Avian Pathology

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/cavp20

Bioefficacy of dietary inclusion of *Nannochloropsis oculata* on *Eimeria* spp. challenged chicks: clinical approaches, meat quality, and molecular docking

Marwa I. Abdel Haleem, Hanem F. Khater, Shimaa N. Edris, Hanan A.A. Taie, Samah M. Abdel Gawad, Nibal A. Hassan, Ali H. El-Far, Yasmeen Magdy & Sawsan S. Elbasuni

To cite this article: Marwa I. Abdel Haleem, Hanem F. Khater, Shimaa N. Edris, Hanan A.A. Taie, Samah M. Abdel Gawad, Nibal A. Hassan, Ali H. El-Far, Yasmeen Magdy & Sawsan S. Elbasuni (2024) Bioefficacy of dietary inclusion of *Nannochloropsis oculata* on *Eimeria* spp. challenged chicks: clinical approaches, meat quality, and molecular docking, Avian Pathology, 53:3, 199-217, DOI: 10.1080/03079457.2024.2312133

To link to this article: https://doi.org/10.1080/03079457.2024.2312133

AVIAN PATHOLOGY
2024, VOL. 53, NO. 3, 199–217
https://doi.org/10.1080/03079457.2024.2312133

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Bioefficacy of dietary inclusion of *Nannochloropsis oculata* on *Eimeria* spp. challenged chicks: clinical approaches, meat quality, and molecular docking

Marwa I. Abdel Haleem [a], Hanem F. Khater[b], Shimaa N. Edris [c], Hanan A.A. Taie [d], Samah M. Abdel Gawad [b], Nibal A. Hassan[e,f], Ali H. El-Far[g], Yasmeen Magdy[h] and Sawsan S. Elbasuni [a]

[a]Department of Avian and Rabbit Diseases, Faculty of Veterinary Medicine, Benha University, Benha, Egypt; [b]Department of Parasitology, Faculty of Veterinary Medicine, Benha University, Benha, Egypt; [c]Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Benha University, Benha, Egypt; [d]Plant Biochemistry Department, National Research Centre, Dokki, Egypt; [e]Department of Biology, Animal Reproduction Research Institute, Pathology Department, Giza, Egypt; [f]College of Science, Taif University, Taif, Saudi Arabia; [g]Department of Biochemistry, Faculty of Veterinary Medicine, Damanhour University, Damanhour, Egypt; [h]Department of Anatomy and Embryology, Faculty of Veterinary Medicine, Benha University, Benha, Egypt

ABSTRACT

Although anticoccidial drugs have been used to treat avian coccidiosis for nearly a century, resistance, bird harm, and food residues have caused health concerns. Thus, *Nannochloropsis oculata* was investigated as a possible coccidiosis treatment for broilers. A total of 150 1-day-old male Cobb broiler chicks were treated as follows: G1-Ng: fed a basal diet; G2-Ps: challenged with *Eimeria* spp. oocysts and fed basal diet; G3-Clo: challenged and fed basal diet with clopidol; G4-NOa: challenged and fed 0.1% *N. oculata* in diet, and G5-NOb: challenged and fed 0.2% *N. oculata*. Compared to G2-Ps, *N. oculata* in the diet significantly ($P < 0.05$) decreased dropping scores, lesion scores, and oocyst shedding. Without affecting breast meat colour metrics, *N. oculata* improved meat quality characters. At 28 days of age, birds received 0.2% *N. oculata* had significantly ($P < 0.05$) higher serum levels of MDA, T-SOD, HDL, and LDL cholesterol compared to G2-Ps. Serum AST, ALT, and urea levels were all decreased when *N. oculata* (0.2%) was used as opposed to G2-Ps. Histopathological alterations and the number of developmental and degenerative stages of *Eimeria* spp. in the intestinal epithelium were dramatically reduced by 0.2% *N. oculata* compared to G2-Ps. Molecular docking revealed a higher binding affinity of *N. oculata* for *E. tenella* aldolase, EtAMA1, and EtMIC3, which hindered glucose metabolism, host cell adhesion, and invasion of *Eimeria*. Finally, *N. oculata* (0.2%) can be used in broiler diets to mitigate the deleterious effects of coccidiosis.

ARTICLE HISTORY
Received 18 August 2023
Revised 3 January 2024
Accepted 20 January 2024

KEYWORDS
Broilers; marine microalgae; lesion scoring; TEM; oxidative stress biomarkers; biochemical parameters

Introduction

The amount of poultry meat produced over the next 10 years is expected to equal half of global bird protein production to fill the food gap caused by expanding populations (de Mesquita Souza Saraiva *et al.*, 2022). Poor practices of some poultry farmers have impaired immune function and spread disease, slowing development and causing substantial economic losses worldwide. Avian coccidiosis is a highly pathogenic and contagious protozoan disease spreading mainly in hot and humid environments (Moryani *et al.*, 2021). Seven highly host- and site-specific *Eimeria* species infecting domestic chickens (*Eimeria acervulina, Eimeria brunetti, Eimeria maxima, Eimeria mitis, Eimeria necatrix, Eimeria praecox*, and *Eimeria tenella*) are recognized as globally ubiquitous (Reid *et al.*, 2014; Saeed & Alkheraije, 2023). Birds become infected after ingestion of sporulated oocysts. Clinical signs of the disease include dysentery, bloody diarrhoea, enteritis, poor growth, drooped wings, emaciation, and decreased production (Habibi *et al.*, 2016; Saeed & Alkheraije, 2023). Furthermore, coccidiosis can result in concurrent infection with bacterial, fungal, and viral pathogens, putting chicken flocks at risk of morbidity and mortality (Moryani *et al.*, 2021).

Coccidiosis can be successfully controlled by the use of vaccinations and anticoccidial medications (Kadykalo *et al.*, 2018; Akanbi & Taiwo, 2020), such as amprolium, clopidol, or halofuginone, which directly affect the metabolism of coccidian species (Kalkal *et al.*, 2021). The extensive use and abuse of medications over the past 40 years has caused various detrimental impacts, including development of drug resistance (Li *et al.*, 2005; Zaheer *et al.*, 2022), toxic effects on bird byproducts when given without considering the suitable duration or dose (Sundar *et al.*, 2017), and food residues harming human health (Mund *et al.*, 2017).

Consumers and poultry farmers are currently seeking natural, safe, and environmentally friendly products for treating coccidiosis in broiler chickens (Abdel Haleem, Abdelnaser *et al.*, 2019; Adjei-Mensah & Atuahene, 2023; Ali *et al.*, 2014, 2015) due to their ability to enhance well-being, reduce the likelihood

CONTACT Marwa I. Abdel Haleem marrwa.mahmoud@fvtm.bu.edu.eg

of specific chronic illnesses, and provide health advantages that extend beyond nutritional value (Durmaz, 2007). Many natural materials, such as herbal extracts, essential oils, symbiotic, and organic acids, have proven their effectiveness as alternatives to chemical anticoccidial drugs in preventing coccidiosis. Microalgae are single-celled, eukaryotic, or prokaryotic primary microorganisms with photosynthetic activity that are used as natural feed additives (Paterson *et al.*, 2023). They contain a variety of amino acids, essential fatty acids, carbohydrates, and lipids, as well as microelements such as minerals, vitamins, polyphenols, flavonoids, carotenoids, and natural antioxidant compounds (Saadaoui *et al.*, 2021; El-Sayed *et al.*, 2022). Algae could exert protective effects against *Eimeria* challenge as a result of improving intestinal integrity and systemic immune responses (Fries-Craft *et al.*, 2021). Microalgae of the genus *Nannochloropsis* have cells ranging in size from 2–4 µm to 3–41.5 µm (Al-Hoqani *et al.*, 2017) and were initially cut off from the coast of Scotland (Ribeiro *et al.*, 2020). There are currently seven known *Nannochloropsis* species, six of which have marine habitats (such as *Nannochloropsis oculata*), and one of which is found in both fresh and brackish waters (Al-Hoqani *et al.*, 2017). Several studies have shown that *Nannochloropsis oculata* (*N. oculata*) has beneficial effects on the palatability and digestion of food; moreover, it has detoxifying, antioxidant, anti-inflammatory, anticancer, and immune stimulant properties (Derner *et al.*, 2006; Colla *et al.*, 2007). *N. oculata* also improved growth, nonspecific immunity, intestinal histomorphometry and resistance to bacterial pathogens and upregulated interleukin-10 gene expression after being added to the diet of marine animals at a rate of 5% or 8% (Md *et al.*, 2018; Abdelghany *et al.*, 2020). Additionally, *N. oculata* is an excellent alternative to conventional protein and eicosapentaenoic acid in animals diets (Lacaz-Ruiz, 2003; Becker, 2007; Abd El-Hack *et al.*, 2023)

Accordingly, this study investigated the potential benefits of incorporating *N. oculata* into chicken feed as an alternative to anticoccidial medication, and its efficacy on the clinical picture, performance, meat quality, serum biochemistry, and intestinal histomorphometry of broiler chicks during coccidiosis challenge.

Materials and methods

Ethical statement

All procedures involving birds complied with the guidelines established by the Animal Welfare Committee at Benha University's Faculty of Veterinary Medicine (BUFVTM 03-02-22).

Birds and experimental design

A total of 150 1-day-old male Cobb broiler chicks were purchased from El-Huda Company (Al-Dier, Qalyubia, Egypt) for hatching. All chicks were fed a broiler starter ration from 0-10 days, a grower ration from 11–24 days, and a finisher ration from 25–35 days (NRC, 1994) (Table 1). Food and water were provided *ad libitum*. The chicks were raised on floor pens bedded by wood shavings in specialized facilities at the Faculty of Veterinary Medicine, Benha University, Egypt, following the best management practices guidelines. The bedding material was covered by plastic sheets from 14–24 days of age to facilitate dropping scoring and collection for oocysts counting. The chicks were provided with all necessary living conditions throughout the experiment, including heaters and humidity.

The chicks were weighed and randomly divided into five groups (six chicks/replicate; five replicates/group) as follows: G1-Ng: fed basal diet without challenge (control negative); G2-Ps: fed basal diet and challenged with *Eimeria* spp. oocysts (control positive); G3-Clo: challenged and fed a basal diet with clopidol (Atco-pharma, Quisna, Egypt) at a rate of 0.9 g/kg following the recommendations of the manufacturer; G4-NOa: challenged and fed a basal diet with *N. oculata* at a rate of 0.1% (1 g/kg diet); and G5-NOb: challenged and fed a basal diet with *N. oculata* at a rate of 0.2% (2 g/kg diet).

Table 1. Composition of basal diets.

	%		
Item	Starter (0–10 d)	Grower (11–24 d)	Finisher (25–35 d)
Yellow corn	56.92	60.22	65.44
Soybean meal 46%	35	33.50	28.20
Corn gluten meal	2.3	–	–
Vegetable oil	0.95	2	2.30
Sodium chloride	0.22	0.26	0.24
DL-Methionine	0.36	0.34	0.34
L-Lysine	0.40	0.22	0.26
Limestone	1.67	1.55	1.44
Mono-calcium phosphate	1.20	1.025	0.90
Sodium bicarbonate	0.30	0.20	0.23
Vit. and mineral premix	0.30	0.30	0.30
Calculated nutritional value			
Crude protein %	23.03	21.02	19.01
MEn (Kcal/kg)	3009.47	3102.61	3198.84
Crude fat %	3.51	4.56	4.99
Lysine %	1.40	1.20	1.10
Lysine digestible %	1.28	1.09	1.00
Methionine %	0.69	0.63	0.60
Methionine digestible %	0.66	0.60	0.58
Methionine + cystine %	1.03	0.95	0.90
Methionine + cystine digestible %	0.94	0.87	0.83
Threonine %	0.95	0.88	0.75
Threonine digestible %	0.83	0.77	0.65
Calcium %	0.96	0.88	0.80
Avail. phosphorus %	0.49	0.44	0.40
Chloride %	0.24	0.23	0.23
Sodium %	0.17	0.16	0.16
Potassium %	0.88	0.85	0.76

Experimental challenge with coccidiosis

The *Eimeria* oocysts used in the current study were collected from commercial broiler houses at Qalyubia Governorate, Egypt (30° 24′ 36″ N, 31° 12′ 36″ E). The oocysts were separated through sieving and sedimentation techniques (Longstaffe, 1984). The culture was inspected under a microscope to distinguish the types of existing oocysts based on morphological characteristics (Al-Gawad *et al.*, 2012). Five *Eimeria* spp. were identified: *E. tenella* (65%), *E. acervulina* (10%), *E. maxima* (10%), *E. necatrix* (10%) and *E. mitis* (5%). Preliminary laboratory challenge of broiler chicks validated the culture's capacity to produce a characteristic picture of coccidiosis (Swayne *et al.*, 2020), and the oocysts were found to be viable. The suspension of fresh sporulated oocysts was stored in a 2.5% potassium dichromate solution in a refrigerator (4°C) until use. On day 14 of the experiment, the number of sporulated oocysts in the culture was counted and identified microscopically. Fresh sporulated oocysts (5×10^3 oocysts/chick) were administered intracrop to groups 2, 3, 4, and 5 with a volume of 1.5 ml (Pop *et al.* 2019). The negative control group received 1.5 ml of normal saline solution as an inoculant.

Nannochloropsis oculata *preparation and evaluation*

N. oculata powder was purchased from the National Research Center, Dokki, Giza, Egypt. The total phenolic content of *N. oculata* powder was determined (Makkar *et al.*, 1997). Its total flavonoid content was estimated (Ordoñez *et al.*, 2006). Standardization in methanol with quercetin (1 mg/ml) yielded a linear relationship ($y = 0.0071x$; $R^2 = 0.9979$). Quercetin equivalents (mg QE/g of dry mass) were used to quantify the total flavonoid content because it is a widely used reference ingredient. Each experiment was performed three times to ensure accuracy.

The carotenoid content of *N. oculata* was estimated spectrophotometrically (Lichtenthaler & Buschmann, 2001), and its condensed tannins were also determined (Glick & Joslyn, 1970).

Clinical parameters

Bird droppings were scored from 0-4 (Morehouse and Barron, 1970) from 0-10 days post-infection (dpi) based on the consistency of the faeces and the presence of mucus and/or blood as follows: a score of 0 indicated normal colour and consistency; a score of 1 indicated mild mucoid to watery droppings; a score of 2 indicated moderate mucoid to watery droppings with abnormal colour; a score of 3 indicated all watery, bloody tinged droppings; and a score of 4 indicated watery bloody droppings. Macroscopic lesion scores of the duodenum, jejunum, and cecum were also observed and recorded at 10 dpi (Ali *et al.*, 2014).

