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# Microalgae: An Exciting Alternative Protein Source and Nutraceutical for the Poultry Sector

### 20 Abstract

Microalgae have garnered a considerable attention as a sustainable substitute as customary feed 21 ingredients for poultry, predominantly due to their extraordinary nutritive profile and purposeful 22 properties. These minuscule organisms are protein rich, retain an ample quantity of essential fatty 23 acids, vitamins, minerals, and antioxidants, thus are capable of improving nutritive value of poultry 24 diets. Microalgae comparatively delivers an outstanding source of protein containing substantial 25 amount of innumerable bioactive complexes, omega-3 fatty acids in addition to the essential amino 26 27 acids (methionine and lysine), crucial for optimal growth and development. Besides nutritional significance, microalgae have considerable immunomodulatory and antioxidant properties that 28 help to reduce oxidative stress and enhance immune status, thereby improving the overall health 29 30 and performance. Additionally, microalgae proved to induce antimicrobial and intestinal health benefits via upregulated gut eubiosis, promoting the colonization and growth of probiotic bacteria 31 and offering protection against infections. These nutraceutical benefits are particularly important 32 33 for sustainable poultry production and reducing the dependence on antibiotic growth promoters 34 (AGPs) to produce antibiotic free food. This review aims to highlights multifaceted advantages of 35 microalgae as a functional feed additive for poultry diet to support sustainable and efficient poultry production. 36

Keywords: Microalgae; Nutraceutical potential; Alternative protein source; Health benefits;poultry

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#### 41 Introduction

42 Poultry farming is vital to the worldwide livestock sector, providing a substantial source 43 of protein and nutrition for growing humans population (Mottet and Tempio, 2017). The demand 44 for poultry products has been gradually increasing due to numerous factors including population increase, rising income levels, and evolving dietary preferences (Clonan et al., 2016; Saeed et al., 45 46 2019; Yatao et al., 2018). The poultry industry constitutes a significant segment of the global agricultural sector, especially in developing nations (Mnisi et al., 2023), where ever rising human 47 population needs the introduction of alternative sources of dietary protein to avoid the hazards 48 related to protein deficits (Mnisi et al., 2023). Poultry plays a crucial role in global food security 49 by providing affordable, high-quality protein in the form of meat and eggs. Poultry is a vital source 50 of meat and eggs containing high-quality proteins, enriched with essential amino acids, vitamins 51 52 and minerals to fulfill the nutritional requirements, vital for addressing malnutrition and combat food security issues in low-income population (Gržinić et al., 2023; Lingala et al., 2024). 53 Additionally, the contribution of poultry in food security extends beyond the nutritional 54 requirements, it plays an important role to enhance the livelihood of low-income population, owing 55 to the economically viable and short production life (Vlaicu et al., 2024). Nonetheless, the 56 57 manufacturing of poultry products demonstrates several obstacles, including environmental degradation, elevated feed costs, and the dissemination of diseases (Mottet and Tempio, 2017). 58 59 Another hurdle which increase feed price is the extravagant feed ingredients, including grains, oilseeds, synthetic chemicals and feed supplements to raise the production cost (Arif et al., 2017; 60 Safdar et al., 2024). To tackle these difficulties, there is increasing interest in identifying alternative 61 feed ingredients that can improve the nutritional quality of poultry diets simultaneously reducing 62 the adverse environmental effects of poultry production (Arain et al., 2022b; Nabi et al., 2020a; 63

Nabi et al., 2020b; Saeed et al., 2018). Poultry feed researchers emphasize for the investigation of
alternative feed ingredients, including novel protein sources, insect-derived feed, algae, and plantbased bioactive substances from the food and agricultural sectors, to substitute expensive
ingredients and attain production objectives (Behan et al., 2024; Nabi et al., 2024).

Microalgae are photosynthetic microorganisms acknowledged as a viable alternative feed 68 component in poultry diets owing to their elevated nutritional content and functional attributes 69 70 (Koyande et al., 2019). Furthermore, microalgae represent a wide assemblage of organisms capable of thriving in various settings, including freshwater (Maltsev and Maltseva, 2021), marine 71 water (Bhuvana et al., 2019), and waste water. They are an abundant source of protein and vital 72 73 fatty acids (Sathasivam et al., 2019), vitamins (Wang et al., 2021), minerals (Uribe-Wandurraga et al., 2019), and source of antioxidants (Gauthier et al., 2020), all these constitute essential 74 elements of a balanced poultry diet (Madeira et al., 2017). While, microalgae have demonstrated 75 the ability to augment the growth and immune response of poultry, in addition to enhancing the 76 quality of poultry products (El-Ghany, 2020). These microscopic photosynthetic organisms are a 77 sustainable and renewable resource, containing higher concentration of essential nutrients, 78 supporting the growth and productive performance of poultry. The lipid fraction, of microalgae 79 particularly omega-3 fatty acids like docosahexaenoic acid (DHA), boost immunity and promoting 80 81 gut health, thus enhances the productivity and nutritional quality of poultry based food such as 82 meat and eggs (Kalia and Lei, 2022).

Present study provides a comprehensive overview of the current understanding on incorporation of microalgae in poultry diets. Moreover, current review offers a comprehensive analysis of the nutritional value, functional features, and advantageous applications of microalgae

in poultry nutrition to improve the health and productivity of poultry. Simultaneously, the
prospective advantages and obstacles related to the use of microalgae in poultry feed should also
be emphasized.

