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Nutrient and Phytochemical Composition of Blue-green Algae (*Spirulina platensis*) as a Potential Feed for Boschveld Chickens

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The study investigated the nutrient and phytochemical composition of blue-green algae (*Spirulina platensis*) as a potential feed for Boschveld chickens. The Official Methods of Analysis (AOAC) were used for nutrient composition determination whereas Quadrupole Time-of-Flight Mass Spectrometer (QTOF-MS) was used for the phenolic compounds. The moisture content of the algae was 3.33%, Crude Protein 21.63%, ADF 57.01%, NDF 69.58%, ash 13.27%, and fat 0.76%. The nutrient components of blue-green algae as determined in the study, exceeded the recommended minimum nutrient requirements for chickens, except for fat, whose composition was below the recommended level. Macro mineral composition of the algae was Ca 0.23%, Mg 0.11%, Na 0.16%, P 0.07%, and K 0.21%. Micro-mineral composition of the algae was Fe 8979ppm, Zn 25ppm, Cu 8ppm, K/Ca+Mg 0.26%, and Mn 25ppm. Phenolic compound analysis indicated an abundance of gallic acid, phenolic acids and flavonoids. The findings of this study revealed that blue-green algae are rich in nutrients and diverse phenolic compounds that are essential for the production of indigenous chicken breeds.

1. Introduction

There is a global demand for livestock commodities and poultry meat (FAO, 2004; Steinfeld, Wassenaar, & Jutzi, 2006). Poultry meat is an important source of protein in the world. Southern Africa and many parts of the developing world, smallholder farmers generally rely on chicken meat to meet their dietary protein requirements (Azimu et al., 2018). According to Manyelo *et al.* (2020), indigenous chicken breeds are mostly kept by farmers due to the demand for organic meat and their tolerance to local are important for the economic, social, and cultural importance of the people of Africa and other developing countries, especially those from poor communities (Manyelo et al., 2020). Intensive indigenous chicken breed production is vital for food and nutrition security of people worldwide (Atela, Mlambo, & Mnisi, 2019). Due to high feeds cost, it is important to identify alternative feed ingredients that are readily available to boost growth and promote health properties. Algae are nutraceutical plants that can be used in the diets of indigenous chickens (Fleurence et al., 1995). Nutrition costs approximately 80–90% of the total cost of production. Therefore, it is the backbone of profitable broiler production (NAFIS, 2017). Therefore, it is important to examine the nutrient composition and metabolomic analysis, of the algae as potential feed for Boschveld chickens.

2. Materials and Methods

2.1. Data Collection and Preparation of the Algae Samples

The study evaluated the nutrient composition and metabolomics properties of the blue-green algae (*Spirulina platensis*). The blue-green algae (*Spirulina platensis*) were harvested at Ozblu (Lepalala berries)

and transported to the University of Limpopo, Animal Production Laboratory. The blue-green algae samples were put into the oven that was set temperature of 40°C for 72 hours to dry. Thereafter, they were grounded using an industrial electric blender (Hamilton Beach Commercial HBF500S series China) and passed through a 1mm measuring sieve. The powder was stored in airtight plastic containers and subjected to proximate analysis as prescribed by AOAC (2012) procedures and analyzed Ash, Crude protein, Moisture, NDF & ADF, Carbohydrates (AOAC, 2012) at the University of Stellenbosch and Cedara, KwaZulu-Natal (KZN) Agricultural and Rural Development Laboratory using Liquid Chromatography - Mass Spectrometer (LCMS).

2.2. Determination of Dry Matter and Moisture Content

Dry matter was determined using drying the sample using the Koster crop tester for 24 hours at 105°C until the weight became constant. Algae moisture content was determined by using the technique outlined by AOAC (2000). A weighing dish was weighed in an oven (LABOTEC-South Africa) after being dried for 72 hours at 40°C to determine the starting weight. Thereafter, the oven-dried algae sample was cooled, grounded, and then put into the previously weighed dish and reweighed to acquire a steady weight. Percentage (%) moisture content was calculated as follows: ‘

Percentage moisture content (%) = $\frac{Wy-Wz}{Wy-Wx} * 100$ where wy =weight of the container with lid and sample before drying, Wz = weight of the container with lid and sample after drying, and Wx = weight of the container with lid.