Oocyst shedding was estimated in fresh faecal samples (five samples per group) collected from 3 and 10 dpi. The faecal samples were stored separately in airtight plastic bags, homogenized, and kept at 4°C. The oocyst count was calculated using a McMaster counting chamber and represented as oocysts per gram faeces (OPG) (Bortoluzzi *et al.*, 2018).

Data on feed intake (FI), body weight (BW), and weight gain (WG) were recorded from day zero to ten dpi. The feed conversion ratio (FCR) per chick was determined (Abdel Haleem, Hassan *et al.*, 2019) as follows:

$$\text{FCR} = \frac{\text{Average FI (g) per chick in period}}{\text{Average WG (g) per chick in the same period}}$$

The following formula (Abdel Haleem, Hassan *et al.*, 2019) was used to obtain the mortality rate:

$$\text{Mortality rate (\%)} = \frac{\text{Number of deaths in a specified period}}{\text{Total population during that period}} \times 100$$

Meat quality characteristics

Fatty acid profile

Five breast muscles *(Musculus pectoralis superficialis)* from each group were dissected. The fatty acid profile of meat was determined using a colourimetric method according to a previously described technique (El-bahr *et al.*, 2021) with some modifications. In brief, samples of chicken breast meat were homogenized and centrifuged to produce a tissue homogenate (1792 × *g* for 15 min). The obtained supernatant was then used to determine the total lipids and total cholesterol using a commercial kit (Stanbio Laboratory Company; Boerne, TX, USA). The total lipid content was extracted from breast flesh samples by centrifuging them at 1792 × *g* for 10 min after vortexing for 2 min in chloroform: methanol (2:1; v/v) solution. Fatty acid methyl esters (FAMEs) were produced from the supernatant using a methanol: sulphuric acid mixture (95:5; v/v) and hexane after the esterification reaction. Gas chromatography (GC; Agilent Technologies, Santa Clara, CA, USA) with an SP2330 column (30 mm × 0.32 mm × 0.2 µm film thickness; Supelco Analytical, St. Louis, MO, USA) and flame ionization detector was used to analyze the FAME hexane extract using a temperature gradient programme, hydrogen as the carrier gas, and a split model. The retention time of the fatty acid standard (Cat. No. 24073, Sigma–Aldrich, St. Louis, MO, USA) was compared to the retention time of the

FAME peaks in Hewlett-Packard ChemStation software (Agilent Technologies Inc.).

Breast meat physicochemical properties

The pH of the chicken breast muscles from each treatment was measured in triplicate using a pH meter (Jenway 3510 pH meter, Cole-Parmer, St. Neots, UK) 24 h *post-mortem* (ultimate pH (pHu)). The measurements were made with a portable pH metre equipped with a glass electrode. To determine the water holding capacity (WHC), the low-speed centrifugation method was used. Briefly, 5 g of intact breast muscle was trimmed and weighed and then placed in a Falcon tube with glass beads at 10,000 × *g* at 5°C for 20 min. After centrifugation, the trimmed breast meat was removed immediately, dried with filter paper and weighed again. The percentage of muscle weight loss after centrifugation was calculated for the WHC (Honikel & Hamm, 1994). The drip and cooking losses (DL&CL) were evaluated (Honikel, 1998). In a closed plastic container, a small cube of chicken breast muscle (60 ± 2 g) was placed over a grid. After 24 and 48 h of storage at 4°C, the drip loss (DL_{24} and DL_{48}) was calculated as a percentage of weight loss during this period. The thawing loss of chicken breast samples was measured (Sun *et al.*, 2019). The frozen meat samples were thawed in the refrigerator at 4°C and then dried using filter paper to remove the surface water. The thawing loss was calculated from the weights of the breast meat samples before (M0) and after (MT) thawing (Xia *et al.*, 2009):

$$\text{Thawing loss (\%)} = (M0 - MT)/M0 \times 100$$

For cooking loss determination (Xia *et al.*, 2012), individually weighed chicken breast samples from each treatment were wrapped in plastic bags and cooked in an 80°C water bath for 1 h until the internal temperature reached 75°C. Then, the samples were suddenly cooled in crushed ice to 5°C and carefully blotted with filter paper before reweighing. Cooking loss was determined by weighing the samples before (M0) and after (Mc) cooking:

$$\text{Cooking loss (\%)} = (M0 - MC)/M0 \times 100$$

The Warner–Bratzler shear force (WBSF) was then calculated using 3343 Universal Test System Mono columns (Instron, Pune, India) (Silva *et al.*, 2017). High performance liquid chromatography (HPLC) (Agilent HP 1200 series apparatus) was used to measure malondialdehyde (MDA) following protocols outlined earlier (El-Bahr *et al.*, 2020).Using a Chroma Meter CR-410 (Konica Minolta Sensing INC., Osaka, Japan), the colour of the meat was measured using the CIE lightness (L*), redness (a*), and yellowness (b*) systems 24 h after slaughter. Meat samples were scanned at five separate sites for colour measurement.

Clinicopathological parameters

Blood samples (five/group) were collected at 14, 21 and 28 days of age from the right jugular vein of the neck using sterile needles to separate serum, which was used for further analyses.

Oxidative stress biomarkers were determined, such as antioxidant enzymes in serum (Biodiagnostic kit's guidelines), serum MDA using a colorimetric technique (Kei, 1978), serum total superoxide dismutase (T-SOD) (Nishikimi *et al.*, 1972) and catalase enzyme (Aebi, 1984).

Serum biochemical parameters were evaluated, such as serum aspartate transaminase (AST) and alanine transaminase (ALT) (Young, 1997), high-density lipoprotein cholesterol (HDL-C) (Lopes-Virella *et al.*, 1977), low-density lipoprotein cholesterol (LDL-C) (Wieland & Seidel, 1983), total cholesterol (Richmond, 1973), triacylglycerol (Fossati & Prencipe, 1982), and creatinine and urea (Tovar *et al.*, 2002).

Transmission electron microscopy (TEM)

Five caeca per group were collected and preserved in glutaraldehyde at 10 dpi for electron microscope examination. The stained grids were examined and photographed using a JEOL, JEM-1400-EXELECTRON MICROSCOPE at Ain Shams University's Central Laboratory using uranyl acetate and lead citrate (Ball *et al.*, 2014).

Molecular docking

Molecular docking of clopidol, as a reference drug, and *N. oculata* bioactive compounds with *E. tenella* aldolase, *E. tenella* apical membrane antigen 1 (EtAMA1), and *E. tenella* microneme protein 3 (EtMIC3) were assessed after retrieval of the three-dimensional structures of ALD, EtAMA1, and EtMIC3 from AlphaFold (https://alphafold.ebi.ac.uk/) protein structure databases, while clopidol and *N. oculata* bioactive compounds were retrieved from LOTUS: Natural Products Online (https://lotus.naturalproducts.net/) and PubChem (https://pubchem.ncbi.nlm.nih.gov/) databases. Three-dimensional structures of *E. tenella* aldolase, EtAMA1, and EtMIC3 were prepared and subjected to molecular docking with *N. oculata* bioactive compounds using Molecular Operating Environment (MOE 2015.10, Chemical Computing Group, Montreal, QC, Canada) software with the induced fit method. Additionally, MOE was used to determine and visualize the protein–ligand interactions (Vilar *et al.*, 2008).

Table 2. Chemical characterization of dried *Nannochloropsis oculata* powder.

Compounds	Total phenols (mg gallic/g D.W.)	Total flavonoids mg Quercetin/gD.W.	Total carotene (mg/g)	Condensed tannins (mg tannic/g D.W.)
	39.59±0.17	8.41±0.17	3.85±0.09	0.45±0.04

Values are given as the mean ($n = 5$) ± standard deviation (absolute value).

Statistical analysis

Analysis of variance (general linear model) was performed on experimental data using the statistical application SPSS version 20.0 for Windows (SPSS, Inc., Chicago, IL, USA). Tukey's *post-hoc* test ($P < 0.05$) was conducted to determine the significance level between the experimental groups.

Results

Phytochemical analyses

The extract of *N. oculata* contained many active compounds, such as total phenols (39.59 mg gallic acid/g), flavonoids (8.41 mg quinstine/g), carotenoids (3.85 mg/g), and condensed tannins (0.45 mg tannic/g) (Table 2).

Clinical evaluation

The positive control (G2-Ps) recorded a remarkable ($P < 0.05$) increase in scores of droppings (0.33–3.00) when compared to the G1-Ng (0.00). The highest score of droppings was recorded in G2-Ps (2.33 and 3.00, respectively) in comparison to the other challenged groups. A profound ($P < 0.05$) decline in dropping score was recorded in G3-Clo at 5 dpi (0.00) and in groups G3-Clo, G4-NOa, and G5-NOb at 6 dpi (1.00, 0.33, and 1.00, respectively) when compared to G2-Ps. G4-NOa recorded a numerical ($P > 0.05$) decline (0.33) in scoring at 6 dpi (Table 3 and Figure 2).

Microscopic examination of the droppings revealed the presence of coccidial oocysts from 4-7 dpi (Figure 1). No oocysts were recorded in the droppings of G1-Ng. The OPG in G2-Ps dramatically increased from that of G1-Ng ($P < 0.05$). A nonsignificantly ($P > 0.05$) number of oocysts was observed in the treated groups compared to G1-Ng. The OPG in the G5-NOb and G3-Clo groups was significantly lower than that of the G2-Ps at 5, 6, and 7 dpi ($P < 0.05$; 4351, 4313.3, and 689.00, respectively). On day 7, G4-NOa had significantly fewer oocysts (31.00) than G2-Ps (689.00) ($P < 0.05$).

The macroscopic findings of the duodenum, jejunum, and caecum were notably ($P < 0.05$) increased (1.20, 1.00, and 2.40, respectively) in G2-Ps compared to G1-Ng (Table 4 and Figure 2). There was a remarkable ($P < 0.05$) decline in the scores of the duodenum, jejunum, and caecum in treated challenged birds (G4-NOa, G5-NOb, and G3-Clo) in comparison to those of G2-Ps. The scores of the duodenum and caecum in groups G4-NOa and G5-NOb were numerically ($P > 0.05$) lower than those of G3-Clo. The jejunum of G4-NOa and G5-NOb showed a remarkable ($P < 0.05$) decline in the lesion when compared to G3-Clo (1.00).

The FI at 10 dpi was not profoundly ($P > 0.05$) different in the challenged groups, whether treated or not, in comparison with the G1-Ng group (840 g); likewise, the situation for BW and WG values during that period of the study. Regarding the FCR, G1-Ng recorded the lowest value (1.72), but this result was not profoundly ($P > 0.05$) different from the challenged and/or treated groups. No mortalities were recorded in the different groups of the experiment during the relevant study period (Table 5).

Meat quality characteristics

The change in fatty acid composition (g/100 g muscle) caused by dietary supplementation with *N. oculata* in the breast muscles of coccidiosis-challenged broilers is shown in Table 6. The concentrations of eicosapentaenoic acid (C20:5n-3, EPA), α-linolenic acid (ALA, C18:3n-3), and total ω −3 polyunsaturated fatty acid (PUFA) in G5-NOb were markedly ($P < 0.05$) elevated in comparison to other groups. However, *N. oculata* microalgae influence EPA levels but do not influence docosahexaenoic acid (C22:6n-3, DHA) levels. The increase in EPA content subsequently increased the

Table 3. Effect of *Nannochloropsis oculata* and clopidol on dropping scoring of *Eimeria* spp. challenged broiler chicks.

Group	3 dpi	4 dpi	5 dpi	6 dpi	7 dpi	8 dpi	9 dpi	10 dpi
G1-Ng	0.00^{c}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{a}	0.00^{b}	0.00^{b}	0.00^{a}
G2-Ps	1.67^{a}	1.67^{a}	2.33^{a}	3.00^{a}	1.00^{a}	1.00^{a}	0.67^{a}	0.33^{a}
G3-Clo	1.67^{a}	0.67^{ab}	0.00^{b}	1.00^{b}	0.67^{a}	0.33^{b}	0.00^{b}	0.00^{a}
G4-NOa	0.33^{bc}	0.67^{ab}	0.67^{ab}	0.33^{b}	1.00^{a}	0.00^{b}	0.00^{b}	0.00^{a}
G5-NOb	1.00^{ab}	0.67^{ab}	1.00^{ab}	1.00^{b}	0.00^{a}	0.00^{b}	0.00^{b}	0.00^{a}
SEM	0.149	0.067	0.221	0.133	0.115	0.115	0.094	0.067
P value	0.015	0.00	0.035	0.001	0.147	0.024	0.072	0.452

Tukey's test represents the least profound differences between different groups at probability $P < 0.05$. $^{a–c}$ Means within a column not sharing a common superscript differ significantly when $P < 0.05$. SEM: standard error of mean. Values are given as the mean ($n = 5$). G1-Ng: fed basal diet without challenge; G2-Ps: challenged with *Eimeria* spp. and fed basal diet; G3-Clo: challenged and fed clopidol (0.9 g/kg); G4-NOa: challenged and fed 0.1% *N. oculata*; G5-NOb: challenged and fed 0.2% *N. oculata*. dpi: day post-infection.