#### 89

# Categories and characteristics of microalgae

Microalgae constitute a complex assemblage of photosynthetic microorganisms inhabiting 90 a variety of aquatic habitats, encompassing both freshwater and marine ecosystems (Saber et al., 91 2022). They are essential to global ecological and biogeochemical cycles, greatly contributing to 92 primary production and serving as the foundation of the food sources for aquatic animals (Lobus 93 and Kulikovskiy, 2023; Quintas-Nunes et al., 2023). These unicellular or multicellular microbes 94 may utilize both organic and inorganic carbon sources for growth and development, rendering 95 them an optimal platform for large-scale biomass production (Chen et al., 2024). The diversity of 96 microalgae exemplifies their flexibility and the extensive variety ranging from unicellular species 97 like Chlorella to multicellular forms like Spirogyra. Their ability to adapt to diverse habitats, including 98 extreme environmental settings (Singh et al., 2023). 99

100 The classification of microalgae is intricate owing to their extensive diversity and the ongoing identification of novel species. They are often categorized according to their pigmentation, 101 cell wall composition, storage products, and various morphological and physiological 102 characteristics (Shaikh et al., 2022). Microalgae are taxonomically categorized into numerous 103 principal groups, including green algae, diatoms, cyanobacteria, and dinoflagellates, among others 104 (de Morais et al., 2016; Masoj idek and Torzillo, 2014). The distinct attributes of each category, 105 including their pigment makeup, cellular architecture, and metabolic pathways, influence their 106 distinctive qualities and prospective applications (Madkour et al., 2023; Masoj idek and Torzillo, 107 108 2014).

Green algae are recognized for their capacity to a mass substantial lipid levels, rendering them a prospective source for biofuel production (Dolganyuk et al., 2020; Khan et al., 2018). Diatoms are acknowledged for their capabilities in wastewater treatment and the synthesis of key biochemical, such as carotenoids and silica (Quintas-Nunes et al., 2023). Cyanobacteria, sometimes referred as blue-green algae, have attained interest for their capacity to synthesize a diverse array of bioactive substances, such as pigments, vitamins, and antioxidants.

# 115 Nutritional composition of microalgae

Microalgae possess the capability to synthesize a diverse array of important substances, such as proteins, lipids, carbohydrates, vitamins, and pigments, which are utilized in the feed, medicinal, and biofuel sectors (Dolganyuk et al., 2020). The scientific investigation of microalgae has attracted heightened interest in recent years owing to its exceptional potential as a sustainable and nutrient-rich source of feed, and valuable bioactive chemicals (Bature et al., 2022).

The nutritional makeup of microalgae can vary considerably based on species, growth 121 122 conditions, and post-harvest processing techniques (Alghazeer et al., 2022). Light intensity, temperature, nutrient availability, and growth strategies can all affect the accumulation of certain 123 nutrients in microalgae cells. The fundamental advantage of microalgae is their remarkable 124 nutritional profile. Microalgae are recognized for their abundance of vital elements, such as 125 proteins, carbs, lipids, vitamins, and minerals (Sanjari et al., 2018). The energy content of 126 127 microalgae biomass is determined by the existence of carbon, oxygen, hydrogen, nitrogen, and other components, rendering them a viable source of renewable energy. Moreover, microalgae 128 have evolved intricate biochemical and physiological mechanisms to thrive in extremely 129 130 competitive environments, resulting in the accumulation of important bioactive chemicals (Rendón-Castrillón et al., 2020). Microalgae can possess a remarkable protein concentration, with 131

certain species up to 70% protein on a dry matter basis (Sanjari et al., 2018). These proteins are often of superior quality, encompassing all essential amino acids necessary to meet the nutritional requirements of animals. Moreover, microalgae serve as a substantial source of omega-3 and omega-6 fatty acids, essential for augmenting immune response and improving the health and productivity of animals. Specific microalgae species are recognized as superior producers of carotenoids, including astaxanthin and lutein, which have strong antioxidant capabilities and are associated with numerous health advantages (Novoveská et al., 2023).

Moreover, microalgae are recognized as a substantial source of lipids, especially 139 triacylglycerol's, which can be utilized for many industrial purposes (de Morais et al., 2016). The 140 lipid content of microalgae is affected by several parameters, including species, growing 141 circumstances, and nutrient availability (Rendón-Castrillón et al., 2020). Besides lipids, 142 microalgae also store considerable quantities of carbohydrates, which can improve their nutritional 143 utility in cattle feed (de Morais et al., 2016). Furthermore, microalgae are distinguished by their 144 pigments, including carotenoids and phycobilipigments, which enhance their vivid coloration and 145 exhibit antioxidant and therapeutic attributes. Moreover, microalgae serve as a superior supplier 146 of important vitamins and minerals, encompassing vitamin A, B-complex vitamins, vitamin C, 147 148 vitamin E, along with minerals such as iron, calcium, and magnesium. Likewise, (Dineshkumar et al., 2017) documented the biochemical and elemental analysis of green algae composition was 149 depicted in Table 1. The nutritional value of microalgae can be augmented by crop optimization 150 and genetic engineering. 151

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154 Table 1: Chemical composition of various species of microalgae.

Species of microalgae	Carbohy drates	Protein	Lipids	Total dietary fiber	References
Chlorella vulgaris	20.99	15.67	41.51	15	(Alfaia et al., 2021; Chia et al., 2015)
Spirulina platensis	30.21	13.3	48.36	14.98	(Seghiri et al., 2019)
Chlorella sorokiniana	35.67	9.9	18.81	13.5	(Chia et al., 2015; Niccolai et al., 2019)
Nannochloropsis oceanica	22.7	24.8	19.1	20	(Zanella and Vianello, 2020)
Scenedesmus obliquus	13.41	4.66	30.38	19.37	(Ferreira et al., 2020)
Dunaliella tertiolecta	21.69	2.87	61.32	9.06	(Araj-Shirvani et al., 2024)
Dunaliella salina	32	57	9	48.82	(Shantkriti et al., 2023)
Scenedesmus dimorphus	36.5	13	28	32.7	(Tapia-López et al., 2024)
Chlorococum humicola	32.5		-	24.5	(Yang et al., 2022)
Chlamydomonas reinhardtii	22.6	64.7	12.6	11.9	(Darwish et al., 2020)
Spirogyra sp.	48.5	13	16	37	(Sriwattana et al., 2024)
Porphyridiumcruentum	48.5	33.5	11.5	21.7	(Castro-Varela et al., 2021)
Dunaliella salina	85.58	8.46	11.47	0.8	(Demirel, 2022)
Arthrospira platensis	42.08	14.95	6.5	42.82	(Markou et al., 2023)
Scenedesmus almeriensis	49.4	24.6	1.58	22.6	(Sánchez-Zurano et al., 2021)
Haematococcus pluvialis	32.59	0.13	3.24	34.56	(Marinho et al., 2021)
Chlorella vulgaris	45.64	59.71	19	21.16	(Ricky et al., 2022)