2.3. Determination of Ash Content

The dried algae ash content was determined by the dry ash method (AgriLASA, 2007). After being dried for one hour at 105°C, the sample dish was chilled and weighed (Wx). The dried (2g) algae sample was placed and weighed again (Wy). The container with algae samples was kept for an hour in a burner at a temperature of 250 C (Furnace E-Range, E300-P4, MET-U-ED South Africa). After that, it was left to cool and then reweighed (Wz). Ash content was calculated as follows:

$$\text{Ash content percentage (\%)} = \frac{Wy-Wz}{Wy-Wx} * 100$$

2.4. Determination of Crude Protein Content

The nitrogen percentage in the sample was multiplied by a factor of 6.25 to account for the conversion of nitrogen to protein given that the majority of proteins contain about 16 % nitrogen, as obtained by the Kjeldahl technique. Protein content in products was determined using a general factor of 6.25 (AOAC, 1990), Crude protein was calculated as follows:

Crude protein=Nitrogen content (sample) x 6.26

2.5. Determination of NDF and ADF

2.5.1. ADF Determination

To determine the ADF, the process outlined by Van Soest, Robertson, & Lewis (1991) was followed. The algae sample (2g) was weighed into a pre-weighed crucible. A known volume of acid detergent solution (typically containing sulfuric acid and detergent) was added to the crucible. The crucible with the sample and solution was placed into a digestion apparatus of fibre analyzers. Then the sample was digested at a temperature of 160°C to 170°C for 2 hours. The crucible’s contents were filtered using a filter crucible that had been pre-weighted, and the left-over residues were rinsed off with hot water to remove acid or traces. The crucible and the residue were left to dry until they reached a consistent weight. The ADF content was calculated using the weight of the residue and was represented by the initial percentage of the sample weight.

2.5.2. NDF Determination

To determine the NDF, the process outlined by Van Soest et al. (1991) was followed. The algae sample (2g) was weighed into a pre-weighed crucible. A known volume of neutral detergent solution (typically containing sodium lauryl sulfate and EDTA) was added to the crucible. The crucible with the sample and solution was placed into a digestion apparatus of fiber analyzers. Then the sample was digested at a temperature of 100 °C to 105°C for 1 hour. After was completed the crucible’s contents were filtered using a filter crucible that had been pre-weighted, and the left-over residues were rinsed off with hot water to remove acid or traces. The crucible and the residue were left to dry until they reached a consistent weight. The NDF content was calculated using the weight of the residue and was represented by the initial percentage of the sample weight.

2.6. Determination of Mineral Element Composition

Mineral element analysis was carried out to quantitatively

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identify the mineral components present in algae. The micro-elements such as iron, zinc, manganese, and copper, as well as the macro-elements such as sodium, calcium, potassium, phosphorus, and magnesium, were identified by technique determined by AOAC (2012), and Shahidi et al. (1999); utilizing the variant 710-ES Series, SMM, Cape Town, South Africa, Inductively Couple Plasma Optical Emission Spectrometer (ICP-OES). The processed samples were weighed and put (2g) of dry ash at 550°C in a muffle furnace in a well-maintained porcelain crucible. The Ash product was dispersed into 5mL of HNO3/HCI/H2O at a ratio of (1:2:3) and briskly cooked on a plate that was heated until the fumes produced vanished. 9 of each crucible residual substance got a deionized water of 5mL which was heated until the solution was of no colour. Whatman No.42 filter paper was used to filter and transfer the solution of mineral one crucible at a time, into a volumetric flask of 100mL. Deionized water was used to adjust the volume to the desired amount. Atomic absorption spectrophotometer elemental samples were estimated using the concentration dry matter proportion that was mg/100 g sample in a 10cm long cell.