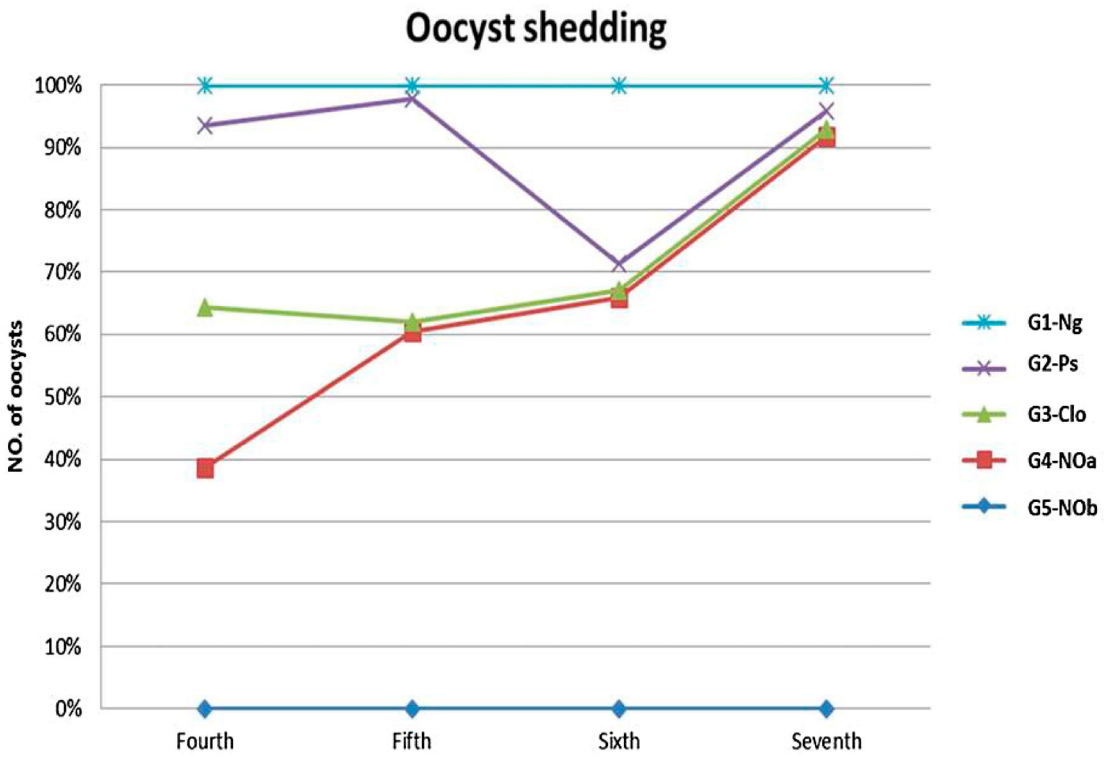

Figure 1. Effect of *Nannochloropsis oculata* and clopidol on oocyst shedding in droppings (OPG) of *Eimeria* spp. challenged broiler chicks.

total PUFA/SFA ratio compared to that in the G1-Ng group. In addition, total EPA+DHA was not notably ($P > 0.05$) altered among the experimental groups, but higher concentrations were recorded in the microalgae-supplemented groups (0.1 and 0.2%) than in the other groups. Regarding meat quality attributes, WHC, DL (24 and 48 h), thawing loss, and cooking loss parameters of broiler breast meat samples dramatically ($P < 0.05$) differed among treatments (Table 7). Treatment with 0.2% *N. oculata* profoundly ($P < 0.05$) affected the WHC. As the microalgae level treatment increased, the WHC increased, leading to improved meat quality. However, a notable ($P < 0.05$) decrease in drip loss (after 24 and 48 h) of breast meat was observed in the G5-NOb group compared to the other groups. The thawing loss and cooking loss showed substantially ($P < 0.05$) the highest values in G3-Clo compared to the other groups (Table 7). However, other meat quality parameters, such as WBSF and colour characteristics (L*, a*, and b*), remained remarkably ($P > 0.05$) altered in all experimental groups. Furthermore, the G4-NOa and G5-NOb groups showed a marked ($P < 0.05$) reduction in MDA in breast meat compared to the G2-Ps group, and nonprofound changes ($P > 0.05$) compared to the G1-Ng and G3-Clo groups (Table 7).

Table 4. Effect of *Nannochloropsis oculata* and clopidol on intestinal lesion scoring of *Eimeria* spp. challenged broiler chicks.

Group/Organ	Duodenum	Jejunum	Caecum
G1-Ng	0.00^{b}	0.00^{c}	0.00^{c}
G2-Ps	1.20^{a}	1.00^{a}	2.40^{a}
G3-Clo	0.40^{b}	0.40^{b}	1.40^{b}
G4-NOa	0.20^{b}	0.00^{c}	0.40^{bc}
G5-NOb	0.00^{b}	0.00^{c}	0.40^{bc}
SEM	0.075	0.049	0.147
P-value	0.00	0.00	0.00

Tukey's test represents the least profound differences between different groups at probability $P < 0.05$. $^{a–c}$ Means within a column not sharing a common superscript differ significantly when $P < 0.05$. SEM: standard error of mean. Values are given as the mean ($n = 5$). G1-Ng: fed basal diet without challenge; G2-Ps: challenged with *Eimeria* spp. and fed basal diet; G3-Clo: challenged and fed clopidol (0.9 g/kg); G4-NOa: challenged and fed 0.1% *N. oculata*; G5-NOb: challenged and fed 0.2% *N. oculata*.

Clinicopathological parameters

Oxidative stress parameters

The MDA values increased gradually in the challenged groups through the period of the experiment (14, 21, and 28 days) and reached 4.28 ± 0.09 nmol/ml after 28 days in G5-NOb, while the control group recorded 2.45 nmol/ml. Both treatments of *N. oculata* extract (0.1 and 0.2%) increased the MDA values, as in the case of G3-Clo. The highest values of MDA were recorded in G5-NOb (2.75 nmol/ml) and G3-Clo (2.70 nmol/ml). The T-SOD value profoundly ($P <$ 0.05) decreased in G2-Ps in comparison with the challenged treated groups. The highest alleviation rate was found in the G3-Clo and G5-NOb groups, followed by the G4-NOa group. Using clopidol and 0.2% *N. oculata* extract in diets resulted in values similar to those of G1-Ng at the end of the experiment (119.42 U/mg), which confirmed the efficiency of the

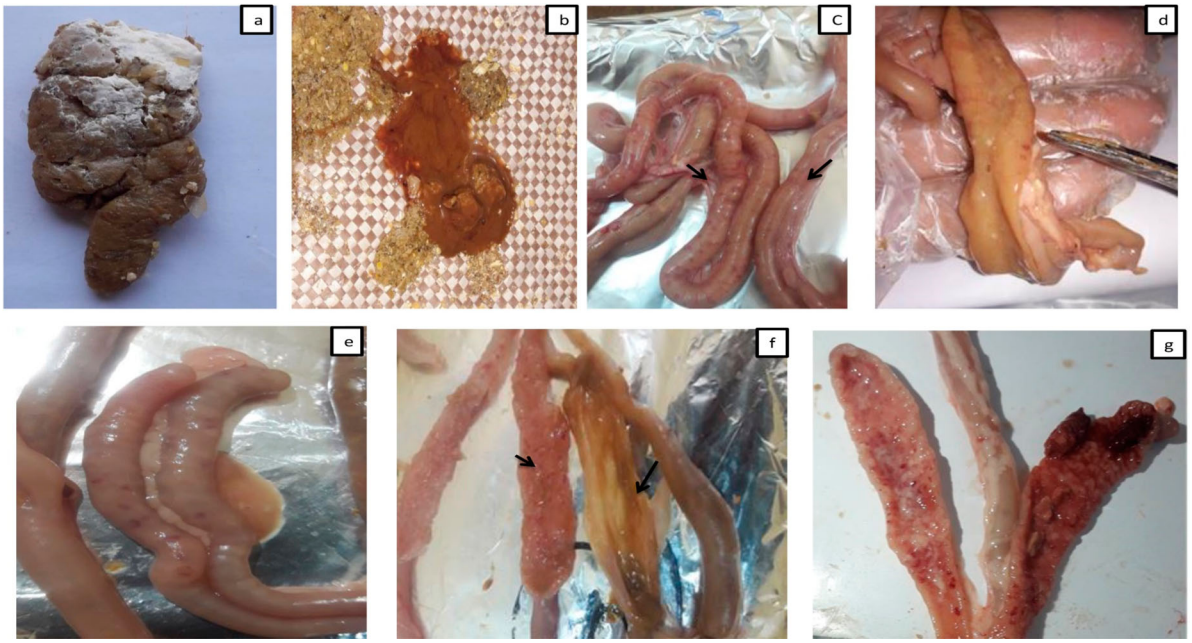

Figure 2. Pathological changes in droppings and the intestinal tract of *Eimeria* spp. challenged birds. a: normal colour and consistency droppings (score 0); b: watery dark red to brown droppings (score 4); c: Numerous petechiae on the serosal surface of the duodenum (right) and jejunum (left); d: mucoid inflammation with petechial haemorrhage on the small intestine mucosal surface; e: numerous red and white foci on the caecum's serosal surface; f: normal caecum mucosa (right), inflamed oedematous mucosa with petechial haemorrhages (left); g: severe haemorrhagic inflammation, mucosal debris, and blood in the caecal lumen, with a thickened wall.

treatments. Concerning catalase results, a slight increase was recorded in the G2-Ps, while no profound ($P > 0.05$) effect was noticed in the other treated challenged groups (Table 8).

Lipid profile

Both total cholesterol and triacylglycerols were dramatically ($P < 0.05$) decreased after challenge with *Eimeria* spp. when compared to G1-Ng at 28 days of age (Table 9). A considerable increase was noted in the levels of total cholesterol and triacylglycerols in both *N. oculata*-treated groups as well as the G3-Clo group. The highest increase in total cholesterol and triacylglycerols was recorded at 28 days in both G5-NOb and G3-Clo, followed by G4-NOa. Similarly, HDL and LDL cholesterol notably ($P < 0.05$) decreased after challenge with *Eimeria* spp. G5-NOb, G4-NOa, and G3-Clo exhibited a promising increase in HDL values at 28 days (65.78, 60.45, and 61.11 mg/dl, respectively) even more than G1-Ng. LDL cholesterol slightly increased as a response to infection with *Eimeria* spp., but no profound ($P > 0.05$) variation was noticed between different treatments.

Table 5. Effect of *Nannochloropsis oculata* and clopidol on the performance of *Eimeria* spp. challenged broiler chicks.

Group/ Parameter	FI (g)	BW (g)	WG (g)	FCR	Mortality (%)
G1-Ng	840[a]	733.33[a]	494.67[a]	1.72[a]	0
G2-Ps	806.67[a]	723.33[a]	477.67[a]	1.73[a]	0
G3-Clo	843.33[a]	723.33[a]	487[a]	1.74[a]	0
G4-NOa	910 [a]	713.33[a]	469.33[a]	1.94[a]	0
G5-NOb	846.67[a]	740.00[a]	489.67[a]	1.74[a]	0
SEM	16.289	10.667	9.133	0.045	–
P value	0.425	0.941	0.906	0.445	–

Tukey's test represents the least profound differences between different groups at probability $P < 0.05$. [a-c] Means within a column not sharing a common superscript differ significantly when $P < 0.05$. SEM: standard error of mean. Values are given as the mean ($n = 5$). G1-Ng: fed basal diet without challenge; G2-Ps: challenged with *Eimeria* spp. and fed basal diet; G3-Clo: challenged and fed clopidol (0.9 g/kg); G4-NOa: challenged and fed 0.1% *N. oculata*; G5-NOb: challenged and fed 0.2% *N. oculata*. FI: feed intake; BW: body weight; WG: weight gain; FCR: feed conversion ratio.

Liver and kidney functions

AST and ALT values in the G2-Ps group were profoundly ($P < 0.05$) increased (almost twofold) after the challenge in comparison to G1-Ng (Table 10). The highest improvement was recorded in G3-Clo, followed by G5-NOb. Regarding kidney function markers, the results revealed that serum urea of the challenged broilers dramatically ($P < 0.05$) increased in G2-Ps in comparison to the G1-Ng group. However, it was markedly ($P < 0.05$) lower in the G3-Clo group than in the G2-Ps group. Additionally, the serum urea content was reduced by 5.17 mg/dl in G5-NOb. No ($P < 0.05$) variations were noted in serum creatinine between different groups during the experimental period.

Transmission electron microscopy

The negative control (G1-Ng) intestinal cells had normal architecture, with well-developed tight junctions connecting them (Figure 3A). Moreover, the enterocyte supranuclear region contained several

Table 6. Effect of *Nannochloropsis oculata* and clopidol on the fatty acid profile of breast meat in *Eimeria* spp. challenged broiler chicks.

Fatty acids (g/100 g) tissue/Groups	G1-Ng	G2-Ps	G3-Clo	G4-NOa	G5-NOb	SEM	*P*-value
Myristic C14:0	0.75^{bc}	0.90^{a}	0.92^{a}	0.71^{b}	0.73^{b}	0.016	0.004
Palmitic C16:0	27.70^{a}	24.23^{c}	23.63^{c}	25.47^{bc}	26.76^{ab}	0.297	0.007
Stearic C18:0	11.80	11.56	12.43	11.26	10.89	0.226	0.325
Myristovaccenic C14:1	0.14	0.14	0.15	0.13	0.14	0.003	0.419
Palmitoleic C16:1	4.26^{a}	3.69^{b}	3.57^{b}	3.98^{ab}	3.84^{ab}	0.064	0.051
Oleic C18:1	30.22	33.25	32.72	30.79	30.20	0.515	0.255
Linoleic (LA) C18:2 (n−6)	14.95^{b}	16.52^{a}	16.64	17.61	16.60	0.221	0.048
Gamma-linolenic (GLA) C18:3 (n-6)	1.93	1.73	1.97	1.73	1.92	0.106	0.913
Eicosadienoic (EDA) C20:2 (n-6)	0.97	0.98	0.92	1.03	1.02	0.028	0.73
Arachidonic (AA) C20:4 (n-6)	3.60^{a}	2.96^{b}	3.00^{b}	3.49^{a}	3.56^{a}	0.037	000
Docosadienoic C22:2 (n-6)	1.37	1.42	1.33	1.57	1.72	0.061	0.326
Adrenic C22:4 (n-6)	1.47	1.74	1.59	1.54	1.68	0.078	0.824
α-Linolenic acid (ALA) C18:3 (n-3)	0.31^{b}	0.35^{ab}	0.33^{b}	0.34^{ab}	0.38^{a}	0.006	0.032
Docosahexaenoic (DHA) C22:6 (n-3)	0.26	0.29	0.27	0.31	0.29	0.01	0.569
Eicosapentaenoic (EPA) C20:5 (n-3)	0.26^{ab}	0.22^{c}	0.23^{bc}	0.26^{abc}	0.28^{a}	0.005	0.031
∑ SFA	40.26^{a}	36.69^{b}	36.98^{b}	37.43^{b}	38.39^{ab}	0.376	0.035
∑∑ MUFA	34.62	37.09	36.43	34.90	34.18	0.519	0.384
∑∑ PUFA	25.13^{b}	26.22^{ab}	26.59^{ab}	27.67^{a}	27.44^{ab}	0.33	0.041
∑∑ n-3 PUFA	0.84^{ab}	0.87^{ab}	0.83^{b}	0.90^{ab}	0.95^{a}	0.015	0.027
∑ n-6 PUFA	24.29^{b}	25.35^{ab}	25.76^{ab}	26.76^{a}	26.48^{ab}	0.316	0.032
PUFA/SFA ratio	0.62^{b}	0.71^{a}	0.72^{a}	0.74^{a}	0.72^{a}	0.011	0.047
∑ EPA+DHA	0.52	0.51	0.51	0.57	0.57	0.013	0.401

Tukey's test represents the least profound differences between different groups at probability $P < 0.05$. $^{a–c}$ Means within a row not sharing a common superscript differ significantly when $P < 0.05$. SEM: standard error of mean. Values are given as the mean ($n = 5$). G1-Ng: fed basal diet without challenge; G2-Ps: challenged with *Eimeria* spp. and fed basal diet; G3-Clo: challenged and fed clopidol (0.9 g/kg); G4-NOa: challenged and fed 0.1% *N. oculata*; G5-NOb: challenged and fed 0.2% *N. oculata*. ∑SAF: total saturated fatty acid; ∑PUFA: total polyunsaturated fatty acid; ∑ MUFA: total monounsaturated fatty acid; Σn−3 PUFA: total omega 3 polyunsaturated fatty acid; and Σn−6 PUFA: total omega 6 polyunsaturated fatty acid.

mitochondria and numerous rough endoplasmic cisternae. Furthermore, the luminal surface of the enterocytes was densely packed with parallel finger-like microvilli. The intestinal cells of G2-Ps showed infection in the caecal epithelium with scant cytoplasm. Moreover, several swollen (or damaged) cytoplasmic organelles and various cytoplasmic inclusions of *Eimeria* oocytes in either mature or first-generation schizonts in a more advanced stage of merozoite formation were recorded (Figure 3B

Table 7. Effect of *Nannochloropsis oculata* and clopidol on breast meat physicochemical properties in *Eimeria* spp. challenged broiler chicks.