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#### **Bioactive constituents and Pharmaceutical properties of microalgae**

Microalgae are acknowledged as a prolific and underutilized source of bioactive chemicals 156 157 with significant potential for many uses. These microscopic photosynthetic organisms may 158 manufacture a variety of primary and secondary metabolites, many of which possess valuable nutritional, medicinal, and industrial characteristics (Alghazeer et al., 2022). Typically, microalgae 159 160 have been cultivated mainly for the synthesis of several recognized beneficial chemicals, including carotenoids, critical omega-3 fatty acids, and phycobilipigments (Quintas-Nunes et al., 2023). 161 Recent study has revealed the significant untapped potential of microalgae, emphasizing the 162 existence of several bioactive compounds with prospective nutraceutical and medicinal 163 applications (Alghazeer et al., 2022; Mobin and Alam, 2017). 164

Microalgae have attracted interest for their capacity to generate a wide variety of bioactive 165 secondary metabolites. These encompass substances possessing antioxidant, anti-inflammatory, 166 antibacterial, and potential anti-cancer activities (Alghazeer et al., 2022; Mobin and Alam, 2017; 167 Saha and Murray, 2018). The chemical composition and quantity of these bioactive molecules can 168 fluctuate considerably based on factors such as species, growth circumstances, and environmental 169 influences (Alghazeer et al., 2022; Quintas-Nunes et al., 2023). Bioactive molecules are typically 170 secondary metabolites, encompassing a diverse array of chemicals such as organic acids, 171 172 carbohydrates, amino acids, peptides, vitamins, growth regulators, antibiotics, enzymes, and 173 poisonous compounds (de Morais et al., 2015; Munaro et al., 2021). The metabolites exhibit a diverse array of biological actions, encompassing anticancer, antiviral, antioxidant, and 174 immunomodulatory properties (Arain et al., 2022a; Du et al., 2024). The possibility exists for the 175 176 discovery of novel medication leads from these metabolites. Cyanobacteria are the most significant source of bioactive chemicals among numerous groups of microalgae (Martínez-Francés and 177

Escudero-Oñate, 2018). In summary, the extensive diversity and unexploited potential of bioactive
chemicals in microalgae signify a promising frontier in nutrition, medicines, and sustainable
resource management.

Microalgae possess numerous pharmaceutical and nutraceutical properties, offering 181 potential benefits for poultry health and performance. Research indicated that microalgae contains 182 bioactive compounds such as carotenoids, omega-3 fatty acids, antioxidants, vitamins, and 183 184 minerals, they are participated to enhance the immune responses and reduce oxidative stress in poultry (Abdelnour et al., 2019) (Figure 1). These bioactive compounds enhance the blood 185 concentration of anti-oxidant enzyme like superoxide dismutase (SOD), glutathione peroxidase 186 187 (GPx) and catalase (CAT), thereby reduced the oxidative stress by improving the antioxidant defense (Emam et al., 2024; Panaite et al., 2023). Furthermore, dietary supplementation of 188 microalgae significantly reduces the oxidative stress by scavenging toxic radicals of reactive 189 oxygen species and also mitigated the negative effects of heat stress in broiler chicken (Chaudhary 190 et al., 2023a). Another study, have revealed that mesobiliverdin IXα (MBV)-enriched microalgae 191 extracts can improve gut health and microbial balance in broilers, increasing beneficial 192 Lactobacillus species and promoting intestinal villi growth. Notably, MBV-SP2 lowered pro-193 inflammatory markers and improved intestinal morphology more effectively than antibiotic 194 195 treatments, highlighting MBV-enriched extracts as a promising alternative for antibiotic-free broiler health management (Chang et al., 2021). Previously it was demonstrated broiler fed diet 196 containing a mixture of *Dunaliella salina* and *Spirulina* (1.0 and 1.5 g/kg), significantly enhanced 197 198 oxidative stress, body weight, feed efficiency, and immune response, in addition to boost the lipid profiles, liver and kidney function (Alghamdi et al., 2024). Additionally, microalgae possess 199 200 antimicrobial and anti-inflammatory properties, helping to combat pathogens and reduce disease

201 incidence (Pagels et al., 2022). As a natural and sustainable feed additive, microalgae present a promising strategy for boosting poultry health and productivity without relying on synthetic drugs 202 (Figure 1). Previously (Balakrishnan et al., 2021), suggested that fatty acid methyl esters derived 203 from marine microalgae effectively inhibit Listeria monocytogenes growth, demonstrating potent 204 antimicrobial properties by disrupting cellular metabolism and membrane integrity. Results also 205 206 confirmed that fatty acid esters suppressed the biofilm formation and improved *Caenorhabditis* elegans survival in infection models, showing potential as a natural feed additive for poultry to 207 control infection and Listeria contamination (Balakrishnan et al., 2021). 208

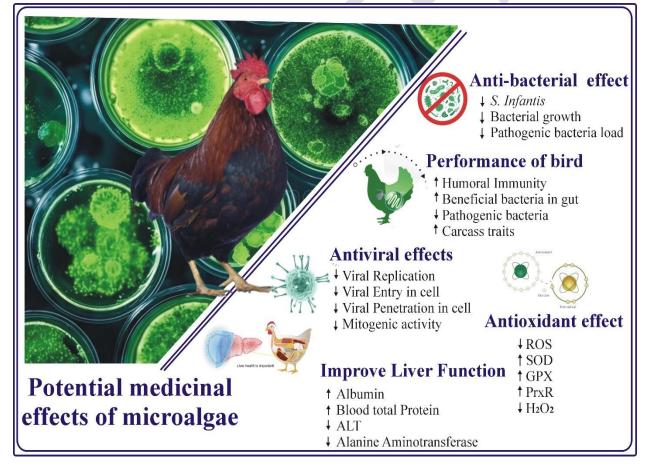


Figure 1: Illustrates the potential positive impact of microalgae on poultry.