The determination of secondary metabolite extracts was prepared from 2g of dry algae material +15 mL of 50% methanol and 1% formic acid dissolved in water with ultrasonication for 1 hour and left standing overnight. This was followed by centrifugation and transfer of the supernatant to a glass vial ready for LC-MS analysis. Samples were then analyzed-through the LC-MS method using a Waters SYNAPT G2 Quadrupole time-of-flight (QTOF) mass spectrometer (MS) connected to a Waters Acquity UPLC (Waters, Milford, MA, USA) for high-resolution UPLC-MS analysis. Electrospray ionization was used in the negative mode with a cone voltage of 15 V, desolvation temperature of 275 ◦C, and desolvation gas at 650 L/h; the rest of the MS settings were optimized for the best resolution and sensitivity. Data was acquired by scanning from 150m/z to 1500m/z in the resolution and the MSE mode.

3. Results

Results of the proximate analysis of blue-green algae are presented in Table 1. Blue-algae had a moisture content of 3.33%, Crude protein of 21.63 %, ADF of 57.01%, NDF of 69.58%, and Ash of 13.27%. Nutrient levels were above the minimum requirements for Boschveld chickens. Blue-green algae Macro mineral composites of Ca, Mg and Na that exceeded the recommended minimum requirements for Boschveld chickens except for P (0.07%), K (0.21%) and Micro mineral composites of Fe 8979 (ppm), Cu (8%) and k/ca+mg (0.26%).

Table 1: Nutrient and Minerals Composition of Blue-green Algae (*Spirulina platensis*).

Nutrient	Content	Minimum Requirements for Boschveld Chickens
Moisture (%)	3.33	-
Crude Protein (%)	21.63	16-20.00
Fat (%)	0.76	3-5.00
ADF (%)	57.01	2-5.00
NDF (%)	69.58	3-7.00
Ash (%)	13.27	6-7.00
Macro Minerals		
Ca (%)	0.23	0.8-1.00
P (%)	0.07	0.6-0.80
Mg (%)	0.11	0.05-0.07
K (%)	0.21	0.6-08
Na (%)	0.16	0.10-0.15
Micro Minerals		
Fe (ppm)	8979.00	50-60
Mn (ppm)	25.00	40-60
Cu (ppm)	8.00	5-7.0
Zn (ppm)	25.00	40-60
K/Ca+Mg (%)	0.26.00	0.15-0.25
Values are the means of triplicate of analyzed blue-green algae (*Spirulina platensis*) Secondary metabolites in blue-green algae (*Spirulina platensis*)		

The phenolic compounds identified in blue-green (*Spirulina platensis*) using LC-MS methods are presented in Table 2, and chromatograms are presented in Figure 1. The study identified, six compounds in the blue-green algae such as Gallic acid Digalloyl-beta-D-glucopyranose, 3,4-dihydroxy-5 (3,4,5-trihydroxybenzoyloxy) benzoic acid, 1,2,3,4,6-Penta-O-galloyl-beta-D-glucose;(+)-1,2,3,4,6-Penta-O-galloyl-beta-D-glucose, 25203464 Hydrolyzable tannins and Alpha-dimorphecolic acid

Table 2: Phenolic Compounds Identified in Blue-green Algae (*Spirulina platensis*) using LC-MS Analysis.

No	RT	Mse Fragments	Identification	Formula	Ontology
1	2,82	169,014	Gallic acid	C6H20H3COOH	Trhydrobenzoic acid
2	3,462	483,07855	1,6-Digalloyl-beta-D-glucopyranose	C20H20O14	Tannins
3	3,742	321,02551	3,4-dihydroxy-5-(3,4,5-trihydroxybenzoyloxy)benzoic acid	C14H10O9	Depsides and depsidones
4	4,594	939,11145	1,2,3,4,6-Penta-O-galloyl-beta-D-glucose;(+)-1,2,3,4,6-Penta-O-galloyl-beta-D-glucose;Pentagalloyl-beta-D-glucose	C41H32O26	Tannins
5	4,763	1091,12207	25203464	C48H36O30	Hydrolyzable tannins
6	9,669	295,22766	Alpha-dimorphecolic acid	C18H32O3	Lineolic acids and derivatives
Abbreviations: Nd = not detected					