Parameters groups	G1-Ng	G2-Ps	G3-Clo	G4-NOa	G5-NOb	SEM	*P* value
Ultimate pH (pH24)	5.38	5.45	5.46	5.49	5.50	0.024	0.538
WHC	89.61^{a}	82.24^{b}	81.97^{b}	82.94^{b}	88.75^{a}	0.927	0.043
DL24	11.70^{a}	12.30^{a}	10.67^{ab}	9.62^{ab}	7.67^{b}	0.884	0.041
DL48	13.20^{a}	12.89^{a}	12.07^{ab}	11.42^{ab}	9.09^{b}	0.857	0.039
Thawing loss	2.21^{b}	2.94^{b}	14.49^{a}	1.43^{b}	0.87^{b}	0.249	0
Cooking loss	7.79^{b}	9.67^{ab}	11.75^{a}	8.59^{b}	8.56^{b}	0.610	0.036
WBSF	3.26	3.59	3.34	3.58	3.38	0.072	0.631
MDA	20.62^{b}	28.02^{a}	22.38^{ab}	20.31^{b}	19.10^{b}	0.912	0.007
CLAB coordinate							
L*	51.78	50.85	51.68	51.33	53.63	1.091	0.510
a*	11.54	11.26	11.32	11.44	11.01	0.264	0.825
b*	8.37	10.33	10.09	8.65	9.97	0.486	0.563

Tukey's test represents the least profound differences between different groups at probability $P < 0.05$. $^{a–c}$ Means within a row not sharing a common superscript differ significantly when $P < 0.05$. SEM: standard error of mean. Values are given as the mean ($n = 5$). G1-Ng: fed basal diet without challenge; G2-Ps: challenged with *Eimeria* spp. and fed basal diet; G3-Clo: challenged and fed clopidol (0.9 g/kg); G4-NOa: challenged and fed 0.1% *N. oculata*; G5-NOb: challenged and fed 0.2% *N.* oculata. WHC: water holding capacity; DL_{24}: drip loss after 24 h; DL_{48}: drip loss after 48 h; WBSF: Warner-Bratzler Shear Force; L*: lightness; a*: redness; b*: yellowness; MDA: malondialdehyde.

Table 8. Effect of *Nannochloropsis oculata* and clopidol on oxidative stress biomarkers in the serum of *Eimeria* spp. challenged broiler chicks.

	MDA (nmol/ml)			T-SOD (U/mg)			Catalase (U/mg)		
	days			days			days		
Group/Parameter	14	21	28	14	21	28	14	21	28
G1-Ng	2.08^{d}	2.45^{c}	2.44^{b}	118.17^{a}	121.42^{a}	119.42^{a}	13.14^{b}	13.15^{b}	12.69^{b}
G2-Ps	2.93^{a}	3.67^{a}	4.28^{a}	100.44^{e}	98.76^{e}	82.93^{d}	14.04^{a}	14.09 ± 0.08^{a}	13.70^{a}
G3-Clo	2.24^{c}	2.58^{c}	2.70^{b}	111.88^{b}	115.07^{b}	118.00^{ab}	13.26^{ab}	12.84 ± 0.10^{bc}	12.65^{b}
G4-NOa	2.54^{ab}	3.11^{b}	3.94^{a}	103.20^{d}	102.44^{d}	97.43^{c}	12.98^{b}	12.56^{c}	12.51^{b}
G5-NOb	2.44^{ab}	2.77^{bc}	2.75^{b}	108.03^{c}	113.65^{c}	117.57^{b}	13.13^{b}	13.23^{b}	12.59^{b}
SEM	0.019	0.050	0.050	0.217	0.183	0.204	0.118	0.074	0.064
P-value	0.00	0.00	0.00	0.00	0.00	0.00	0.110	0.00	0.00

Tukey's test represents the least profound differences between different groups at probability $P < 0.05$. $^{a–c}$ Means within a column not sharing a common superscript differ significantly when ($P < 0.05$). SEM: standard error of mean. Values are given as the mean ($n = 5$).G1-Ng: fed basal diet without challenge; G2-Ps: challenged with *Eimeria* spp. and fed basal diet; G3-Clo: challenged and fed clopidol (0.9 g/kg); G4-NOa: challenged and fed 0.1% *N. oculata*; G5-NOb: challenged and fed 0.2% *N. oculata*. MDA: malondialdehyde; T-SOD: total superoxide dismutase.

Table 9. Effect of *Nannochloropsis oculata* and clopidol on the lipid profile in the serum of *Eimeria* spp. challenged broiler chicks.

	Total cholesterol (mg/dl)			HDL-C (mg/dl)			LDL-C (mg/dl)			Triacylglycerol (mg/dl)		
	days			days			days			days		
Group/Parameter	14	21	28	14	21	28	14	21	28	14	21	28
G1-Ng	162.73^{a}	164.52^{a}	165.26^{a}	60.96^{a}	59.23^{b}	61.12^{b}	36.01^{a}	36.25^{a}	35.60^{a}	99.07^{a}	104.60^{a}	110.09^{ab}
G2-Ps	141.23^{bc}	138.10^{d}	129.07^{d}	48.90^{d}	44.93^{d}	40.78^{c}	22.42^{d}	22.95^{c}	22.72^{d}	75.56^{b}	75.22^{c}	70.44^{c}
G3-Clo	141.94^{b}	145.59^{bc}	163.29^{b}	58.11^{b}	58.65^{b}	61.11^{b}	24.77^{b}	24.27^{b}	25.30^{b}	99.45^{a}	101.26^{b}	110.58^{ab}
G4-NOa	140.11^{c}	144.60^{c}	161.41^{c}	54.71^{c}	54.60^{c}	60.44^{b}	22.40^{d}	22.42^{c}	22.45^{d}	97.50^{a}	100.07^{b}	109.23^{b}
G5-NOb	140.78^{bc}	145.82^{b}	163.67^{b}	58.71^{b}	62.71^{a}	65.77^{a}	23.60^{c}	23.90^{b}	24.08^{c}	98.92^{a}	101.42^{b}	112.14^{a}
SEM	0.238	0.158	0.153	0.151	0.178	0.121	0.118	0.081	0.104	0.298	0.272	0.281
P-value	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Tukey's test represents the least profound differences between different groups at probability $P < 0.05$. $^{a–c}$ Means within a column not sharing a common superscript differ significantly when $P < 0.05$. SEM: standard error of mean. Values are given as the mean ($n = 5$). G1-Ng: fed basal diet without challenge; G2-Ps: challenged with *Eimeria* spp. and fed basal diet; G3-Clo: challenged and fed clopidol (0.9 g/kg); G4-NOa: challenged and fed 0.1% *N. oculata*; G5-NOb: challenged and fed 0.2% *N. oculata*. HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol.

and C). G3-Clo intestinal cells exhibited destruction of enterocyte brush borders as well as the presence of various *Eimeria* spp. developmental stages. The number of protozoan development stages was lower in G3-Clo than in G2-Ps (Figure 3D and E). The intestinal cells of 0.1 and 0.2% *N. oculata* treatments displayed round or elongated mitochondria with little or no structural damage. In G5-NOb, the number of developmental stages of *Eimeria* spp. in the absorptive epithelial lining of intestinal villi was dramatically lower than that in G3-Clo and G4-NOa (Figure 3F and G). Furthermore, degenerated parasitic stages were observed in G5-NOb enterocytes (Figure 3H and I).

Molecular docking

Clopidol exhibited binding energies to *E. tenella* aldolase (Figure 4a), EtAMA1 (Figure 5a), and EtMIC3 (Figure 6a) of −6.45, −4.56, and −4.60 kcal/mol, respectively. *N. oculata* bioactive compounds, violaxanthin, zeaxanthin, canthaxanthin, echinenone, (3s)-6-[(1e,3e,5e,7e,9e,11e,13e,15e,17e)-18-[(4r)-4-hydroxy-2,6,6-trimethylcyclohex-1-en-1-yl]-3,7,12,16-tetramethyloctadeca-1,3,5,7,9,11,13,15,17-nonaen-1-yl]-1,5,5-trimethyl-7-oxabicyclo[4.1.0]heptan −3-ol and luteoxanthin interacted with the *E. tenella* aldolase binding site with binding energies of −6.45 (Figure 4b), −6.88 (Figure 4c), −7.23 (Figure 4d), −6.73 (Figure 4e), −6.75 (Figure 4f), and −6.71 kcal/mol (Figure

Table 10. Effect of *Nannochloropsis oculata* and clopidol on liver and kidney functions in the serum of *Eimeria* spp. challenged broiler chicks.

	AST (U/l)			ALT (U/l)			Urea (mg/dl)			Creatinine (mg/dl)		
	days			days			days			days		
Group/Parameter	14	21	28	14	21	28	14	21	28	14	21	28
G1-Ng	55.75^{e}	56.15^{e}	56.47^{e}	128.20^{d}	129.30^{d}	128.82^{e}	4.25^{a}	4.37^{c}	4.72^{d}	0.38^{b}	0.40^{a}	0.41^{ab}
G2-Ps	85.96^{a}	91.28^{a}	110.03^{a}	151.89^{a}	155.69^{a}	202.95^{a}	4.60^{a}	5.03^{a}	6.40^{a}	0.39^{b}	0.40^{a}	0.42^{ab}
G3-Clo	76.87^{c}	75.97^{c}	63.26^{c}	131.35^{c}	145.66^{b}	142.81^{d}	4.39^{a}	4.92^{ab}	4.82^{cd}	0.41^{a}	0.41^{a}	0.42^{ab}
G4-NOa	82.71^{b}	81.03^{b}	81.37^{b}	142.31^{b}	141.22^{c}	197.62^{b}	4.23^{a}	4.70^{abc}	5.71^{b}	0.39^{b}	0.40^{a}	0.40^{b}
G5-NOb	72.67^{d}	71.12^{d}	61.11^{d}	130.69^{c}	145.86^{b}	153.43^{c}	4.63^{a}	4.51^{bc}	5.17^{c}	0.39^{b}	0.41^{a}	0.42^{a}
SEM	0.224	0.198	0.212	0.162	0.240	0.258	0.592	0.064	0.057	0.002	0.003	0.003
P-value	0.00	0.00	0.00	0.00	0.00	0.00	0.16	0.04	0.00	0.02	0.65	0.12

Tukey's test represents the least profound differences between different groups at probability $P < 0.05$. $^{a–e}$ Means within a column not sharing a common superscript differ significantly when $P < 0.05$. SEM: standard error of mean. Values are given as the mean ($n = 5$). G1-Ng: fed basal diet without challenge; G2-Ps: challenged with *Eimeria* spp. and fed basal diet; G3-Clo: challenged and fed clopidol (0.9 g/kg); G4-NOa: challenged and fed 0.1% *N. oculata*; G5-NOb: challenged and fed 0.2% *N. oculata*. ALT: aspartate transaminase; AST: alanine transaminase.

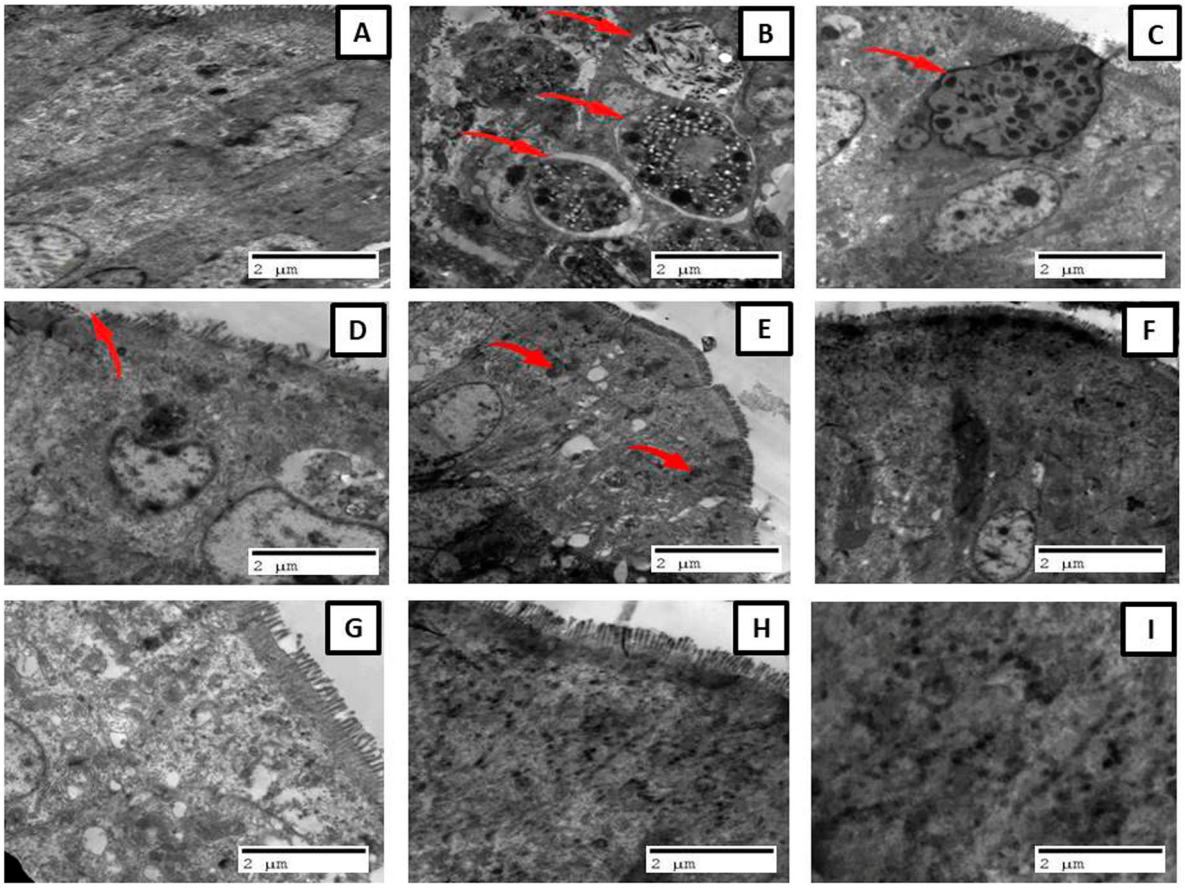

Figure 3. TEM micrograph of the caecum in *Eimeria* spp. challenged birds. A: normal enterocyte content, nucleus, mitochondria, microvilli, and apical junction complex (×2000) in G1-Ng; B, C: several mature microgamonts inside epithelial cells and final merogony, gamont stages (arrow) (1000× & 2500×) in G2-Ps; D, E: the destruction of the brush border of enterocytes as well as a few *Eimeria* stages (arrow) (3000× & 1500×) in G3-Clo; F, G: small number of *Eimeria* stages inside enterocytes (1500× & 1200×) in G4-NOa; H, I: enterocytes showed intact brush border and mitochondria in addition to degenerated parasitic stages (3000× & 5000×) in G5-NOb.