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#### 212 Antioxidant activity

213 Prooxidants are typically **xenobiotic or external compounds**, such as certain chemicals, pollutants, and environmental toxins, they are responsible to promote the oxidative stress by 214 215 increasing the production of free radicals of reactive oxygen species (ROS) and disrupted the body ability to neutralized these toxic agent (Rahal et al., 2014). Reactive molecules of oxygen 216 217 derivatives produced during the process of ROS formation like superoxide anion  $(O_2)$ , hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and hydrogen radicles (OH) (Bergamini et al., 2004). The excessive production 218 219 of these radicals interrupt the normal physiological function at cellular level, leading to cause 220 serious damage and initiated the development of numerous pathological conditions including cancer, cardiovascular and neurodegenerative disorders (Arain et al., 2018). 221

Oxidative stress in poultry refers to the imbalance between the production of ROS and the 222 bird's ability to counteract these harmful molecules with antioxidants. Poultry birds are more 223 susceptible to oxidative stress specially under intensive farming and heat stress conditions 224 particularly in tropical and subtropical regions of the world. Several strategies have been 225 implemented to reduce the oxidative stress such as adopting modern technologies, improved 226 management, shifting of conventional forming practices into the environmental control farming 227 practices and dietary manipulation (Saeed et al., 2017a). Several studies have been reported that 228 nutritional intervention and use phytogenic feed additive (herbs and their derivative) are the 229 230 effective strategy to reduce the incidence of heat stress and mitigate the negative effects of oxidative damage (Arain et al., 2024; Changxing et al., 2018; Hassan et al., 2023; Saeed et al., 231 232 2017b). Furthermore, it was demonstrated that antioxidants supplied through external dietary 233 supplements, they play a crucial role in combating oxidative stress by scavenging ROS and

boosting the activity of antioxidant enzyme in poultry birds (Aboamer et al., 2024; Ashraf et al.,
2024).

236 Microalgae have recently attracted considerable interest for their exceptional potential as 237 a source of vital bioactive chemicals, especially antioxidants. The bioactive substances found in microalgae, such as bioflavonoids, carotenoids, vitamins C and E, biometals, and omega-3 and 6 238 239 fatty acids, are particularly effective to regulate the oxidative stress and boost the anti-oxidant defense (Fernandes et al., 2020b; Kiran and Venkata Mohan, 2021). Microalgae naturally contains 240 numerous antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), 241 glutathione peroxidase (GPX), and peroxiredoxin (PrxR) which play a crucial role in neutralizing 242 the free radicals of ROS. These antioxidants either directly scavenge harmful radicals or regenerate 243 other antioxidants, utilizing the reducing power generated through photosynthesis (Cirulis et al., 244 2013). 245

Multiple studies have documented the existence of a wide range of antioxidant chemicals in several microalgal species, such as *Chlorella, Spirulina*, and *Dunaliella*, among others (Coulombier et al., 2021). The antioxidant capabilities of microalgae stem from their capacity to neutralize free radicals, impede lipid peroxidation, and safeguard cells from damage generated by oxidative stress (Lucas et al., 2020; Saha and Murray, 2018; Sikiru et al., 2019).

Previous research has examined the antioxidant capabilities of *Chlorella vulgaris*, a green microalga recognized for its substantial nutritional benefits and medicinal attributes. *Chlorella vulgaris* exhibits significant antioxidant activity, exceeding that of widely utilized synthetic antioxidants (Sikiru et al., 2019). The antioxidant characteristics of *Chlorella vulgaris* are mainly attributed to the presence of abundant amount of carotenoids, vitamins, and several bioactive substances (Hamouda et al., 2022). Similarly, *Spirulina*, a blue-green microalga, has been 257 thoroughly studied for its antioxidant characteristics, and recognized as a substantial source of phycocyanin, a powerful antioxidant pigment that demonstrates several therapeutic effects, 258 including anti-inflammatory, neuroprotective, and anticancer properties (Alghazeer et al., 2022; 259 Mamani et al., 2020; Sikiru et al., 2019). Similarly, *Caulerpa filiformis*, a green species of algae 260 found in the Sechura Bay area of Peru, renowned for its powerful antioxidant potential, owing to 261 262 the presence of numerous bioactive compounds (Laos et al., 2023). Besides their antioxidant characteristics, microalgae have been explored for possible applications in biofuel generation, 263 wastewater treatment, and animal feed augmentation. The antioxidant capacity of microalgae is 264 well-documented, and their utilization as a source of natural antioxidants presents a promising 265 research avenue with considerable implications for enhancing the health and productivity of 266 livestock animals (Mobin and Alam, 2017). 267

#### 268 Anti-inflammatory activity

Inflammation is a key physiological process essential for the body's defense against detrimental stimuli, like pathogens, damaged cells, or irritants (Rock et al., 2009). This intricate reaction encompasses a series of cellular and molecular activities aimed at eradicating the primary source of cellular harm, removing necrotic cells and tissues affected by the initial damage, and commencing the healing process.