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The results of quantified individual phenolic compounds in blue-green algae (mg/kg) are presented in Table 4. Blue-green algae had high quantities of Alpha-dimorphecolic acid (292.7 mg/kg) with the ontology linoleic acids and derivatives. Followed by Gallic acid (124.8 mg/kg) with ontology tryhydrobenzoic acid, 3,4-dihydroxy-5(3,4,5-trihydroxybenzoyloxy) benzoic acid (118.9 mg/kg) with ontology tannins.

Table 3: Quantified Individual Phenolic Compounds in Plants (mg/kg).

Compound	Ontology	mg/kg
Gallic acid	Trhydrobenzoic acid	124.80
1,6-Digalloyl-beta-D-glucopyranose	Tannins	58.60
3,4-dihydroxy-5-(3,4,5-trihydroxybenzoyloxy)benzoic acid	Depsides and depsidones	118.90
25203464	Hydrolyzable tannins	46.30
1,2,3,4,6-Penta-O-galloyl-beta-D-glucose;(+)-1,2,3,4,6-Penta-O-galloyl-beta-D-glucose;Pentagalloyl-beta-D-glucose	Tannin	102.50
Alpha-dimorphecolic acid	Lineolic acids and derivatives	292.70

Phenolic compounds in blue-green (*Spirulina platensis*) are displayed in the chromatographs, stacked bar chart, Principal Component Analysis (PCA) scatter plot, and Hierarchical clustering (Figure 1, 2, 3 & 4). All compounds were displayed with clear indication of the abundance of Alpha-dimorphecolic acid Gallic acid, followed by 3,4-dihydroxy-5(3,4,5-trihydroxybenzoyloxy)benzoic acid.

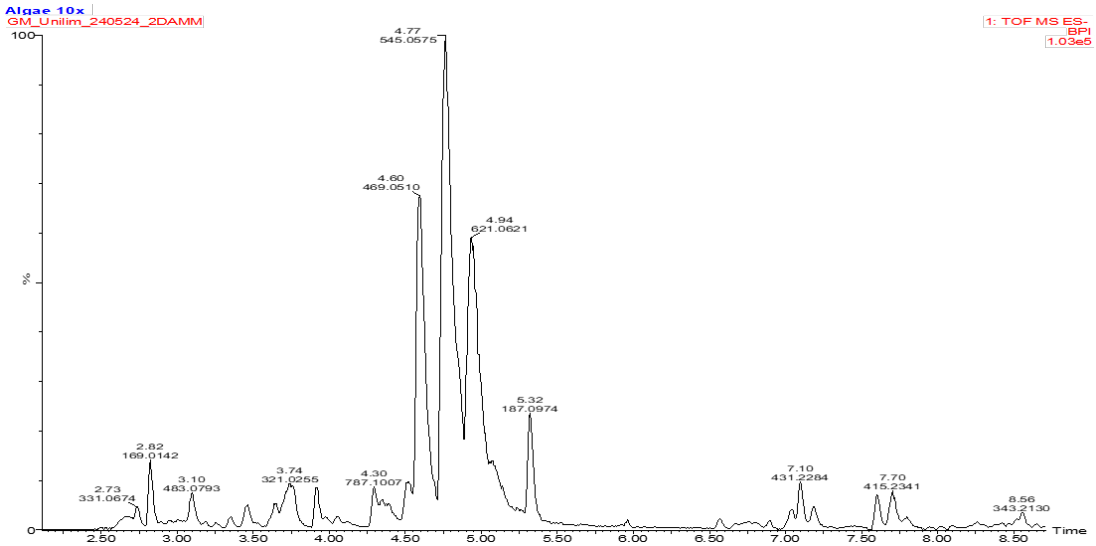

Figure 1: Chromatograms of Blue-green Algae (Spirulina platensis).