4g), respectively. Similarly, the same violaxanthin (−7.33 and −7.28 kcal/mol), zeaxanthin (−8.32 and −7.64 kcal/mol), canthaxanthin (−8.01 and −7.69 kcal/mol), echinenone (−7.89 and −7.43 kcal/mol), (3s)-6-[(1e,3e,5e,7e,9e,11e,13e,15e,17e)-18-[(4r)-4-hydroxy-2,6,6-trimethylcyclohex-1-en-1-yl]-3,7,12,16-tetramethyloctadeca-1,3,5,7,9,11,13,15,17-nonaen-1-yl]-1,5,5-trimethyl-7-oxa-bicyclo[4.1.0]heptan-3-ol (−7.41 and −6.64 kcal/mol), and luteoxanthin (−8.66 and −7.67 kcal/mol) bound with EtAMA1 (Figure 5b–g) and EtMIC3 (Figure 6b–g) binding sites, respectively.

Discussion

A recent trend has emerged to replace the anticoccidial drugs added to poultry feed with natural preparations that are favoured by consumers for their high safety rates and beneficial effects, in response to addressing their negative effects of the overuse and misuse on human or bird health. Therefore, this study evaluated the effect of dietary *Nannochloropsis oculata* as a chemotherapeutic alternative for broilers challenged with coccidiosis.

The biological effectiveness of *Nannochloropsis oculata* is attributed to the presence of proteins, lipids, EPA, PUFAs, as well as antioxidants such as polyphenols, carotenoids, and vitamins (Zanella & Vianello, 2020). In the current study, secondary metabolite levels in *N. oculata* powder matched those found in the ethanolic extract of *Nanochloroposis gaditana* microalgae, with total phenols, flavonoids, and condensed tannins at 41.53 ± 0.42, 19.82 ± 1.66, and 0.35 ± 0.05 mg/g, respectively, as reported by Kherraf *et al.* (2017). Our findings came in agreement with Gessner *et al.*, (2017), who found that the polyphenol content of *N. oculata* was 28 mg gallic acid/100 g. *N. oculata* contained a broad set of antioxidant compounds, as reported previously by Paterson *et al.* (2023).

Experimental birds showed strong reactions to a mixture of *E. tenella, E. acervulina, E. maxima, E. necatrix,* and *E. mitis* oocysts, as evidenced by high dropping scores, severe lesions in the intestine, and a high number of oocysts in the faeces. Similar observations were previously recorded (Swayne *et al.*, 2020). The reported pathological findings in this study are attributed to the host immune system's production of free radical oxidative species in

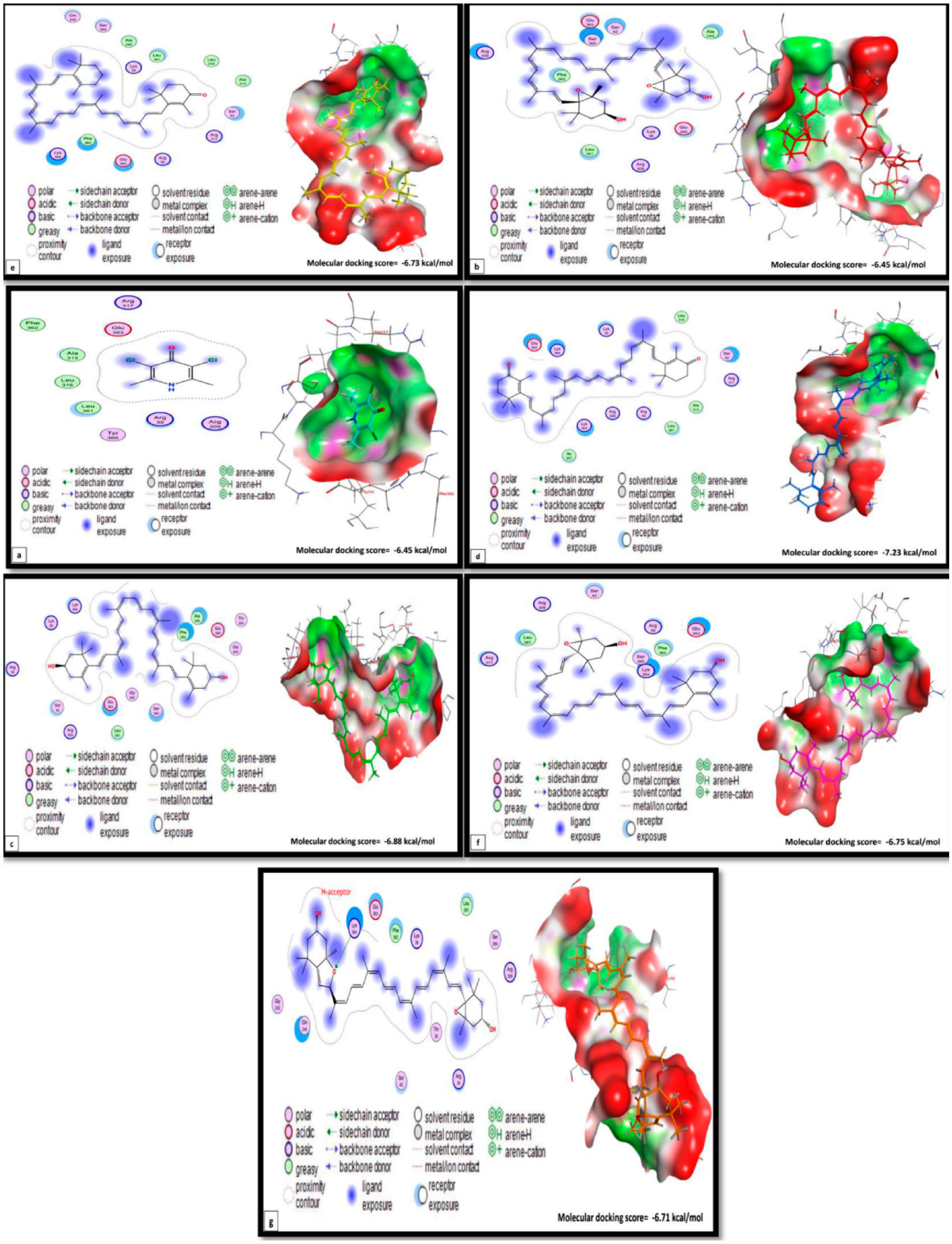

Figure 4. a: *Eimeria tenella* aldolase and clopidol molecular interaction; b: *Eimeria tenella* aldolase and violaxanthin molecular interaction; c: *Eimeria tenella* aldolase and zeaxanthin molecular interaction; d: *Eimeria tenella* aldolase and canthaxanthin molecular interaction; e: *Eimeria tenella* aldolase and echinenone molecular interaction; f: *Eimeria tenella* aldolase and (3s)-6-[(1e,3e,5e,7e,9e,11e,13e,15e,17e)-18-[(4r)-4-hydroxy-2,6,6-trimethylcyclohex-1-en-1-yl]-3,7,12,16-tetramethyloctadeca-1,3,5,7,9,11,13,15,17-nonaen-1-yl]-1,5,5-trimethyl-7-oxabicyclo[4.1.0]heptan-3-ol molecular interaction; g: *Eimeria tenella* aldolase and luteoxanthin molecular interaction.

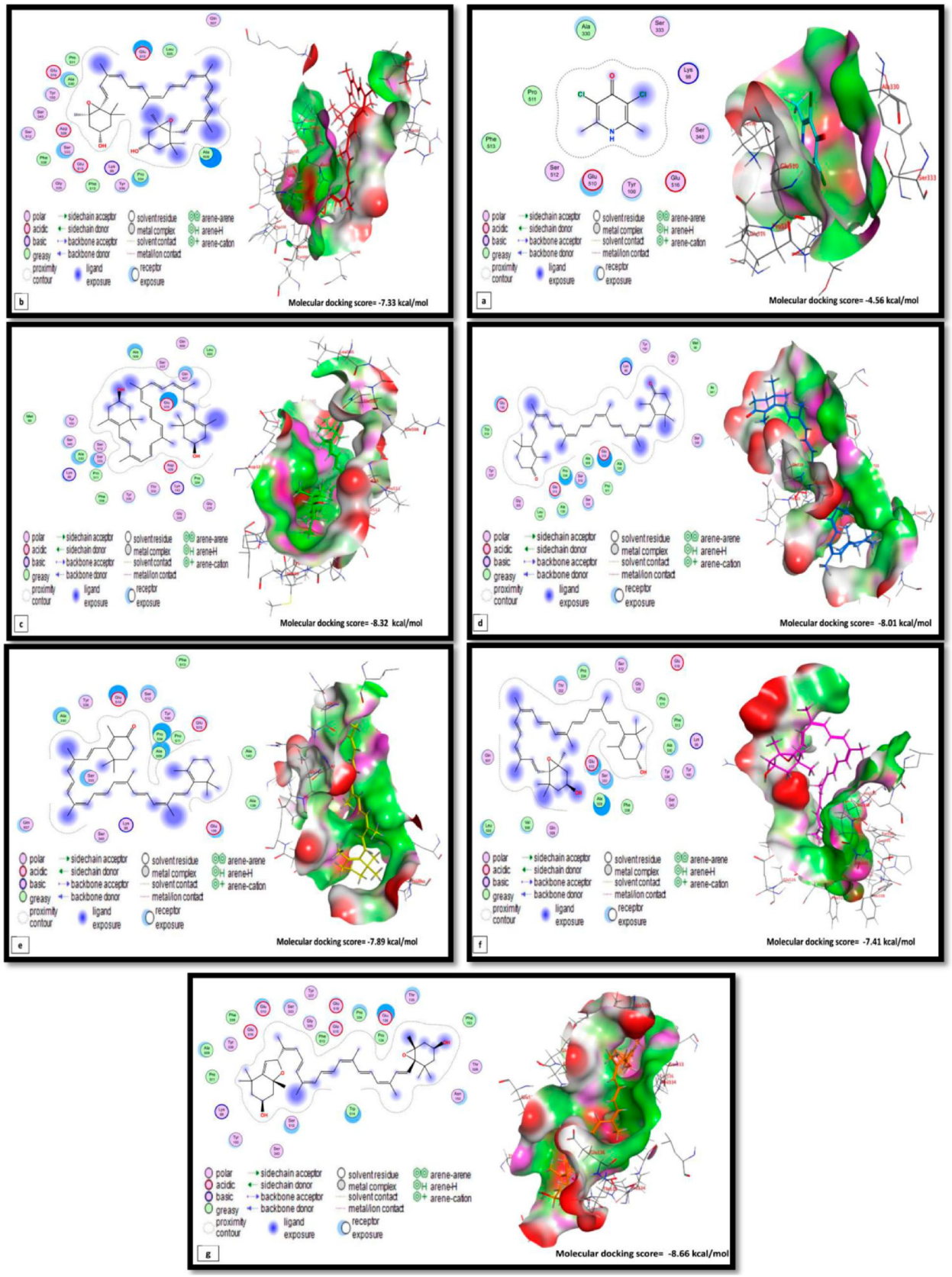

Figure 5. a: *Eimeria tenella* apical membrane antigen 1 (EtAMA1) and clopidol molecular interaction; b: *Eimeria tenella* apical membrane antigen 1 (EtAMA1) and violaxanthin molecular interaction; c: *Eimeria tenella* apical membrane antigen 1 (EtAMA1) and zeaxanthin molecular interaction; d: *Eimeria tenella* apical membrane antigen 1 (EtAMA1) and canthaxanthin molecular interaction; e: *Eimeria tenella* apical membrane antigen 1 (EtAMA1) and echinenone molecular interaction; f: *Eimeria tenella* apical membrane antigen 1 (EtAMA1) and (3s)-6-[(1e,3e,5e,7e,9e,11e,13e,15e,17e)-18-[(4r)-4-hydroxy-2,6,6-trimethylcyclohex-1-en-1-yl]-3,7,12,16-tetramethyloctadeca-1,3,5,7,9,11,13,15,17-nonaen-1-yl]-1,5,5-trimethyl-7-oxabicyclo[4.1.0]heptan-3-ol molecular interaction; g: *Eimeria tenella* apical membrane antigen 1 (EtAMA1) and luteoxanthin molecular interaction.