The anti-inflammatory characteristics of microalgae derived from their diverse secondary metabolites, have demonstrated the ability to regulate immune system and reduced inflammatory reactions (Tolba et al., 2020). Microalgal species such as *Caulerpa filiformis* exhibit significant antioxidant and anti-inflammatory properties, perhaps attributable to their elevated levels of polyphenolic chemical (Pagels et al., 2022). Glycoglycerolipids, a category of glycolipids sourced from microalgae, have emerged as potential anti-inflammatory medicines. These compounds have 280 been shown to possess several biological actions, including anti-inflammatory, antiviral, anticancer, and antibacterial properties, rendering them promising candidates for therapeutic 281 development (Conde et al., 2021). Furthermore, microalgae-derived metabolites, including 282 polyunsaturated fatty acids, carotenoids, and polysaccharides, play a pivotal role in suppressing 283 the synthesis of pro-inflammatory mediators (Choo et al., 2020). These bioactive compounds 284 inhibit the production of cytokines such as TNF- $\alpha$ , IL-1 $\beta$ , and IL-6, which are critical drivers of 285 inflammation (Á vila-Román et al., 2021). Additionally, microalgae metabolites modulate the 286 arachidonic acid pathway, reducing eicosanoid synthesis, including prostaglandins and 287 leukotrienes (Harwood, 2023). This anti-inflammatory action occurs through the downregulation 288 of NF-kB and COX-2 signaling pathways (Nabil-Adam et al., 2023; Talero et al., 2015). These 289 metabolites offer potential therapeutic applications in managing chronic inflammatory conditions 290 and promoting overall health by mitigating the inflammatory cascade effectively. In summary, the 291 anti-inflammatory potential of microalgae represents a promising research domain, with several 292 studies emphasizing the variety of bioactive chemicals generated by these microscopic entities. 293

294 Antimicrobial characteristics

Recent research indicates that microalgae contain distinctive bioactive chemicals that 295 demonstrate significant antibacterial activity, presenting a viable alternative to conventional 296 antimicrobial treatments (Ferrazzano et al., 2020). A multitude of studies has investigated the 297 antibacterial properties of various microalgal species. Cyanobacteria and microalgae are 298 299 recognized as prolific sources of diverse bioactive compounds, such as polyphenols, terpenoids, and carotenoids, which exhibit antimicrobial properties against several pathogenic bacteria 300 (Ferrazzano et al., 2020; Mamani et al., 2020). Furthermore, Chlorella, Spirulina, and Dunaliella 301 302 produce secondary metabolites, including peptides, polysaccharides, phenolics, and fatty acids,

303 that exhibit potent antimicrobial properties (Ilieva et al., 2024; Rojas et al., 2020). The primary mechanism of action behind the antimicrobial effects of these compounds is to disrupt the integrity 304 of microbial cell membranes (Stirk and van Staden, 2022). Additionally, fatty acids like 305 polyunsaturated lipids and certain peptides in microalgae destabilize bacterial and fungal 306 membranes, causing leakage of cellular contents and eventual cause cell death (Surendhiran et al., 307 308 2021). Moreover, microalgae-derived phenolics and flavonoids inhibited the activity of microbial enzymes disturb the metabolic pathways crucial to maintain the normal growth and multiplication 309 of microbial cells (Hassan et al., 2022b). Another mechanism involved to inhibit the bacterial 310 growth, microalgae produce reactive oxygen species and antioxidant compounds (carotenoids and 311 phycocyanin), that create oxidative stress within microbial cells, impairing DNA, proteins, and 312 lipid integrity leading to cause cell death (Hamidi et al., 2019). In addition to the direct anti-313 microbial effects, microalgae also modulate gut microbiota by promoting beneficial microbial 314 growth while suppressing pathogenic species (Ma et al., 2022). This competitive inhibition is 315 attributed to the production of antimicrobial metabolites and improve the immunological status 316 and gut barrier functions, thereby improving the health and proactive performance of livestock 317 318 animals.

The antibacterial capabilities of microalgae can be affected by several factors, including growing conditions, extraction procedures, and specie of organism. A study on *Spirulina platensis* shown that the supercritical fluid extracts of this microalga displayed significant antibacterial activity against gram-positive and gram-negative bacteria, as well as yeasts and fungi (Mendiola et al., 2007). Similarly another study, demonstrated that microalgal species such as *Chlorella*, *Dunaliella*, and *Nostoc* produce bioactive chemicals having with superior antibacterial characteristics (Saha and Murray, 2018). The antibacterial capabilities of microalgae signify a viable direction for additional research and development by utilizing varied bioactive moleculesgenerated by these photosynthetic organisms.

#### 328 Advantageous utilization of microalgae in poultry

#### 329 *Effect on growth performance*

Microalgae possess considerable potential to enhance growth performance and general health in poultry (Table 2). These microorganisms exhibit an extraordinary capacity to flourish under diverse environmental settings, rendering them a versatile and sustainable feedstock alternative. Multiple studies have evidenced the beneficial effects of integrating microalgae into poultry feed on several growth metrics, including body weight, feed conversion ratio, and egg production (Benedetti et al., 2018; de Morais et al., 2016).

Three kinds of microalgae, namely Chlorella vulgaris, Spirulina platensis, and Amphora 336 *coffeaformis*, were reported to positively influence body weight, growth performance, and meat 337 quality in broiler chickens (El-Bahr et al., 2020). The results indicated that microalgae 338 substantially enhanced the profiles of important fatty and amino acids, increased antioxidant levels 339 in breast muscles, and diminished microbial development and oxidative damage (El-Bahr et al., 340 2020). Another study indicated that supplementing broiler diets with microalgae, with or without 341 xylanase, markedly enhanced growth performance, increasing body weight by over 44% and 342 decreasing feed conversion ratio by 6.3%. They further suggested possible advantages for gut 343 health, evidenced by notable alterations in gut health genes and an expanded villi surface area in 344 the microalgae groups (Chaudhary et al., 2023a). Another study examined the effects of Spirulina 345 346 platensis (SP) supplementation in Fayoumi hens, demonstrating that a diet containing 1% SP markedly enhanced growth performance and immunological response. Economically, 0.25% SP 347

