



OPEN

Impacts of algae supplements (*Arthrospira* & *Chlorella*) on growth, nutrient variables, intestinal efficacy, and antioxidants in New Zealand white rabbits

Mohammed F. El Basuni^{1,2✉}, Ahmed A. A. Khattab¹, Salma H. Abu Hafsa³, Islam I. Teiba¹, Nabila E. M. Elkassas⁴, Emad H. El-Bilawy², Mahmoud A. O. Dawood^{5,6} & Safaa Elsayed Salah Atia¹

An 8-week trial to examine the impacts of *Arthrospira platensis* and *Chlorella vulgaris* on the growth, nutrient aspects, intestinal efficacy, and antioxidants of 75 New Zealand white male rabbits (initial body weight = 665.93 ± 15.18 g). Herein the study was designed in one-way ANOVA to compare the effects of the two algae species with two levels of supplementations in the feeds of New Zealand white rabbits. The rabbits were divided into five groups (n = 15/group), where the first group was allocated as the control group (Ctrl) while the second and third groups received *A. platensis* at 300 or 500 mg/kg diet (Ap300 or Ap500). The fourth and fifth groups fed *C. vulgaris* at 300 or 500 mg/kg diet (Ch300 or Ch500). The basal diet rabbits exhibited the lowest values of weight, lipase, protease, and the highest feed conversion ratio, which improved noticeably with algae addition, particularly with Ap500, Ch300, and Ch500. All tested groups showed normal intestinal structure. Amylase potency, hematological indicators, and serum biochemistry revealed non-significant variation except for a higher serum total protein and lower total cholesterol in algal groups. The best GPx existed in groups fed algal diets, while favorable SOD and CAT efficiency occurred at the higher level of *Arthrospira* and both levels of *Chlorella*. In conclusion, incorporating *Arthrospira* or *Chlorella* in the diet of New Zealand white rabbits improved performance, nutrient utilization, intestinal efficacy, and antioxidants. *Arthrospira* (Ap500) and *Chlorella* (Ch300 or Ch500) have almost the same beneficial effect on rabbit performance.

Rabbits are one of the most profitable agricultural sectors involved in offering high-quality animal products with distinct merits, remarkably rapid growth, and sexual maturity with high fertility as well as a high gain of meat in carcass¹. Finding promising tactics to ameliorate organisms' wellbeing and performance is vital for animal production sectors, specifically under stressful circumstances, and the foundation of success is efficient management^{2,3}. Antibiotics were broadly employed as growth promoters, stress relievers, and remedies⁴. The usage of antibiotics as growth stimulants in animal production has been banned since 2006 within the European Union⁵. Antibiotics and other synthetic substances have been phased out in favor of more ecologically friendly methods for enhancing animal health, performance, and, eventually, ensuring the safety and superior quality of animal products⁶.

The foundation for good growth is a well-balanced diet, and one of the most effective approaches to altering an animal's growth is to modify the diet⁷. Utilizing functional feed supplements has become a commonly recognized approach for boosting the performance of animals⁸. Algae has the leverage to be a sustainable fountain

¹Faculty of Agriculture, Tanta University, Tanta 31527, Egypt. ²King Salman International University, South Sinai 46618, Egypt. ³Livestock Research Department, Arid Lands Cultivation Research Institute, City of Scientific Research and Technological Applications, New Borg El-Arab 21934, Alexandria, Egypt. ⁴Animal Production Research Institute, Agricultural Research Center, Dokki, Giza, Egypt. ⁵Department of Animal Production, Faculty of Agriculture, Kafrelsheikh University, Kafr El-Sheikh 33516, Egypt. ⁶The Center for Applied Research On the Environment and Sustainability, The American University in Cairo, Cairo 11835, Egypt. ✉email: mohamed.elbasuni@agr.tanta.edu.eg

of food and energy in the future. The majority of microalgal constituents are carbohydrates, lipids, proteins, minerals, vitamins, and bioactive substances⁹. Algal products in the diet of animals have been demonstrated to boost performance and meat goodness in ruminants and nonruminants. These results are greatly reliant on the form of microalgae and their level in the diet⁹.

Arthrospira (formerly *Spirulina*) and *Chlorella* are the two genera of algae that warrant a more thorough examination for nutritional purposes. Most microalgal protein fractions are stated to have the same or even better quality than typical plant protein fractions¹⁰. *Arthrospira* is well-known as a high-protein basis (60–70% of dry weight) with a high digestibility coefficient, with all essential amino acids accounting for about half of total protein¹¹, essential fatty acids¹², phytopigments (carotene—phycocyanin—phycocyanobilin chlorophyll and xanthophyll)^{13,14}, water and lipid-soluble vitamins (B group, ascorbic acid, A, D, E, K) as well as minerals (Ca, Cr, Cu, Fe, K, Na, P, Se, Zn)¹⁵. Dry *Chlorella* has a 50–60% protein content, making it comparable to other sources, e.g., yeast, soy flour, and milk¹⁶. Also, *Chlorella* biomass provides basic nutrient, pigments, minerals, vitamins, and provitamins¹⁷. Moreover, dry *Arthrospira* and *Chlorella* microalgae contain a significant portion of lipids (up to 80%) and carbohydrates (12–57%)¹⁶. *Arthrospira* and *Chlorella* have been proposed as primary ingredients or dietary supplements to enhance the performance and health of animals. In this sense, rabbits treated with *Arthrospira* exhibited higher growth^{18–23}, meat quality^{18,24}, reproductive performance²⁵, immunity^{18,19,26,27}, and antioxidants^{18,19,21,28,29}. Likewise, rabbits treated with *Chlorella* showed better growth^{30–32}, immunity^{30,33}, and antioxidants^{30,32}.

Considering the high nutritional value of algae, the purpose of the current trial was to contrast the impacts of dry *Chlorella vulgaris* and *Arthrospira platensis* as dietary supplements on the growth, nutrient efficiency, intestinal health, blood indices, and antioxidant capacity in New Zealand white rabbits.

Materials and methods

Isolation of algal species. Two algal species were used in this study, namely, *Chlorella vulgaris* and *Arthrospira platensis*. The green alga *Chlorella vulgaris* was isolated from a site in the Damietta branch (Drainage of sewage Omar Buck for 10 km in the city of Mansoura), while the cyanobacterial *Arthrospira platensis* species was isolated from wadi-elnatron brackish ponds. The isolated algae were developed primarily in a 250-ml Erlenmeyer conical container comprising 100 ml of growth media. For the growth of *Chlorella vulgaris*, Bold's Basal Medium (BBM) with a final pH of 6.3 was used, while *A. platensis* was enriched in spirulina medium. Unialgal strains were acquired by picking up the clonal population from an algal medium agar plate which was obtained by serial dilution of the primary inoculum.

Morphological Identification of algal species. The isolated algal species were identified morphologically according to features described by Deyab et al.³⁴ using a Zeiss (Axiolab 5) light microscope. For more accurate morphological characterization, the isolated species were examined using a JEOL JSM 6510 scanning electron microscope (Figs. 1 and 2).

Cultivation of algal species. All culture media were incubated on an orbital shaker (130 rpm) at 25 ± 2 °C, with a light intensity of 1.2 Klux, and an illumination of 16:8 h for a week with continuous aeration. The separated algal species were grown in a 2L flask, each comprising 1000 ml of medium, and developed under similar circumstances for 21 days to achieve biomass. To obtain dried biomass, thin layers of wet biomass of both algal species were dehydrated using a Binder Hot oven at 60 °C for 12 h. The chemical content of the tested algae supplements was evaluated following standard analysis techniques³⁵.

