mill (1mm screen) and stored in vacuum-sealed bags at -20°C until further analysis. The samples were further analyzed for their chemical composition, *in vitro* evaluation, and antioxidant properties. All processing methods were performed in triplicate for each treatment by using a completely randomized design.

All nutritional parameters of moisture, ash, crude protein (CP), and ether extract (EE) were measured according to AOAC [3]. The neutral detergent fiber (NDF) and acid detergent fibre (ADF) contents were analyzed according to the procedure described by Van-Soest *et al.*, [23].

Additionally, *in vitro* evaluation was conducted by using rumen fluid collected from the local slaughterhouse. Samples were incubated in buffered-rumen fluid at 39°C for 48h by following Menke *et al.*, [13] protocol, with minor modifications. Post-incubation parameters included organic matter digestibility (IVOMD), total volatile fatty acid (VFA), and gas production kinetics (Ørskov and McDonald 1979).

Antioxidant activities of the samples were assessed using three assays: (1) 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, (2) ferric reducing antioxidant power (FRAP), and total polyphenol content (TPC), following the methods described by Singleton *et al.* (Singleton *et al.* 1999), with slight modification.

Data were analysed by analysis of variance ANOVA within the General Linear Model (GLM) procedure of SAS software (Statistical Analysis System software version 9.4; SAS Institute Inc., Cary, NC, USA). Significant differences between means ( $p < 0.05$ ) were compared by using Duncan's multiple range test.

## 3. Results and Discussion

The present study revealed distinct nutritional, fermentation kinetics, and antioxidant profiles among the substrates. The nutritional content of the substrate was presented in Table 1, in which revealed that the CV as the highest yield of the crude protein content (51.80%) and ash concentration (8.40), with minimal fat value (0.85), closely resembling the SBM but with greater

mineral richness (15). In contrast, at the same value of proximate composition of CV and SBM, BSF demonstrated the highest value of crude fat (30.83%) and comparatively lower protein among the substrates, indicating a high-energy feed potential for the ruminants [1,7,19]. Despite a good nutritional background, the true nutritive value of the feed components will be determined by the efficiency rate of nutrient utilization through the mechanism of kinetic ruminal fermentation [18].

**Table 1**

Nutritional composition of *Chlorella vulgaris* (CV), soybean meal (SBM), and black soldier fly (BSF)

Proximate Composition (%)	CV	SBM	BSF
Dry Matter	93.10 ± 0.00 <sup>a</sup>	89.71 ± 0.06 <sup>b</sup>	94.79 ± 1.94 <sup>a</sup>
Ash	8.40 ± 0.00 <sup>a</sup>	6.59 ± 0.11 <sup>b</sup>	7.91 0 ± 0.32 <sup>a</sup>
Crude Protein	51.80 ± 0.12	49.98 ± 0.41	45.01 ± 2.80
Crud Fat	0.85 ± 0.03 <sup>b</sup>	0.85 ± 0.03 <sup>b</sup>	30.83± 3.85 <sup>a</sup>

Results were expressed as mean ± standard deviation and were evaluated by one way-ANOVA and Duncan's test. Different superscripts in the same row indicate significant differences between means (p<0.05).

The efficiency of nutrient utilization in the rumen microbiome is presented in Table 2. The fermentation potential (a+b) of CV and SBM is 55.74 ml/h and 54.22 ml/h, respectively, both significantly exceeding BSF, demonstrating an excellent potential in rumen fermentability compared to BSF. Consistent with these values, in vitro organic matter digestibility (IVOMD) was lowest for BSF (37.75%). The reduced degradability of BSF may be associated with the abundance of lipid content, whereby fatty acids can form hydrophobic coatings on microbial cell membranes, inhibiting microbial colonization and substrate accessibility [14]. However, the fermentation rate of the substrate had revealed that CV had the slowest degradation rate (0.05), likely due to its rigid and recalcitrant cellulosic cell wall structure, characterized by a complex matrix of polysaccharides and glycoproteins, which restricts enzymatic penetration for the extraction of nutritional compounds [8]. Despite its lower gas production and digestibility, BSF yielded the highest total volatile fatty acid (VFA) concentrations and a lower acetate-to-propionate ratio, suggesting a shift in fermentation pathways toward propionate production. This shift may result from the presence of high unsaturated fatty acid content of BSF, which undergoes biohydrogenation to a saturated form less metabolically available in the rumen, thereby lowering degradability while altering VFA profiles.