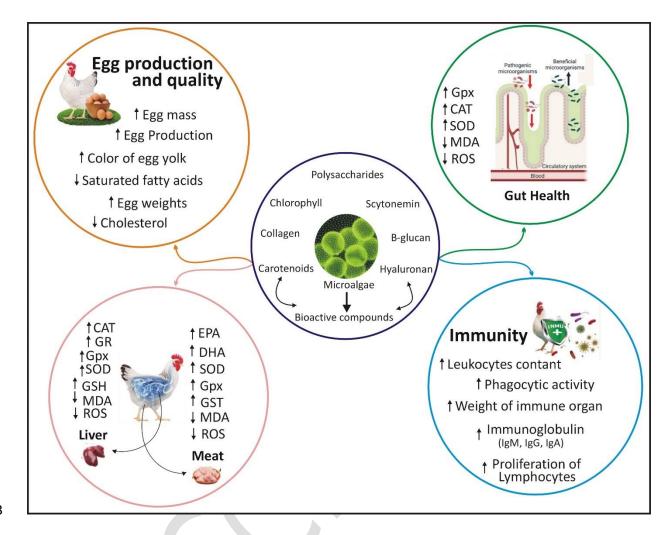
was determined to be the most cost-effective, optimizing production and profitability (Hassan et 348 al., 2022a). Moreover, the pilot investigation indicated that food supplementation with *Tetraselmis* 349 chuii and Porphyridium cruentum microalgae enhanced body weight and gut morphology in 350 broiler chicks. Treated birds exhibited increased villus-height-to-crypt-depth ratios, less thawing 351 weight loss in fillets, and higher nutrient absorption, indicating a viable approach for augmenting 352 growth performance (Šefcová et al., 2021). Supplementation of heat-stressed broilers with 3% 353 microalgae markedly increased final body weight and improved gut health indicators, 354 encompassing antioxidant, immunological, and tight-junction gene expression. Furthermore, 355 microalgae enhanced microbial diversity and fostered good gut bacteria, illustrating its potential 356 to alleviate heat stress in chickens (Chaudhary et al., 2023b). Enhancing the diets of Arbor Acres 357 chicks with DHA-enriched microalgae markedly enhanced growth performance, liver proportion, 358 359 and antioxidant status, while decreasing abdominal fat and blood cholesterol levels. Furthermore, birds that consumed microalgae exhibited enhanced deposition of good fatty acids and decreased 360 oxidative stress relative to the control group (Long et al., 2018). The published research 361 demonstrates that microalgae is a significant and dependable feed element for enhancing the 362 productive performance of chickens (Figure 2). 363

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370	Table 2: Showing the beneficial effect of microalgae on poultry birds
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Type of animal	Type, Dosage and duration	Mechanism	Overall effect	References
Broilers and Layers	Spirulina (1.5, 3.0, 6.0, or 12.0% for nine weeks	<ul> <li>↑ Cellular and Humoral Immunity,</li> <li>↑ Maintaining the Beneficial Intestinal bacteria</li> <li>↓ Pathogenic bacteria</li> <li>↑ Improving the carcass traits</li> </ul>	Improves productive performance of broilers and layers	(El-Ghany, 2020)
Broilers	1-2 % micro algae in diet for 42 days	<ul> <li>↑ superoxide dismutase</li> <li>↓ Polyunsaturated fatty acid</li> <li>↓ malondialdehyd</li> </ul>	Improves Performance, Serum composition, Carcass trait, Antioxidant status, and fatty acid	(El-Bahr et al., 2020)
Broilers	Microalgae <i>Chlorella spp.</i> 0.5- 1.0% of the diet	<ul> <li>↑ Blood total protein, Albumin</li> <li>↑ High-density lipoprotein (HDL)</li> <li>cholesterol</li> <li>↓ Alanine Aminotransferase and ALT</li> <li>↑ Blood Lymphocytes</li> <li>↑ IgA, IgG, and IgM</li> </ul>	<ul> <li>↑ Body weight gain</li> <li>↓ Feed conversion ratio</li> <li>↓ Drip loss</li> <li>↑ Liver function</li> <li>↑ Immunity</li> </ul>	(Abdelnour et al., 2019)
Cornish cross chicks	Docosahexaenoic acid (22:6 n-3)-rich 2% microalgae for day 42	<ul> <li>↑ Breast muscle</li> <li>↑ <u>Meat tenderness</u> and color</li> <li>↓ Incidence of breast muscle striping and myopathy</li> </ul>	Improves Production performance, Breast muscle quality attributes, Lipid profile, and incidence of white striping and myopathy	(Khan et al., 2021)
Isa Brown laying hens	DHA-rich microalga 0.25%, 0.5%, and 1.0% for 40 d	↑ Nutrititive value of hen's eggs	↑ omega-3 content of eggs	(Moran et al., 2019; Moran et al., 2018)
Broiler Chickens	Microalgae <i>Tetraselmis chuii</i> 20 g/kg of feed (2%)	$\downarrow$ S. Infantis caecal load	Antibacterial	(Corrales- Martinez et al., 2022)
Broiler chickens	Microalgal docosahexaenoic acid 2% for 6 weeks	<ul> <li>↑ Body weight gain</li> <li>↓ Cholesterol and triglyceride</li> <li>concentrations in plasma, liver, breast,</li> <li>and thigh</li> <li>↑ Improve tibia breaking strength</li> <li>↑ Total bone volume and bone mineral</li> </ul>	Improves growth performance, tissue lipid profiles, and tibia characteristics	(Kalia et al., 2023)