Chemical profiling of the algal extract using GC–MS. For the extraction of algae, 1 g of freeze-dried biomass for each alga was extracted twice using 10 ml methanol according to Deyab et al.³⁴. To obtain cell-free supernatant, the extracts were centrifuged at 6000 rpm for 20 min, then concentrated using a rotary evaporator at 40 °C. The dried residues were redissolved using 3 ml of methylene chloride and kept at 4 °C until GC–

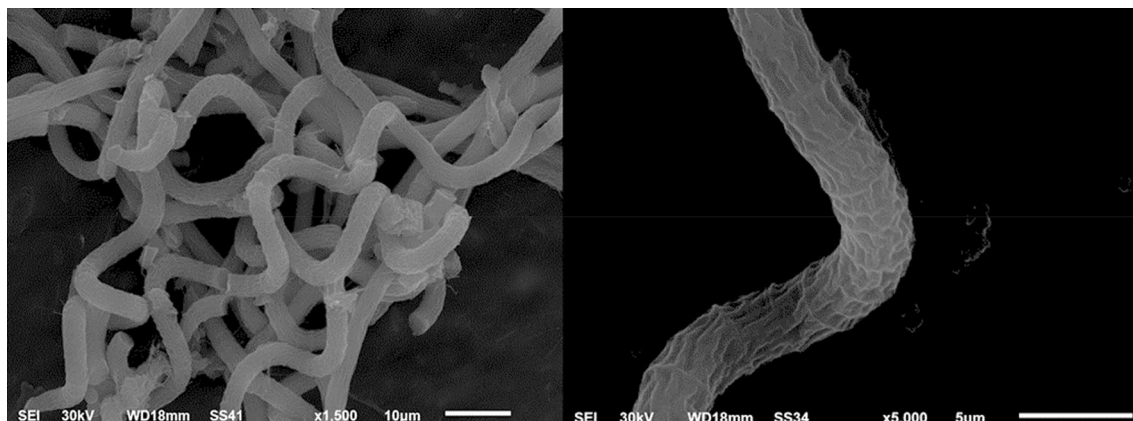


Figure 1. Electron micrograph of *Arthrospira platensis*.

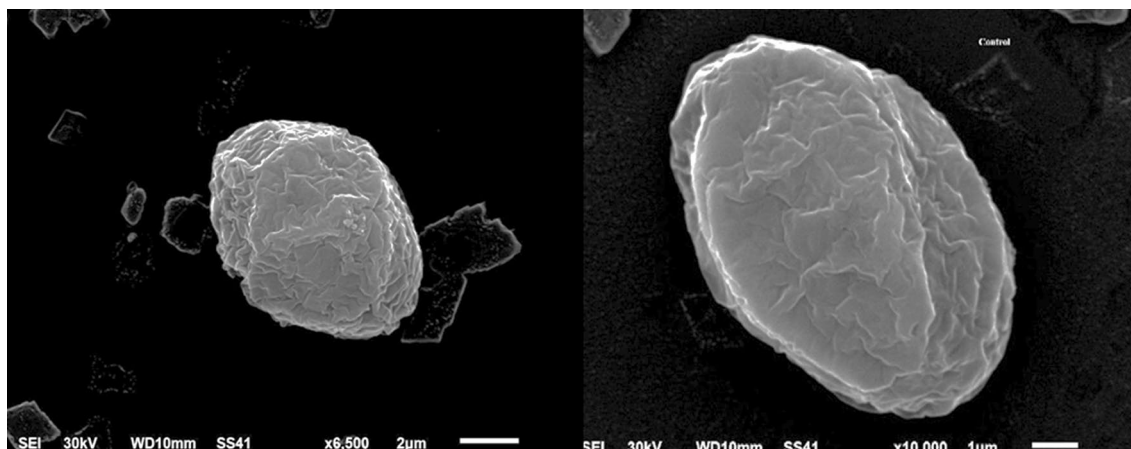


Figure 2. Electron micrograph of *Chlorella vulgaris*.

MS analysis. The crude extracts were analyzed using Varian GC–MS (Varian Chrompack CP-3800 GC/MS/MS-2000, Germany). The GC–MS was equipped with a split-splitless injector in addition to a DB-5.625 GC column (30 m × 0.25 mm i.d., 0.25 μm film thickness). The active chemical compounds were identified by matching their recorded spectra with the data bank mass spectra (Saturn and NIST library databases) provided by the instrument software. The concentration (% content) of the components of the extract was computed by integrating their peak areas in the total ion current (TIC) chromatograms, assuming a unity response by all components.

Animals and management. The experiment was conducted for 8 weeks from December 2020 to January 2021 on a private farm under the supervision of the Animal Production Department, Faculty of Agriculture at Tanta University in Egypt. The ethical committee of the Faculty of Agriculture at Tanta University approved the experimental protocol and all methods in the present study for treating animals for scientific purposes (Approval No. AY₂₀₁₉₋₂₀₂₀/Session 6/2020.01.13). All experiments were performed in accordance with relevant guidelines and regulations. Our reporting of research involving animals follows the recommendations of the ARRIVE guidelines. Seventy-five five weeks old New Zealand white male rabbits were chosen for litter weight at weaning (665.93 ± 15.18 g) and placed into five experimental groups (n = 15/group). All rabbits were maintained separately in galvanized wire pens (35 × 35 × 60 cm) with freely accessible feeders (ad libitum) and a freshwater outlet under the same management and hygienic conditions, namely a regimen of 12 h light and 12 h dark, natural ventilation, an average temperature of 17.29 ± 0.27 °C and a relative humidity of 59.96 ± 0.42 (Table 1).

The rabbits in the reference group (Ctrl) were fed a basal diet with no additions (Table 2), whereas the remainder of the groups were provided a basal diet with 300 or 500 mg of *Arthrospira platensis* (Ap300 or Ap500) or *Chlorella vulgaris* (Ch300 or Ch500).

Performance variables. The weight of the rabbits at the start and conclusion of the trial and the amount of feed consumed were recorded as follows:

$$\text{Weight gain, g/ rabbit} = W_T - W_0$$

Period (December–January)	Temperature °C			Humidity%
	Maximum	Minimum	Average	
1st week	23.29 ± 0.71	14.57 ± 0.20	18.93 ± 0.43	61.71 ± 0.87
2nd week	23.14 ± 0.86	14.57 ± 0.87	18.86 ± 0.68	62.29 ± 0.75
3rd week	21.29 ± 0.29	12.86 ± 0.40	17.07 ± 0.28	60.57 ± 0.65
4th week	20.43 ± 0.43	11.14 ± 0.70	15.79 ± 0.39	61.29 ± 0.71
5th week	21.57 ± 0.48	12.43 ± 0.87	17.00 ± 0.64	59.86 ± 1.06
6th week	24.29 ± 0.87	14.29 ± 0.57	19.29 ± 0.63	61.43 ± 0.78
7th week	20.29 ± 0.64	13.43 ± 0.65	16.86 ± 0.64	58.43 ± 0.84
8th week	19.43 ± 0.61	9.71 ± 0.42	14.57 ± 0.35	54.14 ± 0.59

Table 1. Maximum, minimum, average temperatures, and relative humidity during the experiment (December 2020–January 2021).