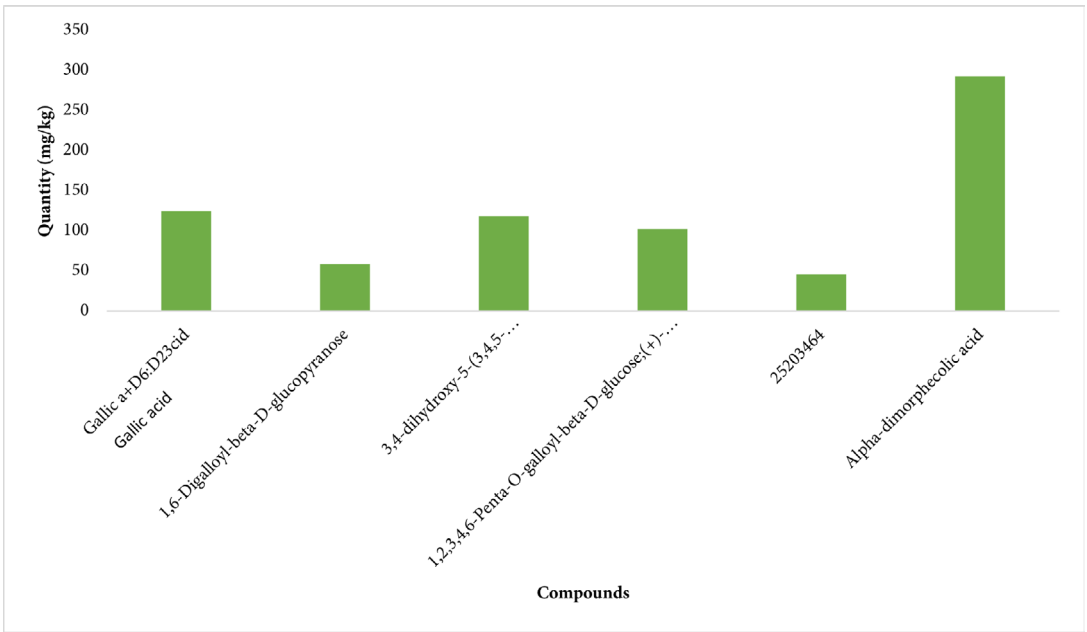

Figure 2: Stacked Bar Graph of Phenolic Compounds Extracted from Blue-green Algae.

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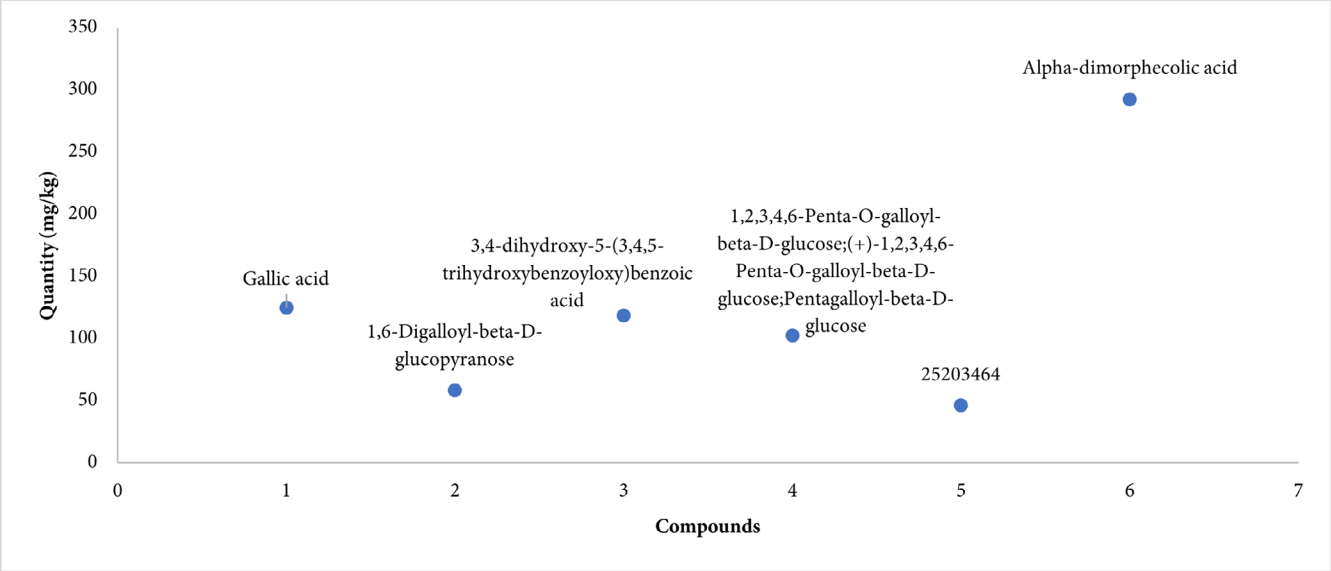