Fayoumi	Microalgae (Spirulina	↑ serum total protein	Improves Growth Performance,	(Hassan et al.,
Broilers	platensis)	↑ globulin	Ingestive Behavior, Hemato-	2022a)
	1% for 8 weeks	↓ serum cholesterol	Biochemical Parameters, and	
		↑ lymphocyte percentage.	Economic Efficiency	
Broiler and	Microalgae	↑ Weight, Feed intake and digestibility	Enhances the nutritive value	(Esakkimuthu et
Layer birds		↑ Increase meat quality	of poultry meat an eggs	al., 2023)
		↑ Improve organoleptic quality of meat		
		↑ Digestibility		
		↑ Egg shell thickness and Egg weight		
Broiler and	Spirulina platensis (0.25–	↓ Oxidative stress	Positive impact on performance	(Abdel-Wareth et
Layer birds	1.0%) and <i>Chlorella vulgaris</i>	↑ Immune response, Growth rates, feed	metrics	al., 2024)
	(1.55  g/kg)	conversion ratios		
		$\uparrow$ Carcass quality, and meat attributes		
<b>N</b> 11		↑ Egg production and Egg quality		
Broiler	S. platensis (10%) &	$\uparrow$ GSH, GPX and SOD	Improves antioxidant status	(Abdel-Wareth et
	Haematococcus pluvialis			al., 2024; Jubie et
D 11	(0.004%)	A D 1 : (2.70/)		al., 2012)
Broilers	Chlorella (vulgaris spp.; CLV)	$\uparrow$ Body weight gain (2.7%)	Improves growth and health of	(Abdelnour et al.,
	(0.5-1.0% of the diet)	$\uparrow$ Feed conversion ratio (lowered by	birds	2019)
		2.8%)		
		↑ Meat color and breast muscle weight (20.1%)		
		$\downarrow$ Drip loss (2.26%) from breast muscle		
		$\uparrow$ IgA (29.7%), IgG (69.1%), and IgM		
		(32.3%)		
Ross-308	1 g/kg diet of <i>Chlorella</i>	$\uparrow$ Essential fatty and amino acids $\downarrow$	Enhance performance and meat	(El-Bahr et al.,
broiler chicks	vulgaris (CV), Spirulina	Microbial growth in breast muscle	quality in broiler chickens	(El Dall et al., 2020)
er sher enters	platensis (SP), and Amphora	$\downarrow$ malondialdehyde (MDA) and protein		
	coffeaformis (AC)	carbonyl (PC) levels, cooking loss &		
		aerobic plate count (APC)		
		↑ Superoxide dismutase (SOD)		
		activities in breast muscle		



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Figure 2: Illustrating the advantageous impact of microalgae on avian species.

# 375 *Effects on meat production and quality attributes*

Integrating microalgae into poultry feed offers the significant benefit of potentially enhancing the nutritional quality of the meat (Martins et al., 2021). In addition, microalgae containing carotenoids and other bioactive compounds that can enhance the color and overall quality of poultry meat. Studies have shown that adding  $\omega$ -3-rich microalgae (*Schizochytrium* JB5) to broiler diets improved the fatty acid composition of breast meat by increasing levels of DHA, oleic acid, and  $\omega$ -3 fatty acids, while decreasing the ratio of  $\omega$ -6 to  $\omega$ -3 and the saturated fat content (Gatrell et al., 2015). Despite these advantages, the growth performance and blood parameters of 383 the poultry were not impacted by the inclusion of microalgae in their diet (Yan and Kim, 2013). Furthermore, a research examined the impact of various microalgae species on broiler chickens. It 384 demonstrated that Spirulina platensis notably enhanced the crucial fatty and amino acid 385 compositions while decreasing microbial growth in the breast muscle (Amer et al., 2024). In 386 addition, all microalgae types decreased the markers of oxidative stress and enhanced meat quality 387 388 by reducing melondialdehyde and protein carbonyl levels, as well as cooking loss (Varzaru et al., 2024). These results indicated that microalgae have the potential to enhance broiler performance 389 and meat quality. On the contrary, a recent study on feeding trials examined the impact of 390 incorporating microalgal biomass into broiler diets. The results revealed that substituting 5-10% 391 of soybean meal with three different microalgal strains led to a reduction in breast weight 392 percentages, while not impacting the overall growth performance or meat quality (Cabrol et al., 393 2022). It is worth noting that the combined use of microalgae did not prevent the loss of breast 394 weight and also influenced gene expressions associated with muscle hypertrophy and atrophy, 395 indicating complex effects on protein metabolism (Sun et al., 2024). In addition, Liu and his 396 collogues studied that laying hens fed diet supplemented with DHA-rich microalgae 397 (Aurantiochytrium sp.) led to a significant enhancement in the n-3 polyunsaturated fatty acid levels 398 399 and lipid health indicators of breast and thigh meat (Liu et al., 2022). The enrichment did not affect meat quality aspects such as tenderness or color, however slight increase in lipid oxidation was 400 observed in cooked and stored meat (Liu et al., 2022). Furthermore, incorporating Hermetia 401 402 *illucens* larval meal or *spirulina* into the poultry diet, showed slightly increase in color and saturated fat content (Gkarane et al., 2020). Similarly, another study reported that spirulina 403 404 supplementation significantly improved the red color of chicken and enhance the umami flavor of 405 meat after storage under oxygen-rich environment (Altmann et al., 2020). Furthermore, dietary

406 incorporation of alternative protein sources such as Spirulina and Hermetia meal to poultry feed demonstrates encouraging outcomes for the quality of meat, especially when combined with 407 408 advanced packaging techniques (Altmann, 2020). Moreover, broiler chicken diets enriched with DHA-rich microalgae and methionine led to a significant decrease in the occurrence of breast 409 muscle white striping and myopathy, along with an improvement in the muscle's n-3 fatty acid 410 411 content (Khan et al., 2021). Nevertheless, the incorporation of methionine resulted in increased meat tenderness and changes in its color, suggesting possible trade-offs in meat quality (Khan et 412 al., 2021). Conclusively, incorporating microalgae into poultry feeds has revealed a substantial 413 potential and great promise in improving efficiency and supporting the creation of premium 414 poultry goods. 415

# 416 *Effect on egg production*

Exploring the use of microalgae in poultry feed has been considered to enhance egg 417 production, as these tiny organisms can provide important nutrients like proteins, lipids, and 418 pigments (Fernandes et al., 2020a). Chlorella vulgaris and Spirulina platensis are an examples of 419 microalgae that contain various bioactive compounds capable of positively impacting the health 420 421 and performance of laying hens. Incorporating microalgae into poultry diets not only boosts egg 422 production but also enhances the nutritional content and sensory characteristics of the eggs (Liu et 423 al., 2020). The presence of abundant amount of carotenoids and fatty acids in microalgae biomass, 424 significantly improve the eggs' color, texture, and overall quality, making them more attractive to consumers (Kalia and Lei, 2022). 425