Ingredients	%	Chemical analysis	Dry Matter Basis
Yellow corn	18	Crude protein (CP)	17.3%
Soybean meal (44%)	15.1	Ether extract (EE)	2.31%
Barley grain	13	Crude fiber (CF)	13.52%
Berseem hay	33	Ash	3.71%
Wheat bran	16.5	DL-Methionine	0.4%
Molasses	2	Lysine	1.03%
Limestone	1.1	Calcium	0.94%
Dicalcium Phosphate	0.5	Total phosphorus	0.45%
Premix*	0.3	Digestible energy	2530 kcal/kg
DL-Methionine	0.1		
Common salt	0.4		
Total	100		

Table 2. The basal diet ingredients and chemical analysis. * Premix provided each kg of feed with Biotin = 0.05 mg; Choline = 250mh; Co = 0.1 mg; Cu = 5 mg; Fe = 50 mg; Folic acid = 3 mg; I = 0.2 mg; Mn = 85 mg; Niacin = 50 mg; Pantothenic acid = 10 mg; Se = 0.1 mg; Vitamin A = 6000 IU; Vitamin B1 = 2 mg; Vitamin B12 = 0.01 mg; Vitamin B2 = 4 mg; Vitamin B6 = 2 mg; Vitamin D3 = 900 IU; Vitamin E = 40 mg; Vitamin K3 = 2 mg; Zn = 50 mg.

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{FI (g)}}{W_T - W_0}$$

where W_T = Final weight; W_0 = Initial weight; FI = Feed intake.

Sampling procedure. After 8 weeks of feeding, 5 rabbits/group were allocated for blood collection and slaughter. Blood samples were drawn without anesthesia from the lateral saphenous superficial vein of the back leg after wetting the fur with alcohol using a 1 ml syringe with heparin for hematological measurements or without anticoagulants to separate the serum. Heparin-treated blood was employed for hematocrit (Ht) quantification using microhematocrit tubes and rotary centrifugation (13,000 rpm for 5 min)³⁶. Non-heparinized blood was centrifuged [3000 rpm undercooling (4 °C) for 10 min] to harvest serum. Hematological and biochemical blood indices were measured using CBC Micros ABX, France automatic analyzer with P500 kinetic & Quality control Diatron Q.C kits according to package guidelines. The liver and small intestine were separated on an ice layer, cleaned with regular saline solution (0.90%; pH 7.5), and subjected directly to the determination of hepatic antioxidants, intestinal structure, and digestive enzyme activities.

Intestinal enzymes and histology assessment. Parts of the collected intestine (duodenum) were finely homogenized in freezing iced NaCl (0.86%) using VEVOR, FSH-2A device, and centrifuged at 8000 rpm for 5 min, 4 °C. The filtrate was employed for the colorimetric detection of amylase and lipase at A_{714} and A_{540} ⁷. Protease potency was measured using a non-specific protease vigor methodology utilizing casein³⁷. For histological evaluation, samples (duodenum, jejunum, ileum) were fixed in a neutral buffered (10% formalin solution) for 72 h, dehydrated in rising grades of ethanol (60–100%), cleared in xylene, embedded in paraffin wax (24 h), and then sectioned with Rotary Microtome 2145, Leica Microsystems at a 3–5 µm in thickness.

Hepatic antioxidants. Liver samples (5 rabbits/treatment) were finely homogenized in cold iced potassium phosphate buffer (pH 7.4, 10% w/v) using VEVOR, FSH-2A device, and centrifuged at 4 °C, 12,000 rpm for 10 min. The filtrate was employed for the colorimetric detection (Jenway UV-Vis spectrophotometer 7415, Staffordshire, UK) of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) at 550, 280, 412 nm using Biodiagnostic and research reagents, Dokki, Giza, Egypt.

Statistical analysis. The study was designed in one-way ANOVA to compare the effects of the two algae species with two levels of supplementations in the feeds of New Zealand white rabbits. The rabbits were divided into five groups. The first group received a diet without either *A. platensis* or *C. vulgaris* (Control group, Ctrl). Conversely, the second group was given a diet with 300 mg/kg of *A. platensis* (Ap300), the third group a diet with 500 mg/kg of *A. platensis* (Ap500), the fourth group a diet with 300 mg/kg of *C. vulgaris* (Ch300), and the fifth group a diet with 500 mg/kg of *C. vulgaris* (Ch500). The data was examined using the IBM® SPSS® Inc., IL, USA program (IBM SPSS Statistics Ver. 26.0). The Shapiro–Wilk and Levene tests were employed to verify variance normality and homogeneity. The outcomes of the one-way ANOVA and Duncan's post hoc test were presented as a mean of three replicates with standard errors.

Approval for animal experiments. The ethical committee of the Faculty of agriculture at Tanta University approved the experimental protocol and all methods in the present study for treating animals for scientific purposes (Approval No. AY₂₀₁₉₋₂₀₂₀/Session 6/2020.01.13). All experiments were performed in accordance with relevant guidelines and regulations. Our reporting of research involving animals follows the recommendations of the ARRIVE guidelines.

Results

Chemical composition of the Algal supplements. *Arthrospira platensis* dry biomass comprises 56.4 ± 3.3 , 6.6 ± 0.6 , and $26.2 \pm 0.98\%$ of protein, lipids, and carbohydrates, compared to *Chlorella vulgaris*'s proportions of 43.6 ± 2.4 , 20.19 ± 1.2 , and $23.8 \pm 0.94\%$. A total of 25 active chemical compounds were characterized in the extracts of both algae. The identified chemical products with their retention time and % peak area of both extracts were shown in Table 3. The chromatograms of both extracts were shown in Fig. 3.

In general, the identified chemical compounds belong to seven major chemical groups, including esters, fatty acids, fatty alcohol, hydrocarbons, ketone, steroids, and terpenes. *Chlorella vulgaris* extract contains more esters (53.35%), fatty compounds (21.82), and hydrocarbons than *Arthrospira platensis* extract (31.00, 1.72, and 20.25, respectively). Meanwhile, *Arthrospira platensis* extract contains more ketone (19.96%), cholesterol (4.64%), and terpenes (20.24%) than *Chlorella vulgaris* extract (5.94, 0.00, and 5.92%, respectively).

Performance variables. Table 4 shows the growth and nutrient efficiency of New Zealand white rabbits fed experimental diets for 8 weeks. Rabbits fed the basal diet exhibited the lowest final weights and weight gains and the highest feed conversion ratio, which improved noticeably with algae addition, particularly with Ap500, Ch300, and Ch500. Feed intake did not change with treatments except for the high level of *Chlorella vulgaris* (Ch500), which showed the lowest FI value.

Intestinal efficiency. Figure 4 shows the intestinal structure of New Zealand white rabbits fed experimental diets for 8 weeks. All rabbit groups showed intact and normal intestinal structures with no pathological alterations such as degeneration, necrosis, hemolysis, edema, congestion, hemorrhages, and hypertrophy. The efficiency of intestinal enzymes is shown in Table 4. A remarkable enhancement in the efficiency of lipase and protease occurred in algal groups compared to the control, while the efficiency of amylase did not change between the experimental groups.

Blood health. Table 5 exhibits the blood profile of New Zealand white rabbits after 8 weeks of feeding trial. Hematological indicators comprising hematocrit (Ht), hemoglobin (Hb), red blood cells (RBCs), and white blood cells (WBCs) showed non-significant variation with dietary treatments. Similarly, serum biochemistry displayed no alteration in glucose, triglyceride, alanine transaminase (ALT), and aspartate transaminase, while a significant alteration occurred in total protein and total cholesterol. Rabbits treated with *Arthrospira* (Ap300 and Ap500) and *Chlorella* (Ch300 and Ch500) exhibited higher total protein and lower total cholesterol compared with the reference group. The lowest level ($P < 0.05$) of cholesterol was found in the blood of rabbits given a high level of *Arthrospira* (Ap500) and both levels of *Chlorella* (Ch300 and Ch500).