Figure 3: Principal Component Analysis (PCA) Scatter Plot of Metabolites of Green-blue Algae.

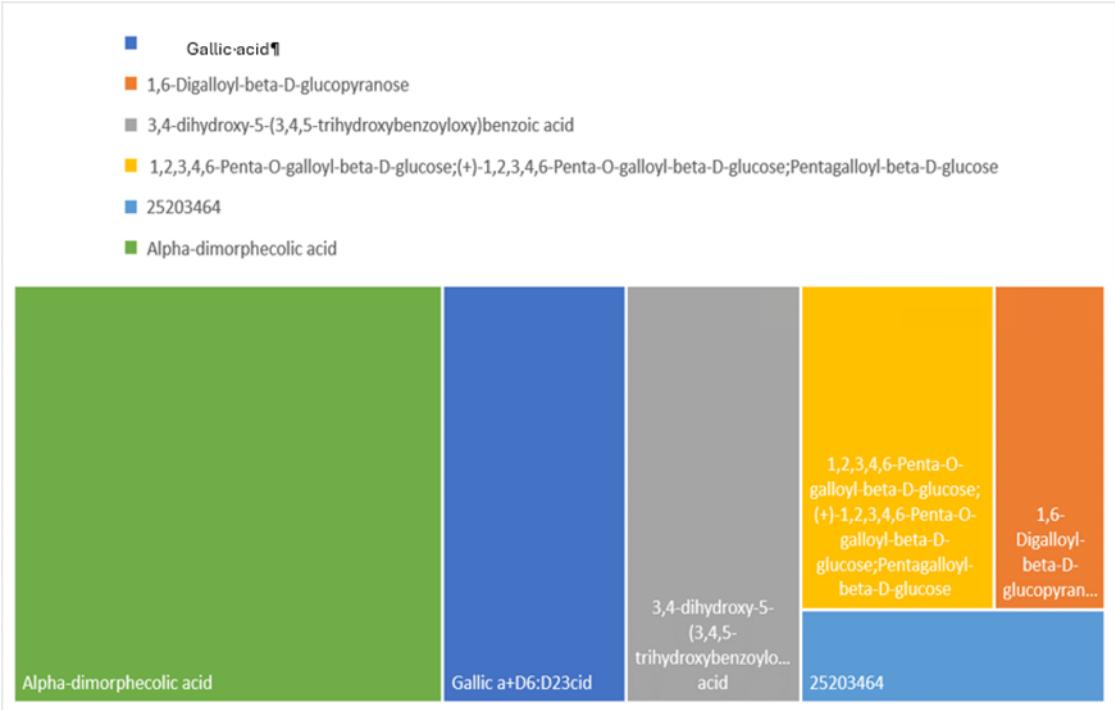

Figure 4: Hierarchical Clustering of Phenolic Compounds in Blue-green Algae.