Research has shown that integrating microalgae into chicken feed can cause a notable rise
in egg output. The active components found in microalgae, like carotenoids and polyunsaturated
fatty acids, can boost the metabolic functions of the birds, improving nutrient absorption and

429 energy effectiveness (Vlaicu et al., 2023). Additionally, superior nutritional value of microalgae helps to boost growth performance and optimize the development of reproductive system of laying 430 hens leading to increased egg production (Martins et al., 2021). Preceding studies have shown that 431 the active components in microalgae, like carotenoids and polyunsaturated fatty acids, can boost 432 the metabolic functions of the birds, improving nutrient absorption and energy effectiveness 433 434 (Emam et al., 2024). In addition, the supplementation of *Spirulina platensis* (SP) to the feed for layers resulted in noticeable enhancements in egg production, egg quality, and indicators of blood 435 health (Abbas et al., 2022). The most effective level of SP inclusion was found to be 9%, with 4.7% 436 of soybean meal being replaced for every 1% of SP, indicating its viability as a cost-effective 437 dietary alternative (Abbas et al., 2022). 438

During a 32-week experiment, the addition of graded levels of All-G-Rich<sup>™</sup> microalgae 439 in layer diets resulted in a notable elevation of docosahexaenoic acid (DHA) levels in egg yolks, 440 with no impact on production performance, egg weight, or shell quality (Ao et al., 2015; Keegan 441 et al., 2019). Simultaneously, dietary supplementation of microalgae oil or fish oil to the diet of 442 laying hens raised DHA levels and n-3 PUFA in eggs, encouragingly both sources were found 443 equally effective (Kaur et al., 2024). Nevertheless, eggs produced from microalgae oil had a better 444 taste and were more generally accepted, indicating that it could be a more favorable choice for 445 446 enriching DHA compared to fish oil (Feng et al., 2020). Likewise, the addition of omega-3-rich microalgae such as Phaeodactylum tricornutum, Isochrysis galbana, and Chlorella fusca to the 447 diets of laying hens resulted in a significant increase in n-3 long-chain polyunsaturated fatty acids 448 449 (LC-PUFA) in egg yolk, particularly docosahexaenoic acid (Lemahieu et al., 2013). It is important to note that *Chlorella fusca* mainly boosted  $\alpha$ -linolenic acid, while *Phaeodactylum* and *Isochrysis* 450 451 demonstrated the highest LC-PUFA efficiency, also improving yolk color through carotenoid

transfer (Lemahieu et al., 2013). Whereas another study demonstrated that enhancing the diets of Japanese quail with *Spirulina* and *Dunaliella* (SD) resulted in better body weight, feed conversion ratio, and fertility, along with remarkable decreases in lipid profile indicators (Abd El-Hack et al., 2024). Despite minimal changes in egg production and quality, the use of SD improved the function of the liver and kidneys, as well as the quails' immunological response (Abd El-Hack et al., 2024). Convincingly, it was noted that adding microalgae to the diet not only enhances egg production but also elevates the nutritional quality of poultry eggs.

# 459 Potential drawbacks of adding microalgae to poultry feed

The widespread use of microalgae in poultry diets has been impeded by various constraints 460 that require significant attention. A key obstacle is the inconsistent quality and makeup of 461 microalgae, which can be impacted by several factors like cultivation conditions, harvesting 462 methods, and processing techniques (Martins et al., 2021). Henceforth, formulating poultry diets 463 that consistently meet the birds' specific nutritional requirements can be challenging due to the 464 variability in ingredients. Additionally, the high expense of producing and processing microalgae 465 may render it economically impractical for large-scale commercial poultry operations, particularly 466 when compared to traditional protein sources like soybean meal and fishmeal (Ciani et al., 2021; 467 Sanjari et al., 2018). 468

Microalgae's potential to cause unwanted sensory characteristics in poultry products, such as off-flavors or texture alterations, presents another limitation (Espinosa-Ramírez et al., 2023). This limitation can significantly hinder consumer acceptance, especially for eggs and meat, which are highly sensitive to flavor and quality. Furthermore, incorporating microalgae into poultry diets can be difficult due to the requirement for specialized feed processing equipment and the 474 possibility of decreased feed intake or digestibility in the birds. Despite these constraints, 475 continuous research and technological progress could potentially surmount the obstacles to the 476 extensive utilization of microalgae in poultry nutrition. Innovative methods of cultivation, such as 477 utilizing wastewater or industrial waste streams as nutrient sources, may aid in cutting production 478 expenses and enhancing the sustainability of feeds based on microalgae.

#### 479 Conclusion

Microalgae holds significant potential as a nutraceutical and alternative protein source for 480 the poultry industry, offering a sustainable, nutrient-rich solution to enhance poultry health and 481 productivity. It is widely accepted that microalgae are a valuable source of essential nutrients, such 482 as proteins, omega-3 fatty acids, vitamins, and antioxidants, which help to boost immune status, 483 enhance nutrient utilization, reduce oxidative stress and prevent infectious disorders, thereby 484 improves health status and overall productive performance. Furthermore, microalgae contribute to 485 486 gut health by modulating gut microbiota and improving intestinal integrity, thus decreasing the incidence of diseases. Additionally, inclusion of microalgae in poultry diets significantly enhance 487 quality of meat and egg by improving the nutrient content and reducing undesirable fatty acids, 488 489 these benefits make them a valuable feed additive for modern poultry to ensure the consistent 490 productivity.

#### 491 **Declarations**

#### 492 Author Statement

493 LXZ, YNJ and JAB: Conceptualization and writing—original draft SH and XYS: methodology
494 and software. FHL and LNM: writing—review and editing. PFW and CXL resources, validation,

495 review and editing. All authors have visualized, read and agreed for the submission and publication

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