Hepatic antioxidants. Figure 5 displays the hepatic superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities in New Zealand white rabbits after 8 weeks of feeding trial. Rabbits fed the basal diet exhibited the poorest antioxidant potency (SOD, CAT, and GPx). The best GPx existed in all groups fed algal diets, while the favored SOD and CAT efficacy appeared at higher *Arthrospira* (Ap500) and both levels of *Chlorella* (Ch300 and Ch500).

Discussion

Stimulating the maximum production of animals necessitates specific procedures to ensure quantity, quality, and animal health⁷. Nutraceuticals feed additives in the animal production business as natural substitutes for antibiotics have risen in importance⁶. Rabbit production is an appropriate agricultural investment because of its low production costs, superior fertility, short generation intervals, and ability to utilize a range of forages¹.

Results of growth variables showed that rabbits fed the basal diet exhibited the poorest performance, which improved noticeably with algae addition, particularly with Ap500, Ch300, and Ch500 (Table 4). The high nutritional value of algae may be one of the reasons for the enhanced performance in animals fed with algae supplements. In this sense, Mahmoud et al.¹⁸ found that soybean substitution by *A. platensis* at levels of 20, 40, and 60% in rabbit feed did not show any negative outcomes and maintained indicators of growth, health, and meat quality. Furthermore, Seyidoglu et al.²⁶ found an enhancement in the immune system of growing rabbits with *A. platensis*-diets. In contrast, Gerencsér et al.³⁸ assumed that *Arthrospira* (5%) and thyme (3%), either alone or in combination, did not substantially alter the growth or health of growing rabbits. *Chlorella* has been suggested to boost the performance and health of animals^{17,30,39}. In a previous study, Hassanein et al.⁴⁰ compared the influence of *Arthrospira* (*Spirulina*) *platensis* and *Chlorella vulgaris* at levels of 0.75 and 1.5 g/kg diet on growing New Zealand white rabbits and concluded that both levels of *A. platensis* improved the growth and reduced liver enzyme, cholesterol and total lipids contents in serum in comparison with *Chlorella vulgaris* supplements. Moreover, An et al.³⁹ demonstrated that adding 0.15% dried *Chlorella vulgaris* powder to Ross broiler chicks' feed considerably

Biomass proximate composition	<i>Arthrospira platensis</i>	<i>Chlorella vulgaris</i>		
Protein	56.4 ± 3.3%	43.6 ± 2.4%		
Lipids	6.6 ± 0.6%	20.19 ± 1.2%		
Carbohydrates	26.2 ± 0.98%	23.8 ± 0.94%		
GC-MS profiling of the algal extract	Molecular formula	Retention time, Min	Peak Area %	
			<i>Arthrospira platensis</i>	<i>Chlorella vulgaris</i>
Esters				
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	C ₁₆ H ₂₂ O ₄	35.1	10.77	1.29
Pentadecanoic acid, 14-methyl-, methyl ester	C ₁₇ H ₃₄ O ₂	35.6	–	0.82
1,2-Benzenedicarboxylic acid, diisooctyl ester (Isooctyl phthalate)	C ₂₄ H ₃₈ O ₄	45.6	2.15	14.17
7-Hexadecenoic acid, methyl ester, (Z)-	C ₁₇ H ₃₂ O ₂	35.9	–	0.82
Hexadecanoic acid, methyl ester (Methyl palmitate)	C ₁₇ H ₃₄ O ₂	37	–	13.5
11-Octadecenoic acid, methyl ester	C ₁₉ H ₃₆ O ₂	38.8	–	0.45
Octadecanoic acid, 1-[(tetradecyloxy)carbonyl]pentadecyl ester	C ₄₈ H ₉₄ O ₄	25.1	2.15	22.3
Pentadecanoic acid, 14-methyl-, methyl ester	C ₁₇ H ₃₄ O ₂	35.3	6.46	–
Ethanoeperoxoic acid, 1-cyano-1-[2-(2-phenyl-1,3-dioxolan-2-yl)ethyl]pentyl ester	C ₁₉ H ₂₅ NO ₅	35.7	7.32	–
1,2-Benzenedicarboxylic acid, diisooctyl ester	C ₂₄ H ₃₈ O ₄	39.1	2.15	–
Total			31.00	53.35
Fatty acid: Pentanoic acid, 4-methyl- (Isocaproic Acid)	C ₆ H ₁₂ O ₂	26	–	15.42
Fatty Alcohol: 3-Methyl-2-(3-methylpentyl)-3-buten-1-ol	C ₅ H ₁₀ O	27.5	1.72	6.4
Total			1.72	21.82
Hydrocarbon				
Eicosane	C ₂₀ H ₄₂	32.5	1.29	–
4-Dodecene, (E)-	C ₁₂ H ₂₄	26.1	–	17.32
Pentadecane	C ₁₅ H ₃₂	33.8	–	6.29
Pentadecane, 7-methyl-	C ₁₆ H ₃₄	30.8	18.96	5.1
Total			20.25	28.71
Ketone				
Propiophenone, 2'-(trimethylsiloxy)-	C ₁₂ O ₁₈ O ₂ Si	21	1.72	–
1,4-Benzenediol, 2,6-bis(1,1-dimethylethyl)-	C ₁₄ H ₂₂ O ₂	18.2	13.94	–
Acetophenone, 2-chloro-	C ₈ H ₇ ClO	32	–	2.54
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	C ₁₇ H ₂₄ O ₃	33.4	4.3	3.4
Total			19.96	5.94
Steroids: Cholesterol	C ₂₇ H ₄₆ O	51.8	4.64	–
Terpenes				
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	34.7	10.34	4.72
Phytol	C ₂₀ H ₄₀ O	38.6	3.44	1.2
3',8,8'-Trimethoxy-3-piperidyl-2,2'-binaphthalene-1,1',4,4'-tetrone	C ₂₈ H ₂₅ NO ₇	46.7	–	–
Squalene	C ₃₀ H ₅₀	38.8	6.46	–
Total			20.24	5.92

Table 3. Chemical constituents of the algal biomass and extracts.

improved growth, blood cell counts, and declined total lipids in serum. According to Abdelnour et al.³⁰, adding 1.0 g of *Chlorella vulgaris* to the diet of growing New Zealand white rabbits could boost their immunological and antioxidant health, as well as reduce blood lipid accumulation. Despite these valuable results and to the authors' best knowledge, so far there are no planned studies that have compared the potentials of *Arthrospira* and *Chlorella* on intestinal histology, digestive enzyme potency, and hepatic antioxidants of New Zealand white rabbits. Consequently, the current trial was designed to cover these parameters.