**Table 2**

Fermentation kinetics profile of *Chlorella vulgaris* (CV), soybean meal (SBM) and black soldier fly (BSF)

Fermentation Kinetics	CV	SBM	BSF
a+b (mL)	55.74 ± 1.29 <sup>a</sup>	54.22 ± 6.50 <sup>a</sup>	29.80 ± 1.11 <sup>b</sup>
c (mL/h)	0.05 ± 0.00 <sup>b</sup>	0.09 ± 0.01 <sup>a</sup>	0.08 ± 0.00 <sup>a</sup>
IVOMD (%)	70.49 ± 3.07 <sup>a</sup>	74.47 ± 5.93 <sup>a</sup>	37.75 ± 0.66 <sup>b</sup>
Total VFA (mM)	41.00 ± 2.55 <sup>c</sup>	72.69 ± 0.45 <sup>b</sup>	90.11 ± 4.44 <sup>a</sup>
Acetic acid/Propionic acid (mM)	3.34 ± 0.40 <sup>a</sup>	3.12 ± 0.24 <sup>a</sup>	1.95 ± 0.18 <sup>b</sup>

Results were expressed as mean ± standard deviation and were evaluated by one way-ANOVA and Duncan's test. Different superscripts in the same row indicate significant differences between means (p<0.05).

a, soluble and very rapidly fraction; b, insoluble but fermentable fraction; a+b, fermentation potential; c, fractional degradation rate of b; in vitro organic matter digestibility; total VFA, total volatile fatty acid

Antioxidant plays an essential role in maintaining health balance and free from diseases. Supplementation of antioxidants from the nutritional (exogenous) components play crucial position

in neutralization of oxidative stress, to protect against the toxic effects [16]. In Table 3, further highlights the exceptional functional value of CV, which recorded the highest DPPH radical scavenging activity, ferric reducing antioxidant power (FRAP), and total phenolic content (TPC) at 81.86%, 0.15 mg AAE/ml, and 291.23 mg GAE/g, potentially offering added health benefits in livestock nutrition due to the abundance of antioxidant profile. In agreement with other studies had found that, *Chlorella vulgaris* enriched with bioactive compounds may exert beneficial physiological effects [11].

**Table 3**

Antioxidant properties of *Chlorella vulgaris*, soybean meal and black soldier fly (BSF)

Antioxidant properties	<i>Chlorella vulgaris</i>	Soybean meal	Black soldier fly
DPPH (%)	81.86 ± 0.85 <sup>a</sup>	66.39 ± 2.67 <sup>b</sup>	36.00 ± 1.44 <sup>c</sup>
FRAP (mg AAE/ml)	0.15 ± 0.00 <sup>a</sup>	0.04 ± 0.01 <sup>b</sup>	0.05 ± 0.01 <sup>b</sup>
TPC (mg GAE/g)	291.23 ± 11.08 <sup>a</sup>	3.93 ± 2.29 <sup>b</sup>	11.62 ± 1.92 <sup>b</sup>

Results were expressed as mean ± standard deviation and were evaluated by one way-ANOVA and Duncan's test. Different superscripts in the same row indicate significant differences between means ( $p < 0.05$ ).

DPPH, DPPH radical scavenging ability; FRAP, ferric reducing antioxidant power; TPC, total phenolic content.

#### 4. Conclusion

Collectively, these findings position CV as a promising sustainable alternative to SBM, matching its protein quality and digestibility while providing superior antioxidant properties, whereas BSF may serve as an energy-dense feed ingredient but requires further processing interventions to enhance its digestibility and rumen fermentation efficiency.

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