4. Discussions

The study revealed that blue-green algae (*Spirulina platensis*) have an absolute mineral composition that has higher ash content, Crude Protein, ADF and NDF. Blue-green algae exceeded the minimal nutrient requirement for fat that is needed by Boschveld, this may be due to the season on which it was collected which was late summer-winter. Scholars reported that harvest done in summer obtained high levels of essential macro and micronutrients, trace minerals, and phenolic compounds. The findings contrast with those of Negreanu-Pîrjol et al. (2011), who found a high percentage of Ash and fats but lower crude protein. The present findings are consistent with the report of Markou, Vandamme, & Muylaert (2014), which indicated that Blue-green algae are rich in both macro and micro minerals such as Ca, Fe, Cu, Na, and Mg minerals, which exceeded the minimum nutrient components, while P and Zn were below the minimum required by chickens. The findings obtained from this study suggest that blue-green algae (*Spirulina platensis*) are a good source of minerals needed in poultry nutrition. Protein supplements are expensive hence needed in animal production, however Markou et al. (2014) reported that algae obtain high percentage of Vitamin B12, C and D2 and are still limited in the

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market. The Study revealed that blue-green algae blue-green algae contained phytochemical compounds such as phenolics and flavonoids of phenolic compounds extracted from blue-green algae (*Spirulina platensis*) indicating the quality (mg/kg) and compounds of metabolites of green-blue algae indicating quality (mg/kg) and compounds (*Spirulina platensis*). All compounds were displayed with clear indication of the abundance of Alpha-dimorphecolic acid Gallic acid, followed by 3,4-dihydroxy-5 (3,4,5-trihydroxybenzoyloxy) benzoic acid in the chromatograms of blue-green algae (*Spirulina platensis*) indicating the total score of algae and the retention time, the stacked bar graph, the principal component analysis (PCA) scatter plot, the Hierarchical clustering. Several studies have reported that there is a correlation between the number of phenolic compounds present on algae and ontology. Phenolic compounds in algae have antioxidant, anti-inflammatory and anti-apoptotic properties that play a crucial role in protecting against oxidative stress (Jimenez-Lopez et al., 2021). Gallic acids can be found in various plants and food, pharmaceuticals and cosmetics industry. Many scholars have reported that phenolic compounds that improve digestive health and protect against oxidative stress, reduce inflammation, immune- boosting in poultry and piglets. Literature also decribed that Gallic acid percentage tolerated by the chicken in a diet is 0,05%. The 1.6- Digalloyl-beta-D-glucopyranose, 3,4-dihydroxy-5 (3,4,5-trihydroxybenzoyloxy) benzoic acid 1-2% in a diet. Prior studies on the dietary supplementation of Alpha-dimorphecolic acid reported improved growth performance and intestinal health as well as jejunal morphology. The supplementation of phenolic compounds improved the caecal microbial and increased mitochondrial activity in broilers. Moreover, there is growing evidence suggesting that these flavonoids can be used in animal health as antioxidants, antiradicals, estrogenic, anti-inflammatory, antiviral, anti-tumoral, anti-diabetic and cytotoxic activities (Ay et al., 2016; Karamać et al., 2019).

5. Conclusions

Blue-green algae (*Spirulina platensis*) are a group of photosynthetic bacteria, that is known for its ability to produce toxins, fix nitrogen, and produce oxygen. Blue-green algae are underutilized despite their number of phenolic compounds and high nutritional value. The present study corroborates that blue-green algae (*Spirulina platensis*) have adequate nutrients components such as Crude Protein, ADF, NDF, and Ash content as well as micro and macro minerals that support Boschveld chickens' production. Furthermore, the presence of blue-green algae (*Spirulina platensis*) highlights its potential for enhancing immunity and gut functions in animals and should be accorded significant attention as an alternative for the replacement of protein in animal diets.

5.1. Acknowledgments

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6.1. Data Availability

All data that were evaluated during the research are available in the manuscript.

6.2. Conflict of Interest

The authors declare that there is no conflict of interest.

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