Likewise, the improvement in weight with algae supplements may be linked to a change in the feed conversion ratio (↓ FCR). The detected reduction in FCR may be linked with the amended intestinal efficiency (Table 4), particularly digestive enzymes (lipase and protease). Several studies have demonstrated that adding algal biomass

or extracts improves growth and nutrient use. In *Arthrospira* impacts regard, Alazab et al.²⁰ found that adding *Spirulina platensis* (SP) to the diet of growing rabbits at a level of 0.6 g/kg diet resulted in considerably better growth performance parameters and enhanced feed conversion ratio in comparison to those provided the low level (0.3 g/kg diet) or those fed a basal diet. Moreover, Aladaileh et al.²¹ highlighted that exogenous supplementation of SP enhanced the growth traits of rabbits subjected to Pb. In addition, Peiretti and Meineri^{22,23} demonstrated that rabbits receiving *Arthrospira* at a level of 10% exhibited higher feed consumption. Regarding the effect of *Chlorella*, Sikiru et al.³¹ noted that dietary implementation of the *Chlorella vulgaris* amidst 200 and 500 mg/kg diet considerably raised the rabbits' weights without substantial alteration in feed intakes, but substantially enhanced feed to gain ratio. In another study by Sikiru et al.³² on New Zealand white rabbits, a significant positive boost in the final body weight and feed intake with the addition of *Chlorella vulgaris*. In contrast to the findings of the current study, no alteration was observed in the growth aspects with the dietary incorporation of *Arthrospira* (*Spirulina*)^{22–24,38} or *Chlorella*³⁰ and this may be due to the different conditions of the experiment.

Blood status is a precise sign of the welfare and health status of animals, hence are direct reflectors of stressors and external stimuli⁴¹. Hematological indicators and serum biochemistry showed non-significant variation except for serum total protein and total cholesterol (Table 5). Rabbits treated with *Arthrospira* and *Chlorella* had higher total protein (TP) and lower total cholesterol than the reference group. The higher levels of TP in rabbits fed algae may suggest an improvement in rabbit health. In this context, Hassan et al.¹⁹ reported an enrichment in plasma total protein in rabbits provided a diet enhanced with Zn-Se- rich *Spirulina* compared to the reference group. A similar improvement in glycoprotein appeared with *Chlorella* treatment³³. The hypocholesterolemic effect of algae could explain the lower cholesterol levels associated with supplementation. In line with the present results, Cheong et al.²⁷ suggested that spirulina consumption can reduce hypercholesterolemic atherosclerosis by lowering total serum cholesterol in New Zealand White rabbits. Also, Hassan et al.¹⁹ found low levels of total cholesterol, LDL- and VLDL-cholesterol in Se-rich *Spirulina* and Zn-Se- rich *Spirulina* groups of New Zealand White male rabbits. Similar impacts on cholesterol were reported with *Chlorella* incorporation. In this regard, Abdelnour et al.³⁰ found a reduction in serum VLDL in the *Chlorella*-treated groups relative to those in the control group.

The oxidative state of the animal is positively related to its immunity and wellbeing⁴². Oxidative stress is caused by an imbalance in the generation and clearance of reactive oxygen species (ROS)⁴³. Several enzymes in the oxidative system, such as SOD, CAT, and GPx aid in the elimination of ROS and the maintenance of cell homeostasis⁴⁴. In the present trial, algae dietary application mediates a substantial rise in SOD, CAT, and GPx activities. This may be due to the unique compositions of *Arthrospira* and *Chlorella* that are rich in effective compounds with an antioxidant impact, e.g., minerals, vitamins, β carotene, β -glucan, linolenic acid, tocopherols, phycocyanin, flavonoids, and phenols. Similar interpretations were reported for New Zealand White rabbits fed *Arthrospira* by Hassan et al.¹⁹ or *Chlorella* by Abdelnour et al.³⁰. Several studies have found enhanced antioxidant enzymes in rabbits fed *Arthrospira*^{21,28,29} and *Chlorella*^{30,32}.

The content of active chemicals in feed additives is mostly responsible for their beneficial effects. The overall results indicated that including *Chlorella* surpassed *Arthrospira* additives in New Zealand white rabbit feeds. These results indicated superior improvements in the growth performance, feed efficiency, and intestinal and blood health of New Zealand white rabbits fed *Chlorella*. These observations could be associated with the content of *Chlorella* and its effect on intestinal health and body immunity in rabbits. The GC–MS analysis of the crude extracts of the two algae showed the presence of 25 chemical substances with known favorable bioactivity on rabbits^{19–21,31} and humans⁴⁵. It is challenging to explain the effects of algae dietary supplements at the level of a single ingredient because algal extracts contain a significant number of active compounds and the best strategy is to classify them into major categories. *Arthrospira* exceeded *Chlorella* in its content of ketone, cholesterol, and terpenes, but *Chlorella* surpassed *Arthrospira* in its content of esters, fatty compounds, and hydrocarbon. Both extracts contain the majority of the active chemicals, albeit in different amounts which amply explain the convergence of the impacts supporting the performance and wellbeing of New Zealand white rabbits. In this context, phytol is a diterpene compound found in almost all crude extracts of the used algae and known for its anticancer and antioxidative properties⁴⁶. The hydrocarbon pentadecane and the fatty acid Pentanoic acid, 4- methyl- are known for their antimicrobial activity⁴⁷ and antitumor activity⁴⁸, as well as a growth promoter⁴⁹.

Conclusions

The present study sheds light on the potential of algae feed additives (*Arthrospira platensis* VS *Chlorella vulgaris*) on the performance and wellbeing of New Zealand white rabbits. Incorporating *Arthrospira platensis* at 500 mg/kg diet or *Chlorella vulgaris* at levels of 300 and 500 mg/kg diet improved growth, nutrient aspects, intestinal enzyme efficiency, blood health, and antioxidants of New Zealand white rabbits. It will be vital for rabbit production in the future to monitor molecular responses to external feeds and/or supplements and to concentrate on obtaining a precise nutritional formula for algal biomass in rabbit feeding without compromising performance or health.

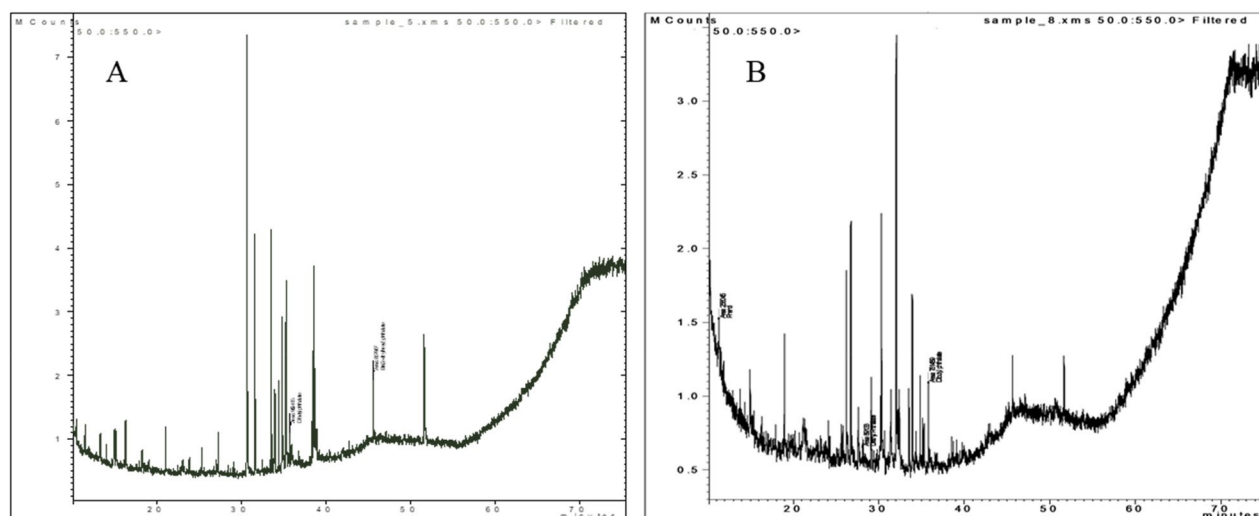


Figure 3. Chromatograms of Algal crude extracts. (A) *Arthrospira platensis* extract and (B) *Chlorella vulgaris* extract.