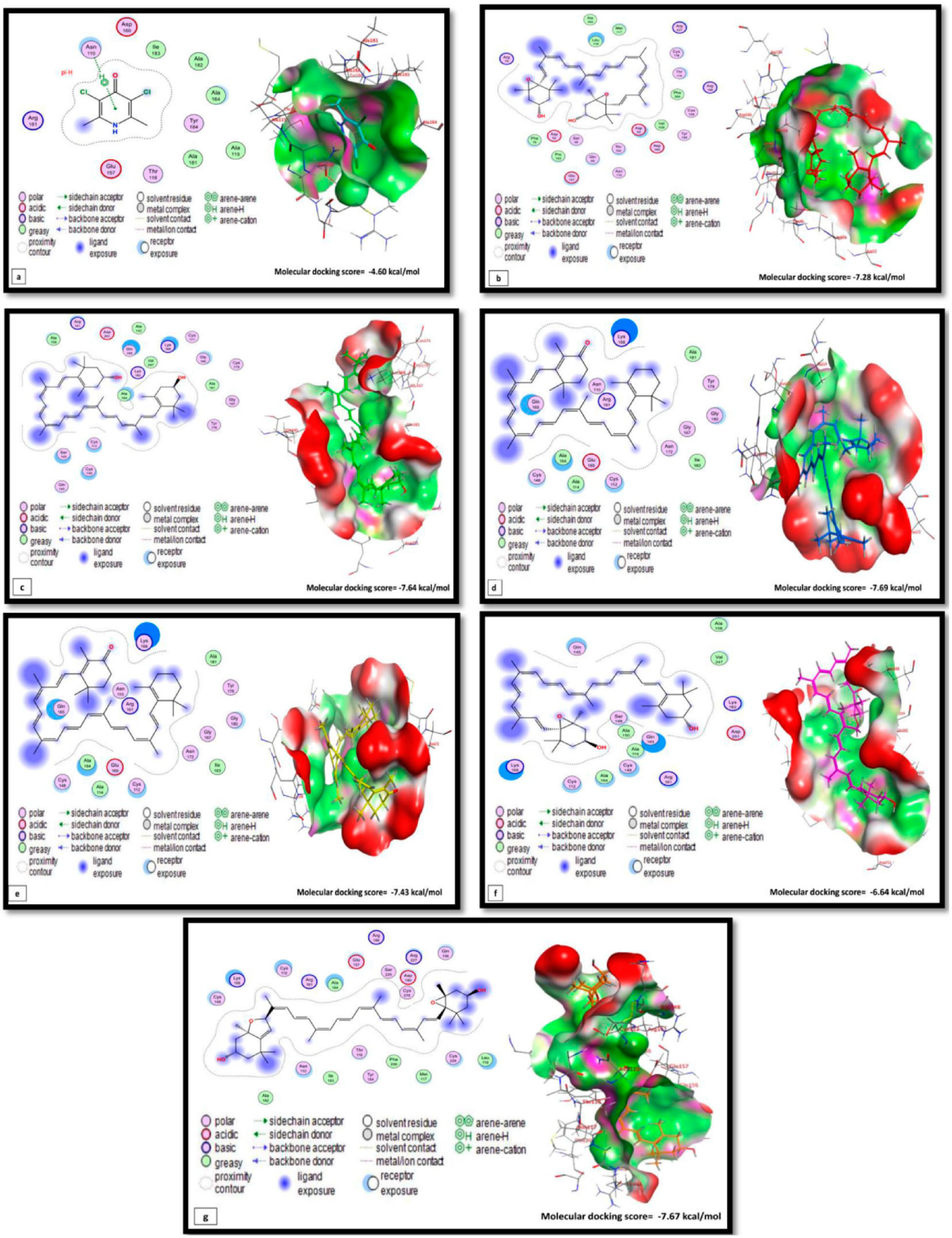

Figure 6. a: *Eimeria tenella* microneme protein 3 (EtMIC3) and clopidol molecular interaction; b: *Eimeria tenella* microneme protein 3 (EtMIC3) and violaxanthin molecular interaction; c: *Eimeria tenella* microneme protein 3 (EtMIC3) and zeaxanthin molecular interaction; d: *Eimeria tenella* microneme protein 3 (EtMIC3) and canthaxanthin molecular interaction; e: *Eimeria tenella* microneme protein 3 (EtMIC3) and echinenone molecular interaction; f: *Eimeria tenella* microneme protein 3 (EtMIC3) and (3s)-6-[(1e,3e,5e,7e,9e,11e,13e,15e,17e)-18-[(4r)-4-hydroxy-2,6,6-trimethylcyclohex-1-en-1-yl]-3,7,12,16-tetramethyloctadeca-1,3,5,7,9,11,13,15,17-nonaen-1-yl]-1,5,5-trimethyl-7-oxabicyclo[4.1.0]heptan-3-ol molecular interaction; g: *Eimeria tenella* microneme protein 3 (EtMIC3) and luteoxanthin molecular interaction.

response to the invasion of *Eimeria* species, which causes pathogenic oxidative stress and an altered ecological oxidative balance (Georgieva *et al.*, 2006).

This study indicated that *N. oculata* (0.1%) improved the droppings of challenged chicks compared to G2-PS. This improvement was comparable to that in the clopidol-treated group. Furthermore, the *N. oculata* (0.1 and 0.2%) resulted in a clear and substantial improvement in the intestinal condition, and this improvement was superior to that of the clopidol-treated group. Compared to the challenged untreated group, the dietary microalgae profoundly reduced the number of shed oocysts, similar to that of the clopidol-treated group. These results are consistent with an earlier study on Nile tilapia fish (*Oreochromis niloticus*) (Abdelghany *et al.*, 2020). The severity of *E. tenella* infections and intestinal lipid peroxidation can be mitigated by antioxidant chemicals (Allen *et al.*, 1998). As a result, *N. oculata* minimized the harmful effects of oxidative stress and brought back the natural balance of oxidation in the birds' intestines after the challenge. This attribution has been previously confirmed in other research (Mamdouh *et al.*, 2021; Abd El-Hamid *et al.*, 2022).

Regarding performance parameters, the current study revealed that at 10 days post-infection, the infection had no apparent impact when compared to G1-Ng; thus, we did not observe any significant effects of interventions on FI, BW, WG and FCR. The present results are supported by several studies (Long *et al.*, 2018; Park *et al.*, 2018), indicating that a period of 10 days following infection may not be adequate to clearly observe the impact of the challenge and treatments on performance.

n3-PUFA are inadequately synthesized by the human body and must therefore be obtained from the diet (Cartoni Mancinelli *et al.*, 2022). In this study, the dietary supplementation of coccidiosis-challenged broilers with *N. oculata* (2.0%) could provide a valuable source of such necessary fatty acids for humans. A similar study recorded that using microalgae (0.1%) led to a significant increase in EPA, DHA, total PUFAs, and arachidonic acid in the breast muscle of broiler chickens (El-Bahr *et al.*, 2020). The higher concentration of EPA in the broiler groups supplemented with microalgae in this study could be due to the fact that *Nannochloropsis* spp. is abundant in omega-3 fatty acids, particularly EPA (Kagan & Matulka, 2015). Furthermore, the amount of EPA+DHA ingested in 100 g of broiler meat fed marine algae met the recommended daily intake mentioned in previous reports (Kang *et al.*, 2005; Molendi-Coste *et al.*, 2011; Aranceta & Pérez-Rodrigo, 2012). In this study, the total PUFA/SFA ratios observably increased in the microalgae-supplemented groups compared to the control negative group, indicating improved fatty acid balance in the investigated tissues.

The present study revealed that the addition of *N. oculata* to the diet resulted in enhanced meat quality indicators, including water holding capacity, drip loss at 24 and 48 h, cooking loss, and thawing loss, in the challenged broiler chicks. In addition, there were no significant differences observed among the dietary interventions in the ultimate pH level, which is consistent with findings from other studies (Rajput *et al.*, 2014; Liu *et al.*, 2020). The WHC assesses the ability of meat to keep its water content, either fully or partially (Huff-Lonergan & Lonergan, 2005). In the present investigation, the WHC increased notably in the microalgae treated challenged birds in comparison to the G2- pos. These findings align with previous studies (Qaid *et al.*, 2021; Rajput *et al.*, 2014) and can be attributed to the microalgae used, which supply the body with omega-3 PUFA. This nutrient aids in the formation of a flexible lipid bilayer membrane in muscle cells, resulting in improved tenderness, juiciness, firmness, and appearance (Mir *et al.*, 2017; Kalbe *et al.*, 2019).

The current study observed a significant decrease in drip loss (at 24 and 48 h) in the G5-NOb group compared to the other treatment groups. Comparable findings were reported in broiler meat (Long *et al.*, 2018) and pig meat (Kalbe *et al.*, 2019). The reduction in drip loss of breast meat which was supplemented with 0.2% *N. oculata* can be explained by the concurrent increase in WHC. The use of microalgae treatment in challenged birds resulted in a decrease in both cooking loss and thawing loss compared to G3-Clo. This finding aligns with previous studies by Khan *et al.* (2021) and Šefcová *et al.* (2021).

The level of tenderness in meat is a crucial factor in assessing its overall quality. An insignificant ($P > 0.05$) change in Warner–Bratzler Shear Force was observed in all experimental groups during the current study. Conversely, an increased dosage of *Eimeria* oocytes significantly decreased the tenderness of meat (Chodová *et al.*, 2018). The absence of differences in muscle shear force among the experimental groups could be attributed to the relatively little variation in final pH observed between the groups.

The colour of meat is often used as an indicator of its freshness and quality (Uhlířová *et al.*, 2018). The inclusion of *N. oculata* in the feed of broilers subjected to coccidiosis challenge did not have a significant impact on the colour metrics ($P > 0.05$), aligning with the results reported by Chodová *et al.* (2018). Based on the MDA analysis, G2-Ps exhibited a significant ($P < 0.05$) increase compared to both the negative control and the groups treated with microalgae. There was no significant difference ($P > 0.05$) in the MDA value between the birds treated with *N. oculata* and the negative control. Prior studies have demonstrated an

elevation in MDA levels in birds when exposed to coccidiosis, which is attributed to oxidative stress (Georgieva *et al.*, 2006; El-maksoud, 2014).

This study revealed that administering of 0.1 or 0.2% *N. oculata* resulted in a notable decrease in the oxidative stress parameters to approach the normal ranges. The dietary *N. oculata* (0.2%) profoundly ($P < 0.05$) increased the total antioxidant capacity compared to the control group. The current results were in agreement with a previous study of Abd El-Hamid *et al.* (2022). The current results could be attributed to the fact that microalgae have many natural active ingredients that can help reduce oxidative stress by boosting antioxidant enzymes and other molecules (Abdelnour *et al.*, 2020; Abd El-Hamid *et al.*, 2022).

The present investigation found that the *N. oculata* treatment effectively minimized the adverse effects on the lipid profile of the challenged birds. Moreover, *N. oculata* (0.1 or 0.2%), improved the quality of the cholesterol by increasing the DHL. These outcomes may be explained by the fact that *N. oculata* contains polysaccharides that inhibit the intestinal absorption of total cholesterol and triacylglycerols (Niewold *et al.*, 2012). Using *N. oculata* in feed of challenged birds has improved HDL-C and decreased LDL-C, possibly due to its anti-hypercholesterolaemia impacts (Andrés *et al.*, 1992; Werman *et al.*, 2003; Komprda, 2012; Kagan *et al.*, 2014; Bendimerad-Benmokhtar *et al.*, 2017).

Serum enzymes (ALT, AST) in the current study had doubled values in G2-Ps in comparison to G1-Ng, reflecting the dangerous effect of the challenge on the liver. Over the course of the experiment, the serum levels of ALT and AST were reduced in *N. oculata* treated birds, indicating its protective effects on the liver cells. These findings are in agreement with previous research (Bhattacharyya & Mehta, 2012; Nacer *et al.*, 2020; Abd El-Hamid *et al.*, 2022; El-Hawy *et al.*, 2022). Serum urea readings were reduced after *N. oculata* intake in the current work, which is indicative of a beneficial effect on the kidneys as stated previously (Nuño *et al.*, 2013; Aboulthana *et al.*, 2018; Nacer *et al.*, 2020).

In this study, G1-Ng showed normal caecal histological architecture as reported by Cukrowska *et al.* (2017) and Clark and Mach (2017). The ultrastructure of the G2-Ps cecal wall revealed histopathological damage, that was consistent with previous studies (Teshfam & Rahbari, 2003; Dai *et al.*, 2005; Bashtar *et al.*, 2010). This damage could be attributed to protozoan-induced mechanical irritation (Singla *et al.*, 2000). The G3-Clo showed a lower number of coccidian developing stages in comparison to G2-Ps. These observations could be attributed to the ability of clopidol to destroy some enterocytes which, in turn, decrease its coccidiostatic activity (Hafeez *et al.*, 2022), also clopidol could increase cell death and rates of *Eimeria* infection of enterocytes (Picard *et al.*, 2013). There was a significant decrease in the number of developmental stages of *Eimeria* spp. and degenerated stages, as well as a normal mitochondrial appearance with mild or no cellular structural damage in G4-NOa and G5-NOb. Similar findings were recorded by Cerezuela *et al.* (2012). The ability of *N. oculata* to improve enterocyte performance and remarkably decline *Eimeria* developmental stages could be attributed to its ability to increase the number of intestinal macrophages (Reyes-Becerril *et al.*, 2013; Levine *et al.*, 2018).

E. tenella aldolase is an essential enzyme for *Eimeria* energy metabolism (Hu *et al.*, 2022). EtAMA1 and EtMIC3 were expressed at high levels in the sporozoite stage, facilitating their invasion into host cells (Jiang *et al.*, 2012; Chen *et al.*, 2021). In the current study, molecular docking revealed a higher binding affinity of *N. oculata* for *E. tenella* aldolase, EtAMA1, and EtMIC3, which hindered glucose metabolism, host cell adhesion, and invasion of *Eimeria*. Therefore, *E. tenella* aldolase, EtAMA1, and EtMIC3 have been considered potential drug targets.

In conclusion, administering 0.2% dried N. *oculata* powder in the diet of broilers challenged with 5×10^3 sporulated oocysts of *Eimeria* spp. from 1 day old had effects that were close to or better than those of clopidol in terms of improving clinical parameters, meat quality, clinicopathological parameters, and histological parameters. We recommend carrying out extensive experiments under field conditions with higher doses of oocyst challenge.

Acknowledgements

The authors would like to expresses sincere appreciation to Ass. Prof.: Mohamed Elbadawy (Department of Pharmacology, Faculty of Veterinary Medicine, Benha University), for his valuable support during the review and editing of the paper.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

The authors did not receive support from any organization for the submitted work.

ORCID

Marwa I. Abdel Haleem http://orcid.org/0000-0003-3161-661X
Shimaa N. Edris http://orcid.org/0000-0002-1703-0981
Hanan A.A. Taie http://orcid.org/0000-0002-2497-3611
Samah M. Abdel Gawad http://orcid.org/0000-0001-7027-9738
Sawsan S. Elbasuni http://orcid.org/0000-0002-2393-9948

References

Abdelghany, M.F., El-Sawy, H.B., Abd El-hameed, S.A.A., Khames, M.K., Abdel-Latif, H.M.R. & Naiel, M.A.E. (2020). Effects of dietary *Nannochloropsis oculata* on growth performance, serum biochemical parameters, immune responses, and resistance against *Aeromonas veronii* challenge in Nile tilapia (*Oreochromis niloticus*). *Fish & Shellfish Immunology*, 107, 277–288.

Abd El-Hack, M.E., Abdel-Moneim, A.-M.E., Shehata, A.M., Mesalam, N.M., Salem, H.M., El-Saadony, M.T. & El-Tarabily, K.A. (2023). Microalgae applications in poultry feed. In E. Jacob-Lopes, M. I. Queiroz, & L. Q. Zepka (Eds.), *Handbook of Food and Feed from Microalgae* (pp. 435–450). Santa Maria- Brazil: Academic press.

Abdel Haleem, M., Abdelnaser, A. & Ali, M. (2019). Effect of synbiotics and essential oils in ameliorating the negative drawbacks of necrotic enteritis and coccidiosis in broiler chicks. *Benha Veterinary Medical Journal*, 36, 187–198.

Abdel Haleem, M., Hassan, A. & Moustafa, M. (2019). Effect of using synbiotics and essential oils on performance parameters and immune response of necrotic enteritis challenged broiler chicks. *Benha Veterinary Medical Journal*, 36, 384–391.

Abd El-Hamid, I.S., Fouda, W.A., Shedeed, H.A., Mostafa, S.A., Elbaz, A.M., Bakr, S.A., Mosa, B.H., Morsy, A.S., Hasan, A.M. & Emam, K.R. (2022). Influence of microalgae *Nannochloropsis oculata* on blood constituents, reproductive performance and productivity in Hi-plus doe rabbits under North Sinai conditions in Egypt. *Journal of Animal Health and Production*, 10, 135–145.

Abdelnour, S.A., El-Saadony, M.T., Saghir, S.A.M., Abd El-Hack, M.E., Al-shargi, O.Y.A., Al-Gabri, N. & Salama, A. (2020). Mitigating negative impacts of heat stress in growing rabbits via dietary prodigiosin supplementation. *Livestock Science*, 240, 104220.

Aboulthana, W.M., El-Feky, A.M., Ibrahim, N.E., Sahu, R.K. & El-Sayed, A.E.K.B. (2018). Evaluation of the pancreatoprotective effect of *Nannochloropsis oculata* extract against streptozotocin-induced diabetes in rats. *Journal of Applied Pharmaceutical Science*, 8, 46–58.

Adjei-Mensah, B. & Atuahene, C.C. (2023). Avian coccidiosis and anticoccidial potential of garlic (*Allium sativum L.*) in broiler production: a review. *Journal of Applied Poultry Research*, 32, 100314.

Aebi, H. (1984). Catalase *in vitro*. In L. Packer (Ed.), *Methods in enzymology* (105th ed., pp. 121–126). Iowa: Academic press.

Akanbi, O.B. & Taiwo, V.O. (2020). The effect of a local isolate and Houghton strain of *Eimeria tenella* on clinical and growth parameters following challenge in chickens vaccinated with IMMUCOX® and LIVACOX® vaccines. *Journal of Parasitic Diseases*, 44, 395–402.

Al-Gawad, A.A., Mahdy, O.A., El-Massry, A.A.N. & Al-Aziz, M.S.A. (2012). Studies on coccidia of Egyptian Balady breed chickens. *Life Science Journal*, 9, 568–576.

Al-Hoqani, U., Young, R. & Purton, S. (2017). The biotechnological potential of *Nannochloropsis*. *Perspectives in Phycology*, 4, 1–15.

Ali, A.L.I.M., Khater, H.F., Seddiek, S.A. & Nada, M.A.I.O. (2015). Comparative efficacy of synbiotic and diclazuril on broilers experimentally infected with *Eimeria acervulina*. *Assiut Veterinary Medical Journal*, 61, 24–33.

Ali, A.M., Seddiek, S.A. & Khater, H.F. (2014). Effect of butyrate, clopidol and their combination on the performance of broilers infected with *Eimeria maxima*. *British Poultry Science*, 55, 474–482.

Allen, P.C., Danforth, H.D. & Augustine, P.C. (1998). Dietary modulation of avian coccidiosis. *International Journal for Parasitology*, 28, 1131–1140.

Andrés, M., Raúl, C., Luis, L. & Mariane, L. (1992). Evaluation of marine microalga *Nannochloropsis sp*. As a potential dietary supplement. Chemical, nutritional and short term toxicological evaluation in rats. *Nutrition Research*, 12, 1273–1284.

Aranceta, J. & Pérez-Rodrigo, C. (2012). Recommended dietary reference intakes, nutritional goals and dietary guidelines for fat and fatty acids: a systematic review. *British Journal of Nutrition*, 107, S8–S22.

Ball, S.J., Pittilo, M.R. & Snow, K.R. (2014). Observations on oocyst development of *Eimeria stiedai* in rabbits. *Acta Parasitologica*, 59, 544–547.

Bashtar, A.R., Abdel-Ghaffar, F., Al-Rasheid, K.A.S., Mehlhorn, H. & Al Nasr, I. (2010). Light microscopic study on *Eimeria* species infecting Japanese quails reared in Saudi Arabian farms. *Parasitology Research*, 107, 409–416.

Becker, E.W. (2007). Micro-algae as a source of protein. *Biotechnology Advances*, 25, 207–210.

Bendimerad-Benmokhtar, S., Bouanane, S., Merzouk, H., Baba Ahmed, F.Z. & Bendaoud, A. (2017). Effects of *Nannochloropsis* fed on serum and tissue lipids metabolism in obese offspring of overfed dams. *Current Nutrition & Food Science*, 15, 72–86.

Bhattacharyya, S. & Mehta, P. (2012). The hepatoprotective potential of Spirulina and vitamin C supplemention in cisplatin toxicity. *Food & Function*, 3, 164–169.

Bortoluzzi, C., Paras, K.L., Applegate, T.J. & Verocai, G.G. (2018). Comparison between McMaster and Mini-FLOTAC methods for the enumeration of *Eimeria maxima* oocysts in poultry excreta. *Veterinary Parasitology*, 254, 21–25.

Cartoni Mancinelli, A., Mattioli, S., Twining, C., Dal Bosco, A., Donoghue, A.M., Arsi, K., Angelucci, E., Chiattelli, D. & Castellini, C. (2022). Poultry meat and eggs as an alternative source of n-3 long-chain polyunsaturated fatty acids for human nutrition. *Nutrients*, 14, 1969.

Cerezuela, R., Fumanal, M., Tapia-Paniagua, S.T., Meseguer, J., Morinigo, M.Á. & Esteban, M.Á. (2012). Histological alterations and microbial ecology of the intestine in gilthead seabream (*Sparus aurata* L.) fed dietary probiotics and microalgae. *Cell and Tissue Research*, 350, 477–489.

Chen, W., Ma, C., Li, G., Jia, Z., Yang, X., Pan, X. & Ma, D. (2021). Specific EtMIC3-binding peptides inhibit *Eimeria tenella* sporozoites entry into host cells. *Veterinary Research*, 52, 24.

Chodová, D., Tůmová, E., Sládková, K., Langrová, I., Jankovská, I., Vadlejch, J., Čadková, Z., Krejčířová, R., Chodov, D., Tůmov, E., Ina, K., Adkov, S., Langrov, I., Jankovsk, I., Cadkov, Z. & Krej, R. (2018). Effects of subclinical *Eimeria tenella* infection on Pectoralis major muscle in broiler chickens Nano chloroposis. *Italian Journal of Animal Science*, 17, 18–21.

Clark, A. & Mach, N. (2017). The crosstalk between the gut microbiota and mitochondria during exercise. *Frontiers in Physiology*, 8.

Colla, L.M., Furlong, E.B. & Costa, J.A.V. (2007). Antioxidant properties of *Spirulina* (*Arthospira*) *platensis* cultivated under different temperatures and nitrogen regimes. *Brazilian Archives of Biology and Technology*, 50, 161–167.

Cukrowska, B., Sowińska, A., Bierła, J.B., Czarnowska, E., Rybak, A. & Grzybowska-Chlebowczyk, U. (2017). Intestinal epithelium, intraepithelial lymphocytes and the gut microbiota – key players in the pathogenesis of

celiac disease. *World Journal of Gastroenterology*, 23, 7505–7518.

Dai, Y., Liu, X., Liu, M. & Tao, J. (2005). The life cycle and pathogenicity of coccicium *Eimeria nocens* (Kotlán, 1933) in domestic goslings. *Journal of Parasitology*, 91, 1122–1126.

de Mesquita Souza Saraiva, M., Lim, K., do Monte, D.F.M., Givisiez, P.E.N., Alves, L.B.R., de Freitas Neto, O.C., Kariuki, S., Júnior, A.B., de Oliveira, C.J.B. & Gebreyes, W.A. (2022). Antimicrobial resistance in the globalized food chain: a One Health perspective applied to the poultry industry. *Brazilian Journal of Microbiology*, 53, 465–486.

Derner, R., Ohse, S., Villela, M., Carvalho, S. & Fett, R. (2006). Microalgas, produtos e aplicações Nanochloroposis. *SciELO Brasil*, 36.

Durmaz, Y. (2007). Vitamin E (α-tocopherol) production by the marine microalgae *Nannochloropsis oculata* (Eustigmatophyceae) in nitrogen limitation. *Aquaculture*, 272, 717–722.

El-Bahr, S., Shousha, S., Shehab, A., Khattab, W., Ahmed-Farid, O., Sabike, I., El-Garhy, O., Albokhadaim, I. & Albosadah, K. (2020). Effect of dietary microalgae on growth performance, profiles of amino and fatty acids, antioxidant status, and meat quality of broiler chickens. *Animals*, 10, 761.

El-bahr, S.M., Shousha, S., Khattab, W., Shehab, A., El-garhy, O., El-garhy, H., Mohamed, S., Ahmed-farid, O., Hamad, A. & Sabike, I. (2021). Impact of dietary betaine and metabolizable energy levels on profiles of proteins and lipids, bioenergetics, peroxidation and quality of meat in Japanese Quail. *Animals*, 11, 117.

El-Hawy, A.S., El-Bassiony, M.F., Abd El-Hamid, I.S., Shedeed, H.A., Fouda, W.A., Ali, S.A.M., Abd-Elazem, R.A.-E., Morsy, A.S. & Emam, K.R.S. (2022). Semen characteristics and blood metabolites of Hi-plus buck rabbits fed on microalgae *Nannochloropsis oculata* meal during the summer season. *World's Veterinary Journal*, 12, 449–458.

El-maksoud, A. (2014). Biochemical effect of coccidia infestation in laying hen. *Benha Veterinary Medical Journal*, 26, 127–133.

El-Sayed, M., El-Azzazi, F., Khalil, H. & El-Gayar, M. (2022). Using marine algae (Nannochloropsis oculata) as natural feed additives to improve reproductive performance in rabbits. *Journal of Animal, Poultry & Fish Production*, 11, 15–23.

Fossati, P. & Prencipe, L. (1982). Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clinical Chemistry*, 28, 2077–2080.

Fries-Craft, K., Meyer, M.M. & Bobeck, E.A. (2021). Algae-based feed ingredient protects intestinal health during Eimeria challenge and alters systemic immune responses with differential outcomes observed during acute feed restriction. *Poultry Science*, 100, 101369.

Georgieva, N.V., Koinarski, V. & Gadjeva, V. (2006). Antioxidant status during the course of *Eimeria tenella* infection in broiler chickens. *The Veterinary Journal*, 172, 488–492.

Gessner, D.K., Ringseis, R. & Eder, K. (2017). Potential of plant polyphenols to combat oxidative stress and inflammatory processes in farm animals. *Journal of Animal Physiology and Animal Nutrition*, 101, 605–628.

Glick, Z., & Joslyn, M. A. (1970). Effect of Tannic Acid and Related Compounds on the Absorption and Utilization of Proteins in the Rat. *The Journal of Nutrition*, 100, 516–520.

Habibi, H., Firouzi, S., Nili, H., Razavi, M., Asadi, S.L. & Daneshi, S. (2016). Anticoccidial effects of herbal extracts on *Eimeria tenella* infection in broiler chickens: *in vitro* and *in vivo* study. *Journal of Parasitic Diseases*, 40, 401–407.

Hafeez, M.A., Sattar, A., Aslam, F., Imran, M., Ashraf, K., Zia, R. & Mehdi, M.M. (2022). Effects of ibuprofen and clopidol alone and in combination on experimentally induced coccidiosis in broiler chickens. *Pakistan Journal of Zoology*, 54, 1369–1377.

Honikel, K. (1998). Reference methods for the assessment of physical characteristics of meat. *Meat Science*, 49, 447–457.

Honikel, K.O. & Hamm, R. (1994). Measurement of water-holding capacity and juiciness. In Pearson, A.M., Dutson, T.R. (Eds.), *Quality Attributes and their Measurement in Meat, Poultry and Fish Products.* Advances in Meat Research, (vol, 9. pp. 125–161). Boston, MA: Springer.

Hu, J., Sun, M., Qi, N., Abuzeid, A.M.I., Li, J., Cai, H., Lv, M., Lin, X., Liao, S. & Li, G. (2022). Inhibitory effect of morin on aldolase 2 from *Eimeria tenella*. *International Journal for Parasitology: Drugs and Drug Resistance*, 20, 1–10.

Huff-Lonergan, E. & Lonergan, S.M. (2005). Mechanisms of water-holding capacity of meat: the role of postmortem biochemical and structural changes. *Meat Science*, 71, 194–204.

Jiang, L., Lin, J., Han, H., Dong, H., Zhao, Q., Zhu, S. & Huang, B. (2012). Identification and characterization of *Eimeria tenella* apical membrane Antigen-1 (AMA1). *PLoS One*, 7, e41115.

Kadykalo, S., Roberts, T., Thompson, M., Wilson, J., Lang, M. & Espeisse, O. (2018). The value of anticoccidials for sustainable global poultry production. *International Journal of Antimicrobial Agents*, 51, 304–310.

Kagan, M.L. & Matulka, R.A. (2015). Safety assessment of the microalgae *Nannochloropsis oculata*. *Toxicology Reports*, 2, 617–623.

Kagan, M.L., Sullivan, D.W., Gad, S.C. & Ballou, C.M. (2014). Safety assessment of EPA-rich polar lipid oil produced from the microalgae *Nannochloropsis oculata*. *International Journal of Toxicology*, 33, 459–474.

Kalbe, C., Priepke, A., Nürnberg, G. & Dannenberger, D. (2019). Effects of long-term microalgae supplementation on muscle microstructure, meat quality and fatty acid composition in growing pigs. *Journal of Animal Physiology and Animal Nutrition*, 103, 574–582.

Kalkal, H., Kumar, P. & Vohra, S. (2021). Advances in control strategies and vaccine development of coccidiosis in poultry. *The Pharma Innovation Journal*, 10, 1084–1090.

Kang, M.J., Shin, M.S., Park, J.N. & Lee, S.S. (2005). The effects of polyunsaturated:saturated fatty acids ratios and peroxidisability index values of dietary fats on serum lipid profiles and hepatic enzyme activities in rats. *British Journal of Nutrition*, 94, 526–532.

Kei, S. (1978). Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. *Clinica Chimica Acta*, 90, 37–43.

Khan, I.A., Parker, N.B., Löhr, C.V. & Cherian, G. (2021). Docosahexaenoic acid (22:6 n-3)-rich microalgae along with methionine supplementation in broiler chickens: effects on production performance, breast muscle quality attributes, lipid profile, and incidence of white striping and myopathy. *Poultry Science*, 100, 865–874.