Parameters	Control	<i>Arthrospira platensis</i>		<i>Chlorella vulgaris</i>	
		Ap300	Ap500	Ch300	Ch500
Initial body weight, g/rabbit	662.67 ± 50.40	671.00 ± 32.45	649.33 ± 35.62	679.67 ± 16.80	667.00 ± 51.63
Final body weight, g/rabbit	1906.00 ± 77.66 ^b	2092.00 ± 57.36 ^{ab}	2236.33 ± 64.92 ^a	2148.67 ± 25.39 ^a	2205.00 ± 62.96 ^a
Body weight gain, g/rabbit	1243.33 ± 27.76 ^b	1421.00 ± 81.64 ^{ab}	1587.00 ± 49.56 ^a	1469.00 ± 41.47 ^{ab}	1538.00 ± 114.53 ^a
Feed intake, g/rabbit/56 day	6114.00 ± 66.11 ^a	6107.00 ± 55.42 ^a	5726.00 ± 109.02 ^{ab}	5891.67 ± 187.72 ^a	5385.33 ± 144.46 ^b
Feed conversion ratio	4.92 ± 0.7 ^a	4.32 ± 0.22 ^b	3.62 ± 0.17 ^c	4.01 ± 0.06 ^{bc}	3.55 ± 0.34 ^c
Amylase	19.44 ± 1.83	23.10 ± 1.49	22.71 ± 2.93	25.34 ± 3.06	21.56 ± 2.49
Lipase	17.71 ± 1.47 ^b	26.95 ± 1.57 ^a	26.33 ± 0.97 ^a	25.17 ± 2.11 ^a	25.90 ± 1.66 ^a
Protease	15.02 ± 2.07 ^b	28.43 ± 2.48 ^a	26.96 ± 2.58 ^a	26.88 ± 2.42 ^a	25.50 ± 2.15 ^a

Table 4. Growth, nutrient efficacy variables, and efficiency of intestinal enzymes of New Zealand white rabbits (initial weight = 665.93 ± 15.18 g) fed experimental diets for 8 weeks. Values are means ± standard errors. Numbers with unique letters vary statistically ($P < 0.05$).

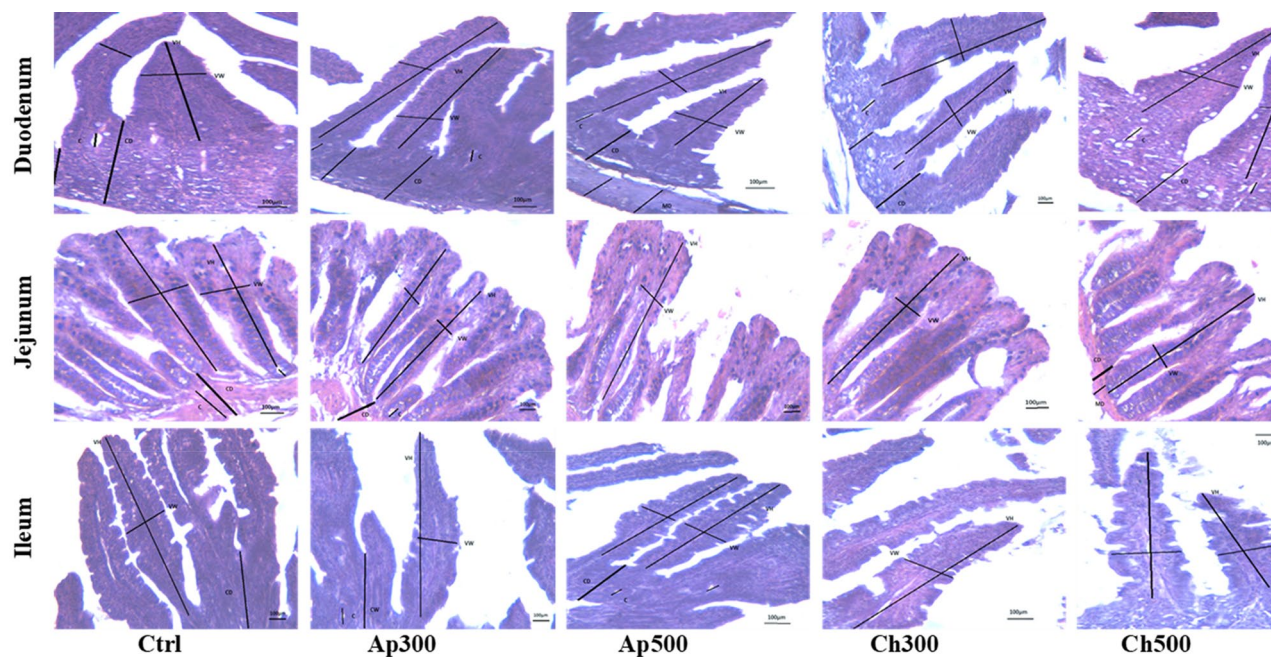


Figure 4. Intestinal structure (duodenum, jejunum, ileum, H&E = 40 X) of New Zealand white rabbits fed experimental diets for 8 weeks. Ctrl = the control group; Ap300 and Ap500 = *Arthrospira platensis* inclusion levels at 300 and 500 mg/kg; Ch300 and Ch500 = *Chlorella vulgaris* inclusion levels at 300 and 500 mg/kg.

Parameter	Control	<i>Arthrospira platensis</i>		<i>Chlorella sp.</i>	
		Ap300	Ap500	Ch300	Ch500
Hematocrit (Ht, %)	28.14 ± 3.76	28.75 ± 2.32	30.80 ± 3.00	30.70 ± 3.86	30.81 ± 2.09
Hemoglobin (Hb, g/dl)	6.78 ± 0.30	6.70 ± 0.56	6.67 ± 0.61	6.80 ± 0.42	6.75 ± 0.33
Red blood cells (RBCs, 10 ⁶ /μl)	1.83 ± 0.14	2.26 ± 0.13	2.33 ± 0.34	2.52 ± 0.32	1.95 ± 0.15
White blood cells (WBCs, 10 ³ /μl)	61.96 ± 5.09	81.89 ± 7.34	77.04 ± 6.70	75.03 ± 5.02	73.20 ± 5.05
Glucose (mg/dl)	95.10 ± 7.89	96.44 ± 9.19	74.98 ± 3.18	88.03 ± 16.58	91.30 ± 11.08
Total protein (TP, g/dl)	5.04 ± 0.23 ^b	6.65 ± 0.13 ^a	6.68 ± 0.26 ^a	6.58 ± 0.08 ^a	6.43 ± 0.14 ^a
Total cholesterol (T-Chol, mg/dl)	137.01 ± 3.66 ^a	119.86 ± 2.58 ^b	114.69 ± 4.29 ^{bc}	107.96 ± 2.05 ^c	105.85 ± 0.80 ^c
Triglyceride (mg/dl)	100.18 ± 3.21	102.73 ± 1.75	95.79 ± 6.47	97.64 ± 5.99	98.53 ± 8.30
Alanine transaminase (ALT, IU/l)	29.43 ± 2.70	32.91 ± 3.44	31.42 ± 2.81	33.29 ± 3.38	28.12 ± 3.18
Aspartate transaminase (AST, IU/l)	57.97 ± 4.92	61.92 ± 3.35	54.54 ± 7.60	59.80 ± 3.52	55.64 ± 4.46

Table 5. Blood profile of New Zealand white rabbits fed experimental diets for 8 weeks. Values are means ± standard errors (n = 3 /replicate). Numbers with various letters differ statistically ($P < 0.05$).