Kherraf, A., Tehami, W., Boufeldja, W., Yahla, I., Dra, G.A., Mansour, I.F.Z. & Benali, M. (2017). Determination of

the nutritional and functional metabolites of *Nanochloropsis gaditana* produced in Algeria and evaluation of its antioxidant activity. *South Asian Journal of Experimental Biology*, 7, 13–20.

Komprda, T. (2012). Eicosapentaenoic and docosahexaenoic acids as inflammation-modulating and lipid homeostasis influencing nutraceuticals: a review. *Journal of Functional Foods*, 4, 25–38.

Lacaz-Ruiz, R. (2003). Espirulina: estudos e trabalhos. *Microbiologia (Madrid, Spain)* (p. 296). São Paulo: Roca.

Levine, R., Horst, G., Tonda, R., Lumpkins, B. & Mathis, G. (2018). Evaluation of the effects of feeding dried algae containing beta-1,3-glucan on broilers challenged with Eimeria. *Poultry Science*, 97, 3494–3500.

Li, G.Q., Kanu, S., Xiao, S.M. & Xiang, F.Y. (2005). Responses of chickens vaccinated with a live attenuated multi-valent ionophore-tolerant *Eimeria* vaccine. *Veterinary Parasitology*, 129, 179–186.

Lichtenthaler, H.K. & Buschmann, C. (2001). Chlorophylls and carotenoids measurement and UV-VIS characterization lichtenthaler 2001. *Current Protocols in Food Analytical Chemistry*, F4.3.1-F4., 1–8.

Liu, B., Jiang, J., Yu, D., Lin, G. & Xiong, Y.L. (2020). Effects of supplementation of microalgae (*Aurantiochytrium* sp.) to laying hen diets on fatty acid content, health lipid indices, oxidative stability, and quality attributes of Nano chloroposis. *Foods*, 9, 1271.

Long, S.F., Kang, S., Wang, Q.Q., Xu, Y.T., Pan, L., Hu, J.X., Li, M. & Piao, X.S. (2018). Dietary supplementation with DHA-rich microalgae improves performance, serum composition, carcass trait, antioxidant status, and fatty acid profile of broilers. *Poultry Science*, 97, 1881–1890.

Longstaffe, J.A. (1984). Helminths, arthropods and protozoa of domesticated animals (7th edition). *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 78, 329.

Lopes-Virella, M.F., Stone, P., Ellis, S. & Colwell, J.A. (1977). Cholesterol determination in high-density lipoproteins separated by three different methods. *Clinical Chemistry*, 23, 882–884.

Makkar, H.P.S., Becker, K., Abel, H. & Pawelzik, E. (1997). Nutrient contents, rumen protein degradability and antinutritional factors in some colour- and white-flowering cultivars of *Vicia faba* beans. *Journal of the Science of Food and Agriculture*, 75, 511–520.

Mamdouh, A.Z., Zahran, E., Mohamed, F. & Zaki, V. (2021). *Nannochloropsis oculata* feed additive alleviates mercuric chloride-induced toxicity in Nile tilapia (*Oreochromis niloticus*). *Aquatic Toxicology*, 238, 105936.

Md, A., Jin, F., Jeong, U.C., Choi, J.K., Lee, D.I., Yu, H.S. & Kang, S.J. (2018). Effects of *Nannochloropsis* concentration in diet on growth, survival and anti-inflammatory cytokine (interleukin-10) production of the sea cucumber *Apostichopus japonicus*. *Turkish Journal of Fisheries and Aquatic Sciences*, 18, 567–575.

Mir, N.A., Rafiq, A., Kumar, F., Singh, V. & Shukla, V. (2017). Determinants of broiler chicken meat quality and factors affecting them: a review. *Journal of Food Science and Technology*, 54, 2997–3009.

Molendi-Coste, O., Legry, V., & Leclercq, I. A. (2011). Why and How Meet n-3 PUFA Dietary Recommendations? *Gastroenterology Research and Practice*, 2011, 1–11.

Morehouse, N. F., & Baron, R. R. (1970). Coccidiosis: Evaluation of coccidiostats by mortality, weight gains, and fecal scores. *Experimental Parasitology*, *28*(1), 25–29. https://doi.org/10.1016/0014-4894(70)90062-7

Moryani, A.A., Rajput, N., Naeem, M., Shah, A.H. & Jahejo, A.R. (2021). Screening of the herbs and evaluation of their combined effects on the health and immunity of coccidiosis challenged broiler chickens. *Pakistan Veterinary Journal*, 41, 228–234.

Mund, M., Khan, U., Tahir, U. & Mustafa, B. (2017). Antimicrobial drug residues in poultry products and implications on public health: a review. *International Journal of Food Properties*, 20, 1433–1446.

Nacer, W., Baba Ahmed, F.Z., Merzouk, H., Benyagoub, O. & Bouanane, S. (2020). Evaluation of the anti-inflammatory and antioxidant effects of the microalgae *Nannochloropsis gaditana* in streptozotocin-induced diabetic rats. *Journal of Diabetes & Metabolic Disorders*, 19, 1483–1490.

Niewold, T.A., Schroyen, M., Geens, M.M., Verhelst, R.S.B. & Courtin, C.M. (2012). Dietary inclusion of arabinoxylan oligosaccharides (AXOS) down regulates mucosal responses to a bacterial challenge in a piglet model. *Journal of Functional Foods*, 4, 626–635.

Nishikimi, M., Appaji Rao, N. & Yagi, K. (1972). The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochemical and Biophysical Research Communications*, 46, 849–854.

NRC. (1994). *Nutrient requirements of poultry*, 9th edn. Washington, DC: National Academy Press.

Nuño, K., Villarruel-López, A., Puebla-Pérez, A.M., Romero-Velarde, E., Puebla-Mora, A.G. & Ascencio, F. (2013). Effects of the marine microalgae Isochrysis galbana and *Nannochloropsis oculata* in diabetic rats. *Journal of Functional Foods*, 5, 106–115.

Ordoñez, A.A.L., Gomez, J.D., Vattuone, M.A. & Isla, M.I. (2006). Antioxidant activities of *Sechium edule* (Jacq.) Swartz extracts. *Food Chemistry*, 97, 452–458.

Park, J.H., Lee, S.I. & Kim, I.H. (2018). Effect of dietary *Spirulina* (*Arthrospira*) *platensis* on the growth performance, antioxidant enzyme activity, nutrient digestibility, cecal microflora, excreta noxious gas emission, and breast meat quality of broiler chickens. *Poultry Science*, 97, 2451–2459.

Paterson, S., Gómez-Cortés, P., de la Fuente, M. A. & Hernández-Ledesma, B. (2023). Bioactivity and digestibility of microalgae *Tetraselmis* sp. and *Nannochloropsis* sp. as basis of their potential as novel functional foods. *Nutrients*, 15, 477.

Picard, M., Shirihai, O.S., Gentil, B.J. & Burelle, Y. (2013). Mitochondrial morphology transitions and functions: implications for retrograde signaling? *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 304, R393–R406.

Pop, L. M., Varga, E., Coroian, M., Nedisan, M. E., Mircean, V., Dumitrache, M. O., Farczádi, L., Fülöp, I., Croitoru, M. D., Fazakas, M., & Gyorke, A. (2019). Efficacy of a commercial herbal formula in chicken experimental coccidiosis. *Parasites and Vectors*, *12*(1). https://doi.org/10.1186/S13071-019-3595-4

Qaid, M. M., Al-Mufarrej, S. I., Azzam, M. M., Al-Garadi, M. A., Alqhtani, A. H., Fazea, E. H., Suliman, G. M., & Alhidary, I. A. (2021). Effect of rumex nervosus leaf powder on the breast meat quality, carcass traits, and performance indices of eimeria tenella oocyst-infected broiler chickens Nano chloroposis. *Mdpi.Com*. https://doi.org/10.3390/ani11061551

Rajput, N., Ali, S., Naeem, M., Khan, M.A. & Wang, T. (2014). The effect of dietary supplementation with the natural carotenoids curcumin and lutein on pigmentation, oxidative stability and quality of meat from broiler

chickens affected by a coccidiosis challenge. *British Poultry Science*, 55, 501–509.

Reid, A.J., Blake, D.P., Ansari, H.R., Billington, K., Browne, H.P., Bryant, J., Dunn, M., Hung, S.S., Kawahara, F., Miranda-Saavedra, D., Malas, T.B., Mourier, T., Naghra, H., Nair, M., Otto, T.D., Rawlings, N.D., Rivailler, P., Sanchez-Flores, A., Sanders, M., Subramaniam, C., Tay, Y.-L., Woo, Y., Wu, X., Barrell, B., Dear, P.H., Doerig, C., Gruber, A., Ivens, A.C., Parkinson, J., Rajandream, M.-A., Shirley, M.W., Wan, K.-L., Berriman, M., Tomley, F.M. & Pain, A. (2014). Genomic analysis of the causative agents of coccidiosis in domestic chickens. *Genome Research*, 24, 1676–1685.

Reyes-Becerril, M., Guardiola, F., Rojas, M., Ascencio-Valle, F. & Esteban, M.Á. (2013). Dietary administration of microalgae *Navicula* sp. affects immune status and gene expression of gilthead seabream (Sparus aurata). *Fish & Shellfish Immunology*, 35, 883–889.

Ribeiro, D.M., Bandarrinha, J., Nanni, P., Alves, S.P., Martins, C.F., Bessa, R.J.B., Falcão-e-Cunha, L. & Almeida, A.M. (2020). The effect of *Nannochloropsis oceanica* feed inclusion on rabbit muscle proteome. *Journal of Proteomics*, 222, 103783.

Richmond, W. (1973). Preparation and properties of a cholesterol oxidase from *Nocardia* sp. and its application to the enzymatic assay of total cholesterol in serum. *Clinical Chemistry*, 19, 1350–1356.

Saadaoui, I., Rasheed, R., Aguilar, A., Cherif, M., Al Jabri, H., Sayadi, S. & Manning, S.R. (2021). Microalgal-based feed: promising alternative feedstocks for livestock and poultry production. *Journal of Animal Science and Biotechnology*, 12, 76.

Saeed, Z. & Alkheraije, K.A. (2023). Botanicals: a promising approach for controlling cecal coccidiosis in poultry. *Frontiers in Veterinary Science*, 10, 1–13.

Šefcová, M.A., Santacruz, F., Larrea-álvarez, C.M., Vinueza-Burgos, C., Ortega-Paredes, D., Molina-Cuasapaz, G., Rodríguez, J., Calero-Cáceres, W., Revajová, V., Fernández-Moreira, E. & Larrea-álvarez, M. (2021). Administration of dietary microalgae ameliorates intestinal parameters, improves body weight, and reduces thawing loss of fillets in broiler chickens: a pilot study. *Animals*, 11, 3601.

Silva, D.R.G., de Moura, A.P.R., Ramos, A.L.S. & Ramos, E.M. (2017). Comparison of Warner-Bratzler shear force values between round and square cross-section cores for assessment of beef longissimus tenderness. *Meat Science*, 125, 102–105.

Singla, L., Juyal, P. & Sandhu, B.S. (2000). Pathology and therapy in naturally *Eimeria stiedae*-infected rabbits. *The Journal of Protozoology Research*, 10, 185–191.

Sun, Q., Chen, Q., Xia, X., Kong, B. & Diao, X. (2019). Effects of ultrasound-assisted freezing at different power levels on the structure and thermal stability of common carp (*Cyprinus carpio*) proteins. *Ultrasonics Sonochemistry*, 54, 311–320.

Sundar, S.T.B., Harikrishnan, T.J., Latha, B.R., Chundra, G.S. & Kumar, T.M.A.S. (2017). Anticoccidial drug resistance in chicken coccidiosis and promising solutions: a review. *Journal of Entomology and Zoology Studies*, 5, 1526–1529.

Swayne, D.E., Boulianne, M., Logue, C.M., Mcdougald, L.R., Nair, V. & Suarez, D.L. (2020). *Diseases of poultry*, 14th edn, Vol. 2. Wiley-Blackwell.

Teshfam, M. & Rahbari, S. (2003). Alteration in small intestinal structure induced by efi 2xperimental subclinical coccidiosis in chicken. *Journal of Applied Animal Research*, 24, 33–39.

Tovar, D., Zambonino, J., Cahu, C., Gatesoupe, F., Vázquez-Juárez, R. & Lésel, R. (2002). Effect of live yeast incorporation in compound diet on digestive enzyme activity in sea bass (Dicentrarchus labrax) larvae. *Aquaculture*, 204, 113–123.

Uhlířová, L., Tůmová, E., Chodová, D., Vlčková, J., Ketta, M., Volek, Z. & Skřivanová, V. (2018). The effect of age, genotype and sex on carcass traits, meat quality and sensory attributes of geese. *Asian-Australasian Journal of Animal Sciences*, 31, 421–428.

Vilar, S., Cozza, G., & Moro, S. (2008). Medicinal Chemistry and the Molecular Operating Environment (MOE): Application of QSAR and Molecular Docking to Drug Discovery. *Current Topics in Medicinal Chemistry,* 8, 1555–1572.

Werman, M., Sukenik, A. & Mokady, S. (2003). Effects of the marine unicellular alga *Nannochloropsis* sp. to reduce the plasma and liver cholesterol levels in male rats fed on diets with cholesterol. *Bioscience, Biotechnology and Biochemistry*, 67, 2266–2268.

Wieland, H. & Seidel, D. (1983). A simple specific method for precipitation of low density lipoproteins. *Journal of Lipid Research*, 24, 904–909.

Xia, X., Kong, B., Liu, Q., & Liu, J. (2009). Physicochemical change and protein oxidation in porcine longissimus dorsi as influenced by different freeze–thaw cycles. *Meat Science*, 83, 239–245.

Xia, X., Kong, B., Liu, J., Diao, X. & Liu, Q. (2012). Influence of different thawing methods on physicochemical changes and protein oxidation of porcine longissimus muscle. *LWT - Food Science and Technology*, 46, 280–286.

Young, D.S. (1997). Effects of drugs on clinical laboratory tests. *Annals of Clinical Biochemistry: International Journal of Laboratory Medicine*, 34, 579–581.

Zaheer, T., Abbas, R.Z., Imran, M., Abbas, A., Butt, A., Aslam, S. & Ahmad, J. (2022). Vaccines against chicken coccidiosis with particular reference to previous decade: progress, challenges, and opportunities. *Parasitology Research*, 121, 2749–2763.

Zanella, L. & Vianello, F. (2020). Microalgae of the genus *Nannochloropsis:* chemical composition and functional implications for human nutrition. *Journal of Functional Foods*, 68, 103919.