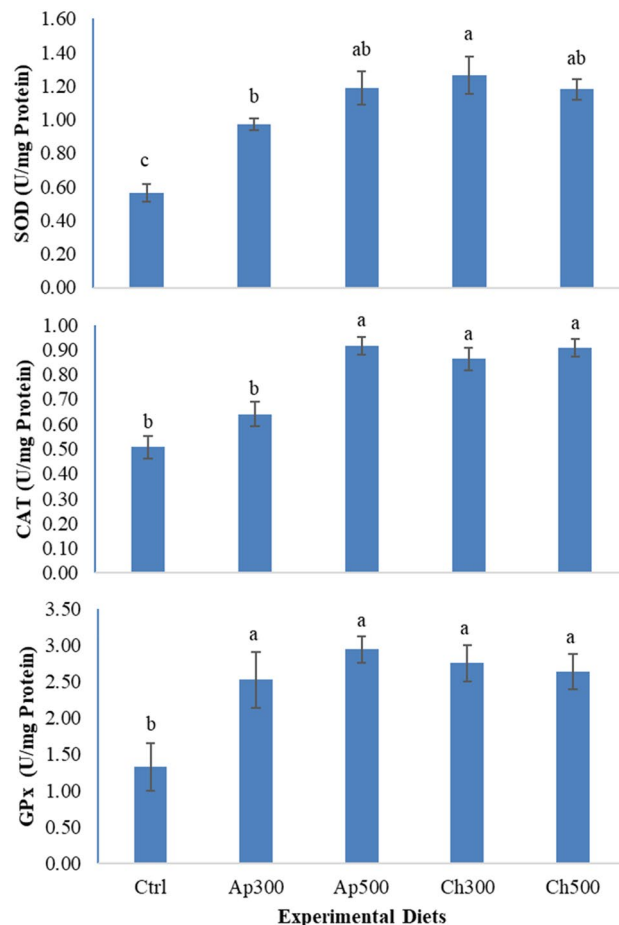


Figure 5. Hepatic superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities in New Zealand white rabbits fed experimental diets for 8 weeks. Ctrl = the control group; Ap300 and Ap500 = *Arthrospira platensis* inclusion levels at 300 and 500 mg/kg; Ch300 and Ch500 = *Chlorella vulgaris* inclusion levels at 300 and 500 mg/kg.

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Received: 14 December 2022; Accepted: 9 May 2023

Published online: 16 May 2023

References

- Gorlov, I. F., Semenova, I. A., Knyazhechenko, O. A., Mosolov, A. A. & Karpenko, E. V. Assessment of the impact of new complex feed additives in the production of rabbit meat. *IOP Conf. Ser. Earth Environ. Sci.* **548**, 82073 (2020).
- Oladimeji, A. M., Johnson, T. G., Metwally, K., Farghly, M. & Mahrose, K. M. Environmental heat stress in rabbits: implications and ameliorations. *Int. J. Biometeorol.* <https://doi.org/10.1007/s00484-021-02191-0> (2021).
- Yue, Z. *et al.* Vitamin A alleviates heat stress-induced damage to hair follicle development in Rex rabbits. *J. Sci. Food Agric.* **102**, 2291 (2021).
- Roth, N. *et al.* The application of antibiotics in broiler production and the resulting antibiotic resistance in *Escherichia coli*: A global overview. *Poult. Sci.* **98**, 1791–1804 (2019).
- Millet, S. & Maertens, L. The European ban on antibiotic growth promoters in animal feed: From challenges to opportunities. *Vet. J.* **187**, 143–144 (2011).
- Abd El-Hack, M. E. *et al.* Essential oils and their nanoemulsions as green alternatives to antibiotics in poultry nutrition: A comprehensive review. *Poult. Sci.* **101**, 101584 (2022).
- Khattab, A. A. A., El Basuini, M. F. M., El-Ratel, I. T. & Fouda, S. F. *Dietary probiotics as a strategy for improving growth performance, intestinal efficacy, immunity, and antioxidant capacity of white Pekin ducks fed with different levels of CP.* *Poultry Science* vol. 100 (Poultry Science Association Inc., 2021).
- Calik, A. *et al.* Dietary non-drug feed additive as an alternative for antibiotic growth promoters for broilers during a necrotic enteritis challenge. *Microorganisms* **7**, 257 (2019).
- Madeira, M. S. *et al.* Microalgae as feed ingredients for livestock production and meat quality: A review. *Livest. Sci.* **205**, 111–121 (2017).
- Becker, E. W. Micro-algae as a source of protein. *Biotechnol. Adv.* **25**, 207–210 (2007).

11. Gutiérrez-Salmeán, G., Fabila-Castillo, L. & Chamorro-Cevallos, G. Nutritional and toxicological aspects of Spirulina (Arthrospira). *Nutr. Hosp.* **32**, 34–40 (2015).
12. Mendes, R. L., Nobre, B. P., Cardoso, M. T., Pereira, A. P. & Palavra, A. F. Supercritical carbon dioxide extraction of compounds with pharmaceutical importance from microalgae. *Inorganica Chim. Acta* **356**, 328–334 (2003).
13. Gong, R., Ding, Y., Liu, H., Chen, Q. & Liu, Z. Lead biosorption and desorption by intact and pretreated spirulina maxima biomass. *Chemosphere* **58**, 125–130 (2005).
14. Bermejo, P., Piñero, E. & Villar, Á. M. Iron-chelating ability and antioxidant properties of phycocyanin isolated from a protean extract of Spirulinaplatisensis. *Food Chem.* **110**, 436–445 (2008).
15. Farag, M. R., Alagawany, M., Abd El-Hac, M. E. & Dhama, K. Nutritional and healthical aspects of Spirulina (Arthrospira) for poultry, animals and human. *Int. J. Pharmacol.* **12**, 36–51 (2015).
16. Chisti, Y. Biodiesel from microalgae. *Biotechnol. Adv.* **25**, 294–306 (2007).
17. Kotrbáček, V., Doubek, J. & Doucha, J. The chlorococcalean alga Chlorella in animal nutrition: a review. *J. Appl. Phycol.* **27**, 2173–2180 (2015).
18. Mahmoud, A. E., Naguib, M. M., Higazy, A. M., Sultan, Y. Y. & Marrez, D. A. Effect of Substitution Soybean by Blue Green Alga Spirulina platensis on Performance and Meat Quality of Growing Rabbits. *Am. J. Food Technol.* **12**, 51–59 (2016).
19. Hassan, F. *et al.* Zinc and/or selenium enriched spirulina as antioxidants in growing rabbit diets to alleviate the deleterious impacts of heat stress during summer season. *Animals* **11**, 756 (2021).
20. Alazab, A. M. *et al.* Effect of Spirulina platensis supplementation in growing Rabbit's diet on productive performance and economic efficiency. *J. Anim. Poult. Prod.* **11**, 325–330 (2020).
21. Aladaileh, S. H. *et al.* Spirulina platensis ameliorates the sub chronic toxicities of lead in rabbits via anti-oxidative, anti-inflammatory, and immune stimulatory properties. *Sci. Total Environ.* **701**, 134879 (2020).
22. Peiretti, P. G. & Meineri, G. Effects of diets with increasing levels of Spirulina platensis on the performance and apparent digestibility in growing rabbits. *Livest. Sci.* **118**, 173–177 (2008).
23. Peiretti, P. G. & Meineri, G. Effects of diets with increasing levels of Spirulina platensis on the carcass characteristics, meat quality and fatty acid composition of growing rabbits. *Livest. Sci.* **140**, 218–224 (2011).
24. Dalle Zotte, A., Sartori, A., Bohatir, P., Rémignon, H. & Ricci, R. Effect of dietary supplementation of Spirulina (Arthrospira platensis) and Thyme (Thymus vulgaris) on growth performance, apparent digestibility and health status of companion dwarf rabbits. *Livest. Sci.* **152**, 182–191 (2013).
25. Abadjieva, D., Shimkus, A., Shimkiene, A., Rashev, P. & Kistanova, E. Transgenerational beneficial effect of Arthrospira (Spirulina) platensis on the rabbit ovaries. *J. Appl. Phycol.* **30**, 1691–1700 (2018).
26. Seyidoglu, N., Galip, N., Budak, F. & Uzabaci, E. The effects of Spirulina platensis (Arthrospira platensis) and Saccharomyces cerevisiae on the distribution and cytokine production of CD4+ and CD8+ T-lymphocytes in rabbits. *Austral J. Vet. Sci.* **49**, 185–190 (2017).
27. Cheong, S. H. *et al.* Spirulina prevents atherosclerosis by reducing hypercholesterolemia in rabbits fed a high-cholesterol diet. *J. Nutr. Sci. Vitaminol. (Tokyo)* **56**, 34–40 (2010).
28. Abdelnour, S. A. *et al.* The beneficial impacts of dietary phycocyanin supplementation on growing rabbits under high ambient temperature. *Ital. J. Anim. Sci.* **19**, 1046–1056 (2020).
29. Sabry, M. M., Abdel-Rahman, R. F., El-Shenawy, S. M., Hassan, A. M. & El-Gayed, S. H. Estrogenic activity of Sage (Salvia officinalis L.) aerial parts and its isolated ferulic acid in immature ovariectomized female rats. *J. Ethnopharmacol.* **282**, 114579 (2022).
30. Abdelnour, *et al.* Impacts of enriching growing rabbit diets with chlorella vulgaris microalgae on growth, blood variables, carcass traits, immunological and antioxidant indices. *Animals* **9**, 788 (2019).
31. Sikiru, A. B., Arangasamy, A., Alemede, I. C., Egena, S. S. A. & Bhatta, R. Dietary supplementation effects of Chlorella vulgaris on performances, oxidative stress status and antioxidant enzymes activities of prepubertal New Zealand White rabbits. *Bull. Natl. Res. Cent.* **43**, 162 (2019).
32. Sikiru, A. B. *et al.* Chlorella vulgaris supplementation effects on performances, oxidative stress and antioxidant genes expression in liver and ovaries of New Zealand White rabbits. *Heliyon* **5**, e02470 (2019).
33. Lesyk, I. V., Fedoruk, R. S. & Dolaichuk, O. P. Immunobiological blood parameters in rabbits after addition to the diet suspensions of chlorella, sodium sulfate, citrate and chromium chloride. *Fiziol. Zh.* **59**, 78–84 (2013).
34. Deyab, M., Mofeed, J., El-Bilawy, E. & Ward, F. Antiviral activity of five filamentous cyanobacteria against coxsackievirus B3 and rotavirus. *Arch. Microbiol.* **202**, 213–223 (2020).
35. AOAC. *Official Methods of Analysis of AOAC International*. (Associations of Analytical Chemists, International, 2007).
36. Goldenfarb, P. B., Bowyer, F. P., Hall, E. & Brosious, E. Reproducibility in the hematology laboratory: the microhematocrit determination. *Am. J. Clin. Pathol.* **56**, 35–39 (1971).
37. Cupp-Enyard, C. Sigma's non-specific protease activity assay—Casein as a substrate. *J. Vis. Exp.* **899**, 1–3 (2008).
38. Gerencsér, Z. *et al.* Effect of dietary supplementation of spirulina (Arthrospira platensis) and thyme (Thymus vulgaris) on apparent digestibility and productive performance of growing rabbits. *World Rabbit Sci.* **22**, 1 (2014).
39. An, B.-K., Kim, K.-E., Jeon, J.-Y. & Lee, K. W. Effect of dried Chlorella vulgaris and Chlorella growth factor on growth performance, meat qualities and humoral immune responses in broiler chickens. *Springerplus* **5**, 718 (2016).
40. Hassanein, H., Arafa, M. M., Abo Warda, M. A. & Abd-Elall, A. Effect of Using Spirulina platensis And Chlorella vulgaris as Feed Additives on Growing Rabbit Performance. *Egypt. J. Rabbit Sci.* **24**, 413–431 (2014).
41. El-Tarabany, M. S., Ahmed-Farid, O. A. & El-Tarabany, A. A. Impact of space allowance on performance traits, brain neurotransmitters and blood antioxidant activity of New Zealand White rabbits. *Prev. Vet. Med.* **163**, 44–50 (2019).
42. El-Gindy, Y., Zeweil, H., Zahran, S., El-Rahman, M. A. & Eisa, F. Hematologic, lipid profile, immunity, and antioxidant status of growing rabbits fed black seed as natural antioxidants. *Trop. Anim. Health Prod.* **52**, 999–1004 (2020).
43. Lee, M. T., Lin, W. C. & Lee, T. T. Potential crosstalk of oxidative stress and immune response in poultry through phytochemicals—A review. *Asian-Australas. J. Anim. Sci.* **32**, 309–319 (2019).
44. Chauhan, S. S., Rashamol, V. P., Bagath, M., Sejian, V. & Dunshea, F. R. Impacts of heat stress on immune responses and oxidative stress in farm animals and nutritional strategies for amelioration. *Int. J. Biometeorol.* **65**, 1231–1244 (2021).
45. Wollina, U. *et al.* Arthrospira Platensis - Potential in Dermatology and Beyond. *Open Access Maced. J. Med. Sci.* **6**, 176–180 (2018).
46. Gliszczynska, A. *et al.* Synthesis of novel phytol-derived γ -butyrolactones and evaluation of their biological activity. *Sci. Rep.* **11**, 4262 (2021).
47. Omage, K., Azeke, M. A. & Omage, S. O. Evaluation of the efficacy of Acalypha wilkesiana leaves in managing cardiovascular disease risk factors in rabbits exposed to salt-loaded diets. *Clin. Phytoscience* **4**, 1 (2018).
48. Essien, E. E., Ogunwande, I. A., Setzer, W. N. & Ekundayo, O. Chemical composition, antimicrobial, and cytotoxicity studies on S. erianthum and S. macranthum essential oils. *Pharm. Biol.* **50**, 474–480 (2012).
49. Martignon, M., Burel, C., Cauquil, L., Combes, S. & Gidenne, T. Impact of feed restriction and fragmented feed distribution on performance, intake behaviour and digestion of the growing rabbit. *Animal* **15**, 100270 (2021).

Acknowledgements

The first author would like to thank all staff members of the Animal Production Department, Faculty of Agriculture, Tanta University, Egypt.

Author contributions

All authors contributed equally to this manuscript.

Funding

Open access funding provided by The Science, Technology & Innovation Funding Authority (STDF) in cooperation with The Egyptian Knowledge Bank (EKB). Open access funding is provided by The Science, Technology & Innovation Funding Authority (STDF) in cooperation with The Egyptian Knowledge Bank (EKB).

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to M.F.E.B.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2023